Modifying Donor Organ Retrieval and Preservation to Enhance Transplant Outcomes

A thesis submitted in fulfilment of the requirements of the University of Sydney for the degree of Doctor of Philosophy

AHMER HAMEED, BSc (Hons), MBBS (Hons), MS

Westmead Clinical School
Faculty of Medicine (Sydney Medical School)
The University of Sydney

Table of Contents

Statement of Originality

I declare that to the best of my knowledge, the content of this thesis is my own work, unless explicitly acknowledged. No aspect of this thesis has previously been submitted for any degree at this or other institutions. Assistance received in preparing any aspect of this thesis has been appropriately acknowledged.

AHMER MOHAMMAD HAMEED

Abstract

The last 1-2 decades have seen remarkable advances in organ procurement and preservation practices, especially with renewed enthusiasm for machine perfusion (MP) technology. However, cold static storage (CS) remains the most popular world-wide approach for the preservation of organs such as the kidneys, liver, and pancreas, largely due to its simplicity. It is clear that CS techniques have limited potential for further improvement, and will likely be supplanted and/or supplemented with MP technologies over the coming years due to the reparative, resuscitative, and assessment capabilities afforded by MP. This is especially important as we increase our utilisation of marginal and/or donation after circulatory death (DCD) organs to meet the ever-increasing demand requirements for transplantation.

This dissertation explores selected aspects of abdominal organ procurement and preservation as targets for improvement and/or modification with the aim to enhance recipient transplantation outcomes. The kidney is used as a model organ for the development and exploration of MP as a means to ameliorate transplant organ ischaemia-reperfusion injury (IRI), including through the targeted delivery of anti-IRI drugs. In contrast, the optimization of CS protocols, including identification of ideal perfusion fluids and *in situ* perfusion routes, forms the basis for liver and pancreas transplantation work in this thesis. Such investigations are necessary to promote uniformity of practice between centres, and allow appropriate comparisons between MP and CS.

The kidney MP work was guided by a systematic review and meta-analysis comparing MP and CS in the clinical and pre-clinical setting. Although hypothermic MP (HMP) was shown to enhance short-term graft outcomes, results were equivocal with respect to graft survival, especially in the DCD setting. Preliminary evidence indicated the potential superiority of normothermic MP (NMP) above HMP or CS, which may be further enhanced by using NMP as a conduit for directed drug delivery to the kidney to ameliorate IRI. We therefore developed and optimized a local NMP set-up using a series of porcine kidneys, which was then utilized to deliver the anti-IRI agent CD47-blocking antibody (αCD47Ab) in a porcine DCD model. The significant potential of this agent was initially confirmed by testing in a murine model of severe warm IRI, including its comparative efficacy to two other promising IRI agents, soluble complement receptor 1 (sCR1), and

recombinant thrombomodulin, and also sCR1 in combination with αCD47Ab. αCD47Ab was successfully delivered to porcine DCD kidneys using NMP, with subsequent downstream positive impacts upon renal perfusion, and some functional and IRI-related parameters.

The clinical utilisation of renal NMP has so far been limited to the UK, and this modality has not been tested in human kidneys in Australasia. Furthermore, the mechanistic basis of brief renal NMP is not entirely clear. Therefore, and as a prelude to a phase I clinical trial, NMP was tested in discarded deceased donor human kidneys. Fifteen kidneys were obtained from 10 donors, and successfully underwent NMP. NMP was especially effective for assessing and improving DCD kidneys discarded for poor macroscopic perfusion at retrieval. Flow cytometry analyses showed evidence of a massive passenger leukocyte efflux during NMP. In paired kidney analyses, one hour of NMP was shown to be superior to CS alone after simulated transplantation using *ex vivo* whole allogeneic blood reperfusion, in terms of renal perfusion and functional parameters. Whole transcriptome RNA sequencing revealed NMP-mediated induction of protective stress and inflammatory-related pathways, in addition to a reduction in cell death pathways. Accordingly, immunofluorescence techniques confirmed a reduction in cell death and IRI in NMP kidneys compared to their CS counterparts.

CS and procurement techniques formed the basis of liver and pancreas transplantation-related studies conducted for this thesis. Firstly, we showed that blood transfusion requirements can be significantly reduced in recipients if the pancreas is retrieved using ultrasonic shears (Harmonic Scalpel), implying a reduction in procedural risk and recipient sensitization. Two systematic reviews and meta-analyses were then conducted to ascertain optimal *in situ* perfusion/preservation fluids, and perfusion routes, during procurement of pancreatic and hepatic allografts. There was a lack of overwhelming evidence favouring any specific preservation fluid, although University of Wisconsin solution will likely remain the solution of choice, especially for the pancreas. Furthermore, in standard criteria donors, aortic-only perfusion was found to produce equivalent liver transplant outcomes in comparison to dual (aorto-portal perfusion). However, existing studies included small patient numbers and short periods of follow-up. We therefore compared aortic and dual perfusion during liver retrieval using the Australia and New Zealand Liver Transplant Registry, which provided a much larger patient cohort with prolonged follow-up. This study confirmed the equivalence of aortic-only and dual perfusion in standard criteria liver donors,

however there was also evidence indicating the superiority of dual perfusion in a subset of suboptimal/higher risk donors.

Overall, this thesis expounds upon the putative benefits of NMP in kidney transplantation, including by directed drug delivery targeting the IRI cascade, and also enhances our understanding of optimal perfusion routes and preservation fluids for the liver and pancreas. The ultimate aim is to facilitate expansion of the donor pool whilst simultaneously enhancing recipient transplantation outcomes through the evidence-based implementation of technologies and techniques in a unified and coordinated manner.

Acknowledgments

My PhD journey is perhaps a little unusual in this part of the world, having deferred my general surgical training immediately prior to its commencement to first pursue a higher research degree. However, I had known for quite some time that this was the path I wished to embark upon. Reaching the end has not been easy, and would not have been possible without the invaluable and unconditional support along the way from many important people in my life.

To Professors Hawthorne and Pleass, I cannot begin to describe or thank you enough for your help and support over these last few years. You both fulfil the roles of mentors in the truest sense of the word, and I would be lucky to achieve even half of what you have (although I could do without the hair loss!). Prof Hawthorne, you went out on a limb and took me on as your student, not knowing what to expect. You knew I had minimal laboratory experience, and no experience working with animals. Yet you stuck with me, guided me heavily to begin with, and then gave me enough autonomy to develop and pursue my own crazy ideas, usually at your own expense! Even though I did not know you personally before starting this PhD, I have never, ever regretted being under your supervision, and hope we can continue our friendship for many years to come. Prof Pleass, you have instilled in me a love for surgery and the desire to always be better for myself and our patients. I am always amazed by your kindness and willingness to teach, even if it is 4 a.m. in the northern-most tip of NSW and you haven't slept properly for days. It has truly been an honour to spend time with you and learn from you, not just about surgery but also humanity, and I doubt I will ever come across someone so skilled and caring, both in and outside of the operating theatre. I truly hope I live up to your expectations, and can make you proud.

To A/Professor Natasha Rogers, you entered my PhD a little later on in the journey, but have made my experience in the lab a true pleasure. Your passion for science is amazing. You always have new ideas, and aren't scared to pursue them. Without your help, especially my final year would not have been this smooth, and I would not have been able to finish all that I did in this time.

To Professor Vincent Lam, my research journey started after I met you as a medical student, knowing I was coming to Westmead as an intern and keen to get a head-start in surgery. Your

advice is worth its weight in gold, and without your ongoing support none of this would have been a reality. To Dr Muzib Abdul-Razak, you always kept me focused on the bigger picture, especially during all of our operating sessions together. Because of you, I know the value of hard work, and what it takes to be worthy of our patients' trust. To Dr Lawrence Yuen, I have always appreciated your skill, speed, and opportunities you have given me over these last few years, and am better for your teaching.

To A/Professor Greg O'Grady, you were the ideal role model for me as a junior doctor – a skilled operator, yet also a prolific researcher, and as such a true academic surgeon. I am grateful that we have managed to keep in touch, and cannot thank you enough for your help, especially for providing the basic hardware I needed to perform my machine perfusion research. To the other consultant surgeons that I have worked with over these few years, in particular Dr Ronald De Roo, A/Professor Jerome Laurence, Dr Tony Pang, Professor Richard Allen, Dr Brendan Ryan, and A/Professor Michael Hollands, thank you for all of your help and teaching. To the transplant surgical fellows and registrars, especially Dr Emma Tulley, Dr Hien Nguyen, Dr Nicholas Cocco, Dr Ian Ng, and Dr Peter Yoon, thank you for working with me and giving me your invaluable (and often frank) advice and friendship.

To the Royal Australasian College of Surgeons, and Sue Pleass, this work could not have been done without your funding support, and I hope you continue to support young surgeons with their research endeavours well into the future.

To Dr Bo Lu, your micro-surgical skills are unsurpassed, and I am indebted to your support and friendship over these few years, especially including our long political chats. To Renan Gaspi, Chris Zhang, and Paul Robertson, you have kept me up on many nights, but it was well worth it in order to spend time with all of you, and I can't think of better people to have to work with at any hour of the day.

To Dr Ross Matthews, and all animal care staff, especially Louie, Arturo, Michelle, Sharon, and Callista, thank you for your support with the extensive animal work I undertook over these last three years.

To Ray Miraziz, you have been extremely patient with me and your selfless advice is the reason the machine perfusion experiments worked at all, let alone as well as they did.

To A/Professor Germaine Wong, you are the most reassuring presence to have in a crowd when talking in front of hundreds of people. Thank you for your advice and support for many aspects of this PhD.

To staff of the Westmead Institute for Medical Research, in particular Virginia James, Dr Hong Yu, and Dr Suat Dervish, you ensured my lab work was up to standard, and offered essential assistance with histology, immunofluorescence, and 3D-printing.

To Dr Ellis Patrick, Hsiufen (Tanya) Chua, Bo Xu, and Joey Lai, thank you for your prompt and expert assistance with the genomic work. I would like to thank Dr Henry Marsh for generously donating sCR1 for my mouse experiments. I would also like to thank Dr Chow Heok P'ng, Dr John-Paul Tung and the Australian Red Cross Blood Service, Abhijit Patekar, Glenda Balderson, and Jane Treloggen and staff members of the NSW Organ and Tissue Donation Service for their help with critical parts of my projects.

To the organ donors and their families – may you continue to inspire us all to be better people and to serve humanity, rather than just the individual.

To the members of my research group, the Centre for Transplant and Renal Research, it has been a pleasure working with each and every one of you. You have truly made my stay here a joy, and have all made important contributions to my work. In particular I would like to acknowledge and thank Heather Burns, Dr Yi Vee Chew, Dr Min Hu, Lindy Williams, Elvira Jimenez-Vera, Dr Negar Talaei Zanjani, Dr Nicole Byrne, Dr Sohel Julovi, Dr Kedar Ghimire, Dr David Liuwantara, Dr Barkha Sanganeria, Ali El-Ayoubi, Dr Qi Liang, Christian Haron, Maryam El-Rashid, and Danny Nguyen-Ngo for their invaluable assistance with laboratory and/or animal work, advice in general, or for your company on long days.

To Yuan Fei Zhao, I look forward to you finishing your PhD and hope you continue to live in Australia. Thank you for all the food, and tolerating my sense of humour. To Titi Chen, your perpetual smile kept me smiling as well, and our corridor chats made my days all the better. To Ankit "Abhijit" Sharma, I can't believe our dads knew each other before we did, but I'm glad to have finally met you and become close friends over these few years. Lunchtime won't be the same without your presence, but I hope we can continue dinner dates

into the future. To Karen Keung, who knew I would become friends with the person whose husband questioned my competence as an intern. Our shared insanity over failed experiments and "level 7" jokes kept me going, and it truly has been a pleasure being friends with you. This last year hasn't been the same without your continued presence.

To my friends Jude, Roshan, and Sahir, I am honoured and extremely grateful to have shared your company, including all of the ups and downs, since our journeys together started as medical students, and cannot wait for what's to come.

To my one remaining grandparent, you always believed in me and I hope to see you soon. And to my grandparents who have passed away, especially my grandfather whom we lost earlier this year – my writing pales in comparison to yours, but I can promise that I tried my best. May you be granted the highest levels of Paradise.

To Sheeza, in you God has given me a gift that I did not deserve, but I cherish and am grateful for every day. I promise to do my best to keep you happy, forever and ever, and may we make the world a better place together, insha'Allah.

To Hiba and Azka, what can I say about the both of you? You have seen me at my best and my worst, and I have seen the same from you. No matter how busy I get or whatever part of the world we may be in, know that you'll always be my little sisters and I will do anything for you.

To my parents, this thesis is dedicated to you. I will never be able to thank you enough for what you have done for me. Starting a new life in a new country is not easy, but you made it ever so easy for all three of us, and did it without any consideration for yourselves. You have never once questioned the major decisions in my life, but have instead put your full weight of support and trust behind me, no matter what. I hope I have made you proud, you deserve nothing but absolute happiness.

I thank God for all of the blessings and people in my life, and pray to be a better person with each day. To God we belong, and to Him we shall return.

Ahmer Hameed

Authorship Attribution Statements

Chapter 2 of this thesis is published as:

> Hameed, A.M., Hawthorne, W.J. & Pleass, H.C. (2016). Advances in organ preservation for transplantation. *ANZ Journal of Surgery*, *87*(12): 976-80

This is a narrative review article that was written by myself, with contributions and editing by Wayne Hawthorne and Henry Pleass.

Chapter 3 of this thesis is published as:

> Hameed, A.M., Pleass, H.C., Wong, G. & Hawthorne, W.J. (2016). Maximizing kidneys for transplantation using machine perfusion: from the past to the future – a comprehensive systematic review and meta-analysis. *Medicine* (Baltimore), *95*(40):e5083

I designed the study (in conjunction with supervisors), collected and analyzed study data, and drafted and revised the article. Other authors made contributions to aspects of study design, data collection/interpretation, and/or article revision, as relevant.

Chapter 4 of this thesis is published as:

> Hameed, A.M., Miraziz, R., Lu, D.B., Warwick, N., El-Ayoubi, A., Burns, H., Chew, Y., Matthews, R., O'Grady, G., Yuen, L., Rogers, N., Pleass, H.C. & Hawthorne, W.J. (2018). Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ Journal of Surgery*, *88*(5): E429-34

I designed the study (in conjunction with supervisors), was the main researcher involved in the performance of all animal, machine perfusion, and laboratory-based experiments, analyzed study data, and drafted/revised the article. Other authors made contributions to aspects of study design, animal experiments, data interpretation, and/or article revision, as relevant.

Chapter 5 of this thesis is published as:

> Hameed, A.M., Dervish, S., Rogers, N., Pleass, H. & Hawthorne, W. (2018). A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transplant International*, Epub ahead of print, DOI: 10.1111/tri.13361

I designed the original concept, look, and specifications of the custom perfusion chamber. This concept was then further modified and 3D-printed in conjunction with Suat Dervish. All experiments conducted using the perfusion chamber were performed primarily by myself, in addition to the writing and revision of this article.

Chapter 6 of this thesis has been submitted for publication as:

Hameed, A.M., Lu, D., Burns, H., Byrne, N., Chew, Y., Julovi, S., Ghimire, K., Talaei Zanjani, N., P'ng, C., Meijles, D., Dervish, S., Matthews, R., Miraziz, R., O'Grady, G., Yuen, L., Pleass, H., Rogers, N. & Hawthorne, W. (2018). CD47-blockade to ameliorate kidney ischemia-reperfusion injury using normothermic machine perfusion – a small and large animal study. *Submitted to Annals of Surgery (under review).*

I designed the study (in conjunction with supervisors) and was involved in the performance of all animal and machine perfusion experiments. Fifty percent of mouse surgeries were conducted by myself, and the other 50% by David Bo Lu (on an alternating basis during each session). All laboratory-based experiments were conducted by myself, with the exception of real-time measurement of reactive oxygen species using cytochrome C and amplex red (performed by Daniel Meijles). After acquiring all relevant data, I was the main author involved in data interpretation, and drafting of the submitted article. Other authors made contributions to aspects of study design, data acquisition and/or interpretation, and/or article revision, as relevant.

Chapter 7 of this thesis has been submitted for publication as:

Hameed, A.M., Lu, D., Patrick, E., Xu, B., Hu, M., Chew, Y., Keung, K., P'ng, C., Gaspi, R., Zhang, C., Robertson, P., Alexander, S., Thomas, G., Laurence, J., De Roo, R., Wong, G., Miraziz, R., O'Grady, G., Yuen, L., Hawthorne, W., Rogers, N. & Pleass, H. (2018). *Manuscript in preparation.*

I designed the study (in conjunction with supervisors), and was involved in a majority of kidney procurement procedures, and subsequently all machine perfusion experiments. I also conducted all laboratory-based work, with the exception of whole transcriptome RNA sequencing, which was performed by Bo Xu. I was involved with all data collection and interpretation, but gained significant assistance for RNA/gene expression data interpretation from Ellis Patrick and Karen Keung, and for flow cytometry analyses from Min Hu and Yi Vee Chew. I drafted a complete version of the article. Other authors made contributions to aspects of study design, data acquisition and/or interpretation, and/or article revision, as relevant.

Chapter 8 of this thesis is published as:

Hameed, A.M., Yu, T., Yuen, L., Lam, V., Ryan, B., Allen, R., Laurence, J., Hawthorne, W. & Pleass, H. (2016). Use of the Harmonic Scalpel in the cold phase recovery of the pancreas for transplantation: the Westmead technique. *Transplant International, 29*(5): 636-38

I designed the study (in conjunction with supervisors), collected and analyzed study data, and drafted and revised the final article. Other authors made contributions to aspects of study design, data interpretation, and/or article revision, as relevant.

Chapter 9 of this thesis is published as:

> Hameed, A.M., Wong, G., Laurence, J.M., Lam, V.W.T., Pleass, H.C. & Hawthorne, W.J. (2017). A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. *HPB, 19*(11): 933-43

I designed the study (in conjunction with supervisors), collected and analyzed study data, and drafted and revised the article. Other authors made contributions to aspects of study design, data collection/interpretation, and/or article revision, as relevant.

Chapter 10 of this thesis is published as:

> Hameed, A.M., Laurence, J.M., Lam, V.W.T., Pleass, H.C. & Hawthorne, W.J. (2017). A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: working toward a unified approach. *Liver Transplantation*, *23*(12): 1615-27

I designed the study (in conjunction with supervisors), collected and analyzed study data, and drafted and revised the article. Other authors made contributions to aspects of study design, data collection/interpretation, and/or article revision, as relevant.

Chapter 11 of this thesis is published as:

> Hameed, A.M., Laurence, J.M., Lam, V.W.T., Pleass, H.C. & Hawthorne, W.J. (2018). Reply to Letter "Liver preservation solutions: Absence of proof is not proof of absence." *Liver Transplantation*, 24(8): 1144-46

This is a Letter to the Editor in response to a Letter written by other authors (Adam *et al*.) regarding the article in Chapter 10. I drafted and revised this letter in conjunction with all other authors.

Chapter 12 of this thesis is published as:

> Hameed, A.M., Pang, T., Yoon, P., Balderson, G., De Roo, R., Yuen, L., Lam, V., Laurence, J., Crawford, M., Allen, R., Hawthorne, W. & Pleass, H. (2018). Aortic versus dual perfusion for retrieval of the liver after brain death – a national registry analysis. *Liver Transplantation*, Epub ahead of print, DOI: 10.1002/lt.25331

I designed the study (in conjunction with supervisors), analyzed study data, and drafted and revised the article. Glenda Balderson provided merged data from the Australia and New Zealand Liver Transplant Registry and the Australia and New Zealand Organ Donation Registry. Tony Pang provided invaluable assistance for complex statistical analyses. Other authors made contributions to aspects of study design, data interpretation, and/or article revision, as relevant.

In all cases, one or more of my PhD supervisors was designated as the corresponding author(s), as per journal convention, and to acknowledge their role as the supervising author(s). I was the first author on all of these papers, indicating my primary role in study design, implementation, analysis, and writing.

In addition to the statements above, permission to include the published material has been granted by the corresponding author(s).

Student Name, Signature, Date

Dr Ahmer Hameed [redacted] 29.11.18

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct, and acknowledge the candidate was the primary contributor to all of the published work. As such, inclusion of all of the aforementioned work in this thesis is wholly warranted.

Supervisor Name, Signature, Date

Prof Wayne Hawthorne [redacted] 28.11.18

Corresponding Authors' Statement

As the corresponding authors of the manuscripts and/or publications included in this thesis, and PhD supervisors for the candidate, we confirm the primary contribution of Ahmer Hameed to all included manuscripts (as outlined in the Authorship Attribution Statements).

Supervisor/Corresponding Author Name, Signature, Date

Prof Wayne Hawthorne  28.11.18

Supervisor/Corresponding Author Name, Signature, Date

Prof Henry Pleass  29.11.18

Supervisor/Corresponding Author Name, Signature, Date

A/Prof Natasha Rogers  29.11.18

Publications During Candidature

Primary author

- Hameed et al. Use of the harmonic scalpel in cold phase recovery of the pancreas for transplantation: the Westmead technique. *Transpl Int 2016, 29(5): 636-38; DOI: 10.1111/tri.12777*
- Hameed et al. Maximizing kidneys for transplantation using machine perfusion: from the past to the future – a comprehensive systematic review and meta-analysis. *Medicine (Baltimore) 2016, 95(40): e5083; DOI: 10.1097/MD.0000000000005083*
- Hameed et al. Advances in organ preservation for transplantation. *ANZ J Surg 2017, 87(12): 976-80; DOI: 10.1111/ans.13713*
- Hameed et al. A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. *HPB (Oxford) 2017, 19(11): 933-43; DOI: 10.1016/j.hpb.2017.07.012*
- Hameed et al. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: working toward a unified approach. *Liver Transpl 2017, 23(12): 1615-27; DOI: 10.1002/lt.24829*
- Hameed et al. Reply to Letter: "Liver preservation solutions: Absence of proof is not proof of absence". *Liver Transpl 2018, 24(8): 1144-46; DOI: 10.1002/lt.25195*
- Hameed et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ J Surg 2018, 88(5): E429-34; DOI: 10.1111/ans.14321*
- Hameed et al. The evolution of kidney transplantation surgery into the robotic era and its prospects for obese recipients. *Transplantation 2018, 102(10): 1650-65; DOI: 10.1097/TP.0000000000002328*
- Hameed et al. Aortic versus dual perfusion for retrieval of the liver after brain death: a national registry analysis. *Liver Transpl 2018 Sept 7, Epub ahead of print; DOI: 10.1002/lt.25331*

- Hameed et al. Techniques to ameliorate the impact of second warm ischaemic time on kidney transplantation outcomes. *Transplant Proc 2018 Sept 8, Epub ahead of print; DOI: 10.1016/j.transproceed.2018.09.003*
- Hameed et al. A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transpl Int 2018 Oct 13, Epub ahead of print; DOI: 10.1111/tri.13361*
- Hameed et al. CD47-blockade to ameliorate kidney ischemia-reperfusion injury using normothermic machine perfusion – a small and large animal study. Manuscript Submitted To: *Annals of Surgery*
- Hameed et al. Brief normothermic machine perfusion rejuvenates discarded human kidneys – the potential for discard reduction and improved outcomes. *Manuscript in preparation*

Other

- Hawthorne, WJ, Hameed, AM, and Pleass, HC. Pancreas retrieval for whole organ and islet cell transplantation. *IntechOpen 2018 July 25 (Book Chapter); DOI: 10.5772/intechopen.75151*
- Shahrestani, S, Hameed, AM, Hitos, K, Pleass, HC, and Hawthorne, WJ. Optimal culture methods and microbial contamination during kidney ex vivo normothermic perfusion. *Transplantation 2018, 102(9): e398-99; DOI: 10.1097/TP.0000000000002302*
- Cocco, A, Shahrestani, S, Cocco, N, Hameed, AM, Yuen, L, Ryan, B, Hawthorne, WJ, Lam, V, and Pleass, HC. Dual kidney transplant techniques: a systematic review. *Clinical Transplantation 2017, 31(8): e13016; DOI: 10.1111/ctr.13016*

Presentations During Candidature

Peer-reviewed abstracts

- Hameed, A., Rogers, N., Lu, B., Hu, M., Chew, Y., Zhang, C., Gaspi, R., Robertson, P., Miraziz, R., Yuen, L, De Roo, R., Laurence, J., Hawthorne, W. & Pleass, H. (2018). Normothermic machine perfusion of discarded human kidneys – the potential for more organs with better outcomes. *Presentation to the 55th Annual Scientific Meeting of the Surgical Research Society of Australasia, Sydney*
- Hameed, A., Pang, T., Yoon, P., Balderson, G., De Roo, R., Yuen, L., Lam, V., Laurence, J., Crawford, M., Allen, R., Hawthorne, W. & Pleass, H. A national registry analysis of aortic versus dual in situ perfusion for retrieval of the DBD liver. *Presentation to the 27th International Congress of The Transplantation Society, Madrid*
- Hameed, A., Rogers, N., Lu, B., Burns, H., Byrne, N., Chew, Y., Sanganeria, B., Julovi, S., Ghimire, K., Miraziz, R., Pleass, H. & Hawthorne, W.J. (2018). Intra-renal delivery of drugs targeting ischemia-reperfusion injury of the kidney using normothermic machine perfusion. *Presentation to the 27th International Congress of The Transplantation Society, Madrid [Poster]*
- Hameed, A., Pang, T., Yoon, P., Balderson, G., De Roo, R., Yuen, L., Laurence, J., Lam, V., Crawford, M., Hawthorne, W. & Pleass, H. (2018). Aortic versus dual perfusion for retrieval of the DBD liver – an analysis of recipient outcomes using the ANZ liver transplant registry. *Presentation to the 36th Annual Scientific Meeting of the TSANZ, Melbourne*
- Hameed, A., Lu, B., Miraziz, R., Burns, H., Rogers, N., Pleass, H. & Hawthorne, W. (2018). Intra-renal delivery of drugs targeting ischaemia-reperfusion injury of the kidney in a rodent model and porcine model of normothermic machine perfusion. *Presentation to the 36th Annual Scientific Meeting of the TSANZ, Melbourne [President's Prize Session]*
- Hameed, A., Rogers, N., De Roo, R., Lu, B., Robertson, P., Zhang, C., Gaspi, R., Miraziz, R., Nguyen, H., Yuen, L., Allen, R., Hawthorne, W. & Pleass, H. (2018).

Normothermic machine perfusion of non-utilized human kidneys – our first 9 cases. *Presentation to the 36th Annual Scientific Meeting of the TSANZ, Melbourne [Poster]*

- Hameed, A., Miraziz, R., Lu, D.B., Rogers, N., Pleass, H.C. & Hawthorne, W. (2017). Modification of donor kidney preservation conditions to enhance transplantation outcomes. *Presentation to the 54th Annual Scientific Meeting of the Surgical Research Society of Australasia, Adelaide*
- Hameed, A., Wong, G., Laurence, J., Lam, V., Pleass, H. & Hawthorne, W. (2017). A systematic review and meta-analysis of cold *in situ* perfusion and preservation of the pancreas and liver for transplantation. *Presentation to the 16th International Congress of the International Pancreas and Islet Transplant Association (IPITA), Oxford*
- Hameed, A., Rogers, N., Lu, B., Miraziz, R., Warwick, N., Wong, G., El-Ayoubi, A., Burns, H., Chew, Y., Pleass, H. & Hawthorne, W. (2017). Normothermic machine perfusion of the kidney prior to transplantation – machine development and optimisation. *Presentation (mini-oral) to the 25th NSW Annual Scientific Meeting of the Australian Society for Medical Research, Sydney*
- Hameed, A., Rogers, N., Warwick, N., Miraziz, R., Wong, G., El-Ayoubi, A., Burns, H., Chew, Y., Pleass, H. & Hawthorne, W. (2017). Developing a normothermic renal perfusion system. *Presentation to the 35th Annual Scientific Meeting of the TSANZ, Brisbane [Poster]*
- Hameed, A., Pleass, H., Wong, G. & Hawthorne, W. (2017). Kidney preservation for the future – a comprehensive systematic review and meta-analysis of machine perfusion in renal transplantation. *Presentation to the 35th Annual Scientific Meeting of the TSANZ, Brisbane [Poster]*
- Hameed, A., Wong, G., Laurence, J., Lam, V., Pleass, H. & Hawthorne, W. (2017). A systematic review and meta-analysis of cold *in situ* perfusion and preservation of the pancreas and liver for transplantation. *Presentation to the 35th Annual Scientific Meeting of the TSANZ, Brisbane*
- Hameed, A.M., Pleass, H. & Hawthorne, W. (2016). Cold *in situ* perfusion prior to liver and pancreas procurement for transplantation – a systematic review and meta- analysis. *Presentation to the 53rd Annual Scientific Meeting of the Surgical Research Society of Australasia, Melbourne*

- Hameed, A.M., Yu, T., Yuen, L., Lam, V., Ryan, B., Allen, R., Laurence, J., Hawthorne, W. & Pleass, H. (2016). Harmonic Scalpel in the rapid procurement of the pancreas for transplantation: Recipient outcomes. *Presentation to the 26th International Congress of The Transplantation Society (TTS), Hong Kong*

Invited talks

- Hameed, A.M. (2018). Rise of the machines – the future of organ preservation and transplantation. *Presentation to the 50th Annual Scientific Meeting of the Australasian College of Biomedical Scientists*
- Hameed, A.M. (2018). Expanding the kidney donor pool using machine perfusion. *Presentation to the Asia-Pacific Histocompatibility and Immunogenetics Association 2018 Conference [Plenary]*
- Hameed, A.M. (2018). Normothermic machine perfusion of discarded human kidneys – Update from an Australia-first study. *Presentation to the NSW Organ and Tissue Donation Symposium, Sydney*
- Hameed, A.M. (2018). Abdominal donor organ preservation – machine perfusion of the kidney and liver. *Presentation to the 87th Annual Scientific Congress of the Royal Australasian College of Surgeons*
- Hameed, A.M (2017). Ex vivo normothermic kidney perfusion – back to the future. *Presentation to the NSW Organ and Tissue Donation Symposium, Sydney*
- Hameed, A.M. with Pleass, H. (2016). State-of-the-art – current kidney machine perfusion literature. *Presentation to the TSANZ Machine Perfusion Workshop/Meeting, Melbourne*

Funding and Awards

- Travel Grant (2018) – 55th Annual Scientific Meeting of the Surgical Research Society of Australasia, Sydney
- Prize for Best Poster Presentation (2018) – Westmead Hospital Week (Westmead Association)
- TTS International Transplantation Science Mentor-Mentee Award (2018) – 27th International Congress of the Transplantation Society, Madrid
- TSANZ Early Career Researcher Award (2018) – 36th Annual Scientific Meeting of the TSANZ
- John Loewenthal Project Grant (2018-19) with W. Hawthorne, H. Pleass, N. Rogers, P. Boughton, G. Wong – Normothermic machine perfusion of the donor kidney – better organs, better outcomes, $200,000 – Royal Australasian College of Surgeons
- Post-graduate Research Support Scheme grants (2016-18) – Sydney University
- Westmead Association Research Travel Grant recipient (2017) – Westmead Hospital (Westmead Association)
- TSANZ Young Investigator Award (2017) – 35th Annual Scientific Meeting of the TSANZ
- Sir Roy McCaughey Surgical Research Scholarship (2017-8) – Royal Australasian College of Surgeons
- Australian Postgraduate Award (2016) – Australian Government

Commonly Used Abbreviations

ALT – Alanine aminotransferase

AST – Aspartate aminotransferase

ATP – Adenosine triphosphate

BMI – Body mass index

CI – Confidence interval

CIT – Cold ischaemic time

COD – Cause of death

CrCl – Creatinine clearance

CS – Cold (static) storage

CVA – Cerebrovascular accident

DBD – Donation after brain death

DCD – Donation after circulatory death

DGF – Delayed graft function

DM – Diabetes mellitus

DRI – Donor risk index

ECD – Expanded criteria donor

ESRF – End-stage renal failure

FeNa – Fractional excretion of sodium

GRADE – Grading of Recommendations, Assessment, Development and Evaluations

HMP – Hypothermic machine perfusion

HTK – Histidine-tryptophan-ketoglutarate

IGL – Institute Georges Lopez

IRI – Ischaemia-reperfusion injury

ITBL – Ischaemic-type biliary lesions

IVC – Inferior vena cava

KDPI – Kidney donor profile index

KDRI – Kidney donor risk index

MELD – Model for end-stage liver disease

MP – Machine perfusion

NMP – Normothermic machine perfusion (warm perfusion, WP)

NRP – Normothermic regional perfusion

PDF – Primary graft dysfunction

PNF – Primary non-function

PRBCs – Packed red blood cells

QoL – Quality of life

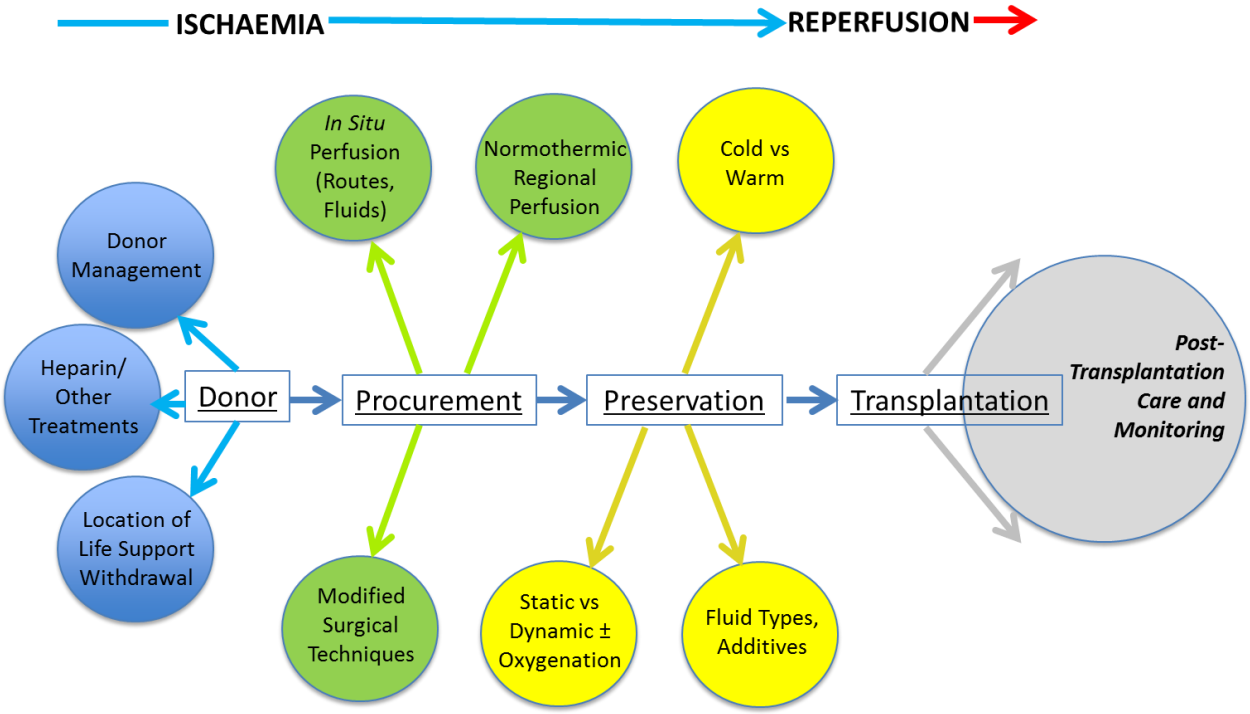
ROS – Reactive oxygen species

SDC – Supplemental digital content

SWIT – Second warm ischaemic time (anastomotic time)

UW – University of Wisconsin

WIT – Warm ischaemic time



PART 1 – GENERAL INTRODUCTION

Chapter 1 - Introduction

Organ transplantation is a life-saving and life-altering process for thousands of recipients worldwide each year. The immediate post-implantation period is characterized by the complexities of the ischaemia-reperfusion cascade and the alloimmune response, the severity of which largely determines immediate and longer term graft function. Indeed, improvements in methods to modulate ischaemia-reperfusion and alloimmunity comprise the majority of transplantation research. The ultimate goals of such research are the amelioration of ischaemia-reperfusion and induction of tolerance to promote life-long graft survival.

However, transplantation outcomes are influenced by events that occur well in advance of the transplantation procedure itself. Transplantation requires a suitable organ donor, whom may be deceased or, in certain circumstances, living. The donor must be managed appropriately, with management strategies dictated by evidence-based guidelines, local laws, and resource availability. The organ must then be procured safely and effectively, avoiding anatomical damage and minimizing any warm ischaemic insult. There-in, it is the responsibility of the procurement team to ensure appropriate storage and preservation of the graft during transport to the recipient centre. Organ transplantation must then proceed in a technically sound and timely manner to avoid major surgical complications that may compromise the graft and/or patient. After implantation commences the life-long process of medical management of the patient, including the institution of tailored immunosuppression regimens.

This dissertation focuses on the defined phase in transplantation starting from surgical procurement of the major abdominal organs, the kidney, liver, and pancreas, to the point of transplantation, and how certain processes can be modified and/or improved to enhance recipient graft function. This exploration will occur in the context of the increasing use of more marginal organs to help reduce ongoing donor organ shortages. Preservation of the deceased donor kidney will form the core of this thesis, investigating the modulation of ischaemia-reperfusion injury (IRI) using pharmacologic agents and machine perfusion (MP). Selected aspects of liver and pancreas procurement and preservation will also be analyzed with respect to their impacts on post-transplantation outcomes.

1.1 The need for organ transplantation, organ supply & demand, and maximizing the donor pool

Organ transplantation represents the best viable long-term treatment option for diseases such as end-stage renal failure (ESRF) and type I diabetes mellitus (DM). Liver transplantation, in the context of hepatic failure, is an immediately life-saving procedure.

The acceptance and growth in transplantation as a therapeutic option is justified by both short and long-term clinical outcome data, and patient quality of life (QoL) parameters. In a systematic review incorporating data from close to two million patients, Tonelli *et al*. compared renal transplantation outcomes to those achieved with long-term dialysis[1]. The authors showed significantly reduced mortality and cardiovascular complications, in addition to enhanced QoL, in transplanted patients. Similarly, successful pancreatic transplantation, or replacement of beta-cell function through islet cell transplantation, reduces patient mortality and undoubtedly improves QoL as the need for exogenous insulin therapy and further complications from DM are minimized.[2] Furthermore, analyses of liver transplantation have shown a realistic possibility of long-term survival and superior QoL outcomes.[3, 4]

The reality however is that there is a perpetual shortage of available organs with respect to demand.[5-8] This disparity is not only reflected in transplant wait times, but also in proportions of patients with end-stage organ disease removed from active waiting lists, and/or dying whilst wait-listed. As an example, median waiting time for renal transplantation in Australia is 2-3 years, with more than 1000 patients on the waiting list during any one time.[9] Of particular relevance and oft-forgotten is that these statistics will still grossly misrepresent the true shortage of donor organs. This is because only a small percentage of patients with end-stage organ disease are actively placed on waiting lists due to relatively strict eligibility criteria to account for donor shortages.

The ongoing shortage of donor organs has necessitated the implementation of multiple strategies to address this deficiency. These encompass a host of domains, including interventions aimed at (i) the education of relevant stakeholders and the public at large regarding transplantation, in association with policies regarding donation consent and allocation (also including regulated live donor and/or paired organ exchange programs); (ii) enhancing the pre-procurement management

of potential donors to minimize organ deterioration; (iii) improving procurement and preservation techniques such that graft outcomes are optimized and discard rates are minimized; and (iv) widen the number of potential organs through (a) the loosening of organ acceptance criteria, such as donation after circulatory death (DCD) and expanded criteria donor (ECD) organs, and (b) exploring alternative sources for donor allografts, primarily through research into xenotransplantation and in some cases stem-cell derived sources.

1.2 Organ procurement, preservation, and transplantation – major concepts

1.2.1 DECEASED DONORS – TYPES AND DEFINITIONS

Deceased donor organ donation can occur after the irreversible cessation of brain function or following circulatory arrest. The former places donors in the donation after brain death (DBD) category, whilst the latter classifies them as donation after circulatory death (DCD) donors.

DCD donors can be further sub-classified based on the pattern/nature of circulatory arrest. The primary classification system in this regard was developed after a consensus meeting in Maastricht, The Netherlands.[10] Overall there are five DCD donor categories.[11, 12] DCD I to V donors are defined by death on arrival (I), failed resuscitation (II), cardiac/circulatory arrest awaited (III), circulatory arrest in a DBD donor (IV), or unexpected circulatory arrest in a patient who has critical illness (V), respectively.[11] Controlled DCDs encompass category III and IV donors in which life support measures are withdrawn in a controlled/planned manner, whilst Maastricht I, II and V donors are 'uncontrolled'.[13] The majority of DCD donation occurs in a controlled fashion, and uncontrolled DCD donation is limited to a few countries. Currently within the Australian and UK setting, only Maastricht III or IV donors can proceed to donation.[14, 15]

Expanded criteria donors (ECD) are named as such due to an anticipated shorter graft life-span after transplantation in comparison to standard criteria donor (SCD) grafts. ECDs can incorporate both DCD or DBD donors. Within the sphere of kidney transplantation, ECD terminology has largely been supplanted by the related concepts of the kidney donor risk index (KDRI) and kidney donor profile index (KDPI). However, ECD and high KDPI will be used interchangeably in this thesis due to ongoing use of ECD in the literature. The KDRI and KDPI

estimate risk of graft failure relative to other donor kidneys.[16, 17] The Australian KDPI score incorporates donor age, history of hypertension or DM, height/weight, cause of death (COD) as stroke, terminal creatinine level, and DCD pathway within overall scoring.[16] A high KDPI generally equates to a score >80-85%.[18, 19] The older 'ECD' definition for kidney donors defined ECD as a donor either greater than 59 years old, or between the ages of 50 and 59 years and having at least two of the following three variables – death due to a cerebrovascular accident, a history of hypertension, and/or a terminal serum creatinine that exceeds 1.5 mg/dl.[12]

Liver ECD definitions vary, with no clear consensus, but once again encompass higher risk donors. Donor-related risk factors that may be associated with graft failure can include increased age, hypernatraemia, hepatitis B or C, transaminitis as a marker of ischaemic damage, and/or macrosteatosis.[20, 21] The Donor Risk Index (DRI), and in Europe the Eurotransplant DRI (ET-DRI), attempt to more formally define the contribution of donation-related factors to subsequent graft failure.[22, 23] These indices include within the risk scores such factors as advanced donor age, donor COD, the use of partial grafts, ischaemic times, DCD pathway, and graft shipping.

A consensus ECD definition also does not yet exist for pancreas transplantation, although it can be argued that a donor who does not fit within 'standard' acceptance criteria can be considered an ECD.[24] This may include donors above 45-50 years of age, with/without obesity, and dying due to cerebrovascular accident, and/or circulatory death.[24-26] The Pancreas Donor Risk Index (PDRI) was proposed in 2010, and considers parameters such as elevated donor age (> 28 years), body mass index > 24, COD as stroke, cold ischaemic time > 12 hours, and the DCD pathway as risk factors for graft loss.[27] An alternative score was developed for the Eurotransplant region, the Pre-procurement Pancreas Allocation Suitability Score (P-PASS), incorporating purely donor-related factors, including intensive care unit stay, and donor amylase and lipase levels.[28]

1.2.2 DECEASED DONORS – UTILISATION AND OUTCOMES BY TYPE

DBD donors continue to represent the highest proportion of organ donors in Australia, USA, and Europe. However, the landscape of organ donation with respect to acceptable donor risk factors has changed significantly over the past few years in order tackle the problem of organ shortages. The greatest change has been in the proportion of ECD and DCD donor organs utilized. In

Australia in 2017, 70% of all donors resulted from the DBD pathway, whilst 30% of donors were from the DCD category; in comparison, the corresponding percentages for 2009 were 83% and 17% for DBD and DCD donors, respectively.[29] In contrast, DCD donors represent 17-18% of all deceased donors in the USA, and a much larger 39% in the UK.[15, 30] In general, donor age has also seen a significant increase in Australia over the last decade, and comorbidities such as DM, hypertension, and smoking are much more prevalent in donors today compared to even 10 years ago.[31]

Kidney transplantation outcomes from DCD and ECD/higher KDPI donors are not equivalent to those achieved from SCD DBD donors. Even before reaching the stage of transplantation, there is a significantly greater risk of discarding grafts retrieved from DCD and/or higher KDPI donors.[32-34] DCD and ECD kidneys confer a higher risk of early graft loss due to primary non-function (PNF) or vascular thrombosis.[35-37] Delayed graft function (DGF) rates, most commonly defined as the requirement for dialysis in the first week post-transplantation, are significantly higher in both DCD and ECD kidneys.[37-41] DGF in turn is associated with greater hospital length-of-stay and costs.[42-44]

The relationship between the use of marginal and/or DCD kidneys and long-term graft survival is more complicated. A large multi-institutional study from the UK showed equivalent 5-year graft survival in DCD and DBD kidneys.[38] However amongst both DBD and DCD donors, increasing donor age, cold ischaemic times (CITs), and stroke as a COD were associated with graft failure.[38] A more recent large cohort study from the Netherlands showed similar 10-year graft and patient survival rates after kidney transplantation from DCD or DBD donors.[37] Other studies similarly show no impact on long-term graft survival,[45-47] although there may be a trend towards inferior graft survival in the higher risk donors after a 10 year period.[48] Regardless, the presence of multiple risk factors within the same donor such as increasing age and/or comorbidities reduces graft survival.[44]

Difficulties arise when considering the impact of DGF on graft and patient survival in DCD kidney transplantation. A paired kidney study comparing survivals in DCD kidney pairs with and without DGF showed a significantly higher risk of graft loss in the DGF grafts.[49] Similarly,

increased risk of graft loss was shown in paediatric recipients of DCD grafts with subsequent DGF.[50] In contrast, other authors suggest that the occurrence of DGF has no subsequent impact on graft survival in DCD transplantation.[51-53] Another large paired registry analysis conversely suggested that DGF adversely impacts on graft survival, but only in the first post-transplant year.[54] Duration of DGF and/or functional recovery of renal filtration may be a more important consideration and determinant of subsequent graft loss.[55, 56] Another reason for differences between studies may be attributed to the alternate definitions of DGF used (dialysis-based versus creatinine clearance-based). However, the negative impact of DGF on DBD transplant survival appears to be much clearer in comparison to its impacts on DCD kidney transplants.[37, 52, 53] Importantly, even when accounting for increased DGF, DCD and/or ECD kidney transplantation continues to offer a significant survival advantage, improved access to transplantation, improved QoL, and cost advantages when compared to remaining on dialysis.[19, 35, 57-59]

The DCD and/or ECD categories have a clearer impact upon graft and patient survival after liver transplantation. A large multi-centre cohort study from the UK showed a higher graft loss conferred upon DCD livers.[60] This trend is also shown in other studies, including meta-analyses.[61-63] Much of this difference is probably attributable to a greater incidence of ischaemic-type biliary lesions (ITBL) in DCD liver transplants.[61-63] Indeed, DCD livers that do not develop ITBL likely have similar survivals compared to matched DBD livers.[64] 'Expanded' criteria livers, characterized by such factors as increased donor age, ischaemic time greater than 8 hours, and macrosteatosis, tend to have worse outcomes.[22, 23, 65] Younger DCD donor livers appear to perform better in comparison to older DBD livers and therefore careful selection of DCD donors and an emphasis on keeping ischaemic times short can significantly close any gaps in outcomes between DCD and DBD livers.[66, 67]

Within the realm of pancreatic transplantation, long-term success equivalent to that seen from DBD donors can be achieved with DCD pancreases.[68-70] A large cohort study from the UK confirmed this finding, although DCD pancreases were used from significantly younger donors compared to the DBD cohort.[25] A recently published study investigated the medium-term comparative efficacy of selective ECD pancreatic allografts, utilizing donors with an age of 50-

60 years and/or BMI of 30-34. In comparison to SCD organs, ECD pancreases had a very similar one-year graft survival.[71]

1.2.3 MULTI-ORGAN PROCUREMENT

Organ procurement requires efficient and safe organ dissection and removal to facilitate effective transplantation outcomes. The rapidity and exact nature of the retrieval process is significantly impacted by whether the donor is within the DBD or DCD subclass. Procurement can be undertaken for single organs or multiple organs from the same patient; both abdominal and thoracic organs may be obtained from a donor, if indicated and suitable. Furthermore, donor organs can be retrieved individually, or in some cases can be removed from the donor in an *en bloc* fashion, after which they are separated on the back-table.

The standard multi-organ DBD donor proceeds utilizing a method originally described by Starzl, which was later modified by the same author into a rapid procurement technique.[72, 73] Preliminary dissection of organs is minimized, and the procurement procedure proceeds in a more stream-lined fashion. Procurement occurs in the 'warm' and 'cold' phases, representing the period before and after the cold *in situ* perfusion/flush, respectively. Primary steps in this procedure, which are facilitated by mobilization of the large bowel and small intestinal mesentery, include:

- A general laparotomy, inspecting the abdomen for pathology that would contra-indicate donation (e.g. cancer);
- Dissection of the supracoeliac aorta;
- Dissection of the aorta at its bifurcation into the common iliac arteries;
- Distal aortic cannulation with or without portal venous cannulation;
- Minor preliminary organ dissection (warm phase), in particular involving dissection/identification of vital structures that may easily be damaged in the cold phase (e.g. ureters, portal structures);
- Supracoeliac aortic cross-clamping and cold *in situ* perfusion (~2-4 degrees Celsius) via the aortic cannula (and portal vein, if applicable); blood/perfusion fluid is vented via the inferior vena cava (IVC) after cannulation or into the thoracic cavity following transection;

- Cold dissection and removal of relevant organs, along with suitable lengths of supplying arteries and draining veins; aberrant anatomy must be accounted for; and
- Back-table dissection and perfusion of organs prior to subsequent storage and transportation.[73, 74]

Modifications to Starzl's methods exist, involving variable levels of preliminary organ and vessel dissection in the warm phase.[74] Although no systematic evidence exists, Brockmann *et al*. found no significant evidence of organ compromise when multi-organ retrieval of the liver, pancreas and kidneys is undertaken.[75] Furthermore, rapid procurement techniques with minimal warm-phase dissection do not tend to impair graft function.[75] Additionally, *en bloc* organ removal, in contrast to separate dissection and removal, is likely associated with improved liver and pancreas graft outcomes.[75]

In contrast, DCD organ procurement requires the rapid administration of cold *in situ* perfusion as an initial step, such that the organs' warm ischaemic insult is minimized. Casavilla, from Starzl's group in Pittsburgh, described this 'super-rapid' technique in 1995.[76] It entails a swift laparotomy, exposure of the distal aorta, followed by immediate cannulation and cold perfusion.[76] Cold phase dissection of the organ(s) of interest is then undertaken prior to their removal. Alternative techniques that may be considered in the DCD setting, in particular the potential use of ante-mortem interventions, are considered in section 1.4.

1.2.4 IN SITU ORGAN PERFUSION AND STATIC METHODS OF PRESERVATION

A cold *in situ* systemic vascular flush must be undertaken during organ procurement in order to induce rapid organ cooling, remove static red blood cells (RBCs), and provide an appropriate substrate for subsequent organ preservation. Cooling of the organ must be achieved such that its metabolic rate is dropped in the absence of a blood supply; each 10 degree Celsius reduction in temperature causes an approximately two-fold reduction in enzyme activity.[77] The type of fluid used for the *in situ* organ flush is generally subsequently used for organ storage during transportation to the transplant centre.

<u>Perfusion/preservation fluids</u>

Cold organ preservation fluids should ideally minimize and/or reverse the following cellular and subcellular processes occurring within the organ of interest secondary to an absent blood supply (also see Section 1.3):

- Disrupted ionic pumps and ion accumulation and/or depletion, with additional downstream effects;
- Altered redox potentials;
- Cellular oedema;
- Acidosis;
- Accumulation of reactive oxygen species (ROS), including in mitochondria;
- Adenosine triphosphate (ATP) depletion; and,
- Disruption of glycolytic pathways.[77-79]

Current static hypothermic preservation solutions can broadly be classified as (i) intracellular versus extracellular or intermediate, based largely upon the solution's potassium content, and/or (ii) low viscosity versus high viscosity solutions.[80] Common components include colloid and/or impermeants to counteract cellular oedema, antioxidants for protection against ROS generation, ATP precursors to allow replenishment upon reperfusion, and buffers to retard the acidosis attendant with organ ischaemia.[80] University of Wisconsin (UW) solution is arguably the most well-known and commonly utilized fluid. Other popular preservation fluids include histidine-tryptophan-ketoglutarate (HTK, or Custodiol), Celsior, Eurocollins, Marshall's (Ross/Hyperosmolar Citrate), and Institute Georges Lopez (IGL)-1.[78]

Perfusion routes, techniques and volumes, and static storage during transportation

Abdominal organ perfusion prior to procurement is primarily undertaken via the aorta, with the option of undertaking additional portal venous perfusion ('dual' perfusion) for liver retrievals. Unfortunately to date there has been no uniformity in guidelines regarding whether the aortic or dual perfusion route should be employed.[81-83] Furthermore, another potential variation exists in the use of a 'pre-flush' whereby a fluid that is not the final preservation fluid is utilized in the systemic flush prior to the final flush to allow for adequate clearance of PRBCs. Perfusion volume depends on the solution utilized (e.g. much higher volumes required for equilibration of

HTK), and is partially determined by the perfusionist/surgeon based on the resistance to fluid flow and perceived content of blood within the perfused effluent visualized from the venting site. The back-table provides an additional site for final perfusion of the organ prior to transport to the recipient centre.[74]

Following *in situ* and back-table perfusion, the majority of organs undergo cold (static) storage (CS) whereby they are bagged and/or boxed in cold preservation solution, surrounded by at least one more layer of ice slush.[84-86] This helps maintain the organ(s) in a suitable, hypothermic microenvironment in preparation for transport to the transplantation centre. Alternative preservation approaches, in particular dynamic and/or normothermic methods, are introduced in section 1.4.

What is the best perfusion fluid, volume, and route to use?

Perfusion fluid types, route(s), and volumes for abdominal transplant organs largely seem to be dependent on individual transplant retrieval unit preference in the context of the exact organ(s) being retrieved. Indeed, organ flush protocols vary significantly between centres with respect to all of these parameters, and there is certainly no worldwide consensus.[81-83, 87, 88]

The most commonly utilized static preservation solutions for deceased donor kidneys are UW, HTK, Celsior, Eurocollins, and Marshall's. Systematic evaluations of their comparative efficacies largely fail to demonstrate inferiority of one fluid type over another with the exception of Eurocollins, which may be responsible for higher rates of DGF.[89-92] One registry analysis also purported reduced kidney transplant survival associated with the use of HTK, although this is an isolated finding.[93] The choice of perfusion and static preservation solution is likely more relevant in the context of liver and pancreas transplantation. Perhaps the greatest controversy is in the comparison between UW and HTK. Some studies have failed to show a graft survival difference for either organ, using either preservation solution.[94-96] Later, larger registry analyses have shown a higher risk of pancreatic and hepatic graft failure when HTK was used in comparison to UW.[97-99]

Paramount in the setting of multi-organ retrieval, the perfusion fluid that is chosen must not compromise outcomes for any of the procured organs. As for what the ideal solution is for all organs, the current literature and guidelines are also not entirely clear, and significant further work is required in this area. The situation becomes further complicated by the increasing use of dynamic preservation strategies during transportation or in the pre-implantation setting, which will be an important focus of this dissertation.

1.3 Damage to the donor organ during storage and transplantation

The function of a transplant organ is determined by donor, recipient, and preservation-related factors. Organ function in the original donor is usually superior to what is achieved upon transplantation as the donor organ suffers two major interacting insults after procurement and implantation – (i) an antibody and cell-mediated alloimmune response to the graft (this is beyond the scope of this thesis); and (ii) ischaemia-reperfusion injury (IRI). The severity of IRI itself is a function of (i) the time to transplantation and restoration of sanguinous oxygenated perfusion in the recipient; (ii) organ temperature dynamics during storage; and (iii) the preservation conditions and/or substrates utilized. Ischaemic injury can either be 'warm' or 'cold', and in effect primes the organ for further damage upon reperfusion in the recipient.

1.3.1 ISCHAEMIC TIMES AND DEFINITIONS

Ischaemic times encountered during the donation/transplantation process are outlined in Figure 1.

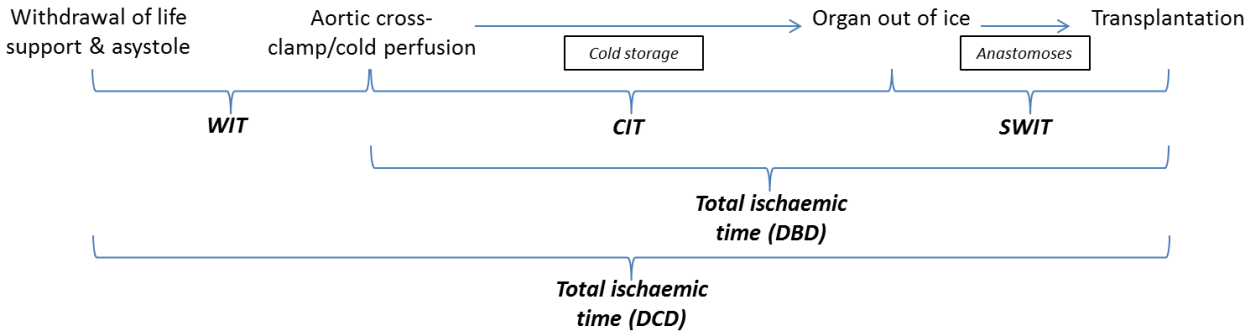


Figure 1. Ischaemic intervals encountered in organ transplantation. Note that definitions of the initial warm ischaemic time (WIT) vary, and may be measured from the time of withdrawal of life support, or from the time of asystole and pronunciation of death, or after pre-defined patient vital criteria. The period between aortic cross-

clamping and cold *in situ* perfusion and subsequent anastomosis may be partially replaced by normothermia or subnormothermia as part of dynamic machine perfusion techniques. In DCD donors, the time prior to aortic cross-clamp/cold perfusion may also incorporate a period of artificial re-institution of the patient's circulation using normothermic regional perfusion. CIT – cold ischaemic time; SWIT – second warm ischaemic time

Exact definitions of ischaemic times are somewhat heterogeneous in the literature, in particular with respect to the first warm ischaemic time (WIT) in DCD donors.[100, 101] Most commonly, the WIT is defined as the period from extubation (withdrawal of life support) to institution of cold *in situ* perfusion.[101] Alternative definitions may describe the WIT as the time from asystole to cold perfusion, or the time after the blood pressure or oxygen saturation drops below a pre-defined level until cold perfusion (i.e. the 'functional warm ischaemic period').[101] Some authors have suggested splitting the WIT into two phases – (i) phase I, representing the time from extubation to asystole, and (ii) phase II, denoting the time from asystole to cold perfusion.[101] Use of the additional sub-phase of functional warm ischemia, alternatively defined based on blood pressure or saturation measurements, is potentially more useful and impactful upon an organ's subsequent function.[102]

Another important consideration with respect to defined ischaemic intervals is that they do not consider the initial warm ischaemic insult suffered by DBD donor organs. This warm ischaemic insult is secondary to the haemodynamic disturbances and inflammatory activation commonly present in these donors, in addition to potential exposure of the organs to warm ischaemia during retrieval and dissection, and priming of DBD organs to further immune-related damage upon reperfusion.[103-108]

The kidney, liver, and pancreas all have different tolerance to cold and warm ischaemic periods. Generally, in the context of controlled DCD procurement, the initial WIT (from time of extubation) should not exceed 30-45 minutes for the liver, and 45-60 minutes for the kidney and pancreas, otherwise there is an increased risk of transplant graft dysfunction.[109] These values are not absolute however, and particularly in the UK units may wait up to 2 hours after the 'functional' warm ischaemic threshold is reached before abandoning kidney retrieval.[110] Generally recommended CITs for DCD livers, pancreases, and kidneys are less than 10 hours, 18

hours, and 24 hours, respectively, although there is considerable variation between jurisdictions.[109, 110]

1.3.2 ISCHAEMIA-REPERFUSION INJURY (IRI)

Organ ischaemia commences upon the cessation of effective circulation within the donor. In the absence of intervening dynamic oxygenated perfusion, the ischaemic interval ceases once the arterial clamps are released during the transplantation procedure. At this point, the reperfusion phase comes into effect with an influx of oxygen, leukocytes, complement, and other plasma mediators. Together, the cumulative insult that is derived is known as ischaemia-reperfusion injury (IRI). IRI is a complex cascade that represents the intersection of multiple injurious pathways, and its severity can impact upon short and long-term graft function.[103, 111-113] A schematic representation of IRI in organ transplantation is presented in Fig. 2.

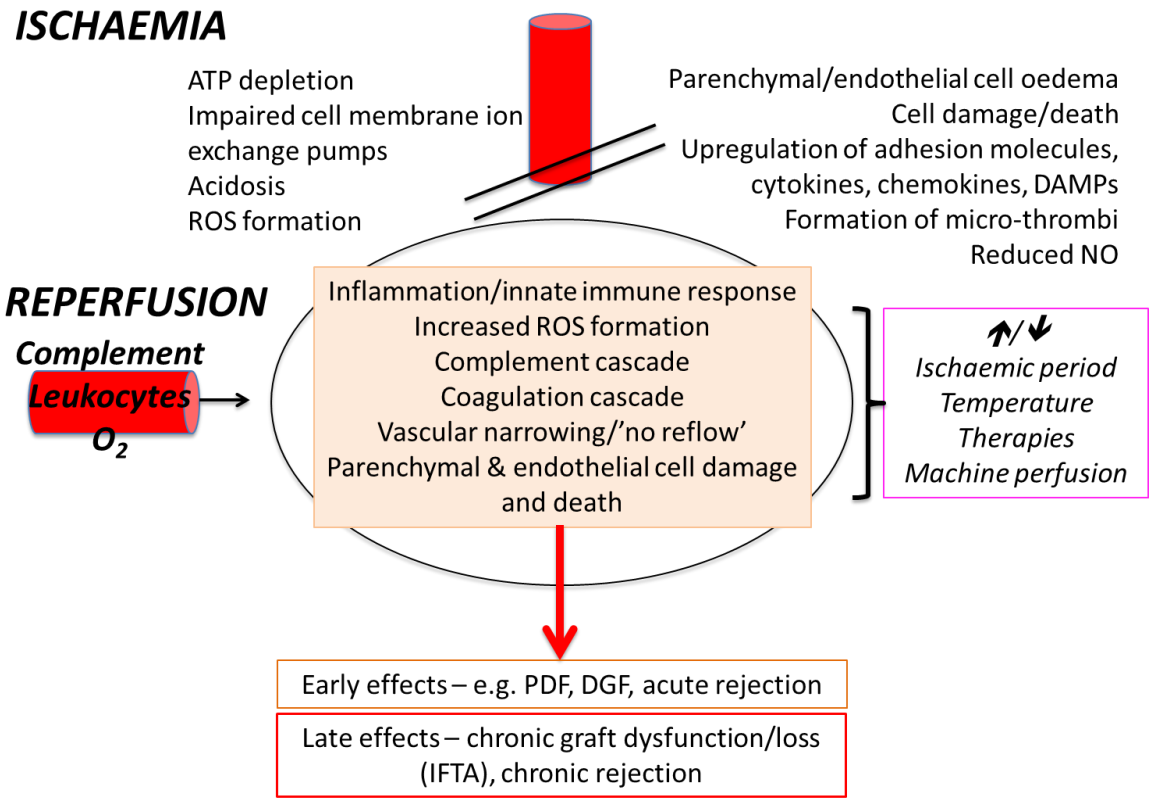


Figure 2. The ischaemia-reperfusion injury cascade. ATP – adenosine triphosphate; DGF – delayed graft function; DAMP – danger-associated molecular patterns; IFTA – interstitial fibrosis and tubular atrophy; NO – nitric oxide; PDF – primary graft dysfunction; ROS – reactive oxygen species

Disconnection of an organ's arterial supply naturally induces absolute ischaemia and hypoxia. ATP stores are depleted, and anaerobic metabolism is induced. There is attendant acidosis, malfunction of membrane ATP-dependent ion exchange pumps, including the Na^+/K^+ transporter, in addition to intracellular calcium accumulation, and the formation of reactive oxygen species (ROS). Endothelial and parenchymal cellular oedema and injury is induced, in addition to the increased expression of adhesion molecules such as ICAM-1, release of damage-associated molecular patterns (DAMPs), and the up-regulation of gene expression related to inflammatory and hypoxic signaling.[114, 115] These changes during the ischaemic phase prime the organ for subsequent reperfusion injury when sanguinous perfusion is restored.

The reperfusion process paradoxically induces further damage as it brings with it allogeneic blood containing reactive innate and adaptive immune cells, along with other injury-provoking mediators such as complement components, coagulation factors, and plasma immunoglobulins. There is also an acute inflammatory immune response dominated by innate immune cells, which induces local damage.[114] ROS formation is amplified with the re-introduction of oxygen. Leukocyte adhesion and diapedesis, along with platelet binding and activation of coagulation, contribute to a local 'no reflow' phenomenon and microvascular dysfunction. Angiogenic induction in the local environment is inhibited, contributing to a chronic relative hypoxia. The adaptive immune response is also activated in response to IRI. Endothelial and parenchymal cell injury may result in cell death via necrosis, apoptosis, and/or induction of autophagy-related pathways.[103, 113-115]

It is important to note that hypothermia does not completely attenuate IRI, and indeed may be damaging in of itself.[112, 116, 117] It is very difficult to isolate the potential deleterious effects of hypothermia from the general IRI process, but it is nonetheless clear that the CS period is not benign in of itself.[116] Although the organ's metabolic rate is reduced at lower temperatures, some metabolic processes nonetheless continue and deplete stores of ATP. Enzyme function and protein conformation is sub-optimal, impairing critical subcellular processes that continue in hypothermia.[77] A prolonged period of hypothermic organ storage can be highly deleterious, especially in combination with period(s) of warm ischaemia and the inevitable reperfusion in the recipient.[112] Indeed, a critical determinant of cold ischaemic injury may be its coupling to warm

reperfusion; if this occurs abruptly, as is generally the case in transplantation, there is evidence for mitochondrial stress, dysfunction, and induction of apoptotic pathways.[116-118]

1.3.3 CLINICAL SIGNIFICANCE OF IRI

The primary aim of organ preservation strategies is the minimization of IRI-related damage to the graft, which is pictorially depicted in Fig. 3, and is a function of preservation time and temperature, amongst other factors.

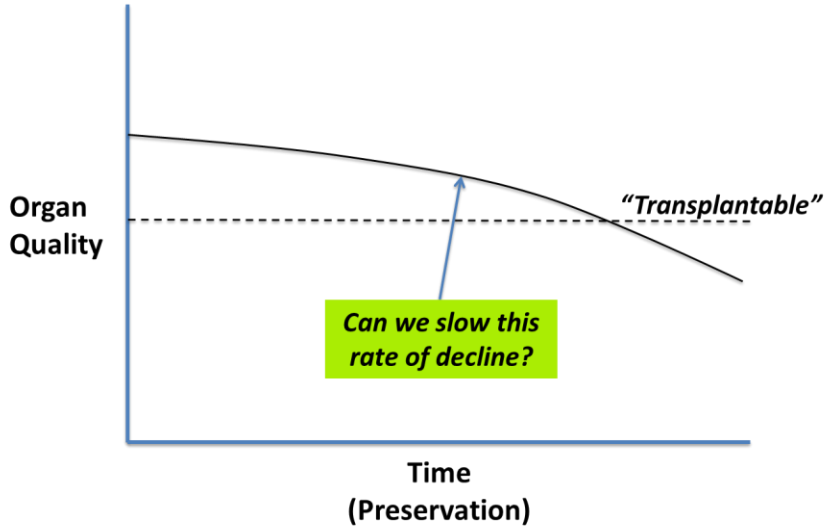


Figure 3. Organ procurement and preservation practices impact organ quality at transplant. The aim of preservation approaches between procurement and transplantation are to minimize the rate of decline of organ quality. This may be achieved by minimizing storage time, modulating temperature, modifying preservation solutions, and using technologies such as machine perfusion, in addition to other approaches.

The manifestation of IRI in the graft depends on the severity of the insult. Within the kidney, severe IRI with associated acute tubular necrosis may manifest as DGF. Potential delayed/longer-term effects are mediated by activation of the adaptive immune response and progressive tubular trophy and interstitial fibrosis due to epithelial-to-mesenchymal cell transition, with subsequent impacts on graft survival.[103, 111] Liver transplant IRI may present as primary graft dysfunction (PDF) and/or primary non-function (PNF), biliary injury and ITBL, and impaired graft survival.[119] Pancreatic graft IRI is naturally associated with graft pancreatitis,

which also serves as a risk factor for graft thrombosis.[120, 121] Transplant graft IRI may also increase graft immunogenicity and contribute to episodes of acute and/or chronic rejection.[111, 122]

The clinical severity and manifestations of graft IRI are not uniform, and are modified by donor and recipient factors, in addition to the duration of ischaemia, temperature during ischaemia, anti-IRI therapeutic delivery, the nature of perfusion/preservation solution(s) utilized, and the use of other technologies such as machine perfusion (MP). The duration of anastomoses, i.e. the second WIT (SWIT), is also critical to subsequent graft function, as a prolonged period will increase the severity of the IRI hit. All of these factors will be discussed in detail over the course of this dissertation.

1.3.4 PHARMACOLOGIC AMELIORATION OF IRI IN CLINICAL TRANSPLANTATION

The transplant and IRI literature is replete with pre-clinical studies investigating the role of different pharmacologic/therapeutic agents in the amelioration of IRI-related injury. Multiple reviews have been published investigating the role of such agents, especially in the context of kidney and liver transplantation.[111, 113, 123-126]

Broadly, the pathophysiologic processes within the IRI cascade targeted by these agents most commonly include oxidative and/or mitochondrial stress, inflammation/leukocyte influx, the complement cascade, the coagulation cascade, and local vascular abnormalities.[127-136] This includes the utilization of newer, experimental drugs, monoclonal antibodies, repurposing of existing drugs, and other experimental techniques such as the use of small interfering RNAs (siRNAs) and microRNAs (miRNAs).

Very few agents have made it past the pre-clinical phase and been tested in the human transplantation setting, and close to none are being used regularly. It is clear that the majority of pre-clinical work has been lost in translation, a transplant-specific version of the 'Valley of Death'.[137, 138] Reasons for this are multi-factorial, and are related to such factors as: (i) uncertain or poor clinical efficacy of these agent(s) despite promise in animal testing; (ii) ethical considerations related to the systemic treatment of donors (especially in the DCD setting); (iii) inherent difficulties related to the translation of drugs to allow the systemic treatment of

recipients, including the large costs associated with drug development and trials; and (iv) difficulties with conducting clinical trials in transplantation.[138, 139]

Newer approaches must be utilized to try and ameliorate transplant organ IRI using therapeutic agents. One such approach, the role of MP for drug delivery, will be discussed further in Part 2 of this dissertation.

1.4 Modern strategies to enhance organ procurement and preservation

Once a potential organ donor patient is identified, there are many potential therapies and/or management protocols that can possibly be instituted to minimize graft damage and help optimize function post-transplantation. However, none of these can be implemented without express consideration of the ethical and legal considerations specific to the local setting, and also taking into account the donor's and/or donor's family's wishes. This is especially pertinent in our own state (New South Wales, Australia) with regards to restrictions on the use of ante-mortem interventions in DCD donors.[140]

1.4.1 DBD DONOR MANAGEMENT

Specific considerations need to be made for DBD donors. In conjunction with a rising intracranial pressure, DBD donors have complex cardiovascular and respiratory changes, a systemic inflammatory response, and changes to systemic hormones secondary to pituitary failure.[141, 142] As such, these areas serve as potential therapeutic targets that can be reversed prior to organ retrieval. The primary goal of DBD donor management is to achieve and maintain the donor's physiologic parameters as close to normal as possible.[141] Pituitary failure may be compensated for by use of hormonal resuscitation, which may include the administration of steroids, thyroid hormones, insulin, and desmopressin.[143] However, the individual role(s) of each of these agents is not well-defined, and most studies that have been conducted have been retrospective in nature and/or of low-quality.[144-146] Targeting of circulatory changes is perhaps more essential, and can be achieved through the use of intravenous fluids and vasopressors.[143] Achievement of donor management goals may increase the number of transplantable organs from each individual DBD donor.[147] Overall donor management goals may include the following:

- Mean arterial pressure 60-100 mmHg;
- Central venous pressure 4-10 mmHg;
- Ejection fraction > 50%;
- Use of one or less vasopressor at a low dose;
- Maintenance of a normal pH;
- Maintenance of normal pulmonary function (P_aO_2:FiO_2 > 300); and
- Maintenance of normal urine output (>0.5-3 ml/kg/hr), serum sodium, and glucose levels.[141, 143, 147]

Another potential pre-procurement intervention of note in DBD donors is the use of therapeutic hypothermia. A study by Niemann *et al*. compared DBD kidney transplant outcomes from donors either externally cooled to 34-35°C or maintained at a normal temperature of 36.5-37.5°C.[148] DGF rates were significantly reduced in the hypothermic group, with this effect most pronounced in ECD donors.[148]

1.4.2 DCD DONORS – HEPARIN AND NORMOTHERMIC REGIONAL PERFUSION

DCD organ donors present unique challenges but also opportunities within the pre- and intra-procurement phases of donation. Local customs, laws, and policies are a major determinant of whether certain strategies to enhance function of the DCD organ can be successfully implemented. Furthermore, the type of approach utilized is also dependent upon the type of DCD pathway, i.e. controlled or uncontrolled.

The controlled DCD situation affords the ideal opportunity for the delivery of ante-mortem interventions, if permitted under the legislation of the governing jurisdiction. Administration of ante-mortem heparin is a simple yet very effective therapy that is likely to improve outcomes from DCD organ transplantation.[149] Prior to its use, consideration must be made that therapeutic intravenous heparin can theoretically accelerate death in potential DCD donors with concomitant intra-cranial haemorrhage, although there is no clinical evidence for this.[150, 151] Ante-mortem heparin is estimated to reduce the increased risk of graft thrombosis in DCD pancreas transplantation.[70] Evidence for or against the use of heparin is DCD kidney transplantation is sparse, although it is hypothesized to reduce DGF by minimizing formation of microthrombi.[152,

[153] Ante-mortem heparin is also deemed to be beneficial for DCD liver grafts, and is likely superior to the administration of tissue plasminogen activator post-arrest.[154, 155]

Ante-mortem femoral vessel cannulation is used by certain centres in the controlled DCD setting, allowing for rapid institution of cold *in situ* perfusion as soon as death is declared.[109, 149, 153] Abdominal regional perfusion (ARP) is one technique that may take advantage of such cannulae to reinstitute an artificial donor circulation in a manner that is similar to extracorporeal membrane oxygenation (ECMO) technology. ARP can be performed under hypothermic conditions (hypothermic regional perfusion, HRP), or more commonly under normothermia (normothermic regional perfusion, NRP).[156] HRP has only been used in the context of kidney transplantation, and although some promising results have been obtained, the combined clinical experience with this technique is relatively sparse.[156] NRP in contrast has been more extensively utilized, including for kidneys, the liver, and pancreas. NRP has potential benefits in both controlled and uncontrolled DCD donors, and may be administered via femoral or more centrally placed cannulae. NRP may facilitate more objective graft assessment prior to organ recovery, in addition to graft repair and amelioration of IRI.[157, 158] Beneficial effects have been shown with respect to graft outcomes and utilization rates in controlled DCD kidney, liver, and pancreas transplantation without preceding (ante-mortem) heparinization.[157, 159] NRP is perhaps more innovative and incrementally useful in the uncontrolled DCD setting, and is usually performed after vascular cannulation, heparin administration, and simultaneous external cardiac compressions and ventilation.[159] NRP is especially beneficial with respect to the reduction of ITBL rates in DCD liver transplantation.[159]

1.4.3 ORGAN PRESERVATION POST-PROCUREMENT

The comparative use of different cold *in situ* preservation solutions and techniques was been introduced in section 1.2.4, and will be expanded upon in Part 2 of this thesis. The utilization of *ex vivo* organ perfusion techniques (i.e. MP) in the pre-implantation period is a major focus of this dissertation, especially in the context of kidney transplantation, and will be explored in detail over the course of Part 1. MP in of itself is a useful strategy to target IRI, and its efficacy can potentially be enhanced by using MP as a direct delivery method for anti-IRI agent(s) to the donor organ of interest. A detailed discussion regarding MP has been deliberately omitted from

this introductory chapter in order to avoid unnecessary repetition and redundancy, but is included through the course of Part 2 of this dissertation.

1.5 Aims and introduction to the research conducted for this PhD

The perpetual shortage of donor organ supply with respect to demand necessitates ongoing strategies and research to close this gap. The aims of such research should be twofold – (i) to increase the number of organs available for transplantation; and (ii) to simultaneously improve their outcomes in recipients. As a result of increasing requirements for organs such as the liver, kidney, and pancreas, there has been increasing use of DCD and/or ECD kidneys locally and overseas. These organs are more susceptible to IRI incurred during procurement, transportation, and implantation. We therefore need improved procurement and preservation techniques to optimize the use of these organs. Identification of best-practice in these areas and further research gaps will also help streamline surgical procurement and preservation techniques, and allow future work to occur in a more unified fashion. Fig. 4 summarizes the work conducted for this dissertation in the context of IRI, and the cycle of donor organ procurement, preservation, and transplantation.

PART 2 – The Kidney

The kidney, liver, and pancreas are all commonly procured and transplanted abdominal organs, with the potential to increase life expectancy and improve quality of life. However, the kidney is the most prolific organ with respect to procurement and transplantation rates worldwide, and also serves as an ideal and convenient model to test potential advances in organ preservation, which may then be extrapolated to other organs. Therefore, the kidney is the primary organ of focus for the initial section of this thesis. In particular, advances in deceased donor kidney preservation techniques and the re-emergence of MP preservation are emphasized as a potential means to improve the number and quality of kidney transplants from DCD and higher KDPI donors.

Chapter 2 explores the latest advances in the preservation of abdominal and thoracic organs, expanding upon the utilization of MP techniques in the general field of organ transplantation.

This then sets the scene for **Chapter 3**, which consists of a systematic review and meta-analysis comparing MP to traditional CS in the setting of deceased donor kidney transplantation. Here we show the superiority of hypothermic MP (HMP) over CS with respect to the occurrence of DGF, but equivocal results regarding graft survival and also in DCD transplantation in general. This is supplemented by a systematic exploration of pre-clinical studies investigating the utility of dynamic modalities that have to date seen limited clinical use, including oxygenated HMP and normothermic MP (NMP). The significant potential of NMP with respect to graft resuscitation, assessment, and as a means for direct pharmacologic treatment of the kidney is identified and emphasized.

As a result of the findings from the systematic review, and a resolution from a multi-disciplinary Transplantation Society of Australia and New Zealand Machine Perfusion workshop to pursue the investigation of NMP in preference to HMP for kidney preservation, work from **Chapter 4** was commenced. A porcine model of NMP was developed and optimized using modified cardiopulmonary bypass technology, adapted from existing set-ups in the UK and Canada. As part of this process, a customized 3D-printed perfusion chamber was developed to facilitate renal NMP without cannulating the renal vein; this is outlined in **Chapter 5**.

Simultaneously, the tremendous potential ability of NMP to act as a drug-delivery portal for the kidney was recognized. This is especially relevant in the local climate where systemic donor interventions in the DCD setting are not permitted. As such, a mouse IRI model comparing three well-known IRI-targeting agents was established. The feasibility and efficacy of drug delivery by NMP was then investigated by delivering the most efficacious drug from the murine experiments to porcine DCD kidneys using NMP. The results from these experiments are outlined in **Chapter 6**.

Finally, as a prelude to the implementation of NMP in the clinical setting, experience with human kidney NMP was required. This work is outlined in **Chapter 7**, and employs discarded and/or non-utilized human kidneys. Not only does this work demonstrate the feasibility, safety, and efficacy of this technique in the local setting, but also explores the mechanistic basis for the

potential success of brief pre-implantation NMP, the comparable use of autologous or banked blood for NMP, and also leukocyte extravasation from the graft during NMP.

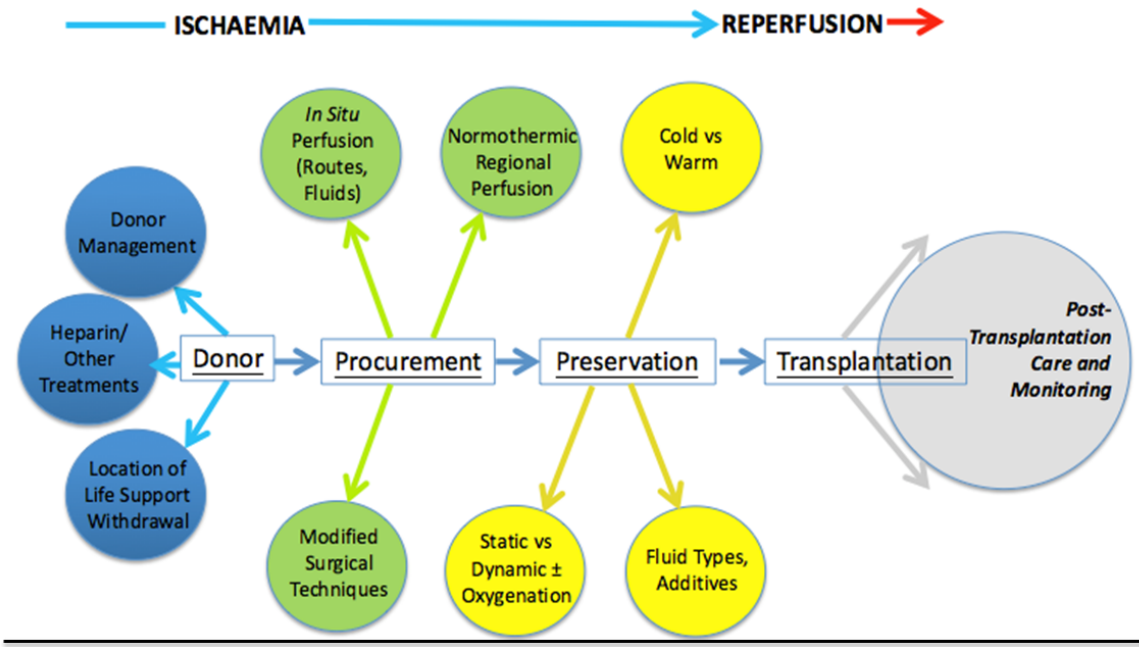
PART 3 – The Liver and Pancreas

Abdominal organs such as the kidneys, liver, and pancreas are often procured in concert in the multi-organ donor setting. The procurement techniques and preservation fluids used need to take into account any subsequent impacts on the post-transplantation outcomes of all of these organs. This part highlights these concepts, especially in the context of deceased donor liver and pancreas procurement/preservation, which require their own special consideration.

Chapter 8 outlines our unique method for recovery of the pancreas in multi-organ donors using ultrasonic shears. The impact of this technique on blood loss and transfusion requirements, in particular, is explored in pancreas transplant recipients.

Chapters 9 and 10 then proceed to convey the results of two systematic reviews and meta-analyses that attempt to fill gaps in our knowledge regarding the most effective perfusion/preservation fluids, routes, and volumes for retrieval and storage of the pancreas and liver, respectively. These reviews generated interest within the transplant community and prompted a Letter to the Editor; our reply Letter is included in **Chapter 11**.

The liver systematic review and meta-analysis identified a significant evidence gap with respect to the use of aortic-only or dual (aortic and portal venous) *in situ* perfusion during liver retrieval. All existing articles either had insufficient patient numbers, or limited periods of follow-up. As such, a large national registry analysis with prolonged follow-up was conducted comparing liver transplantation outcomes after aortic or dual perfusion in Australia. Details and results of this analysis form the basis of **Chapter 12**.



	Procurement & Transplantation	Preservation
GENERAL		Advances in organ preservation narrative review
KIDNEY		Kidney machine perfusion systematic review & meta-analysis Optimization of a normothermic machine perfusion system (porcine model) and 3D-printed chamber Mouse ischemia-reperfusion drug treatment model with subsequent administration by machine perfusion in porcine kidneys Discarded human kidney normothermic machine perfusion paper
LIVER	*In situ* perfusion & subsequent preservation of the liver after retrieval – systematic review & meta-analysis and Reply to letter to the editor	
&	Aortic versus dual perfusion of the liver during retrieval & its impact on transplant outcomes – registry analysis	
PANCREAS	Pancreas retrieval using ultrasonic shears & its impact on recipient blood loss	*In situ* perfusion & subsequent preservation of the pancreas after retrieval – systematic review & meta-analysis

Figure 4. Summary of research conducted for this thesis and its relation to organ procurement, preservation, and transplantation. The top panel indicates potential strategies that may be targeted/improved at each step to enhance transplantation outcomes. The bottom panel indicates some papers published as part of this PhD in the context of organ type and organ procurement, preservation, and transplantation.

1.6 References

1. Tonelli M, Wiebe N, Knoll G, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant*. 2011; 11: 2093-2109.
2. Gruessner RWG, Gruessner AC. The current state of pancreas transplantation. *Nat Rev Endocrinol*. 2013; 9: 555-562.
3. Jain A, Reyes J, Kashyap R, et al. Long-Term Survival After Liver Transplantation in 4,000 Consecutive Patients at a Single Center. *Ann Surg.* 2000; 232: 490-500.
4. Desai R, Jamieson NV, Gimson AE, et al. Quality of life up to 30 years following liver transplantation. *Liver Transplant*. 2008; 14: 1473-1479.
5. Girlanda R. Deceased organ donation for transplantation: Challenges and opportunities. *World J Transplant*. 2016; 6: 451-459.
6. Wall SP, Plunkett C, Caplan A. A potential solution to the shortage of solid organs for transplantation. *JAMA*. 2015; 313: 2321-2322.
7. Abouna GM. Organ shortage crisis: problems and possible solutions. *Transplant Proc.* 2008; 40: 34-38.
8. Chapman JR, Kanellis J. Kidney donation and transplantation in Australia: more than a supply and demand equation. *Med J Aust.* 2018; 209: 242-243.
9. ANZDATA Registry. 40th Report, Chapter 6: Australian Transplant Waiting List. 2018. Available at: http://www.anzdata.org.au/v1/index.html. Accessed October 1, 2018.
10. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. *Transplant Proc.* 1995; 27: 2893-2894.
11. Ridley S, Bonner S, Bray K, Falvey S, Mackay J, Manara A. UK guidance for non-heart-beating donation. *Br. J Anaesth.* 2005; 95: 592-595.
12. Rao PS, Ojo A. The Alphabet Soup of Kidney Transplantation: SCD, DCD, ECD—Fundamentals for the Practicing Nephrologist. *Clin J Am Soc Nephrol.* 2009; 4: 1827-1831.
13. Manara AR, Murphy PG, O'Callaghan G. Donation after circulatory death. *Br J Anaesth.* 2012; 108: i108-i121.
14. Organ and Tissue Authority. National Protocol for Donation after Cardiac Death - July 2010. Canberra: Australian Government - Organ and Tissue Authority, 2010. Available at: https://donatelife.gov.au/sites/default/files/DCD%20protocol%20020311-0e4e2c3d-2ef5-4dff-b7ef-af63d0bf6a8a-1.PDF. Accessed October 10, 2018.
15. NHS Blood and Transplant. Deceased Donation - Donation after Circulatory Death 2018. Available at: https://www.odt.nhs.uk/deceased-donation/best-practice-guidance/donation-after-circulatory-death/. Accessed October 31, 2018.
16. TSANZ. A Guide to the Australian Kidney Donor Profile Index (KDPI) 2016. Available at: https://www.tsanz.com.au/standalonepages/documents/AustralianKDPIINFOv1.0.pdf. Accessed March 5, 2017.
17. OPTN. A Guide to Calculating and Interpreting the Kidney Donor Profile Index (KDPI). USA: The Organ Procurement and Transplantation Network, 2012. Available at: https://optn.transplant.hrsa.gov/resources/guidance/kidney-donor-profile-index-kdpi-guide-for-clinicians/. Accessed November 5, 2018.
18. Parker WF, Thistlethwaite JR, Jr., Ross LF. Kidney Donor Profile Index Does Not Accurately Predict the Graft Survival of Pediatric Deceased Donor Kidneys. *Transplantation*. 2016; 100: 2471-2478.
19. Jay CL, Washburn K, Dean PG, Helmick RA, Pugh JA, Stegall MD. Survival Benefit in Older Patients Associated With Earlier Transplant With High KDPI Kidneys. *Transplantation*. 2017; 101: 867-872.

20. Tector AJ, Mangus RS, Chestovich P, et al. Use of Extended Criteria Livers Decreases Wait Time for Liver Transplantation Without Adversely Impacting Posttransplant Survival. *Ann Surg*. 2006; 244: 439-450.
21. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. *Am J Transplant*. 2015; 15: 161-169.
22. Braat AE, Blok JJ, Putter H, et al. The Eurotransplant donor risk index in liver transplantation: ET-DRI. *Am J Transplant*. 2012; 12: 2789-2796.
23. Feng S, Goodrich NP, Bragg-Gresham JL, et al. Characteristics Associated with Liver Graft Failure: The Concept of a Donor Risk Index. *Am J Transplant*. 2006; 6: 783-790.
24. Fridell JA, Stratta RJ. Expanding the Pancreas Donor Pool. *Curr Transplant Rep*. 2014; 1: 100-112.
25. Muthusamy ASR, Mumford L, Hudson A, Fuggle SV, Friend PJ. Pancreas Transplantation From Donors After Circulatory Death From the United Kingdom. *Am J Transplant*. 2012; 12: 2150-2156.
26. Proneth A, Schnitzbauer AA, Holub I, et al. Extended pancreas donor program – The EXPAND study, a prospective multicentre trial. *Transplantation*. 2016; 100: S1-S929.
27. Axelrod DA, Sung RS, Meyer KH, Wolfe RA, Kaufman DB. Systematic Evaluation of Pancreas Allograft Quality, Outcomes and Geographic Variation in Utilization. *Am J Transplant*. 2010; 10: 837-845.
28. Kopp WH, de Vries E, de Boer J, et al. Donor risk indices in pancreas allocation in the Eurotransplant region. *Transpl Int*. 2016; 29: 921-929.
29. Donatelife. Australian Donation and Transplantation Activity Report 2017. Canberra: Australian Government - Organ and Tissue Authority, 2017. Available at: https://donatelife.gov.au/sites/default/files/2017%20Australian%20Donations%20and%20Transplantation%20Activity%20Report.pdf. Accessed October 12, 2018.
30. AOPO. Organ Procurement and Transplantation Network (OPTN) Data 2018. Available at: http://www.aopo.org/related-links-data-on-donation-and-transplantation/. Accessed October 31, 2018.
31. ANZOD Registry. Annual Report, Section 4: Deceased Organ Donor Profile 2017. Available at: Available at www.anzdata.org.au. Accessed October 31, 2018.
32. Marrero WJ, Naik AS, Friedewald JJ, et al. Predictors of Deceased Donor Kidney Discard in the United States. *Transplantation*. 2017; 101: 1690-1697.
33. Reese PP, Harhay MN, Abt PL, Levine MH, Halpern SD. New Solutions to Reduce Discard of Kidneys Donated for Transplantation. *J Am Soc Nephrol*. 2016; 27: 973-980.
34. Mohan S, Chiles MC, Patzer RE, et al. Factors leading to the discard of deceased donor kidneys in the United States. *Kidney Int*. 2018; 94: 187-198.
35. Hamed MO, Chen Y, Pasea L, et al. Early graft loss after kidney transplantation: risk factors and consequences. *Am J Transplant*. 2015; 15: 1632-1643.
36. Snoeijs MG, Winkens B, Heemskerk MB, et al. Kidney transplantation from donors after cardiac death: a 25-year experience. *Transplantation*. 2010; 90: 1106-1112.
37. Schaapherder A, Wijermars LGM, de Vries DK, et al. Equivalent Long-term Transplantation Outcomes for Kidneys Donated After Brain Death and Cardiac Death: Conclusions From a Nationwide Evaluation. *EClinicalMedicine*. 2018; *Epub ahead of print*. DOI: 10.1016/j.elinm.2018.09.007

38. Summers DM, Johnson RJ, Allen J, et al. Analysis of factors that affect outcome after transplantation of kidneys donated after cardiac death in the UK: a cohort study. *Lancet*. 2010; 376: 1303-1311.
39. Lebranchu Y, Halimi JM, Bock A, et al. Delayed graft function: risk factors, consequences and parameters affecting outcome-results from MOST, A Multinational Observational Study. *Transplant Proc.* 2005; 37: 345-347.
40. Koning OHJ, Ploeg RJ, van Bockel JH, et al. Risk factors for delayed graft function in cadaveric kidney transplantation: A Prospective Study of Renal Function and Graft Survival after Preservation with University of Wisconsin Solution in Multi-Organ Donors1. *Transplantation*. 1997; 63: 1620-1628.
41. de Sandes-Freitas TV, Felipe CR, Aguiar WF, Cristelli MP, Tedesco-Silva H, Medina-Pestana JO. Prolonged Delayed Graft Function Is Associated with Inferior Patient and Kidney Allograft Survivals. *PLOS ONE*. 2015; 10: e0144188.
42. Muth BL, Astor BC, Turk J, et al. Outpatient Management of Delayed Graft Function Is Associated With Reduced Length of Stay Without an Increase in Adverse Events. *Am J Transplant*. 2016; 16: 1604-1611.
43. Matas AJ, Gillingham KJ, Elick BA, et al. Risk factors for prolonged hospitalization after kidney transplants. *Clin Transplant*. 1997; 11: 259-264.
44. Saidi RF, Elias N, Kawai T, et al. Outcome of kidney transplantation using expanded criteria donors and donation after cardiac death kidneys: realities and costs. *Am J Transplant*. 2007; 7: 2769-2774.
45. Chen G, Wang C, Ko DS, et al. Comparison of outcomes of kidney transplantation from donation after brain death, donation after circulatory death, and donation after brain death followed by circulatory death donors. *Clin Transplant*. 2017; 31.
46. Nicholson ML, Metcalfe MS, White SA, et al. A comparison of the results of renal transplantation from non-heart-beating, conventional cadaveric, and living donors. *Kidney Int.* 2000; 58: 2585-2591.
47. Weber M, Dindo D, Demartines N, Ambühl PM, Clavien P-A. Kidney Transplantation from Donors without a Heartbeat. *N Engl J Med.* 2002; 347: 248-255.
48. Barlow AD, Metcalfe MS, Johari Y, Elwell R, Veitch PS, Nicholson ML. Case-matched comparison of long-term results of non-heart beating and heart-beating donor renal transplants. *Br J Surg.* 2009; 96: 685-691.
49. Lim WH, McDonald SP, Russ GR, et al. Association between delayed graft function and graft loss in donation after cardiac death kidney transplants - a paired kidney registry analysis. *Transplantation*. 2017. 101: 1139-1143.
50. Lim WH, McDonald SP, Kennedy SE, Larkins N, Wong G. Association Between Slow and Delayed Graft Function With Graft Outcomes in Pediatric and Adolescent Deceased Donor Kidney Transplant Recipients. *Transplantation*. 2017; 101: 1906-1912.
51. Le Dinh H, Weekers L, Bonvoisin C, et al. Delayed graft function does not harm the future of donation-after-cardiac death in kidney transplantation. *Transplant Proc*. 2012; 44: 2795-2802.
52. Nagaraja P, Roberts GW, Stephens M, et al. Influence of delayed graft function and acute rejection on outcomes after kidney transplantation from donors after cardiac death. *Transplantation*. 2012; 94: 1218-1223.
53. Singh RP, Farney AC, Rogers J, et al. Kidney transplantation from donation after cardiac death donors: lack of impact of delayed graft function on post-transplant outcomes. *Clin Transplant*. 2011; 25: 255-264.

54. Gill J, Dong J, Rose C, Gill JS. The risk of allograft failure and the survival benefit of kidney transplantation are complicated by delayed graft function. *Kidney Int.* 2016; 89: 1331-1336.
55. Lee J, Song SH, Lee JY, et al. The recovery status from delayed graft function can predict long-term outcome after deceased donor kidney transplantation. *Scientific reports*. 2017; 7: 13725-13725.
56. Giral-Classe M, Hourmant M, Cantarovich D, et al. Delayed graft function of more than six days strongly decreases long-term survival of transplanted kidneys. *Kidney Int.* 1998; 54: 972-978.
57. Mirshekar-Syahkal B, Summers D, Bradbury LL, et al. Local Expansion of Donation After Circulatory Death Kidney Transplant Activity Improves Waitlisted Outcomes and Addresses Inequities of Access to Transplantation. *Am J Transplant*. 2017; 17: 390-400.
58. Snyder RA, Moore DR, Moore DE. More donors or more delayed graft function? A cost-effectiveness analysis of DCD kidney transplantation. *Clin Transplant*. 2013; 27: 289-296.
59. Snoeijs MG, Schaubel DE, Hené R, et al. Kidneys from donors after cardiac death provide survival benefit. *J Am Soc Nephrol*. 2010; 21: 1015-1021.
60. Callaghan CJ, Charman SC, Muiesan P, Powell JJ, Gimson AE, van der Meulen JHP. Outcomes of transplantation of livers from donation after circulatory death donors in the UK: a cohort study. *BMJ Open*. 2013; 3.
61. Skaro AI, Jay CL, Baker TB, et al. The impact of ischemic cholangiopathy in liver transplantation using donors after cardiac death: the untold story. *Surgery*. 2009; 146: 543-552.
62. O'Neill S, Roebuck A, Khoo E, Wigmore SJ, Harrison EM. A meta-analysis and meta-regression of outcomes including biliary complications in donation after cardiac death liver transplantation. *Transpl Int*. 2014; 27: 1159-1174.
63. Jay CL, Lyuksemburg V, Ladner DP, et al. Ischemic cholangiopathy after controlled donation after cardiac death liver transplantation: a meta-analysis. *Ann Surg.* 2011; 253: 259-264.
64. Croome KP, Lee DD, Perry DK, et al. Comparison of longterm outcomes and quality of life in recipients of donation after cardiac death liver grafts with a propensity-matched cohort. *Liver Transplant*. 2017; 23: 342-351.
65. Feng S, Lai JC. Expanded criteria donors. *Clin Liver Dis*. 2014; 18: 633-649.
66. Scalea JR, Redfield RR, Foley DP. Liver transplant outcomes using ideal donation after circulatory death livers are superior to using older donation after brain death donor livers. *Liver Transplant*. 2016; 22: 1197-1204.
67. Dubbeld J, van Hoek B, Ringers J, et al. Biliary Complications After Liver Transplantation From Donation After Cardiac Death Donors: An Analysis of Risk Factors and Long-term Outcome From a Single Center. *Ann Surg.* 2015; 261: e64.
68. Fernandez LA, Di Carlo A, Odorico JS, et al. Simultaneous pancreas-kidney transplantation from donation after cardiac death: successful long-term outcomes. *Ann Surg.* 2005; 242: 716-723.
69. Bellingham JM, Santhanakrishnan C, Neidlinger N, et al. Donation after cardiac death: a 29-year experience. *Surgery*. 2011; 150: 692-702.
70. Shahrestani S, Webster AC, Lam VW, et al. Outcomes From Pancreatic Transplantation in Donation After Cardiac Death: A Systematic Review and Meta-Analysis. *Transplantation*. 2017; 101: 122-130.
71. Proneth A, Schnitzbauer AA, Schenker P, et al. Extended Pancreas Donor Program-The EXPAND Study: A Prospective Multicenter Trial Testing the Use of Pancreas Donors Older Than 50 Years. *Transplantation*. 2018; 102: 1330-1337.

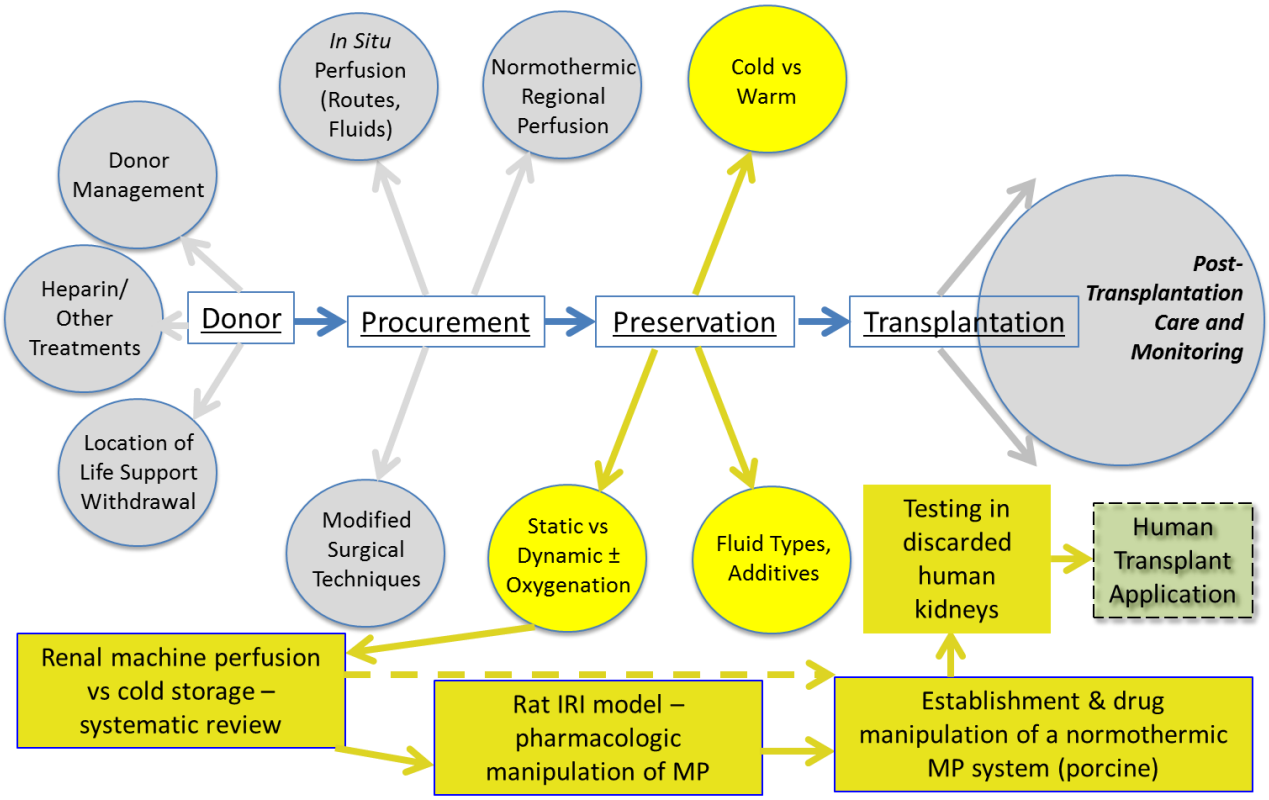
72. Starzl TE, Hakala TR, Shaw BW, Jr., et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet.* 1984; 158: 223-230.
73. Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multuple organ harvesting. *Surg Gynecol Obstet.* 1987; 165: 343-348.
74. Oniscu GC, Forsythe JLR, Fung JJ. Abdominal organ retrieval and transplantation bench surgery. Chichester, West Sussex: John Wiley & Sons, 2013.
75. Brockmann JG, Vaidya A, Reddy S, Friend PJ. Retrieval of abdominal organs for transplantation. *Br J Surg.* 2006; 93: 133-146.
76. Casavilla A, Ramirez C, Shapiro R, et al. Experience with liver and kidney allografts from non-heart-beating donors. *Transplantation*. 1995; 59: 197-203.
77. Churchill TA. Organ Preservation for Transplantation. Functional Metabolism: John Wiley & Sons, Inc.; 2005:pp. 529-555.
78. Guibert EE, Petrenko AY, Balaban CL, Somov AY, Rodriguez JV, Fuller BJ. Organ Preservation: Current Concepts and New Strategies for the Next Decade. *Transfus Med Hemother*. 2011; 38: 125-142.
79. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*. 2014; 515: 431-435.
80. Bon D, Chatauret N, Giraud S, Thuillier R, Favreau F, Hauet T. New strategies to optimize kidney recovery and preservation in transplantation. *Nat Rev Nephrol*. 2012; 8: 339-347.
81. TSANZ. Guidance Document - Surgical Technique for Deceased Donor Abdominal Organ Procurement (ATCA-TSANZ Guidelines G003/2015). Sydney, Australia: TSANZ, 2015. Available from: https://www.tsanz.com.au/standalonepages/document-download.asp. Accessed April 10, 2018.
82. Zalewska K, Ploeg R. National Standards for Organ Retrieval from Deceased Donors (NORS Retrieval Standards). Bristol, UK, 2014.
83. Eurotransplant Foundation. Eurotransplant Manual. Leiden: Netherlands: Eurotransplant 2016. Available from: https://www.eurotransplant.org/cms/index.php?page=et_manual. Accessed September 15, 2018.
84. Opelz G, Dohler B. Multicenter analysis of kidney preservation. *Transplantation*. 2007; 83: 247-253.
85. Lee CY, Mangino MJ. Preservation methods for kidney and liver. *Organogenesis*. 2009; 5: 105-112.
86. Yuan X, Theruvath AJ, Ge X, et al. Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. *Transpl Int*. 2010; 23: 561-570.
87. Hameed AM, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: working towards a unified approach. *Liver Transplant*. 2017. 23: 1615-1627.
88. Hameed AM, Wong G, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. *HPB (Oxford)*. 2017; 19: 933-943.
89. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. Preservation solutions for static cold storage of kidney allografts: a systematic review and meta-analysis. *Am J Transplant*. 2012; 12: 896-906.
90. Bellamy CA, Nicely B, Mattice BJ, Teaster R. Comparative analysis of clinical efficacy and cost between University of Wisconsin solution and histidine-tryptophan-ketoglutarate. *Prog Transplant*. 2008; 18: 166-171.

91. Bond M, Pitt M, Akoh J, Moxham T, Hoyle M, Anderson R. The effectiveness and cost-effectiveness of methods of storing donated kidneys from deceased donors: a systematic review and economic model. *Health Technol Assess.* 2009; 13: iii-iv, xi-xiv, 1-156.
92. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. A National Registry Analysis of Kidney Allografts Preserved With Marshall's Solution in the United Kingdom. *Transplantation.* 2016; 100: 2447-2452.
93. Stewart ZA, Lonze BE, Warren DS, et al. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival of deceased donor kidney transplants. *Am J Transplant*. 2009; 9: 1048-1054.
94. Alonso D, Dunn TB, Rigley T, et al. Increased pancreatitis in allografts flushed with histidine-tryptophan-ketoglutarate solution: a cautionary tale. *Am J Transplant*. 2008; 8: 1942-1945.
95. Englesbe MJ, Moyer A, Kim DY, et al. Early pancreas transplant outcomes with histidine-tryptophan-ketoglutarate preservation: a multicenter study. *Transplantation*. 2006; 82: 136-139.
96. Mangus RS, Fridell JA, Vianna RM, et al. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution in extended criteria liver donors. *Liver Transplant*. 2008; 14: 365-373.
97. Adam R, Delvart V, Karam V, et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant*. 2015; 15: 395-406.
98. Stewart ZA, Cameron AM, Singer AL, Dagher NN, Montgomery RA, Segev DL. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation. *Am J Transplant*; 9: 217-221.
99. Stewart ZA, Cameron AM, Singer AL, Montgomery RA, Segev DL. Histidine-Tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. *Am J Transplant*. 2009; 9: 286-293.
100. Wells A, Watson C, Jamieson N, Bradley JA. Which time is it? A suggestion for unambiguous nomenclature in transplantation. *Am J Transplant*. 2007; 7: 1315-1316.
101. Bernat JL, D'Alessandro AM, Port FK, et al. Report of a National Conference on Donation after Cardiac Death. *Am J Transplant.* 2006; 6: 281-291.
102. Bradley JA, Pettigrew GJ, Watson CJ. Time to death after withdrawal of treatment in donation after circulatory death (DCD) donors. *Curr Opin Organ Transplant*. 2013; 18: 133-139.
103. Ponticelli C. Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant*. 2014; 29: 1134-1140.
104. Kunzendorf U, Hohenstein B, Oberbarnscheid M, et al. Duration of Donor Brain Death and its Influence on Kidney Graft Function. *Am J Transplant.* 2002; 2: 292-294.
105. Pratschke J, Tullius SG, Neuhaus P. Brain death associated ischemia/reperfusion injury. *Ann Transplant*. 2004; 9: 78-80.
106. Dziodzio T, Biebl M, Pratschke J. Impact of brain death on ischemia/reperfusion injury in liver transplantation. *Curr Opin Organ Transplant.* 2014; 19: 108-114.
107. Atkinson C, Floerchinger B, Qiao F, et al. Donor Brain Death Exacerbates Complement-Dependent Ischemia/Reperfusion Injury in Transplanted Hearts. *Circulation*. 2013; 127: 1290-1299.
108. Watts RP, Thom O, Fraser JF. Inflammatory signalling associated with brain dead organ donation: from brain injury to brain stem death and posttransplant ischaemia reperfusion injury. *J Transplant*. 2013; 2013: 521369-521369.

109. Reich DJ, Mulligan DC, Abt PL, et al. ASTS recommended practice guidelines for controlled donation after cardiac death organ procurement and transplantation. *Am J Transplant*. 2009; 9: 2004-2011.
110. Summers DM, Watson CJ, Pettigrew GJ, et al. Kidney donation after circulatory death (DCD): state of the art. *Kidney Int*. 2015; 88: 241-249.
111. Salvadori M, Rosso G, Bertoni E. Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World J Transplant*. 2015; 5: 52-67.
112. Kosieradzki M, Rowinski W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. *Transplant Proc.* 2008; 40: 3279-3288.
113. Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation--from bench to bedside. *Nat Rev Gastroenterol Hepatol*. 2013; 10: 79-89.
114. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest*. 2011; 121: 4210-4221.
115. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. *Nat Med.* 2011; 17: 1391-1401.
116. Salahudeen AK. Cold ischemic injury of transplanted kidneys: new insights from experimental studies. *Am J Physiol Renal Physiol*. 2004; 287: F181-187.
117. Sammut IA, Burton K, Balogun E, et al. Time-dependent impairment of mitochondrial function after storage and transplantation of rabbit kidneys. *Transplantation*. 2000; 69: 1265-1275.
118. Schopp I, Reissberg E, Luer B, Efferz P, Minor T. Controlled Rewarming after Hypothermia: Adding a New Principle to Renal Preservation. *Clin Transl Sci*. 2015; 8: 475-478.
119. Bilzer M, Gerbes AL. Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J Hepatol*. 2000; 32: 508-515.
120. Mayer H, Schmidt J, Thies J, et al. Characterization and reduction of ischemia/reperfusion injury after experimental pancreas transplantation. *J Gastrointest Surg*. 1999; 3: 162-166.
121. Troppmann C, Gruessner AC, Benedetti E, et al. Vascular graft thrombosis after pancreatic transplantation: univariate and multivariate operative and nonoperative risk factor analysis. *J Am Coll Surg.* 1996; 182: 285-316.
122. Zhao H, Alam A, Soo AP, George AJT, Ma D. Ischemia-Reperfusion Injury Reduces Long Term Renal Graft Survival: Mechanism and Beyond. *EBioMedicine*. 2018; 28: 31-42.
123. Saat TC, van den Akker EK, Ijzermans JNM, Dor FJMF, de Bruin RWF. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? *J Transl Med*. 2016; 14: 20.
124. Yang W, Chen J, Meng Y, Chen Z, Yang J. Novel Targets for Treating Ischemia-Reperfusion Injury in the Liver. *Int J Mol Sci*. 2018; 19: 1302.
125. Jaeschke H, Woolbright BL. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. *Transplant Rev*. 2012; 26: 103-114.
126. Guan L-Y, Fu P-Y, Li P-D, et al. Mechanisms of hepatic ischemia-reperfusion injury and protective effects of nitric oxide. *World J Gastrointerest Surg*. 2014; 6: 122-128.
127. Riedemann NC, Ward PA. Complement in ischemia reperfusion injury. *Am J Path*. 2003; 162: 363-367.
128. Banz Y, Rieben R. Role of complement and perspectives for intervention in ischemia-reperfusion damage. *Ann Med*. 2012; 44: 205-217.
129. Loubele ST, ten Cate H, Spronk HM. Anticoagulant therapy in critical organ ischaemia/reperfusion injury. *Thromb Haemost.* 2010; 104: 136-142.

130. Schulz R, Kelm M, Heusch G. Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc Res.* 2004; 61: 402-413.
131. Yang Q, He G-W, Underwood MJ, Yu C-M. Cellular and molecular mechanisms of endothelial ischemia/reperfusion injury: perspectives and implications for postischemic myocardial protection. *Am J Transl Res*. 2016; 8: 765-777.
132. Lutz J, Thürmel K, Heemann U. Anti-inflammatory treatment strategies for ischemia/reperfusion injury in transplantation. *J Inflamm.* 2010; 7: 27.
133. Kezic A, Stajic N, Thaiss F. Innate Immune Response in Kidney Ischemia/Reperfusion Injury: Potential Target for Therapy. *J Immunol Res*. 2017; 2017: 6305439.
134. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol*. 2015; 6: 524-551.
135. Kalogeris T, Bao Y, Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. *Redox Biol*. 2014; 2: 702-714.
136. Weiss JB, Eisenhardt SU, Stark GB, Bode C, Moser M, Grundmann S. MicroRNAs in ischemia-reperfusion injury. *Am J Cardiovasc Dis*. 2012; 2: 237-247.
137. Butler D. Translational research: crossing the valley of death. *Nature*. 2008; 453: 840-842.
138. O'Neill S, Gallagher K, Hughes J, Wigmore SJ, Ross JA, Harrison EM. Challenges in early clinical drug development for ischemia-reperfusion injury in kidney transplantation. *Expert Opin Drug Discov*. 2015; 10: 753-762.
139. DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D costs. *J Health Econ.* 2016; 47: 20-33.
140. NSW Government. Discussion Paper: The use of ante mortem (before death) interventions for organ donation in NSW. Sydney, NSW: NSW Government, 2016. Available from: https://www.health.nsw.gov.au/legislation/Documents/discussion-paper-organ-donation.pdf. Accessed November 10, 2018.
141. McKeown DW, Bonser RS, Kellum JA. Management of the heartbeating brain-dead organ donor. *Br J Anaesth.* 2012; 108: i96-i107.
142. Ranasinghe AM, Bonser RS. Endocrine changes in brain death and transplantation. *Best practice & research. Clin Endocrinol Metab*. 2011; 25: 799-812.
143. Mundt HM, Yard BA, Krämer BK, Benck U, Schnülle P. Optimized donor management and organ preservation before kidney transplantation. *Transpl Int*. 2016; 29: 974-984.
144. Rech TH, Moraes RB, Crispim D, Czepielewski MA, Leitao CB. Management of the brain-dead organ donor: a systematic review and meta-analysis. *Transplantation*. 2013; 95: 966-974.
145. Macdonald PS, Aneman A, Bhonagiri D, et al. A systematic review and meta-analysis of clinical trials of thyroid hormone administration to brain dead potential organ donors. *Crit Care Med.* 2012; 40: 1635-1644.
146. Dupuis S, Amiel J-A, Desgroseilliers M, et al. Corticosteroids in the management of brain-dead potential organ donors: a systematic review. *Br J Anaesth.* 2014; 113: 346-359.
147. Malinoski DJ, Patel MS, Daly MC, Oley-Graybill C, Salim A. The impact of meeting donor management goals on the number of organs transplanted per donor: results from the United Network for Organ Sharing Region 5 prospective donor management goals study. *Crit Care Med.* 2012; 40: 2773-2780.
148. Niemann CU, Feiner J, Swain S, et al. Therapeutic Hypothermia in Deceased Organ Donors and Kidney-Graft Function. *N Engl J Med.* 2015; 373: 405-414.
149. Algahim MF, Love RB. Donation after circulatory death: the current state and technical approaches to organ procurement. *Curr Opin Organ Transplant*. 2015; 20: 127-132.

150. Manara AR, Murphy PG, O'Callaghan G. Donation after circulatory death. *Br J Anaesth*. 2012; 108: i108-i121.
151. Neyrinck A, Van Raemdonck D, Monbaliu D. Donation after circulatory death: current status. *Curr Opin Anaesth*. 2013; 26: 382-390.
152. Shemie SD, Baker AJ, Knoll G, et al. National recommendations for donation after cardiocirculatory death in Canada: Donation after cardiocirculatory death in Canada. *CMAJ*. 2006; 175: S1-S1.
153. D'Alessandro AM, D'Alessandro AM, Hoffmann RM, et al. Successful extrarenal transplantation from non-heart-beating donors. *Transplantation*. 1995; 59: 977-982.
154. Hessheimer AJ, Vendrell M, Munoz J, et al. Heparin but not tissue plasminogen activator improves outcomes in donation after circulatory death liver transplantation in a porcine model. *Liver Transplant*. 2018; 24: 665-676.
155. Cao Y, Shahrestani S, Chew HC, et al. Donation After Circulatory Death for Liver Transplantation: A Meta-Analysis on the Location of Life Support Withdrawal Affecting Outcomes. *Transplantation*. 2016; 100: 1513-1524.
156. Hessheimer AJ, Garcia-Valdecasas JC, Fondevila C. Abdominal regional in-situ perfusion in donation after circulatory determination of death donors. *Curr Opin Organ Transplant*. 2016; 21: 322-328.
157. Oniscu GC, Randle LV, Muiesan P, et al. In situ normothermic regional perfusion for controlled donation after circulatory death--the United Kingdom experience. *Am J Transplant*. 2014; 14: 2846-2854.
158. Hessheimer AJ, Billault C, Barrou B, Fondevila C. Hypothermic or normothermic abdominal regional perfusion in high-risk donors with extended warm ischemia times: impact on outcomes? *Transpl Int*. 2015; 28: 700-707.
159. Tsui SSL, Oniscu GC. Extending normothermic regional perfusion to the thorax in donors after circulatory death. *Curr Opin Organ Transplant*. 2017; 22: 245-250.



PART 2 – THE KIDNEY

Chapter 2

Advances in organ preservation for transplantation

Ahmer Hameed
Wayne Hawthorne
Henry Pleass

As published in the ANZ Journal of Surgery 2017, 87(12): 976-80; DOI: 10.1111/ans.13713

2.1 Abstract

Organ transplantation provides the best available therapy for a myriad of medical conditions, including end-stage renal disease, hepatic failure, and type I diabetes mellitus. The current clinical reality is however that there is a significant shortage of organs available for transplantation with respect to the number of patients on organ waiting lists.

As such, methods to increase organ supply have been instituted, including improved donor management, organ procurement and preservation strategies, living organ donation, transplantation education, and the increased utilization of donation after circulatory death and expanded criteria donors. In particular, especially over the last decade we have witnessed a significant change in the way donor organs are preserved, away from static cold storage methods to more dynamic techniques centred on machine perfusion.

This review highlights the current state and future of organ preservation for transplantation, focusing on both abdominal and thoracic organs. In particular, we focus on machine perfusion preservation of renal, hepatic, pancreatic, cardiac and lung allografts, also noting relevant advances in Australasia. Machine perfusion of organs after procurement holds considerable promise, and has the potential to significantly improve graft viability and function post-transplantation, especially in donors in whom acceptance criteria have been expanded.

2.2 Introduction

The field of organ transplantation continues to push the boundaries between the possible and impossible, allowing successful function in grafts that would previously have been deemed non-viable. Such advances have been necessitated by the continuing gap between organ supply and demand, despite overall increases in transplantation rates.

In particular, we have seen a significant shift towards the utilisation of organs from donation after circulatory death (DCD) and expanded criteria (ECD) donors, as compared to standard criteria donation after brain death (DBD) donors. These organs theoretically have a higher chance of short and/or longer-term dysfunction, owing to an increased duration of warm ischaemic insult and/or suboptimal pre-donation function due to higher donor age and comorbidities.

A major contributing factor to the expansion of the donor pool has been the modification and enhancement of the organ preservation process post-procurement. Not only have there been advancements in preservation solution(s) used, but we have also seen a significant shift away from the traditional paradigm of static cold (hypothermic) organ storage (CS). In fact currently, there is a worldwide push towards dynamic organ storage, such as the use of machine perfusion (MP), potentially in association with perfusate oxygenation and normothermia.

2.3 Mechanistic basis and uses of MP

The primary aims of conventional organ preservation methods are the reduction of the organ's metabolic rate whilst simultaneously storing the allograft in an environment that minimises cellular oedema and ischaemic damage. Hypothermic *in situ* perfusion is currently the mainstay of allograft preservation, and is instituted during the procurement process upon cannulation and perfusion of the aorta, and is some cases the portal vein and/or pulmonary arterial system, with chilled organ preservation solution. Topical sterile saline ice slush is usually used to provide a supplemental source of hypothermia, thereby further reducing organ cellular processes and thus substrate requirements.

Traditionally, the CS organs are bathed in preservation fluid inside sterile plastic bags, which varies by the type of organ and centre preference, prior to transportation to the recipient centre. MP, however, entails cannulation of the organ's vascular inflow such that it is mechanically perfused with the preservation solution during storage, with natural venting and re-cycling of

the perfusate via its venous outflow. In contrast to MP, once organs are bagged for CS the amount of preservation solution maintained within the graft is significantly reduced owing to a collapse of its vasculature; this subsequently impairs the extracellular excretion of waste products.[1] MP further allows for improved maintenance of organ ATP levels, and ameliorates endothelial damage and swelling and thus enhances post-transplantation vascular perfusion.[2-4]

Transplantation centres differ regarding the timing and nature of MP utilised, often dependent on the availability and/or transportability of MP apparatus to retrieval hospitals. As such, MP is often combined with brief periods of CS, although the ideal perfusion period and timing of perfusion is still debated.[5] Furthermore, exact perfusion parameters are far from well-defined. The benefits of perfusate oxygenation and warming are not yet well-established, and are the subject of ongoing clinical trials.[6] Interestingly, MP also allows direct perfusion of the organ of interest with pharmacotherapies targeting the ischaemia-reperfusion process, although the best combination of such therapies is not yet known.[7]

Another potential use of MP of interest to transplant surgeons is in the assessment of graft viability and quality prior to transplantation, especially through the analysis of perfusion resistance and flow, and measurable biomarkers within the perfusate.[8-10] Although these parameters provide some indication of subsequent graft function, at this stage they cannot be used as the only basis for not clinically using the organ.

2.4 Abdominal Organs

2.4.1 ABDOMINAL REGIONAL PERFUSION

Abdominal regional perfusion (ARP) entails the application of modified cardiopulmonary bypass (CPB) technology *in vivo* during organ procurement. Vascular access is usually either obtained peripherally, with simultaneous balloon occlusion of the thoracic aorta, or centrally in association with clamping of the thoracic or supracoeliac aorta, with subsequent perfusion of abdominal organs using the donor's own blood.[11] Isolation of the abdominal circulation from the supra-diaphragmatic aorta aims to ensure that cerebral blood-flow is not restored and thus any potential possibility of auto-resuscitation is avoided. The most promising area of the application of ARP is in the DCD setting, especially in uncontrolled donors that would benefit the most from this resuscitative bridge prior to organ removal.[12]

A systematic review by Shapey and Muiesan showed that hepatic allograft function in

uncontrolled DCDs after ARP is still suboptimal when compared to DBD donors, whilst subsequent kidney survival may in fact be better in comparison to DBD and non-ARP kidneys.[12] Potentially the greatest utility of this technique however is in the fact that it provides a possible mechanism to increase organ availability by resuscitating and allowing the assessment of organs that would otherwise not be procured or transplanted.[13]

Future application of ARP still requires considerable refinement if its use is to be expanded however, not only to make it more cost effective, but also to address the important potential ethical controversies surrounding its implementation.[11,14] Indeed, within the Australasian setting, there are also significant legal barriers to its widespread utilisation, especially regarding the inability to institute donor treatment prior to the declaration of death and the lack of permissibility to procure uncontrolled DCD (Maastricht category I, II and V) grafts.

2.4.2 DECEASED DONOR KIDNEY PRESERVATION

Kidney preservation over the last half century has almost come full-circle. MP was commonly utilised in the 1970s, and was later supplanted by CS as a result of evidence contradicting its efficacy in addition to the significant costs incurred by MP.[15] There has been a push back towards MP of kidneys over the last decade, however, due to the aforementioned increased use of DCD and ECD kidneys.

Standard MP apparatus consist of a reservoir of preservation solution, which is utilised as the source of perfusate for the kidney that is pumped via the renal artery. Temperature and flow characteristics can be monitored and controlled, and some machines also allow direct oxygenation of the perfusion solution. Modern MP apparatus such as the LifePort® kidney transporter are of small enough size and weight such that they can be transported by car or plane during the organ procurement process.[16]

A multi-centre trial in Europe compared MP to CS preservation for matched pair kidneys.[17] MP preservation significantly reduced the incidence of delayed graft function (DGF), defined as the need for dialysis in the first week after transplantation, and increased one-year graft survival. Later subgroup analyses and follow-up studies showed significantly lower DGF rates in DCD and ECD kidneys, with higher three-year graft survivals in ECD but not DCD kidneys.[18-20] Systematic reviews and meta-analyses comparing CS to MP for kidney transplantation confirm lower rates of DGF, with better graft survival in only ECD but not

DCD renal grafts.[21-23]

Hypothermia has long been an essential component of kidney, and indeed any organ's, pre-implantation storage, allowing for reduced organ metabolism during the period in which it has no blood supply. MP has allowed this concept to be turned on its head, as it allows the continuous provision of oxygen and metabolic substrates directly to the kidney. Initial experimental work in animals showed that the maintenance of kidneys during storage in a normothermic environment using MP was beneficial to post-transplantation graft function, helping to maintain the organs in closer to physiological circumstances and avoiding cold ischaemic injury.[24] More recently, Nicholson and Hosgood applied this technique to human ECD kidneys, with significantly reduced rates of DGF.[25] There is still a pressing need for future research and modifications in this area, including in the ascertainment of ideal MP times, the role of oxygenation, and the nature of, and potential additives to, the preservation solution.

Currently within the Australasian setting, MP of kidneys is only being utilised in one centre in Brisbane.[26] Due to accumulating evidence regarding the effectiveness of the technique, especially in more marginal organ donors, momentum is developing toward its potential expansion to other centres.

2.4.3 DECEASED DONOR LIVER PRESERVATION

Liver allograft preservation from deceased donors is most commonly undertaken using traditional CS. However, liver donation rates face the same supply and demand gap, with an increased need for viable donor organs and therefore push to utilise more ECD and DCD donors. The proportion of DCD donors within Australia for all organs has now exceeded 30% and is anticipated to increase further; 5-10% of liver transplants are from DCD donors.[23] DCD livers suffer close to 20% incidence of ischaemic cholangiopathy, lower graft survivals, and are at higher risk of requiring re-transplantation.[27,28] Hence, there is an even greater need to improve liver preservation from this donation pathway.

In contrast to renal MP studies, there is significantly less published literature regarding the efficacy of hepatic MP in humans. Guarrera *et al*. from the USA presented the first clinical data for liver transplantation after hypothermic MP of DBD livers.[29] These authors utilised dual portal vein and hepatic artery perfusion and showed reduced hospital stays and serum injury markers in the MP group when compared to CS. Early allograft dysfunction rates appeared

lower in the MP group, although this only approached statistical significance. A group from Switzerland later employed hypothermic oxygenated MP ("HOPE") for eight DCD livers, delivered only through the portal vein.[30] Highly promising results were obtained, with good early graft function and the absence of ischaemic cholangiopathy in any patient six months post-transplantation. Significantly, these high-risk DCD livers performed no worse than matched DBD livers that underwent CS. A randomized control trial comparing HOPE to CS is currently underway.[30]

Normothermic MP of the liver is also being actively investigated, with preliminary results from a European trial demonstrating the feasibility of this technique.[31] Evidence from animal models also shows that normothermic liver perfusion holds considerable promise for the future of liver preservation.[32,33]

2.4.4 DECEASED DONOR PANCREAS PRESERVATION

The use of the pancreas as a donor organ aims to confer beta-cell function to the diabetic recipient, and is unique as either the whole organ or islets isolated from the organ may potentially be transplanted.

DCD pancreatic grafts represent a very small proportion of donated pancreata in Australia.[23] We recently showed that these grafts have similar survival compared to those from DBD donors.[34] Graft thrombosis rates are however higher in the DCD subset; importantly, this risk can be significantly reduced with the provision of antemortem heparin to the donor.[34]

Like all organs, donor pancreata are most commonly preserved in CS solutions. A variation to CS was developed in the form of the 'two-layer method', whereby the donor pancreas was stored at the interface of Euro-Collins solution and an oxygenated perfluorochemical.[35] This was later applied to humans, replacing Euro-Collins with University of Wisconsin solution, although there were no statistically significant improvements seen compared to conventional CS[36]. More clinical studies have been performed regarding the two-layer method for islet isolation, however its utility has been questioned for this purpose as well.[37]

Pancreatic allograft MP preservation is still in its infancy, with no current studies analysing effects of this technique in human recipients. Part of the reason for this is the 'low flow' nature of the pancreas, with fears that the pressures generated by MP will confer barotrauma to the

organ. Leeser *et al*. did however employ MP for pancreas preservation prior to islet isolation in four human pancreata without subsequent transplantation.[38] Islet yield and *in vitro* function appeared to be better in the MP group compared to CS controls.

An interesting alternative dynamic preservation option is known as persufflation. This method involves oxygen gas perfusion of the pancreas delivered through its arterial inflow, and preliminary studies have indicated that it can improve pancreatic histology and adenosine triphosphate levels.[39,40]

Islet cell transplantation isolation and transplantation in particular suffers from suboptimal donor organ preservation, with a lack of improvements in this area likely contributing to the static transplantation rates in Australia.[41] It remains to be seen whether techniques such as MP and/or persufflation will allow further advances in this area.

2.5 Thoracic Organs

2.5.1 DECEASED DONOR HEART AND LUNG PRESERVATION

DCD grafts represent an important subset of transplanted abdominal organs, yet up until very recently DCD cardiac donation did not exist. Factors contributing to this included the obvious difficulties in the assessment of cardiac function after circulatory cessation, in addition to the ethical issues surrounding revival of the non-functioning heart and the subsequent debate regarding how death is defined.

A group is Sydney was the first in the world to report on the successful human transplantation of DCD hearts after MP preservation.[42] The preservation system utilised was the Organ Care System™, perfusing the cardiac graft with the donor's blood under normothermic conditions. *Ex vivo* perfusion in this fashion allowed resuscitation of the heart, and thence graft function and viability could subsequently be assessed prior to transplantation. DCD heart transplantation has since been successfully conducted in the United Kingdom, albeit using a modified method in which the heart was revived *in situ* using normothermic regional perfusion prior to explantation.[43]

Lung allograft transplantation from DCD donors commenced well before DCD heart transplantation. Snell *et al*. published the early experiences of a unit in Melbourne, reporting good lung function in all eight DCD lung recipients with a mean follow-up of 311 days;

standard CS preservation strategies were utilised in these lungs.[44] MP in lung transplantation, or *ex vivo* lung perfusion (EVLP), was first used for the *ex vivo* assessment of DCD lung function prior to transplantation.[45] Machuca *et al*. extended the use of normothermic EVLP up to 18 hours, with significantly shorter hospital stays in DCD lungs preserved by EVLP compared to standard methods.[46] There is significant potential for the expansion of the utility of EVLP beyond mainly a role in lung graft assessment to the possible modification of pulmonary surfactant, amongst other factors.[47]

2.6 Conclusions

Organ preservation techniques are advancing in an attempt to increase the potential donor organ pool and ensure adequate graft function in marginal DCD and ECD organs. MP preservation has been successfully utilised in both animal and clinical models for most abdominal and thoracic transplantable organs, with encouraging results. Abdominal organ perfusion to date has largely been hypothermic, however research is being conducted into the utility of normothermic, oxygenated perfusion systems. DCD and ECD transplantation is becoming more prevalent, with Australia leading this field in cardiac transplantation. With further time and research, we will likely see the expansion of MP methods for abdominal organ preservation in Australasia, with refinements to the process contributing to even better transplantation outcomes.

2.7 References

1. Churchill TA. Organ Preservation for Transplantation. *Functional Metabolism: Regulation and Adaptation.* Hoboken: John Wiley & Sons, Inc., 2005; 529-555.
2. Vekemans K, Liu Q, Brassil J, Komuta M, Pirenne J, Monbaliu D. Influence of flow and addition of oxygen during porcine liver hypothermic machine perfusion. *Transplant Proc.* 2007; 39: 2647-2651.
3. Maathuis MH, Manekeller S, van der Plaats A, et al. Improved kidney graft function after preservation using a novel hypothermic machine perfusion device. *Ann Surg.* 2007; 246: 982-988.
4. Gracia-Sancho J, Villarreal G, Jr., Zhang Y, et al. Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. *Transplantation*. 2010; 90: 142-149.
5. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transplant Int.* 2015; 28: 665-676.
6. COPE Consortium. Trials. Oxford: Consortium for Organ Preservation in Europe. Available from: http://cope-eu.com/work%20programme/trials.html. Accessed July 4, 2016.
7. Polyak MM, Arrington BO, Stubenbord WT, et al. The influence of pulsatile preservation on renal transplantation in the 1990s. *Transplantation*. 2000; 69: 249-258.
8. Balfoussia D, Yerrakalva D, Hamaoui K, Papalois V. Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. *Exp Clin Transplant*. 2012; 10: 87-100.
9. Bhangoo RS, Hall IE, Reese PP, Parikh CR. Deceased-donor kidney perfusate and urine biomarkers for kidney allograft outcomes: a systematic review. *Nephrol Dial Transplant*. 2012; 27: 3305-3314.
10. van Smaalen TC, Hoogland ER, van Heurn LW. Machine perfusion viability testing. *Curr Opin Organ Transplant*. 2013; 18: 168-173.
11. Hessheimer AJ, Billault C, Barrou B, Fondevila C. Hypothermic or normothermic abdominal regional perfusion in high-risk donors with extended warm ischemia times: impact on outcomes? *Transplant Int*. 2015; 28: 700-707.
12. Shapey IM, Muiesan P. Regional perfusion by extracorporeal membrane oxygenation of abdominal organs from donors after circulatory death: a systematic review. *Liver Transplant*. 2013; 19: 1292-1303.
13. Hessheimer AJ, Garcia-Valdecasas JC, Fondevila C. Abdominal regional in-situ perfusion in donation after circulatory determination of death donors. *Curr Opin Organ Transplant*. 2016; 21: 322-328.
14. Bernat JL, Bleck TP, Blosser SA, et al. Circulatory death determination in uncontrolled organ donors: a panel viewpoint. *Ann Emerg Med.* 2014; 63: 384-390.
15. Opelz G, Terasaki PI. Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. *Transplantation*. 1982; 33: 64-68.
16. Organ Recovery Systems. LifePort Kidney Transporter 2016. Available from: http://www.organ-recovery.com/lifeport-kidney-transporter. Accessed May 25, 2016.
17. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2009; 360: 7-19.
18. Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial S, Machine Preservation Trial Study G. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2012; 366: 770-771.
19. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg.* 2010; 252: 756-764.

20. Treckmann J, Moers C, Smits JM, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. *Transplant Int*. 2011; 24: 548-554.
21. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. *Br J Surg.* 2013; 100: 991-1001.
22. Deng R, Gu G, Wang D, et al. Machine perfusion versus cold storage of kidneys derived from donation after cardiac death: a meta-analysis. *PLoS One*. 2013; 8: e56368.
23. ANZOD Registry. Australia and New Zealand Organ Donation Registry Report 2015. Available from: http://www.anzdata.org.au/anzod/v1/reports.html. Accessed May 1, 2016.
24. Brasile L, Stubenitsky BM, Booster MH, Arenada D, Haisch C, Kootstra G. Hypothermia--a limiting factor in using warm ischemically damaged kidneys. *Am J Transplant*. 2001; 1: 316-320.
25. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013; 13: 1246-1252.
26. Queensland Department of Health. Health Policy Advisory Committee on Technology – Technology Overview – New and Emerging Organ Perfusion Systems. HealthPACT Emerging Health Technology. Herston, Queensland, 2014. Available from: https://www.health.qld.gov.au/healthpact/docs/briefs/WP186.pdf. Accessed July 4, 2016.
27. Monbaliu D, Pirenne J, Talbot D. Liver transplantation using Donation after Cardiac Death donors. *J Hepatol.* 2012; 56: 474-485.
28. Cao Y, Shahrestani S, Chew HC, et al. Donation After Circulatory Death for Liver Transplantation: A Meta-Analysis on the Location of Life Support Withdrawal Affecting Outcomes. *Transplantation*. 2016; 100: 1513-1524.
29. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. *Am J Transplant*. 2010; 10: 372-381.
30. Dutkowski P, Schlegel A, de Oliveira M, Mullhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. *J Hepatol*. 2014; 60: 765-772.
31. Ravikumar R, Jassem W, Mergental H, et al. Liver Transplantation After Ex Vivo Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial. *Am J Transplant.* 2016; 16: 1779-1787.
32. Fondevila C, Hessheimer AJ, Maathuis MH, et al. Superior preservation of DCD livers with continuous normothermic perfusion. *Ann Surg.* 2011; 254: 1000-1007.
33. Brockmann J, Reddy S, Coussios C, et al. Normothermic perfusion: a new paradigm for organ preservation. *Ann Surg.* 2009; 250: 1-6.
34. Shahrestani S, Webster AC, Lam VW, et al. Outcomes From Pancreatic Transplantation in Donation After Cardiac Death: A Systematic Review and Meta-Analysis. *Transplantation*. 2017; 101: 122-130.
35. Kawamura T, Kuroda Y, Suzuki Y, et al. Seventy-two-hour preservation of the canine pancreas by the two-layer (Euro-Collins' solution/perfluorochemical) cold storage method. *Transplantation*. 1989; 47: 776-778.
36. Matsumoto S, Kandaswamy R, Sutherland DE, et al. Clinical application of the two-layer (University of Wisconsin solution/perfluorochemical plus O2) method of pancreas preservation before transplantation. *Transplantation*. 2000; 70: 771-774.
37. Kin T, Mirbolooki M, Salehi P, et al. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation*. 2006; 82: 1286-1290.
38. Leeser DB, Bingaman AW, Poliakova L, et al. Pulsatile pump perfusion of pancreata before human islet cell isolation. *Transplant Proc.* 2004; 36: 1050-1051.

39. Scott WE, 3rd, O'Brien TD, Ferrer-Fabrega J, et al. Persufflation Improves Pancreas Preservation When Compared With the Two-Layer Method. *Transplant Proc.* 2010; 42: 2016-2019.
40. Scott WE, 3rd, Weegman BP, Ferrer-Fabrega J, et al. Pancreas oxygen persufflation increases ATP levels as shown by nuclear magnetic resonance. *Transplant Proc.* 2010; 42: 2011-2015.
41. ANZIPTR. Australia & New Zealand National Pancreas Transplant Registry Report 1984-2013. 2014. Available from: http://anziptr.org/wp-content/uploads/2015/09/ANZIPTR_2015-_revised.pdf. Accessed July 3, 2016.
42. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet*. 2015; 385: 2585-2591.
43. Messer S, Axell R, Colah S, et al. Functional assessment of the donor heart following circulatory death and clinical transplantation. *J Heart Lung Transplant.* 2016; 35: S79-S80.
44. Snell GI, Levvey BJ, Oto T, et al. Early Lung Transplantation Success Utilizing Controlled Donation After Cardiac Death Donors. *Am J Transplant.* 2008; 8: 1282-1289.
45. Steen S, Sjöberg T, Pierre L, Liao Q, Eriksson L, Algotsson L. Transplantation of lungs from a non-heart-beating donor. *Lancet*. 2001; 357: 825-829.
46. Machuca TN, Mercier O, Collaud S, et al. Lung transplantation with donation after circulatory determination of death donors and the impact of ex vivo lung perfusion. *Am J Transplant.* 2015; 15: 993-1002.
47. Snell GI, Levvey BJ, Westall GP. The Changing Landscape of Lung Donation for Transplantation. *Am J Transplant.* 2015; 15: 859-860.

Chapter 3

Maximizing kidneys for transplantation using machine perfusion: from the past to the future – a comprehensive systematic review and meta-analysis

Ahmer Hameed
Henry Pleass
Germaine Wong
Wayne Hawthorne

As published in Medicine (Baltimore) 2016, 95(40): e5083; DOI: 10.1097/MD.0000000000005083

3.1 Abstract

Objective: To elucidate the benefits of machine perfusion (MP) preservation with and without oxygenation, and/or under normothermic conditions, when compared to static cold storage (CS) prior to deceased donor kidney transplantation.

Background: The two main options for renal allograft preservation are CS and MP. There has been considerably increased interest in MP preservation of kidneys, however conflicting evidence regarding its efficacy and associated costs have impacted its scale of clinical uptake. Additionally, there is no clear consensus regarding oxygenation, and hypo- or normothermia, in conjunction with MP, and its mechanisms of action are also debated.

Methods: Clinical (observational studies and prospective trials) and animal (experimental) articles exploring the use of renal MP were assessed (EMBASE, Medline and Cochrane databases). Meta-analyses were conducted for the comparisons between hypothermic MP (HMP) and CS (human studies) and normothermic MP (WP) compared to CS or HMP (animal studies). The primary outcome was allograft function. Secondary outcomes included graft and patient survival, acute rejection and parameters of tubular, glomerular and endothelial function. Subgroup analyses were conducted in expanded criteria (ECD) and donation after circulatory (DCD) death donors.

Results: A total of 101 studies (63 human and 38 animal) were included. There was a lower rate of delayed graft function in recipients with HMP donor grafts compared to CS kidneys (RR 0.77; 95% CI 0.69-0.87). Primary non-function (PNF) was reduced in ECD kidneys preserved by HMP (RR 0.28; 95% CI 0.09-0.89). Renal function in animal studies was significantly better in WP kidneys compared to both HMP (standardized mean difference [SMD] of peak creatinine -1.66; 95% CI -3.19 to -0.14) and CS (SMD of peak creatinine -1.72; 95% CI -3.09 to -0.34). MP improves renal preservation through the better maintenance of tubular, glomerular and endothelial function and integrity.

Conclusions: HMP improves short-term outcomes after renal transplantation, with a less clear effect in the longer-term. There is considerable room for modification of the process to assess whether superior outcomes can be achieved through oxygenation, perfusion fluid manipulation, and alteration of perfusion temperature. In particular, correlative experimental (animal) data provides strong support for more clinical trials investigating normothermic MP.

3.2 Introduction

The optimal long-term treatment option for end-stage renal disease remains kidney transplantation. On a worldwide basis, access and referral for transplantation is limited; in those patients referred for transplantation, there is an imbalance between the supply and demand for suitable organs.[1] In the USA alone, the median time to deceased donor renal transplantation is approximately three to four years.[2] This organ deficit has prompted the adoption of different strategies in order to increase the availability of kidneys for transplantation. One approach of considerable importance is the increasing utilization of donation after circulatory death (DCD) and expanded criteria donors (ECD), which must supplement the standard criteria, donation after brain death (DBD) kidneys.[1,3]

The growing demands for DCD and ECD kidneys must be balanced with their perceived suboptimal post-transplant function. There are higher rates of delayed graft function (DGF) for both DCD and ECD kidneys, and higher discard rates and by definition poorer survival in the ECD subset, when compared to standard criteria DBD kidneys.[4-10] Further improvements to the organ procurement and preservation process are therefore essential in order to improve marginal donor kidney quality.

Although cold static storage (CS) is still the most commonly utilized method for renal preservation, machine perfusion (MP) provides an important alternative. CS largely supplanted MP in the 1980s due to a lack of evidence with regards to improvement in transplantation outcomes and the large associated costs.[11-13] MP has seen a resurgence in the last decade due to the changing donor profile and advancements in perfusion solutions and technology.[14]

Indeed, application of MP is still not widespread, with conflicting evidence even in recent years regarding its utility.[15,16] Furthermore, there is minimal clinical data regarding the utility of evolving modifications to the MP process, and its mechanisms of action are also poorly understood. In particular, the use of warm (normothermic) perfusion (WP), oxygenation or pharmacotherapies has largely been the subject of experimental (animal) studies.

The aims of this systematic review and meta-analysis were therefore to: (i) describe ways in which MP is currently utilized; (ii) provide an updated and comprehensive analysis of the effect of hypothermic MP (HMP) on post-transplant graft function in deceased donor kidney transplantation; and (iii) explore experimental (animal) literature to (a) investigate the utility of

normothermic (WP) and/or oxygenated MP, and (b) understand the mechanisms of action of MP preservation.

3.3 Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was utilized in the completion of this review.[17] The review protocol was registered with the PROSPERO International Prospective Register of Systematic Reviews (March, 2016; registration number – CRD42016037100).[18]

3.3.1 ELIGIBILITY

Inclusion criteria

Clinical (human) studies consisted of randomized control trials (RCT) or prospective (non-randomized) and observational studies, and were included in the presence of MP data. Experimental (animal) studies by their nature are prospective, and were included in the presence of comparative data either between different types of MP, and/or MP and an alternative form of preservation. Both English and non-English articles were considered, utilizing a translator if necessary. Only published works, and not conference abstracts, were included; although there is some evidence to suggest that grey literature exclusion can contribute to publication bias[19], these abstracts were all assessed and deemed to have either insufficient data or quality for inclusion.

Exclusion criteria

Clinical/human studies were excluded if less than 10 patients were in the MP group, or there was significant data and/or patient overlap between two or more published studies, and/or there was insufficient data with regards to delayed graft function (DGF), primary non-function (PNF), or graft/patient survival. These parameters were chosen as they were the most commonly and uniformly reported in the studies analyzed. For animal studies, an article was excluded if there was no appropriate control group for comparison, and/or there was a lack of a reperfusion period (either *ex vivo* or *in vivo*) after MP preservation. All studies prior to 1980 were excluded. This publication year reflects a time after which there was a distinct shift in the type of perfusion machines and perfusion solutions used.

3.3.2 SEARCH STRATEGY

The EMBASE, Medline and Cochrane (1980 to December 2015) databases were searched using Ovid, with key search terms including "kidney or renal" and "machine perfusion" (see Table, Supplemental Digital Content [SDC] 1, for complete strategy). In an effort to include all eligible studies, a manual literature search was also conducted using any potential articles' bibliographies, in addition to reference lists from other reviews.

3.3.3 DATA COLLECTION

Data was extracted from each article by two independent reviewers utilizing a pre-determined template; a third reviewer was consulted if necessary for any disagreements.

Clinical (human) data

Human data was analyzed for the extraction of the following: date of publication and study period; study type (i.e. prospective or retrospective); kidney allocation; study center(s); patients in MP and CS groups; stratification of MP and CS patients by DBD, DCD and ECD status; MP characteristics, including the use of oxygenation and preservation temperature; perfusion machine(s) used; and the preservation solution(s) used in CS and MP groups. Quantitative data was extracted for – the incidence of DGF and primary non-function (PNF), 1-year graft and patient survival in the whole cohort, acute rejection rates, and post-transplant renal function (CrCl in ml/min and serum creatinine in mg/dl). DGF was defined as the need for dialysis in the 1st week after transplantation[20]. Only six studies either utilized an alternate definition of DGF, or did not define DGF.

Hazard ratios (HR) for graft survival were calculated, when possible, using the methods described by Tierney *et al*.[21]

Although the "ECD" graft description is not as descriptively useful as a high Kidney Donor Profile Index (KDPI) donor kidney, ECD is used in this manuscript as it is the most commonly utilized term in the included literature.

Experimental (animal) data

Study parameters collected for animal data included: date of publication, institution(s) involved, animal/species employed, weight range of animals, experimental procedure(s)/model employed (study groups, DCD or DBD, *ex vivo* perfusion or transplantation after preservation, experimental period), number of animals in each group, cold/warm ischemic times (CIT/WIT),

perfusion machine and settings used, preservation/perfusion solution(s) used, additives to preservation/perfusion solution(s), temperature of preservation/perfusion, and the use of oxygen. Study outcomes consisted of renal function parameters (peak creatinine in mg/dl, creatinine clearance (CrCl) in ml/min,), renal tubule parameters (fractional excretion of sodium (Na) (FeNa); enzymatic markers of tubular damage), glomerular parameters (proteinuria), endothelial injury parameters, markers of inflammation, oxidative stress markers, microcirculatory tissue perfusion post-preservation, oxygen consumption, histology, and animal survival.

The standardized mean difference (SMD) was calculated between comparator groups for peak creatinine, CrCl, FeNa and survival using an effects size calculator.[22]

3.3.4 BIAS ASSESSMENT

Clinical (human) data

Bias assessment of prospective cohort studies included in the meta-analyses was performed using the Newcastle-Ottawa quality assessment scale for cohort studies.[23] RCT study quality was assessed using the Cochrane Collaboration's tool.[24]

Experimental (animal) data

Animal experimental studies have several important differences in comparison to clinical studies. As such, SYRCLE's risk of bias tool for animal studies was instead utilized to assess the quality of animal data included in meta-analyses.[25]

3.3.5 SYNTHESIS AND ANALYSIS OF RESULTS

Observational (retrospective) human studies, in conjunction with prospective studies, were collated to systematically summarize the current parameters of MP utilization clinically. Observational studies were not included in subsequent formal quantitative analyses.

Similarly, animal studies comparing HMP and CS were only utilized to explore mechanisms of MP preservation. As there are multiple human studies focusing on the comparison between HMP and CS, animal studies for this comparator group were not formally meta-analyzed in order to avoid additional heterogeneity.

3.3.6 META-ANALYSES

In general, the HMP or WP groups were considered the intervention group when compared to CS; the intervention group was WP when compared to HMP, and oxygenated HMP when compared to non-oxygenated HMP. In the event of multiple experimental groups and one control group, each different experimental group was compared with the control group and analyzed as a separate study.

Human (clinical) data

Only prospective studies were included in meta-analyses. As only one study utilized WP[26] it could not be separately analyzed. Therefore, studies comparing HMP to CS were meta-analyzed. Further subgroup analyses for HMP versus CS in DCD and ECD donors were undertaken. In the event that one article presented the results from a sub-group of a larger study, the ECD or DCD donor results were only included in subgroup analyses. Forest plots denoting relative risk (RR) were constructed for DGF and PNF; HR was utilized in graft survival plots.

Animal (experimental) data

Meta-analyses were undertaken for studies comparing WP to CS or HMP, and oxygenated HMP to non-oxygenated HMP. All WP studies employed a DCD model so further subgroup analyses could not be undertaken. Forest plots were created for the SMD of relevant quantitative parameters.

Meta-analyses were performed for the above comparator groups using Comprehensive Meta-Analysis Version 2.2 (Biostat, Inc., New Jersey, USA). The I^2 statistic was used to analyze study heterogeneity, with values $\geq 50\%$ indicating high levels of heterogeneity. In these cases, a random effects model was used; otherwise, a fixed effects model was employed. Publication bias was assessed using funnel plots. A p-value < 0.05 denotes statistical significance, and meta-analysis results are presented with 95% confidence intervals (CI).

3.4 Results

3.4.1 SUMMARY CLINICAL AND EXPERIMENTAL STUDY CHARACTERISTICS

Both human and animal studies were analyzed in the formulation of this systematic review, with human studies used in comparisons between HMP and CS, and animal articles utilized for the analysis of oxygenated HMP, WP and the mechanisms of MP. In total, 63 human and 38 animal studies met inclusion criteria for which data was extracted for both quantitative and

qualitative analyses. Figure 1 outlines the study selection process. Baseline study characteristics are outlined in SDC 2 and 3 (Tables), whilst Table 1 summarizes preservation and perfusion parameters for all studies.

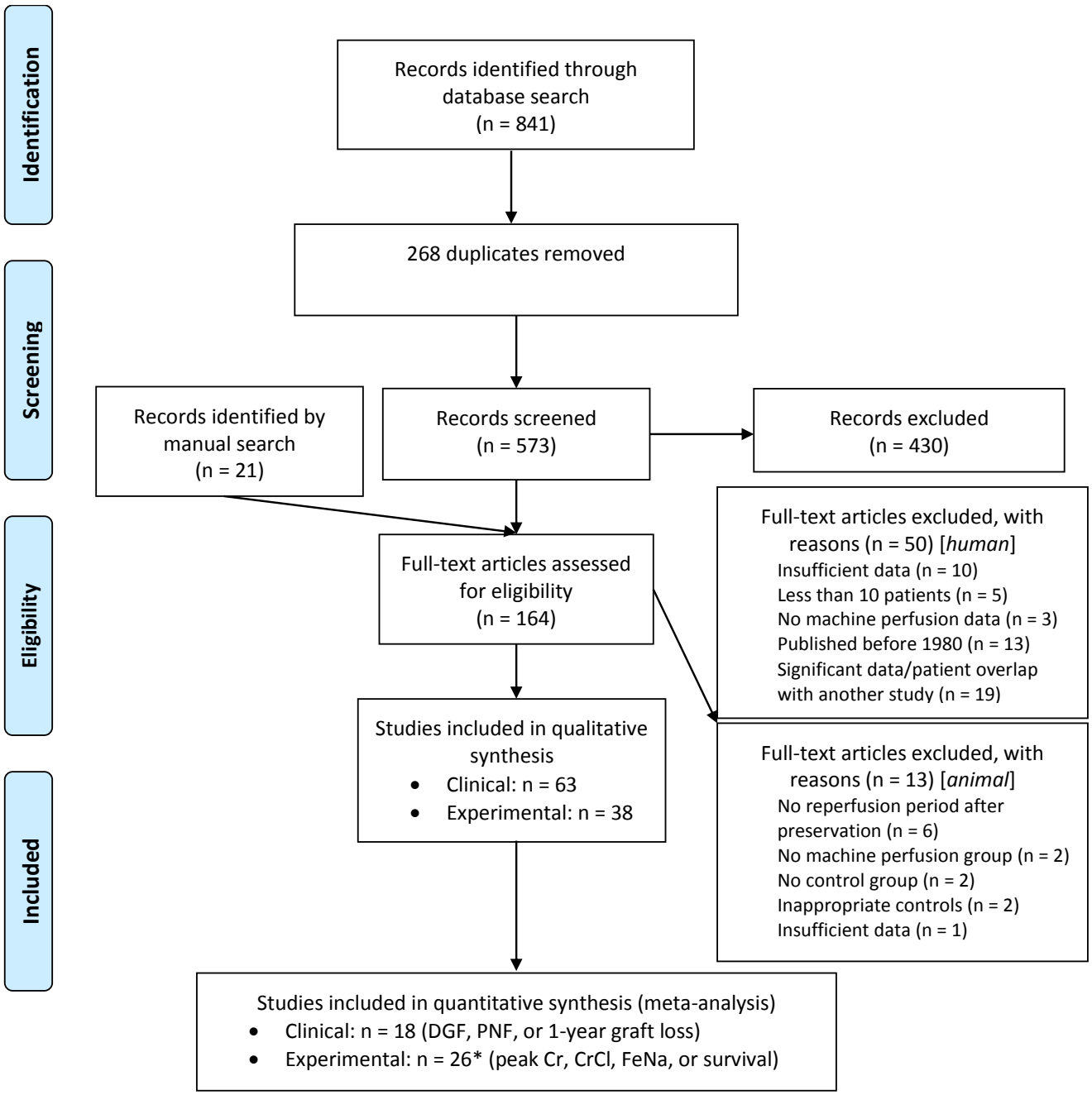


Figure 1. Study selection flow diagram.

Cr – creatinine; CrCl – creatinine clearance; DGF – delayed graft function; FeNa – fractional excretion of sodium; PNF – primary non-function.

*In articles that compared more than two treatment groups, each comparator group pair was treated as a separate experiment for the purposes of the meta-analysis.

Table 1. Summary human and animal study perfusion and preservation characteristics^

Humans	**Preservation solution [*n* studies]**[ζ¥]	**Additives to perfusion solution**^^[ζ]	**Perfusion machine [*n* studies]**[ζ]	**Storage/perfusion temperature [range *°C*]**[ζ]	**Use of Oxygen, *n* studies**[ζ]
CS	EC [14] HTK [3] Other [4] UW [15]	N/A	N/A	Hypothermic	Nil
MP	Plasma/albumin-based [16] Other [3]^^^ UW [32]	α-Ketoglutarate L-Arginine N-Acetylcysteine Papaverine PEG-SOD Phentolamine Prostacyclin PGE1 Verapamil	Gambro [4] LifePort [16] Other [5] Waters (RM3 or MOX-100) [31]	Hypothermic [1-8] Normothermic [34.6]***	4 [3 x HMP; 1 x WP]
Animals	**Preservation solution [*n* studies]**[ζ¥]	**Additives to perfusion solution**[ζ]^^	**Perfusion machine [*n* studies]**[ζ]	**Storage/perfusion temperature [range *°C*]**[ζ]	**Use of Oxygen, *n* studies [pO_2 *mmHg*]**[ζ]
CS	HTK [9] HOC [3] Other [2] UW** [11]	N/A	N/A	Hypothermic	Nil
HMP	Albumin-based [3] Custodiol-N/dextran[κ] [5] HTK [3] Other [4] UW** [21]	Alanine[β] Aspartate[β] Deferoxamine[β] Glycine[β] L-arginine[β] PEG[γ]	Belzer [2] Gambro [3] LifePort [10] Other [5] Waters (RM3 or MOX-100) [8]	Hypothermic [0-8]	19 [150-800]
WP	Blood [6][α] Custodiol-N/dextran [2] EMS medium [5] Other [1]	Components of EMS media[Ϯ] FGF Sodium nitroprusside	EMS technology [4] IOPS[ε] [4] Other [3]	Subnormo/normo-thermic [20-38]	14 [150-700]

CS – cold (static) storage; EC – Euro-Collins; EMS – exsanguinous metabolic support; FGF – fibroblast growth factor; HMP – hypothermic machine perfusion; HOC – hyperosmolar citrate; HTK – histidine–tryptophan–ketoglutarate; IOPS – isolated organ perfusion system; MP – machine perfusion; PEG – polyethylene glycol; PEG-SOD – polyethylene glycol-superoxide dismutase; PGE1 – prostaglandin E1; UW – University of Wisconsin; WP – warm (normothermic, machine) perfusion

[ζ] Where recorded

[¥] Each study may have used > 1 perfusion/preservation solution (for different experimental groups)

^Excluding subgroups in study counts

^^ In addition to 'standard' additives such as insulin, penicillin and dexamethasone, as instructed by manufacturers of UW solution – see UW product sheet[137]

^^^ Plasma-free packed red cells + Ringer's solution used in WP study

* Excludes any potential CS solution used prior to MP

** Includes Kidney Perfusion Solution (KPS) 1, Belzer Machine Perfusion Solution (MPS), Belzer II MPS

*** n = 1 study

[α] In some cases leukocyte-depleted

[β] Part of Custodiol-N solution

[γ] As part of Institut Georges Lopez (IGL)-1 solution (substitute for hydroxyl ethyl starch in extracellular UW solution)[138]

[ε] Based on pediatric cardiopulmonary bypass apparatus

[†] See Brasile *et al*.[56]

[κ] Modified form of HTK

3.4.2 HUMAN (CLINICAL) DATA

MP parameters for deceased human donor kidney preservation (All Studies)

University of Wisconsin (UW)-based MP solutions were the most commonly utilized preservation solutions in human MP (Table 1). Perfusion fluid was pumped through kidneys using Waters or LifePort MP apparatus in most cases (Table 1). Pulsatile perfusion was employed in the vast majority of studies; only two (3.2%) articles specified the use of non-pulsatile MP.[27,28] Median perfusion pressure was 50 mmHg (range 30-60 mmHg) in HMP articles, whilst the one WP study used pressures of 52-70 mmHg.[26]

Pharmacologic manipulation of the perfusate was minimal, with only eight (12.7%) human studies entertaining the addition of non-standard additives (Table 1), and four (6.3%) of articles utilizing oxygenated MP. All but one human study utilized HMP; in the WP study the perfusate was warmed to a temperature of 32-36°C.[26]

The duration and location of placement of kidneys on the machine varied between centers. In particular, 18 of 63 (28.6%) of articles specified the use of CS in conjunction with MP; in these cases, MP was usually commenced upon arrival to the recipient center. Kidneys that underwent MP tended to have greater median CITs compared to CS kidneys (23.4 versus 19.5 hours, respectively) (see Table, SDC 2), largely reflecting the use of MP as a possible means to extend preservation times.

Meta-analyses (Prospective Studies)

Eighteen studies were included in the human meta-analysis, out of which 11 (61.1%) articles were RCTs, and seven (38.9%) studies were prospective but non-randomized (prospective cohorts). As there was only one study comparing WP to CS, WP could not be directly compared to other preservation methods using the human studies.

Forest plots of selected meta-analyses are shown in Figure 2, with all results tabulated in SDC 4.

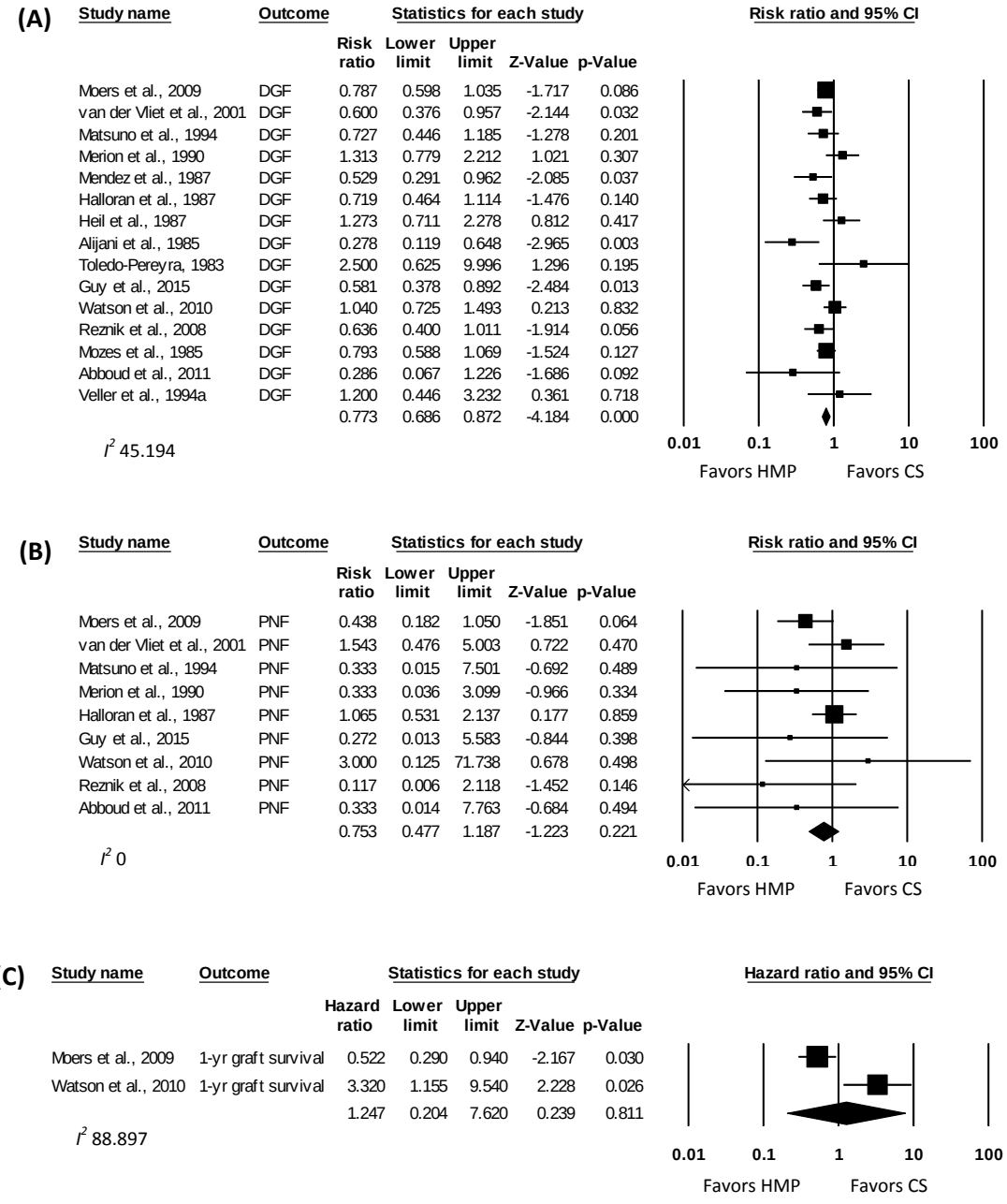


Figure 2. Forest plots comparing DGF (A), PNF (B) and 1-year graft loss (C) for all studies comparing HMP to CS – *human* studies. Data expressed as RR (for DGF and PNF) and HR (for graft loss) ± 95% CI. Different analyses within the same study are denoted by an alphabetical letter suffix (e.g. "a").

Human studies displayed the short-term advantages of MP when compared to CS. The RR (unadjusted) of DGF for HMP versus CS studies was 0.77 (95% CI 0.69-0.87; $p < 0.001$).

Within the DCD kidney subgroup, the RR of DGF was 0.78 (95 % CI 0.66-0.91; p = 0.002), whilst it was 0.67 for ECD donors (95% CI 0.42-1.08; p = 0.097). It should be noted that only two studies were available for the ECD comparison. A significant difference in PNF rates between HMP and CS was only detected in the ECD cohort (RR 0.28, 95% CI 0.09-0.89; p = 0.031).

The medium to long-term effects of MP were less clear. With respect to graft failure rates within the first year, there was no difference between HMP and CS overall (HR 1.25, 95% CI 0.20-7.62; p = 0.81). Insufficient data precluded HR calculations for further subgroup analyses, or for the comparison of patient survival between the HMP and CS groups.

Meta-analysis Publication bias and Heterogeneity (Prospective Studies)

Visual assessment of funnel plots displayed no significant asymmetry when comparing HMP to CS for the DGF parameter. There was only mild asymmetry in favor of positive studies for studies comparing PNF (see Figure, SDC 5, for funnel plots). Study heterogeneity was low for a majority of parameters (see Table, SDC 4).

Trends in one-year graft loss and patient survival (Prospective Studies)

Meta-analyses for graft loss/survival at one year could only be conducted in two studies. In one of these studies by Moers *et al*., graft loss at one year was significantly higher in the CS group compared to HMP (HR 0.52; p = 0.03); this finding was maintained in the ECD (HR 0.35; p = 0.02) but not DCD subgroups (HR 1.29; p = 0.7) in subsequent expansions of the study cohorts.[16,29,30] Graft loss (survival) data for the one year time-point were available in eight further prospective studies. Although there were no statistically significant differences between HMP and CS, there was a trend towards higher survival after HMP in four studies, including one article investigating ECD kidneys.[31-34] In contrast, although still underpowered to produce statistical significance, two studies indicated higher survival in CS kidneys, with one of these studies analyzing DCD kidneys.[35,36]

There were seven prospective studies with results available for patient survival one year post-transplant.[15,16,29-32,34] Median survivals were 94.9% (range 80.6-97%) for HMP kidneys, and 96.7% (range 77.7-100%) for CS kidneys. No study reported statistically significant differences between either preservation method.

Nicholson and Hosgood presented the only human study exploring the use of WP for renal preservation.[26] The WP cohort impressively had 100% one year graft and patient survival rates, although there were only 18 patients in the WP group.

Graft rejection (Prospective Studies)

Acute graft rejection rates were not statistically comparable owing to variable definitions and immunosuppression. Rejection rates were no different in the multi-center trial by Moers *et al*. (13.7% for CS versus 13.1% for MP).[16] In contrast, three prospective studies showed a strong trend toward lower rates of acute rejection in the HMP group, although this did not reach significance.[15,37,38]

Risk of bias assessment (Prospective Studies)

The risk of bias assessment of cohort studies is summarized in SDC 6 (Figure). Six out of 8 domains in the assessment scale were adequately covered in at least 60% of studies. Comparability of cohorts in study design or analysis was less adequately covered, as a proportion of studies did not appropriately account for factors such as organ ischemic times. SDC 7 (Table) displays the risk of bias assessment for the included RCTs upon utilization of the Cochrane Collaboration's bias tool.[24] Across studies, it can be seen that there is a low risk of bias in at least three of the domains. Within the domains of blinding and allocation concealment, however, at least half of the studies were at risk of selection and performance bias.

3.4.3 ANIMAL (EXPERIMENTAL) DATA

MP characteristics (All Studies)

In stark contrast to human studies, 30 of 38 (78.9%) animal articles utilized oxygenated MP. Furthermore, WP, including subnormothermic MP, was used in 14 (36.8%) of the included animal studies (see Table, SDC 3). As such, further quantitative analyses regarding oxygenated and/or WP were undertaken in animal studies.

Meta-analyses (Oxygenated HMP and WP Studies)

There were 10 distinct animal data-sets utilized in the meta-analyses that compared CS to WP, whilst 11 studies were included that compared HMP to WP and five studies were available for the comparison between oxygenated and non-oxygenated HMP.

Figure 3 displays forest plots of selected meta-analyses, with results tabulated in SDC 8.

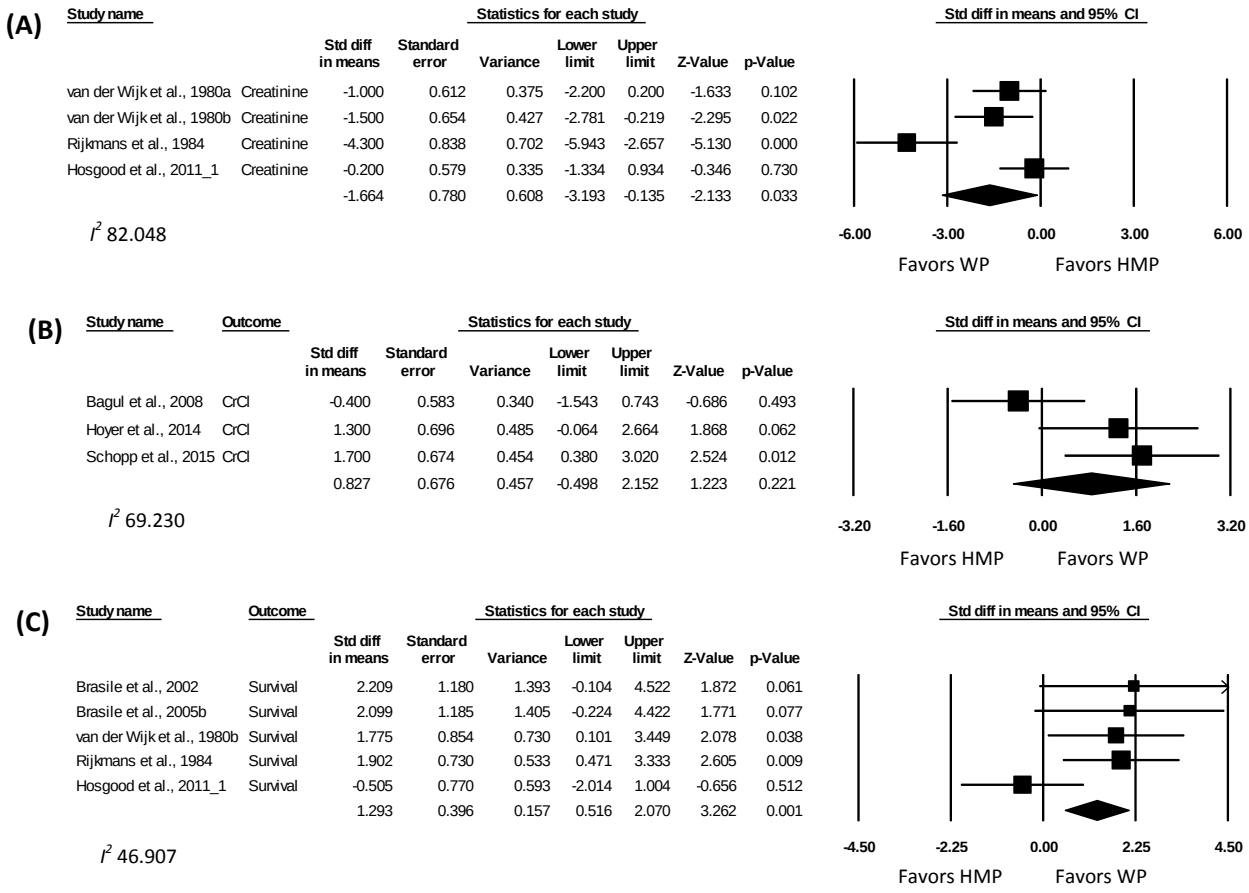


Figure 3. Forest plots comparing peak creatinine (A), peak CrCl (B), and survival (C) for WP compared to HMP – *animal* studies. Data presented as SMD ± 95% CI. Different analyses within the same study are denoted by an alphabetical letter suffix (e.g. "a").

Post-preservation renal function in animal experiments was assessed using the parameters of peak creatinine, CrCl and FeNa, and animal survival during the experimental period. Peak creatinine values were significantly lower in animal groups utilizing WP (SMD -1.72, 95% CI -3.09 to -0.34; p = 0.014) when compared to CS. The SMD of peak serum creatinine levels in the WP group was also significantly lower when compared to the HMP group (-1.66, 95% CI -3.19 to -0.14; p = 0.033). There was no significant difference however between peak creatinine levels in the oxygenated HMP versus non-oxygenated HMP group (SMD -0.39, 95% CI -1.85 to 1.08; p = 0.60), however there were only 2 studies eligible for this comparison.[39,40] However, the SMD of peak CrCl between the WP and HMP (0.83, 95% CI -0.50 to 2.15; p = 0.22) and CS (2.08, 95% CI -1.83 to 6.00; p = 0.22) groups was not significantly different.

FeNa could not be compared between WP and other groups due to an insufficient number of studies. Importantly, pooled FeNa was significantly lower in studies comparing oxygenated to non-oxygenated HMP (SMD -1.54; 95% CI -2.54 to -0.54; p = 0.002).

Animal survival in such studies is a reflection of maintenance of renal function as opposed to actual survival *per se* as the vast majority of deaths reflected euthanasia after manifestation of features of renal failure. Importantly, WP once again demonstrated its superiority over HMP (SMD 1.29; 95% CI 0.52-2.07; p = 0.001). There was not enough data to analyze this parameter for WP compared to CS groups.

Meta-analysis Publication bias and Heterogeneity (WP Studies)

Analysis of funnel plots did not display significant asymmetry when comparing peak creatinine between WP and the HMP or CS groups (see Figure, SDC 9, for funnel plots). Study heterogeneity was high for most parameters (see Table, SDC 8).

Mechanisms of action of MP – tubules, glomeruli and endothelium (All Studies)

The animal studies outlined comparisons between experimental and control groups for a wide range of parameters that could not be meta-analyzed due to significant variability in reporting between different studies. These functional indicators are displayed in Table 2, and can broadly be characterized into those relating to tubular, glomerular or endothelial function or damage, oxidative stress, levels of inflammation, micro-circulatory tissue perfusion, and oxygen consumption. Histology was not included in this analysis due to wide variability in the reporting of histological criteria. Broadly, improved tubular function with a reduction in tubular injury, improved glomerular function, and reduced endothelial injury seemed to be evident after the utilization of HMP compared to CS. Furthermore, HMP appeared to improve renal cortical micro-circulation. There was no obvious advantage for any experimental group regarding markers of inflammation or oxidative stress. Furthermore, with the exception of higher oxygen consumption in all three studies comparing WP to CS, no clear differences could be elucidated between the other experimental and control groups (Table 2).

Table 2. Tubular, glomerular & endothelial function and damage in *animal* studies*

	CS vs HMP [*n* studies/total]	**HMP vs WP [*n* studies/total]**	**CS vs WP [*n* studies/total]**	**HMP no-O_2 vs HMP-O_2 [*n* studies/total]**
Tubules	1. Lower FeNa: • CS – 0/8 • HMP – 8/8 2. Higher serum/urine tubular damage markers**: • CS – 7/11 • HMP – 0/11	1. Lower FeNa: • HMP – 0/2 • WP – 1/2 2. Higher serum/urine tubular damage markers**: • HMP – 0/3 • WP – 1/3	1. Lower FeNa: • CS – 1/4 • WP – 3/4 2. Higher serum/urine tubular damage markers**: • CS – 2/4 • WP – 1/4	1. Lower FeNa: • No-O_2 – 0/2 • O_2 – 0/2 2. Higher serum/urine tubular damage markers**: • No-O_2 – 2/5 • O_2 – 0/5
Glomeruli	Lower proteinuria: • CS – 1/6 • HMP – 4/6	Lower proteinuria: • HMP – 0/1 • WP – 0/1	Lower proteinuria: • CS – NR • WP – NR	Lower proteinuria: • No-O_2 – 0/1 • O_2 – 1/1
Endothelium	Higher injury markers ***: • CS – 3/5 • HMP – 1/5	Higher injury markers ***: • HMP – 0/1 • WP – 0/1	Higher injury markers ***: • CS – 1/3 • WP – 0/3	Higher injury markers ***: • No-O_2 – 1/1 • O_2 – 0/1
Inflammation	Increased inflammatory markers^: • CS – 1/5 • HMP – 2/5	Increased inflammatory markers^: • HMP – 0/2 • WP – 1/2	Increased inflammatory markers^: • CS – 0/2 • WP – 1/2	Increased inflammatory markers^: • No-O_2 – 0/1 • O_2 – 0/1
Oxidative stress	Elevated markers of oxidative stress^^: • CS – 2/4 • HP – 2/4	Elevated markers of oxidative stress^^: • HMP – 1/1 • WP – 0/1	Elevated markers of oxidative stress^^: • CS – 0/1 • WP – 1/1	Elevated markers of oxidative stress^^: • No-O_2 – 1/2 • O_2 – 2/2^^^
Microcirculation[κ]	Better cortical microcirculation[λ]: • CS – 0/4 • HMP – 4/4	Better cortical microcirculation[λ]: • HMP – NR • WP – NR	Better cortical microcirculation[λ]: • CS – NR • WP – NR	Better cortical microcirculation[λ]: • No-O_2 – 0/1 • O_2 – 0/1
O_2 consumption	Higher O_2 consumption[μ]: • CS – 0/5 • HMP – 0/5	Higher O_2 consumption[μ]: • HMP – 0/2 • WP – 1/2	Higher O_2 consumption[μ]: • CS – 0/3 • WP – 3/3	Higher O_2 consumption[μ]: • No-O_2 – 0/3 • O_2 – 0/3

CS – cold (static) storage; HMP – hypothermic machine perfusion; NR – not recorded; WP – warm (machine) perfusion

* Number of studies for each respective outcome included only if *statistically significant difference* recorded in each study (see meta-analyses for pooled outcomes for FeNa); in studies where there were > 2 study groups, study outcome(s) only included for comparable groups

** Markers measured (references): alanine aminopeptidase[45, 115]; aspartate aminotransferase[40, 113, 128]; gamma-glutamyl transpeptidase[59]; lactate dehydrogenase[39, 59, 67, 113, 118, 129, 132, 134]; liver fatty acid binding protein[47, 58]; N-acetyl-β-D-glucosaminidase[45, 115]

*** Endothelial injury markers (references): endothelin-1[47, 113, 114, 132, 134]; thrombomodulin[39]; von Willebrand factor[45, 116, 128]

^ Inflammatory markers (references): high mobility group protein B1[116]; intercellular adhesion molecule 1[116]; interleukin-6[132, 133]; myeloperoxidase activity[113]; nuclear factor kappa B1[114]; toll-like receptor 4[116]; tumor necrosis factor α[59, 133]

^^ Free radical damage/oxidative stress markers (references): 8-isoprostane[113, 118, 132]; malondialdehyde[70]; oxidized to total glutathione ratio[115]; thiobarbituric acid reactive substances[45, 68]; unspecified lipid peroxidation products[39]

^^^ In Gallinat et al.[39], lipid peroxidation products significantly lower in the no oxygen group during preservation (perfusion), with the opposite true after transplantation; in Hoyer et al.[66], markers of oxidative damage were also measured during preservation (perfusion), and were lower in the no oxygen group

ᵡ Assessed as mean cortical erythrocyte flux 10 minutes post-reperfusion by Laser Doppler flowmetry

ᵡ Studies included – [39, 43, 45, 47, 116]

ᵘ Studies included – [44, 58, 59, 67, 113, 118, 132, 134]

Risk of bias assessment (All Studies)

Animal study bias assessment was performed using SYRCLE's assessment tool[25] and is summarized in SDC 10 (Figure). Overall, there were very few domains in which there was clearly a high risk of bias. In 6 out of the 10 parameters however, bias assessment was largely unclear as the domains could not be analyzed from the available study data.

3.5 Discussion

This systematic review and meta-analysis provides a comprehensive and up-to-date insight into the current published literature regarding MP preservation of renal grafts prior to transplantation in the clinical setting. Animal data was included to explore modifications to MP that are as yet grossly under-explored in human studies, namely WP and oxygenated MP, in addition to allowing the development of a greater mechanistic understanding of MP.

We show a definite reduction in DGF post-HMP preservation for renal allografts in humans when compared to CS, including in DCD and ECD kidneys. PNF appeared to be reduced in the ECD subset. There was not enough data to give sufficient power to comparisons of one year graft survival by meta-analysis, and subgroup analyses could not be conducted for this parameter. One year patient survival was comparable amongst the different studies. We obtained mixed results regarding the benefits of oxygenated HMP. Furthermore, although there was only one human study that employed WP[26], multiple animal studies showed its advantages over both CS and HMP kidneys in terms of post-transplantation creatinine levels and animal

survival. Animal study results showed mechanisms for improved allograft function in MP kidneys, including better tubular and glomerular function, and less endothelial damage.

Increased demands for donor kidneys have necessitated the use of more marginal organs for transplantation. Indeed, any method such as MP that will increase the pool of usable kidneys can benefit developing and developed countries alike, especially due to the often prohibitively high costs associated with long-term dialysis, and should be explored further.[1] A detailed economic analysis by Wight *et al.*, albeit from 2003, showed that MP is likely to be more effective than CS in the long-term, with an economic benefit more pronounced when MP preservation is applied to DCD kidneys.[41] Whilst Groen *et al*. in 2012 could not make the same conclusion for DCD transplants due to insufficient numbers, these authors found reduced costs after MP in the ECD subset, largely due to a reduced need for post-transplantation dialysis and hospital bed-stays.[42]

Mechanistically, MP reduces preservation-related damage and aids renal recovery through a variety of mechanisms. ATP levels, and thus energy homeostasis, are better preserved in perfused kidneys.[43,44] Tubular and glomerular integrity seems to be aided by MP, an assertion that is supported by the reduction in markers of tubular damage and improved tubular and glomerular function seen after MP as compared to CS (Table 2). Furthermore, MP ensures better reperfusion of grafts as measured by cortical microcirculation; this is likely related to a reduction in endothelial damage and swelling[43,45] (Table 2). The flow cessation itself in CS as compared to MP likely contributes to the increased endothelial dysfunction in CS grafts.[46] The pulsatile aspect of MP likely has an important effect on the maintenance of endothelial integrity, as pulsatile-perfused kidneys compared to non-pulsatile MP have been shown to have higher renal vascular flow, reduced expression of endothelin-1, and increased expression of the vasoprotective kruppel-like factors and nitric oxide.[47] We did not however find significant support for less inflammation and oxidative stress in the HMP group (Table 2), although recent evidence suggests that apoptosis and inflammation may be reduced in HMP through up-regulation of aldehyde dehydrogenase 2 and reduction in expression of nuclear factor-κB and matrix metalloproteinase 9.[48,49]

In congruence with previous systematic reviews[8,50-52] our data shows that DGF is undoubtedly reduced in patients undergoing MP compared to CS. We additionally showed the possibility of reduced PNF after HMP preservation of ECD kidneys. In contrast to Jiao *et al*.[53] however, we

could not find statistical evidence for improved graft survival in the ECD cohort, due to a lack of available HR data that could subsequently be pooled. Furthermore, statistical methods in the former study are flawed, with survival analyses conducted using OR instead of HR; in addition, two out of the three studies in their survival analysis had significant patient overlap.[53] Perhaps most pertinently however, the pivotal large-scale and multi-center RCT performed by Moers *et al*. showed significantly improved graft survival in HMP patients, with this survival advantage still present after three years in DBD and especially ECD kidneys, but not in kidneys from DCD donors.[16,54,55]

Whilst Moers and colleagues' study provides evidence regarding the efficacy of machine perfusion as it is utilized currently, our analysis of all retrospective and prospective MP studies in humans to date show that it is still employed in a very limited fashion, with considerable room for modification to maximize the potentials of this technique. In particular, temperature modification, oxygenation and pharmacologic manipulation of perfusion solutions are all in their infancy with regard to human renal preservation via MP.

The inclusion of animal data has allowed this review to capture the possible future of MP, as this experimental work has not yet caught up with application to the clinic. In particular, a reasonable deduction can be made regarding the applicability and potential success of WP, which currently has little human data. WP reverses the pivotal concept of hypothermia in organ preservation, sustaining normal metabolic rates with an oxygenated red blood cell-based perfusate. Compared to CS and HMP kidneys, WP kidneys had significantly lower peak creatinine and better survival (Figure 3; also see Table, SDC 8). Nicholson & Hosgood utilized WP in human ECD kidney grafts, and also reported lower rates of DGF compared to CS.[26] WP potentially reduces the possibility of irreversible cold-induced metabolic disruption in addition to reducing ischemia-reperfusion injury upon commencement of normothermic reperfusion *in vivo*.[48,56,57]

An alternative to WP at body temperature is the concept of subnormothermic MP, successfully utilized here in two studies.[58,59] Subnormothermic perfusion helps avoid the injuries induced by cold ischemia without necessitating a significant change in perfusion equipment or solutions.[59] In addition, it guards against the pitfalls inherent to an immediate temperature shift from hypothermia to body temperature upon post-anastomotic reperfusion.[58]

The perfusion solution and its additives potentially have a major impact on the effectiveness of kidney preservation. UW or a modified form of UW was the most commonly employed solution for CS and MP in both animal and human studies (Table 1), which is not surprising considering its proven efficacy.[49] Although there is considerable ongoing research into pharmacological manipulation of organ preservation solutions, surprisingly few studies utilized additives to try and change graft outcomes (Table 1). Pathophysiological targets for these additives include free-radical injury, endothelial damage and vasoconstriction, the complement cascade, and apoptosis.[60-64] These processes were in some cases targeted as part of new perfusion solutions, including Custodiol-N, Vasosol, and Exsanguinous Metabolic Support (EMS) media.[60,62,64,65] It is difficult to ascertain individual effects of each pharmacologic agent, as few studies undertook direct comparisons between them. Guerrera *et al*. compared Vasosol solution, which contains vasodilatory agents such as prostaglandin E1 (PGE1) and nitroglycerin, and the anti-oxidant N-acetylcysteine, to UW (Belzer MPS), and showed significant lower DGF rates in the Vasosol group.[64] The addition of PGE1 to UW was also shown to be effective in another study.[62] Other pharmacological therapies that may be incorporated into renal preservation are reviewed by Chatauret *et al*.[66]

Oxygenation is also a pharmacologic intervention that can be applied to HMP. Its use was much more prevalent in animal studies, with comparisons showing significantly lower FeNa in the oxygenated HMP compared to non-oxygenated HMP group (see Table, SDC 8). The absence of a statistical difference with regards to peak creatinine may be explained by the fact that there were only two studies for comparison.[39,40] Active oxygenation of the perfusate may potentially increase the generation of reactive oxygen species (see Table 2), although this was not supported post-transplantation in the study by Gallinat *et al*.[39] In contrast, the use of oxygen during HMP is purported to restore adequate mitochondrial and cellular homeostasis prior to reperfusion.[67,68] An alternative to oxygenated MP is the use of persufflation, through which oxygen can be delivered to the kidneys directly through its vasculature. Suszynski *et al*. summarize the utility of persufflation for renal preservation;[69] this technique was compared to CS and HMP by Treckmann *et al*., with persufflated kidneys having significantly lower creatinine levels post-transplantation compared to HMP.[70]

Limitations of this review include the suboptimal comparability of HMP and CS cohorts within the human studies. This was largely due to the fact that CIT for human MP kidneys was higher than that for CS kidneys (see Table, SDC 2), which is not surprising given that MP is often

used as a means to extend the period of preservation. Furthermore, a not insignificant proportion of RCTs suffered from features of selection bias due to poor blinding and allocation concealment. Additionally, it is difficult to tease out the impact of MP solutions on the overall effect of MP, as a variety of solutions were utilized that were usually different to the CS control. Animal studies, although informative, were quite heterogeneous and difficult to formally assess for bias. We attempted to minimize bias by excluding all retrospective studies from the meta-analyses, and in order to account for any study heterogeneity a random effects model was employed to help reduce type I error.

In summary, we have shown distinct short-term advantages in the use of MP over CS for the preservation of renal allografts, especially with regards to the reduction of DGF. ECD graft recipients may benefit further from a reduction in PNF rates. In the medium to long-term, there is likely a survival and cost advantage for ECD kidneys that have undergone MP in this way. Although results from animal studies should be interpreted with more caution, they show some mechanistic advantages to the use of oxygenated MP, and distinct functional improvements upon the use of normothermic perfusion; this should provide a further stimulus for MP oxygenation and WP human trials. We strongly encourage additional exploration and enhancement of the MP preservation technique, through a variety of modifications based on the presented experimental evidence, which may improve its short and long-term efficacy.

3.6 References

1. Garcia GG, Harden P, Chapman J, World Kidney Day Steering C. The global role of kidney transplantation. *Lancet.* 2012; 379: e36-e38.
2. United States Renal Data System. *USRDS annual data report: Epidemiology of Kidney Disease in the United States.* Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2015.
3. Gridelli B, Remuzzi G. Strategies for Making More Organs Available for Transplantation. *N Engl J Med.* 2000; 343: 404-410.
4. Metzger RA, Delmonico FL, Feng S, Port FK, Wynn JJ, Merion RM. Expanded criteria donors for kidney transplantation. *Am J Transplant.* 2003; 3: 114-125.
5. Cho YW, Terasaki PI, Cecka JM, Gjertson DW. Transplantation of Kidneys from Donors Whose Hearts Have Stopped Beating. *N Engl J Med.* 1998; 338: 221-225.
6. Saidi RF, Elias N, Kawai T, et al. Outcome of Kidney Transplantation Using Expanded Criteria Donors and Donation After Cardiac Death Kidneys: Realities and Costs. *Am J Transplant.* 2007; 7: 2769-2774.
7. Rao PS, Ojo A. The Alphabet Soup of Kidney Transplantation: SCD, DCD, ECD—Fundamentals for the Practicing Nephrologist. *Clin J Am Soc Nephrol.* 2009; 4: 1827-1831.
8. Bathini V, McGregor T, McAlister VC, Luke PP, Sener A. Renal perfusion pump vs cold storage for donation after cardiac death kidneys: a systematic review. *J Urol.* 2013; 189: 2214-2220.
9. Dutkowski P, Schlegel A, de Oliveira M, Mullhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. *J Hepatol.* 2014; 60: 765-772.
10. Klein AS, Messersmith EE, Ratner LE, Kochik R, Baliga PK, Ojo AO. Organ donation and utilization in the United States, 1999-2008. *Am J Transplant.* 2010; 10: 973-986.
11. Opelz G, Terasaki PI. Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. *Transplantation.* 1982; 33: 64-68.
12. Sheil AG, Drummond JM, Rogers JH, Boulas J, May J, Storey BG. A controlled clinical trial of machine perfusion of cadaveric donor renal allografts. *Lancet.* 1975; 2: 287-290.
13. Rosenthal JT, Herman JB, Taylor RJ, Broznick B, Hakala TR. Comparison of pulsatile machine perfusion with cold storage for cadaver kidney preservation. *Transplantation.* 1984; 37: 425-426.
14. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transpl Int.* 2015; 28: 665-676.
15. Watson CJ, Wells AC, Roberts RJ, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant.* 2010; 10: 1991-1999.
16. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2009; 360: 7-19.
17. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA StatementThe PRISMA Statement. *Ann Intern Med.* 2009;151(4):264-269.
18. Centre for Reviews and Dissemination. PROSPERO: International prospective register of systematic reviews. 2015; http://www.crd.york.ac.uk/prospero/prospero.asp. Accessed March, 2016.
19. McAuley L, Pham B, Tugwell P, Moher D. Does the inclusion of grey literature influence estimates of intervention effectiveness reported in meta-analyses? *Lancet.* 2000; 356: 1228-1231.

20. Mallon DH, Summers DM, Bradley JA, Pettigrew GJ. Defining delayed graft function after renal transplantation: simplest is best. *Transplantation.* 2013; 96: 885-889.
21. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials.* 2007; 8: 1-16.
22. Wilson D. Practical Meta-Analysis Effect Size Calculator. 2001; http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php. Accessed February, 2016.
23. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scal (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2014; http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, 2016.
24. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* 2011; 343:d5928.
25. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol.* 2014; 14:43.
26. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant.* 2013; 13: 1246-1252.
27. Matsuno N, Konno O, Mejit A, et al. Application of machine perfusion preservation as a viability test for marginal kidney graft. *Transplantation.* 2006; 82: 1425-1428.
28. Matsuno N, Konno YN, Jyojima Y, et al. Machine perfusion preservation for kidney grafts with a high creatinine from uncontrolled donation after cardiac death. *Transplant Proc.* 2010; 42: 155-158.
29. Treckmann J, Moers C, Smits JM, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. *Transpl Int.* 2011; 24: 548-554.
30. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg.* 2010; 252: 756-764.
31. Mozes M, Finch W, Reckard C, Merkel F, Cohen C. Comparison of cold storage and machine perfusion in the preservation of cadaver kidneys: A prospective, randomized study *Transplant Proc.* 1985; 17: 1474-1477.
32. Abboud I, Antoine C, Gaudez F, et al. Pulsatile perfusion preservation for expanded-criteria donors kidneys: Impact on delayed graft function rate. *Int J Artif Organs.* 2011; 34:513-518.
33. Mendez R, Mendez RG, Koussa N, Cats S, Bogaard TP, Khetan U. Preservation effect on oligo-anuria in the cyclosporine era: a prospective trial with 26 paired cadaveric renal allografts. *Transplant Proc.* 1987; 19: 2047-2050.
34. Halloran P, Aprile M. A randomized prospective trial of cold storage versus pulsatile perfusion for cadaver kidney preservation. *Transplantation.* 1987; 43: 827-832.
35. van der Vliet JA, Kievit JK, Hene RJ, Hilbrands LB, Kootstra G. Preservation of non-heart-beating donor kidneys: a clinical prospective randomised case-control study of machine perfusion versus cold storage. *Transplant Proc.* 2001; 33: 847.
36. Toledo-Pereyra LH. Renal hypothermic storage with a new hyperosmolar colloid solution. *Bol Asoc Med P R.* 1983; 75: 347-350.
37. Matsuno N, Sakurai E, Tamaki I, Uchiyama M, Kozaki K, Kozaki M. The effect of machine perfusion preservation versus cold storage on the function of kidneys from non-heart-beating donors. *Transplantation.* 1994; 57: 293-294.

38. Reznik ON, Bagnenko SF, Loginov IV, et al. Machine perfusion as a tool to select kidneys recovered from uncontrolled donors after cardiac death. *Transplant Proc.* 2008; 40:1023-1026.
39. Gallinat A, Paul A, Efferz P, et al. Role of oxygenation in hypothermic machine perfusion of kidneys from heart beating donors. *Transplantation.* 2012; 94: 809-813.
40. Thuillier R, Allain G, Celhay O, et al. Benefits of active oxygenation during hypothermic machine perfusion of kidneys in a preclinical model of deceased after cardiac death donors. *J Surg Res.* 2013; 184: 1174-1181.
41. Wight J, Chilcott J, Holmes M, Brewer N. The clinical and cost-effectiveness of pulsatile machine perfusion versus cold storage of kidneys for transplantation retrieved from heart-beating and non-heart-beating donors. *Health Technol Assess.* 2003; 7: 1-94.
42. Groen H, Moers C, Smits JM, et al. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. *Am J Transplant.* 2012; 12: 1824-1830.
43. Minor T, Sitzia M, Dombrowski F. Kidney transplantation from non-heart-beating donors after oxygenated low-flow machine perfusion preservation with histidine-tryptophan-ketoglutarate solution. *Transpl Int.* 2005; 17: 707-712.
44. Yland MJ, Todo S, Zhu Y, et al. An automated and portable low-flow pulsatile perfusion system for organ preservation. *Transpl Int.* 1996; 9: 535-540.
45. Maathuis MH, Manekeller S, van der Plaats A, et al. Improved kidney graft function after preservation using a novel hypothermic machine perfusion device. *Ann Surg.* 2007; 246:982-991.
46. Gracia-Sancho J, Villarreal G, Jr., Zhang Y, et al. Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. *Transplantation.* 2010; 90: 142-149.
47. Gallinat A, Fox M, Luer B, Efferz P, Paul A, Minor T. Role of pulsatility in hypothermic reconditioning of porcine kidney grafts by machine perfusion after cold storage. *Transplantation.* 2013; 96: 538-542.
48. Salahudeen AK. Cold ischemic injury of transplanted kidneys: new insights from experimental studies. *Am J Physiol Renal Physiol.* 2004; 287: F181-187.
49. James H. Southard MDa, Folkert O. Belzer MD. ORGAN PRESERVATION. *Annu Rev Med.* 1995; 46: 235-247.
50. Lam VW, Laurence JM, Richardson AJ, Pleass HC, Allen RD. Hypothermic machine perfusion in deceased donor kidney transplantation: a systematic review. *J Surg Res.* 2013; 180: 176-182.
51. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. *Br J Surg.* 2013; 100: 991-1001.
52. Wight JP, Chilcott JB, Holmes MW, Brewer N. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. *Clin Transplant.* 2003; 17: 293-307.
53. Jiao B, Liu S, Liu H, Cheng D, Cheng Y, Liu Y. Hypothermic Machine Perfusion Reduces Delayed Graft Function and Improves One-Year Graft Survival of Kidneys from Expanded Criteria Donors: A Meta-Analysis. *PLoS ONE.* 2013; 8: e81826.
54. Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial S, Machine Preservation Trial Study G. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2012; 366: 770-771.

55. Gallinat A, Moers C, Smits JM, et al. Machine perfusion versus static cold storage in expanded criteria donor kidney transplantation: 3-year follow-up data. *Transpl Int.* 2013; 26: E52-53.
56. Brasile L, Stubenitsky BM, Booster MH, Arenada D, Haisch C, Kootstra G. Hypothermia--a limiting factor in using warm ischemically damaged kidneys. *Am J Transplant.* 2001; 1: 316-320.
57. Stubenitsky BM, Booster MH, Brasile L, et al. Negative effect of cold ischemia on initial renal function. *ASAIO J.* 2000; 46: 60-61.
58. Schopp I, Reissberg E, Luer B, Efferz P, Minor T. Controlled Rewarming after Hypothermia: Adding a New Principle to Renal Preservation. *Clin Transl Sci.* 2015;8(5):475-478.
59. Hoyer DP, Gallinat A, Swoboda S, et al. Subnormothermic machine perfusion for preservation of porcine kidneys in a donation after circulatory death model. *Transpl Int.* 2014; 27: 1097-1106.
60. Minor T, Paul A, Efferz P, Wohlschlaeger J, Rauen U, Gallinat A. Kidney transplantation after oxygenated machine perfusion preservation with Custodiol-N solution. *Transpl Int.* 2015; 28: 1102-1108.
61. Stratta RJ, Moore PS, Farney AC, et al. Influence of pulsatile perfusion preservation on outcomes in kidney transplantation from expanded criteria donors. *J Am Coll Surg.* 2007; 204: 873-884..
62. Polyak MM, Arrington BO, Stubenbord WT, et al. The influence of pulsatile preservation on renal transplantation in the 1990s. *Transplantation.* 2000; 69: 249-258.
63. Guarrera JV, Polyak MM, Arrington B, et al. Pushing the envelope in renal preservation; improved results with novel perfusate modifications for pulsatile machine perfusion of cadaver kidneys. *Transplant Proc.* 2004; 36: 1257-1260.
64. Guarrera JV, Polyak M, O'Mar Arrington B, Kapur S, Stubenbord WT, Kinkhabwala M. Pulsatile machine perfusion with Vasosol solution improves early graft function after cadaveric renal transplantation. *Transplantation.* 2004; 77: 1264-1268.
65. Brasile L, Stubenitsky B, Haisch CE, Kon M, Kootstra G. Potential of repairing ischemically damaged kidneys ex vivo. *Transplant Proc.* 2005; 37: 375-376.
66. Chatauret N, Thuillier R, Hauet T. Preservation strategies to reduce ischemic injury in kidney transplantation: pharmacological and genetic approaches. *Curr Opin Organ Transplant.* 2011; 16: 180-187.
67. Koetting M, Frotscher C, Minor T. Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. *Transpl Int.* 2010; 23: 538-542.
68. Hoyer DP, Gallinat A, Swoboda S, et al. Influence of oxygen concentration during hypothermic machine perfusion on porcine kidneys from donation after circulatory death. *Transplantation.* 2014; 98: 944-950.
69. Suszynski TM, Rizzari MD, Scott WE, Tempelman LA, Taylor MJ, Papas KK. Persufflation (or Gaseous Oxygen Perfusion) as a Method of Organ Preservation. *Cryobiology.* 2012; 64: 125-143.
70. Treckmann J, Nagelschmidt M, Minor T, Saner F, Saad S, Paul A. Function and quality of kidneys after cold storage, machine perfusion, or retrograde oxygen persufflation: results from a porcine autotransplantation model. *Cryobiology.* 2009; 59:19-23.
71. Gallinat A, Moers C, Treckmann J, et al. Machine perfusion versus cold storage for the preservation of kidneys from donors >/= 65 years allocated in the Eurotransplant Senior Programme. *Nephrol Dial Transplant.* 2012; 27: 4458-4463.

72. Gill J, Dong J, Eng M, Landsberg D, Gill JS. Pulsatile perfusion reduces the risk of delayed graft function in deceased donor kidney transplants, irrespective of donor type and cold ischemic time. *Transplantation.* 2014; 97: 668-674.
73. Opelz G, Dohler B. Multicenter analysis of kidney preservation. *Transplantation.* 2007; 83: 247-253.
74. Sung RS, Christensen LL, Leichtman AB, et al. Determinants of discard of expanded criteria donor kidneys: impact of biopsy and machine perfusion. *Am J Transplant.* 2008; 8: 783-792.
75. Kosieradzki M, Danielewicz R, Kwiatkowski A, et al. Rejection rate and incidence of acute tubular necrosis after pulsatile perfusion preservation. *Transplant Proc.* 1999; 31: 278-279.
76. Gage F, Ali M, Alijani MR, et al. Comparison of static versus pulsatile preservation of matched-paired kidneys. *Transplant Proc.* 1997; 29: 3644-3645.
77. Merion RM, Oh HK, Port FK, Toledo-Pereyra LH, Turcotte JG. A prospective controlled trial of cold-storage versus machine-perfusion preservation in cadaveric renal transplantation. *Transplantation.* 1990; 50: 230-233.
78. Jaffers GJ, Banowsky LH. The absence of a deleterious effect of mechanical kidney preservation in the era of cyclosporine. *Transplantation.* 1989; 47: 734-736.
79. Heil JE, Canafax DM, Sutherland DE, Simmons RL, Dunning M, Najarian JS. A controlled comparison of kidney preservation by two methods: machine perfusion and cold storage. *Transplant Proc.* 1987; 19: 2046.
80. Alijani MR, Cutler JA, DelValle CJ, et al. Single-donor cold storage versus machine perfusion in cadaver kidney preservation. *Transplantation.* 1985; 40: 659-661.
81. Forde JC, Shields WP, Azhar M, et al. Single centre experience of hypothermic machine perfusion of kidneys from extended criteria deceased heart-beating donors: a comparative study. *Ir J Med Sci.* 2016; 185: 121-125.
82. Burgos Revilla FJ, Hevia V, Diez V, et al. Machine perfusion: initial results in an expanded criteria donor kidney transplant program. *Transplant Proc.* 2015; 47: 19-22.
83. Dion MS, McGregor TB, McAlister VC, Luke PP, Sener A. Hypothermic machine perfusion improves Doppler ultrasonography resistive indices and long-term allograft function after renal transplantation: a single-centre analysis. *BJU Int.* 2015; 116: 932-937.
84. Guy A, McGrogan D, Inston N, Ready A. Hypothermic machine perfusion permits extended cold ischemia times with improved early graft function. *Exp Clin Transplant.* 2015; 13 :130-137.
85. Wszola M, Kwiatkowski A, Domagala P, et al. Preservation of kidneys by machine perfusion influences gene expression and may limit ischemia/reperfusion injury. *Prog Transplant.* 2014; 24: 19-26.
86. Chueh SC, Sankari BR, Lipscomb L, Modak A, Castello MG, Avallone EJ. The benefits of pulsatile machine perfusion of standard criteria deceased donor kidneys at a geographically remote transplant center. *ASAIO J.* 2014; 60: 76-80.
87. Wszola M, Kwiatkowski A, Diuwe P, et al. One-year results of a prospective, randomized trial comparing two machine perfusion devices used for kidney preservation. *Transpl Int.* 2013; 26: 1088-1096.
88. Sedigh A, Tufveson G, Backman L, Biglarnia AR, Lorant T. Initial experience with hypothermic machine perfusion of kidneys from deceased donors in the Uppsala region in Sweden. *Transplant Proc.* 2013; 45: 1168-1171.

89. Cannon RM, Brock GN, Garrison RN, Smith JW, Marvin MR, Franklin GA. To pump or not to pump: a comparison of machine perfusion vs cold storage for deceased donor kidney transplantation. *J Am Coll Surg.* 2013; 216: 625-634.
90. Cannon RM, Brock GN, Garrison RN, Marvin MR, Franklin GA, Davis EG. Machine perfusion: not just for marginal kidney donors. *Am Surg.* 2015; 81: 550-556.
91. Hoogland ER, de Vries EE, Christiaans MH, Winkens B, Snoeijs MG, van Heurn LW. The value of machine perfusion biomarker concentration in DCD kidney transplantations. *Transplantation.* 2013; 95: 603-610.
92. Ciancio G, Gaynor JJ, Sageshima J, et al. Machine perfusion following static cold storage preservation in kidney transplantation: donor-matched pair analysis of the prognostic impact of longer pump time. *Transpl Int.* 2012; 25: 34-40.
93. Cantafio AW, Dick AA, Halldorson JB, Bakthavatsalam R, Reyes JD, Perkins JD. Risk stratification of kidneys from donation after cardiac death donors and the utility of machine perfusion. *Clin Transplant.* 2011; 25: E530-540.
94. Lodhi SA, Lamb KE, Uddin I, Meier-Kriesche HU. Pulsatile pump decreases risk of delayed graft function in kidneys donated after cardiac death. *Am J Transplant.* 2012; 12: 2774-2780.
95. Patel SK, Pankewycz OG, Nader ND, Zachariah M, Kohli R, Laftavi MR. Prognostic utility of hypothermic machine perfusion in deceased donor renal transplantation. *Transplant Proc.* 2012; 44: 2207-2212.
96. Ciancio G, Gaynor JJ, Sageshima J, et al. Favorable outcomes with machine perfusion and longer pump times in kidney transplantation: a single-center, observational study. *Transplantation.* 2010; 90: 882-890.
97. Kwiatkowski A, Wszola M, Kosieradzki M, et al. The early and long term function and survival of kidney allografts stored before transplantation by hypothermic pulsatile perfusion. A prospective randomized study. *Ann Transplant.* 2009;14: 14-17.
98. Moustafellos P, Hadjianastassiou V, Roy D, et al. The influence of pulsatile preservation in kidney transplantation from non-heart-beating donors. *Transplant Proc.* 2007; 39: 1323-1325.
99. Balupuri S, Mantle D, Mohamed M, et al. Machine perfusion and viability assessment of non-heart-beating donor kidneys-a single-centre result. *Transplant Proc.* 2001; 33: 1119-1120.
100. Kumar MS, Samhan M, al Sabawi N, et al. Preservation of cadaveric kidneys longer than 48 hours: comparison between Euro-Collins solution, UW solution, and machine perfusion. *Transplant Proc.* 1991; 23: 2392-2393.
101. Barry JM, Metcalfe JB, Farnsworth MA, Bennett WM, Hodges CV. Comparison of intracellular flushing and cold storage to machine perfusion for human kidney preservation. *J Urol.* 1980; 123: 14-16.
102. Plata-Munoz JJ, Muthusamy A, Quiroga I, et al. Impact of pulsatile perfusion on postoperative outcome of kidneys from controlled donors after cardiac death. *Transpl Int.* 2008; 21: 899-907.
103. Matsuoka L, Shah T, Aswad S, et al. Pulsatile perfusion reduces the incidence of delayed graft function in expanded criteria donor kidney transplantation. *Am J Transplant.* 2006; 6: 1473-1478.
104. Buchanan PM, Lentine KL, Burroughs TE, Schnitzler MA, Salvalaggio PR. Association of lower costs of pulsatile machine perfusion in renal transplantation from expanded criteria donors. *Am J Transplant.* 2008; 8: 2391-2401.
105. Kootstra G, Kievit J, Heineman E. The non heart-beating donor. *Br Med Bull.* 1997; 53: 844-853.

106. Sy G, Jr., Toledo-Pereyra LH, Dienst SG, Oh HK. Are there any important predicting factors of renal function during hypothermic pulsatile perfusion for transplantation? *Am Surg.* 1980; 46: 340-343.
107. Kwiatkowski A, Danielewicz R, Kosieradzki M, et al. Six-year experience in continuous hypothermic pulsatile perfusion kidney preservation. *Transplant Proc.* 2001; 33: 913-915.
108. Veller MG, Botha JR, Britz RS, et al. Renal allograft preservation: a comparison of University of Wisconsin solution and of hypothermic continuous pulsatile perfusion. *Clin Transplant.* 1994; 8: 97-100.
109. Elec FI, Lucan C, Ghervan L, et al. Ex-vivo perfusion machines in kidney transplantation. The significance of the resistivity index. *Clujul Medical.* 2014; 87: 27-29.
110. Schold JD, Kaplan B, Howard RJ, Reed AI, Foley DP, Meier-Kriesche HU. Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. *Am J Transplant.* 2005; 5: 1681-1688.
111. Barber WH, Deierhoi MH, Phillips MG, Diethelm AG. Preservation by pulsatile perfusion improves early renal allograft function. *Transplant Proc.* 1988; 20: 865-868.
112. Sellers MT, Gallichio MH, Hudson SL, et al. Improved outcomes in cadaveric renal allografts with pulsatile preservation. *Clin Transplant.* 2000; 14: 543-549.
113. Hosgood SA, Mohamed IH, Bagul A, Nicholson ML. Hypothermic machine perfusion after static cold storage does not improve the preservation condition in an experimental porcine kidney model. *Br J Surg.* 2011; 98: 943-950.
114. Gallinat A, Efferz P, Paul A, Minor T. One or 4 h of "in-house" reconditioning by machine perfusion after cold storage improve reperfusion parameters in porcine kidneys. *Transpl Int.* 2014; 27: 1214-1219.
115. Codas R, Thuillier R, Hauet T, Badet L. Renoprotective effect of pulsatile perfusion machine RM3: pathophysiological and kidney injury biomarker characterization in a preclinical model of autotransplanted pig. *BJU Int.* 2012; 109: 141-147.
116. Gallinat A, Paul A, Efferz P, et al. Hypothermic reconditioning of porcine kidney grafts by short-term preimplantation machine perfusion. *Transplantation.* 2012; 93: 787-793.
117. Schreinemachers MC, Doorschodt BM, Florquin S, et al. Pulsatile perfusion preservation of warm ischaemia-damaged experimental kidney grafts. *Br J Surg.* 2010; 97: 349-358.
118. Hosgood SA, Yang B, Bagul A, Mohamed IH, Nicholson ML. A comparison of hypothermic machine perfusion versus static cold storage in an experimental model of renal ischemia reperfusion injury. *Transplantation.* 2010; 89: 830-837.
119. La Manna G, Conte D, Cappuccilli ML, et al. An in vivo autotransplant model of renal preservation: cold storage versus machine perfusion in the prevention of ischemia/reperfusion injury. *Artif Organs.* 2009; 33: 565-570.
120. Manekeller S, Leuvenink H, Sitzia M, Minor T. Oxygenated machine perfusion preservation of predamaged kidneys with HTK and Belzer machine perfusion solution: an experimental study in pigs. *Transplant Proc.* 2005; 37: 3274-3275.
121. Lindell SL, Compagnon P, Mangino MJ, Southard JH. UW solution for hypothermic machine perfusion of warm ischemic kidneys. *Transplantation.* 2005; 79: 1358-1361.
122. Nicholson ML, Hosgood SA, Metcalfe MS, Waller JR, Brook NR. A comparison of renal preservation by cold storage and machine perfusion using a porcine autotransplant model. *Transplantation.* 2004; 78: 333-337.
123. Hansen TN, D'Alessandro A, Southard JH. Reduced renal vascular injury following warm ischemia and preservation by hypothermic machine perfusion. *Transplant Proc.* 1997; 29: 3577-3579.

124. Booster MH, Wijnen RM, Yin M, et al. Enhanced resistance to the effects of normothermic ischemia in kidneys using pulsatile machine perfusion. *Transplant Proc.* 1993; 25: 3006-3011.
125. McAnulty JF, Ploeg RJ, Southard JH, Belzer FO. Successful five-day perfusion preservation of the canine kidney. *Transplantation.* 1989; 47: 37-41.
126. Brasile L, Stubenitsky BM, Booster MH, et al. The potential of repairing organs ex vivo. *Transplant Proc.* 2002; 34: 2625.
127. Brasile L, Stubenitsky BM, Booster MH, et al. Overcoming severe renal ischemia: the role of ex vivo warm perfusion. *Transplantation.* 2002; 73: 897-901.
128. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg.* 2008; 95: 111-118.
129. Stubenitsky BM, Booster MH, Brasile L, Araneda D, Haisch CE, Kootstra G. Exsanguinous metabolic support perfusion--a new strategy to improve graft function after kidney transplantation. *Transplantation.* 2000; 70: 1254-1258.
130. van der Wijk J, Slooff MJ, Rijkmans BG, Kootstra G. Successful 96- and 144-hour experimental kidney preservation: a combination of standard machine preservation and newly developed normothermic ex vivo perfusion. *Cryobiology.* 1980; 17: 473-477.
131. Rijkmans BG, Buurman WA, Kootstra G. Six-day canine kidney preservation. Hypothermic perfusion combined with isolated blood perfusion. *Transplantation.* 1984; 37: 130-134.
132. Hosgood SA, Patel M, Nicholson ML. The conditioning effect of ex vivo normothermic perfusion in an experimental kidney model. *J Surg Res.* 2013; 182: 153-160.
133. Hosgood SA, Barlow AD, Yates PJ, Snoeijs MG, van Heurn EL, Nicholson ML. A pilot study assessing the feasibility of a short period of normothermic preservation in an experimental model of non heart beating donor kidneys. *J Surg Res.* 2011; 171: 283-290.
134. Patel M, Hosgood S, Nicholson ML. The effects of arterial pressure during normothermic kidney perfusion. *J Surg Res.* 2014; 191: 463-468.
135. Metcalfe MS, Waller JR, Hosgood SA, Shaw M, Hassanein W, Nicholson ML. A paired study comparing the efficacy of renal preservation by normothermic autologous blood perfusion and hypothermic pulsatile perfusion. *Transplant Proc.* 2002; 34: 1473-1474.
136. Lindell SL, Muir H, Brassil J, Mangino MJ. Hypothermic Machine Perfusion Preservation of the DCD Kidney: Machine Effects. *J Transplant.* 2013; 7.
137. Bridge to Life. Belzer UW Cold Storage Solution Instructions. 2016; http://www.bridgetolife.com/belzer-uw-cold-storage-solution-instructions/. Accessed March 2, 2016.
138. Badet L, Petruzzo P, Lefrancois N, et al. Kidney preservation with IGL-1 solution: a preliminary report. *Transplant Proc.* 2005; 37: 308-311.

Chapter 4

Extra-corporeal normothermic machine perfusion of the porcine kidney – working towards future utilisation in Australasia

Ahmer Hameed
Ray Miraziz
David Lu
Neil Warwick
Ali El-Ayoubi
Heather Burns
Yi Vee Chew
Ross Matthews
Greg O'Grady
Lawrence Yuen
Natasha Rogers
Henry Pleass
Wayne Hawthorne

As published in the ANZ Journal of Surgery 2018, 88(5): E429-34; DOI: 10.1111/ans.14321

4.1 Abstract

Introduction: The ongoing supply-demand gap with respect to donor kidneys for transplantation necessitates the increased use of higher kidney donor profile index (KDPI) and/or circulatory death donor (DCD) kidneys. Machine perfusion (MP) preservation has become increasingly popular as a means to preserve such organs. Human data regarding normothermic kidney MP (NMP) is in its infancy, and such a system has not been established in the Australasian clinical setting.

Methods: Modified cardio-pulmonary bypass technology was utilized to develop a viable NMP kidney perfusion system using a porcine DCD model. System development and optimization occurred in two stages, with system components added in each experiment to identify optimal perfusion conditions.

Results: Device functionality was demonstrated by the successful perfusion of and urine production by, eight porcine kidneys. Urine production diminished in the presence of colloid in the perfusate. Pressure-controlled (compared to flow-controlled) perfusion is preferable as a safe perfusion pressure range can be maintained. More physiologic perfusion conditions are achieved if oxygenation is provided by an oxygen/carbon dioxide mixture compared to 100% oxygen.

Discussion: A viable and reproducible NMP system was established and tested in porcine kidneys, which was able to simulate graft function extra-corporeally. Further work is required to identify the most optimal perfusion conditions. Prior to its utilization in clinical transplantation, the system should be tested in non-transplanted human kidneys.

4.2 Introduction

Organ transplantation is the optimal option for the management of end-stage renal disease. The ever-increasing gap between kidney supply and demand necessitates expansion of the organ donor pool through increased utilization of higher kidney donor profile index (KDPI) kidneys, including donation after circulatory death (DCD) and expanded criteria donor (ECD) kidneys. Such kidneys have a significantly higher risk of discard, delayed graft function, and overall graft loss.[1-4] This has provided the stimulus for the adoption of novel organ perfusion and preservation strategies, such as machine perfusion (MP), as a means to rejuvenate kidneys, minimize kidney discard, and improve graft function.[5, 6]

Normothermic MP (NMP) has potential distinct advantages over the more commonly employed hypothermic MP (HMP). NMP effectively 'restarts' the graft *ex vivo* and may allow more accurate prediction of graft viability by assessing adequacy of perfusion, renal blood flow parameters, and urine production.[7] Nicholson and Hosgood were the first to publish an observational study investigating the use of NMP in human ECD kidneys; the delayed graft function (DGF) rate was remarkably low (5.6%) in the NMP group in comparison to 36.2% for CS kidneys.[8] More importantly, kidneys initially underwent cold static storage (CS), and later only had NMP for 1 hour during the immediate pre-implantation period.

The use of renal NMP has not yet been reported in the Australasian transplantation setting. A recent Machine Perfusion Workshop run by the Transplantation Society of Australia and New Zealand (TSANZ) discussed the merits of nationwide implementation of HMP, trialling of NMP, or continuation of the current gold standard (CS). There was strong interest in NMP, and a watch-and-wait approach with regards to further international trials of NMP was adopted.[9] The primary purpose of this study was to undertake technical development and determine the feasibility for a customized NMP system using porcine kidneys, with a staged introduction of core NMP components. This project was performed in anticipation of the increased use of NMP for higher KDPI kidneys, and as a prelude to its testing in human donor kidneys.

4.3 Methods

4.3.1 ANIMALS

Westran pigs (Westmead Transplant, Westmead, NSW, Australia) were utilised for these experiments. All protocols were approved by the Western Sydney Local Health District Animal Ethics Committee, in accordance with the Guidelines for Animal Welfare outlined by the National Health and Medical Research Council.

4.3.2 DEVICE AND PERFUSION DETAILS

The normothermic machine perfusion (NMP) device (Supplemental Digital Content [SDC] 1) was adapted from similar device descriptions,[8, 10, 11] and utilised existing cardiopulmonary bypass (CPB) technology. SDC 2 outlines device components and baseline perfusion solution details.

4.3.3 SYSTEM OPTIMIZATION

As the primary purpose of this study was to undertake technical development and optimization of the NMP process using pre-defined stages (consisting of two kidney perfusions per stage), statistical powering was not required. Study stages and the gradual introduction or alteration of core system components are summarized in Fig. 1.

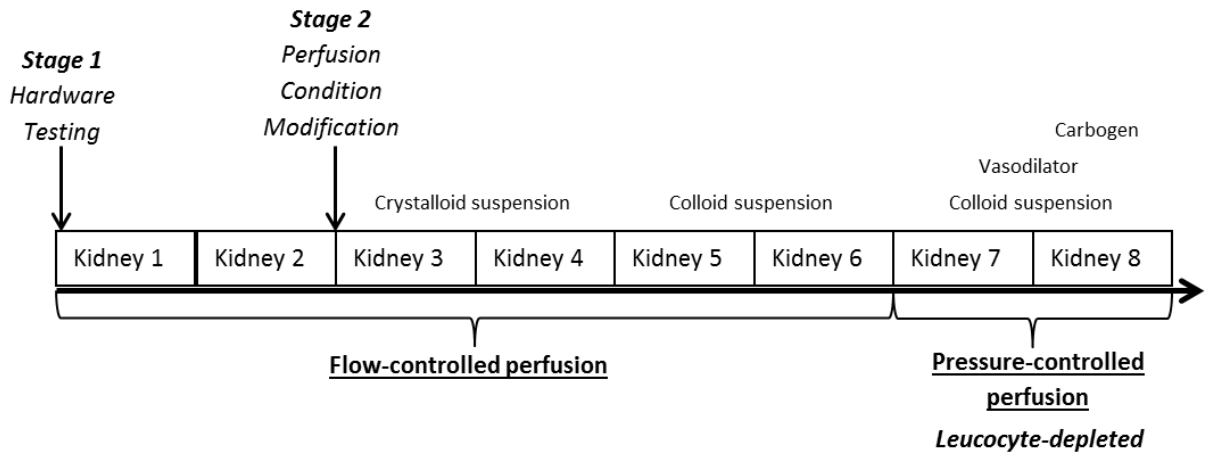


Figure 1. Experimental/system optimization protocol for kidney NMP.

Stage 1 – Hardware Testing

In the first subset of experiments, kidneys (n = 2) were retrieved from a 110 kg female Westran pig and sequentially placed onto the NMP device to test device functionality and feasibility. Kidneys were retrieved in a standard fashion after circulatory arrest was induced by exsanguination and abdominal perfusion was conducted using heparinized University of Wisconsin (UW) solution (see SDC 2 for full details). NMP was conducted over a period of 10

mins at 37°C; pump flow rate was arbitrarily set at 0.25 L/min, and oxygen (100%) was diffused through the membrane oxygenator at 2 L/min.

Stage 2 – NMP device testing in a DCD model of porcine kidney retrieval

In the second set of experiments, kidneys (n = 6) were retrieved from Westran pigs using a donation after circulatory death (DCD) model (based on the methods of Kaths *et al.*[11, 12]), with 30 mins warm ischaemia time (WIT) and close to 24 hrs of cold ischaemia time (CIT) (SDC 3). The kidneys were perfused on the NMP circuit for 60 mins each. Certain modifications to the NMP process were made between respective pigs to help identify ideal perfusion conditions (Fig. 1), and are outlined in detail in SDC 4.

4.3.4 SAMPLES

Wedge biopsies were taken immediately prior to and after cessation of NMP, and stained with hematoxylin and eosin (H&E). Appropriate blood samples were taken at the commencement and conclusion of NMP (i.e. 0 and 60 minutes), whilst urinary assessment was undertaken utilizing the 60 min urinary sample.

4.4 Results

4.4.1 STAGE 1

Both left and right kidneys displayed patchy perfusion after retrieval and back-table flushing (Fig. 2 A & B, respectively). WIT was < 5 minutes for each kidney, whilst the CIT was 2.5 hours and 4 hours for the left and right kidney, respectively. Both left and right kidneys displayed a homogenous perfusion appearance, and commenced urine production after 1-2 minutes (Fig. 2). Urine output (UO) over the 10 minute period was approximately 300 ml and 260 ml for the left and right kidney, respectively.

4.4.2 STAGE 2

Donor and retrieval details

Kidneys from five pigs were utilized; these included two males and three females, with a median weight of 70 kg (range 67.5-90 kg). WIT was controlled at 30 minutes. Median CIT was 22.5 hours (range 20-25.8 hours). NMP was undertaken for one hour.

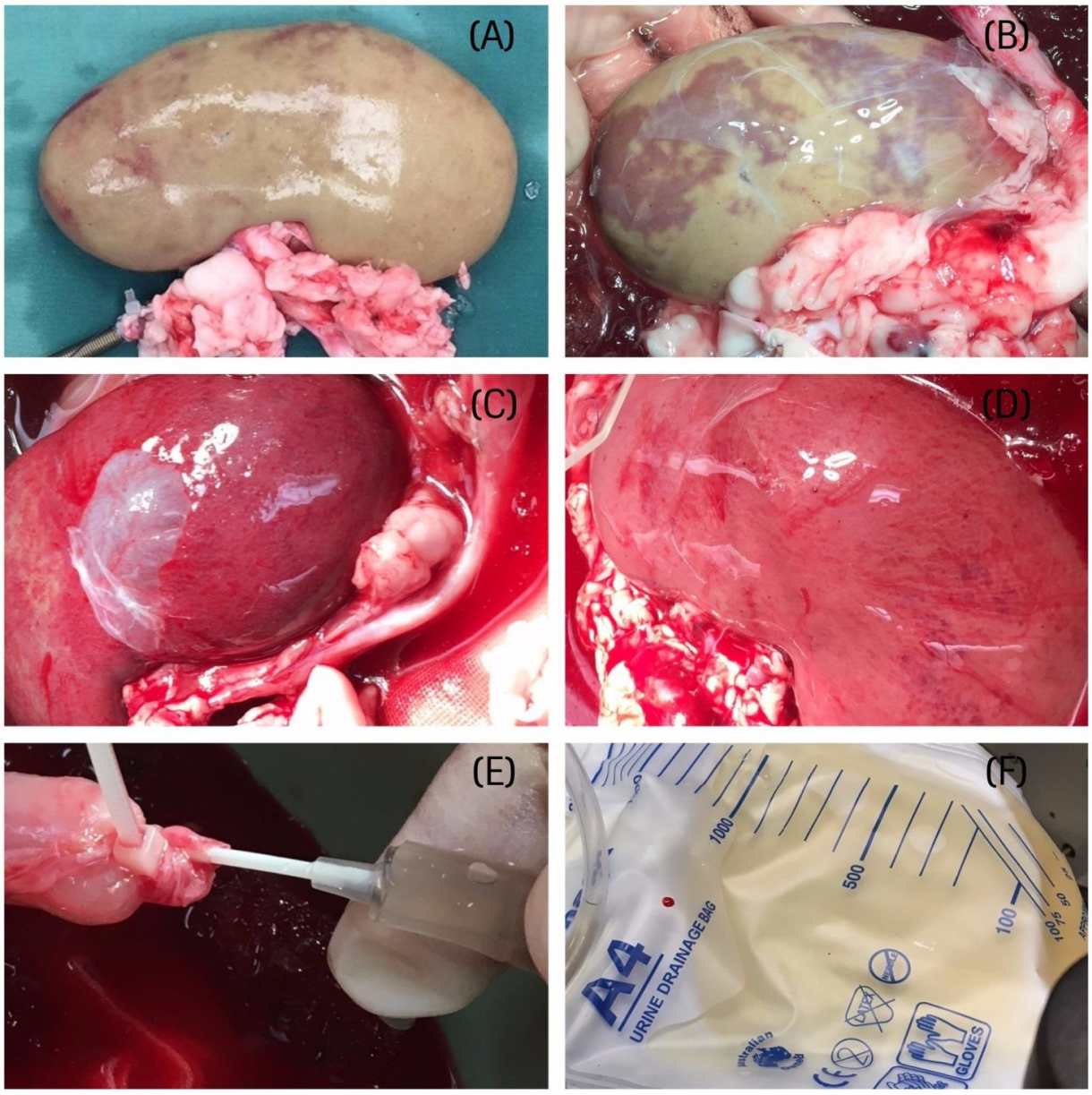


Figure 2. NMP of kidneys 1 and 2. (A, B) Kidney appearance after retrieval and backtable perfusion; (C, D) corresponding kidney appearance at end-NMP; n.b. homogenous/uniform perfusion with NMP (cyst on left kidney); and (E, F) urine production into ureteric cannula and collection bag.

Retrieval and NMP characteristics

These are summarized in SDC 5. Post-retrieval kidney macroscopic appearance after cold perfusion was generally poor, likely due to the prolonged WIT. In general, this could not be salvaged by NMP 24 hours later, regardless of perfusion conditions, and was reflected by dropping flow rates and raised intra-renal resistance (IRR) during the final 20 minutes of NMP (SDC 6, B &

C). This was especially the case in kidneys 7 and 8: flow parameters improved during the first half of NMP (SDC 6, B), but abruptly dropped after this point (see Histology results, below). Furthermore, lactate was measured at 0 and 60 minutes in kidneys 5, 6 and 8, without evidence of clearance over one hour (median 16 mmol/L at 0 minutes [range 8.2-19 mmol/L], compared to a median of 18 mmol/L at 60 minutes [range 11.7-21 mmol/L]).

Flow vs pressure-controlled perfusion

All flow-controlled kidneys had notable, gross oedema and tense capsules, which was not seen in the pressure-controlled kidneys. Representative pressure, flow and IRR measurements are depicted in SDC 6. In the flow-controlled kidney it can be seen that even at a flow rate of 0.25 L/min, a higher pressure of 220 mmHg was achieved; this was not the case in pressure-controlled kidneys.

Histologic changes

Due to the severity of the ischaemic insult, all histologic sections at end-CS showed evolution from changes of ischaemic tubular damage to gross glomerular and tubular disruption, loss of architecture, dilatation of peritubular capillaries, and microthrombi within the glomeruli at end-NMP (Fig. 3). This potentially explains the abrupt drop in flows for kidney 8, with tubular debris and glomerular disruption during NMP.

Urinary parameters

All kidneys produced urine, with appearance varying from clear to blood-stained. Total UO did not directly correlate with the degree of macroscopic perfusion during NMP (SDC 5). One-hour UO was lower once colloid was added to the perfusion solution (median 250 ml, range 100-500 ml) compared to the use of crystalloid alone (median 2180 ml, range 810-3550 ml).

Fractional excretion of sodium, as a measure of tubular function, could not be measured as creatinine was not added to the isolated system. Nevertheless, the kidneys in which urinary electrolytes were measured (kidneys 5-8) displayed tubular function, with a median urinary sodium of 122.5 mmol/L (range 120-129 mmol/L) in comparison to 137.5 mmol/L in the baseline perfusion solution (range 137-138 mmol/L).

Leucocyte depletion

The median white cell count (WCC) in pigs during whole blood retrieval was 4.9 x 10^9/L (range 4.6-7.6 x 10^9/L). The median WCC count after leucocyte depletion by centrifugation and washing of PRBCs was 0.7 x 10^9/L (range 0.6-0.7 x 10^9/L) in comparison to 0.3 x 10^9/L (range 0.2-0.4 x 10^9/L) when a filter was utilized as an additional step.

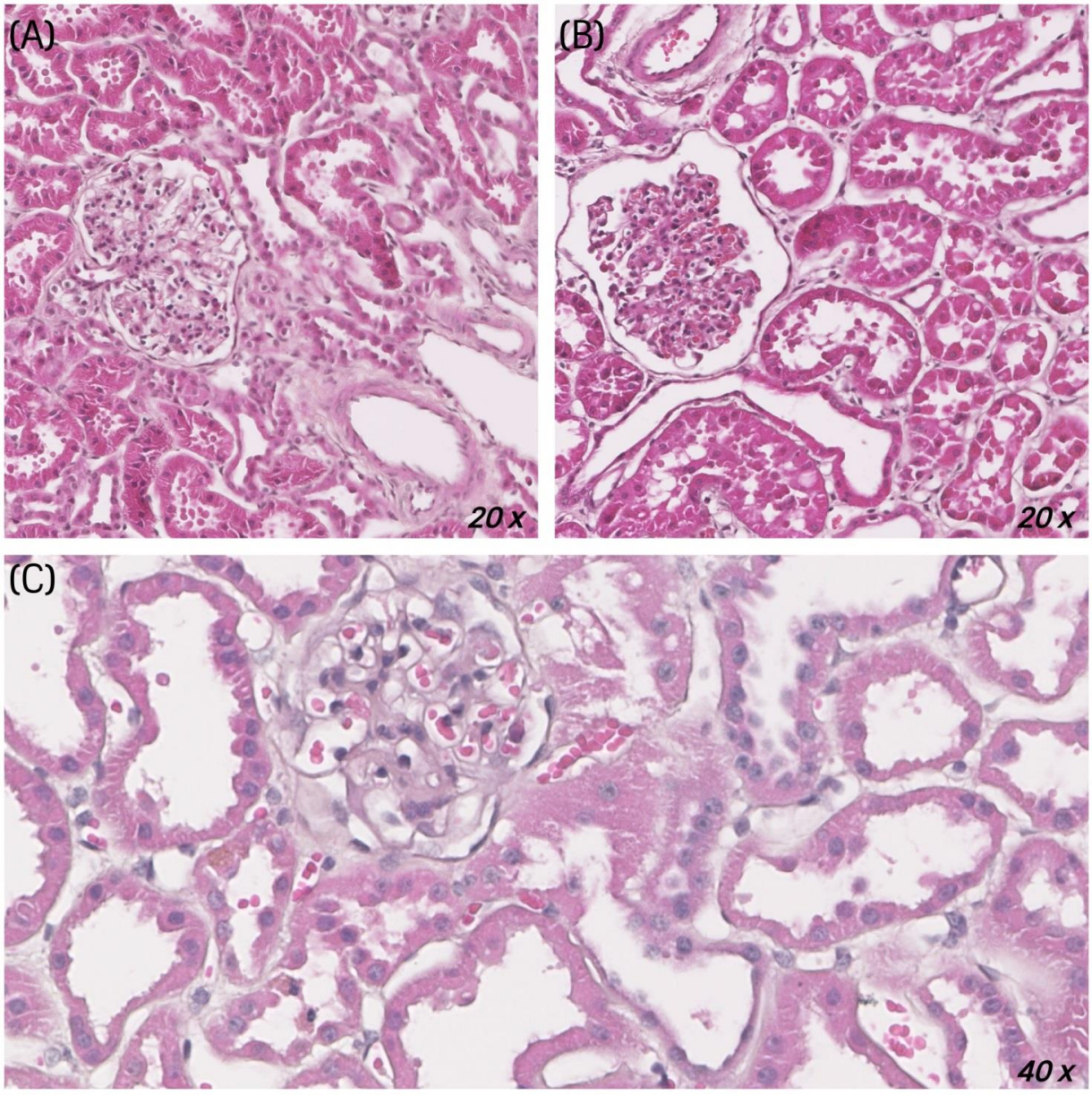


Figure 3. Histologic changes after NMP, H&E sections. (A) End-CS (pre-NMP) [kidney 8]; (B) End-NMP, with glomerular microthrombi, tubular flattening, interstitial oedema and dilatation of peritubular capillaries, and retraction of the glomerulus within Bowman's capsule [kidney 8]; and (C) End-NMP of a flow-controlled kidney [kidney 3], with interstitial oedema; red blood cells are also seen in the tubular lumen, peritubular capillaries and glomerular capillary loops, indicative of loss of basement membrane integrity.

Acid-base homeostasis and partial pressure of O_2/CO_2

Kidney 8, in which carbogen was used instead of 100% O_2, was the only kidney in the series that was able to improve perfusate pH from 0-60 minutes of NMP (pH at 0 minutes 6.97, and at 60 minutes 7.04).

Partial pressure of O_2 and CO_2 in kidney 7, where 100% O_2 was utilized, was 477 mmHg and < 5 mmHg at 0 minutes, respectively, in comparison to the more physiologic 111 mmHg and 38 mmHg, respectively, for kidney 8.

Renin-angiotensin-aldosterone (RAA) system

Neural autoregulatory capacity is lost in the isolated kidney. The RAA system also plays an essential role in the regulation of renal tubular function and blood flow. Aldosterone was detectable in the perfusate of kidneys 5-8 at both 0 and 60 mins (median 182 pmol/L at 0 minutes and 136 pmol/L at 60 minutes; reference interval 32-654 pmol/L). Renin was also measured but not detected at any stage (< 2 mIU/L for all kidneys; reference interval 2.8-39.9 mIU/L).

Pump-related haemolysis

Plasma free haemoglobin (Hb) levels were measured as an indicator of haemolysis during NMP in kidney 8; the measured level at the start of NMP was 1.21 g/L (reference < 0.05 g/L), which dropped to 0.79 g/L at the conclusion of NMP. The drop in free Hb levels indicates there was no significant haemolysis attributable to the use of a roller pump; the high values at the commencement of NMP likely indicate RBC lysis during storage and processing.

4.5 Discussion

Normothermic machine perfusion of the renal allograft has the potential to significantly alter graft viability, assessment, and outcome, especially for higher KDPI kidneys that may either be discarded prior to transplantation or suffer from inferior graft function once transplanted. Kidney preservation techniques in Australia, particularly with respect to the uptake of MP preservation, have significantly lagged behind Europe and the USA. This has led us to develop and test a preliminary NMP device using porcine kidneys as described. Further development of this model will allow us to expand our investigation of optimal perfusion settings, timing, and

solutions/additives, with the ultimate aim of device testing initially in non-transplanted human kidneys prior to translating NMP into a clinical program.

A severe DCD model with a prolonged CIT was utilized in this experimental set-up to help ascertain the extent of injury that can potentially be reversed using NMP. Whilst our current NMP apparatus and perfusion constituents were able to perfuse porcine donor kidneys and successfully simulate graft appearance upon potential transplantation, the long WIT and CIT resulted in significant damage to the kidneys that could not effectively be reversed by the NMP set-up. This may well have been the result of microthrombi, as intravenous heparin was deliberately not used to better mimic the clinical legal requirement of no ante-mortem interventions within New South Wales and most other local jurisdictions; ante-mortem heparin may in fact protect against thrombotic complications in the transplant setting.[13] Thereafter, ischaemic damage sustained during storage primed these porcine kidneys for tubulo-glomerular disruption upon perfusion on the circuit.

Overall, both advantageous and deleterious perfusion conditions and parameters were identified during the optimization process. The danger of flow-based perfusion settings was demonstrated, with high arterial and intra-graft pressures, and consequent graft oedema. This was also explored in greater detail by Mancina *et al*.[14] The ideal pressure setting is not clearly defined, and will differ for pigs and humans. In a porcine NMP model that served as a prelude to the clinical NMP model used by Nicholson and colleagues, a mean arterial perfusion pressure of 75 mmHg in comparison to 55 mmHg resulted in significantly less endothelial injury in the higher pressure group, whilst allowing better perfusion parameters and urine production.[15] Earlier work by the same group showed that pressures up to 95 mmHg may in fact sustain superior renal function during NMP.[16]

The fluid in which PRBCs are suspended and supplemented with can vary significantly. Differences can be elucidated between the Toronto and Cambridge experiences – in particular, the Canadian group utilizes STEEN solution, which contains human serum albumin, to stabilize perfusate oncotic pressures and minimize graft oedema, whilst this is lacking in the UK perfusion cocktail.[11, 17] Lack of albumin in the perfusion fluid may also promote endothelial cell apoptosis, although this requires further investigation in the context of NMP.[18] Presence of colloid in the

perfusate also affects total UO; after its addition into our perfusion fluid, cumulative UO from the system dropped.

One hour of NMP was chosen for this pilot study, although there is evidence that continuous/prolonged NMP may be more advantageous than the one-hour perfusion period.[10, 19] The primary reason for this is the clinical applicability and potential for translation if 1-3 hours of NMP is utilized – this can be performed at the recipient centre during the period in which the patient is being prepared for surgery. In contrast, the costs and logistical issues associated with prolonged perfusion periods may initially be hard to justify in any local clinical program.

There is considerable potential for Australasian transplant programs to progress kidney transplantation outcomes and research through the implementation of NMP as part of carefully conducted clinical trials. A randomized control trial comparing one hour of pre-implantation NMP to CS alone for DCD kidney transplants is currently underway in the UK.[20] Logistically, a similar trial in Australia would be possible to implement. Alternatively, another highly fruitful trial would involve the head-to-head comparison of NMP and HMP (oxygenated) prior to transplantation, as there is currently no clinical data for this. Such trials could likely be instituted after the safety, feasibility, and/or efficacy of NMP is demonstrated in pre-clinical models and human kidneys. NMP also provides an excellent opportunity to deliver pharmacological, cellular or genetic therapies prior to the second insult of reperfusion during transplantation. The legal framework in Australia currently significantly restricts drug therapies delivered to the donor to preserve graft function for the recipient. However, NMP can potentially bypass this consideration by allowing direct treatment of the donor graft itself.

The current spike in interest and research into normothermic perfusion of the kidney heralds an exciting time in transplantation research, with the potential for significant improvements in clinical transplantation outcomes. As this technique continues to be investigated at various centres overseas, this is the perfect opportunity for establishing and testing NMP systems in the Australasian setting such that our patient outcomes and advances keep pace with the rest of the world, and also allowing a contribution to the collective knowledge regarding the efficacy, ideal conditions and settings, and mechanisms of action of NMP.

4.6 References

1. Kayler LK, Magliocca J, Zendejas I, Srinivas TR, Schold JD. Impact of cold ischemia time on graft survival among ECD transplant recipients: a paired kidney analysis. *Am J Transplant.* 2011; 11: 2647-56.
2. Saidi RF, Elias N, Kawai T, Hertl M, Farrell ML, Goes Net al. Outcome of kidney transplantation using expanded criteria donors and donation after cardiac death kidneys: realities and costs. *Am J Transplant.* 2007; 7: 2769-74.
3. Lim WH, McDonald SP, Russ GR, Chapman JR, Ma MK, Pleass Het al. Association between delayed graft function and graft loss in donation after cardiac death kidney transplants – a paired kidney registry analysis. *Transplantation.* 2017; 101: 1139-43.
4. Singh RP, Farney AC, Rogers J, Zuckerman J, Reeves-Daniel A, Hartmann Eet al. Kidney transplantation from donation after cardiac death donors: lack of impact of delayed graft function on post-transplant outcomes. *Clin Transplant.* 2011; 25: 255-64.
5. Hameed AM, Hawthorne WJ, Pleass HC. Advances in organ preservation for transplantation. *ANZ J Surg.* 2016; Epub ahead of print, DOI 10.1111/ans.13713
6. Hameed AM, Pleass HC, Wong G, Hawthorne WJ. Maximizing kidneys for transplantation using machine perfusion: from the past to the future: A comprehensive systematic review and meta-analysis. *Medicine.* 2016; 95: e5083.
7. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. *Br J Surg.* 2015; 102: 1433-40.
8. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant.* 2013; 13: 1246-52.
9. TSANZ Annual Newsletter 2016 [PDF on internet]. Sydney: TSANZ. Available from: https://www.tsanz.com.au/documents/2016Newsletter.pdf. Accessed August 15, 2017.
10. Kaths JM, Cen JY, Chun YM, Echeverri J, Linares I, Ganesh Set al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. *Am J Transplant.* 2017; 17: 957-69.
11. Kaths JM, Spetzler VN, Goldaracena N, Echeverri J, Louis KS, Foltys DBet al. Normothermic Ex Vivo Kidney Perfusion for the Preservation of Kidney Grafts prior to Transplantation. *J Vis Exp.* 2015; 101: e52909.
12. Kaths JM, Echeverri J, Goldaracena N, Louis KS, Yip P, John Ret al. Heterotopic Renal Autotransplantation in a Porcine Model: A Step-by-Step Protocol. *J Vis Exp.* 2016; 108: 53765.
13. Shahrestani S, Webster AC, Lam VW, Yuen L, Ryan B, Pleass HCet al. Outcomes From Pancreatic Transplantation in Donation After Cardiac Death: A Systematic Review and Meta-Analysis. *Transplantation.* 2017; 101: 122-30.
14. Mancina E, Kalenski J, Paschenda P, Beckers C, Bleilevens C, Boor Pet al. Determination of the preferred conditions for the isolated perfusion of porcine kidneys. *Eur Surg Res.* 2015; 54: 44-54.
15. Patel M, Hosgood S, Nicholson ML. The effects of arterial pressure during normothermic kidney perfusion. *J Surg Res.* 2014; 191: 463-8.
16. Hosgood S, Harper S, Kay M, Bagul A, Waller H, Nicholson ML. Effects of arterial pressure in an experimental isolated haemoperfused porcine kidney preservation system. *Br J Surg.* 2006; 93: 879-84.

17. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct.* 2017; 3: e140.
18. Zoellner H, Hofler M, Beckmann R, Hufnagl P, Vanyek E, Bielek Eet al. Serum albumin is a specific inhibitor of apoptosis in human endothelial cells. *J Cell Sci.* 1996; 109: 2571-80.
19. Kaths JM, Echeverri J, Linares I, Cen JY, Ganesh S, Hamar Met al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant.* 2017. Epub ahead of print, DOI 10.1111/ajt.14294
20. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open.* 2017; 7(1).

Chapter 5

A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney

Ahmer Hameed
Suat Dervish
Natasha Rogers
Henry Pleass
Wayne Hawthorne

As published in the Transplant International 2018 OCT 13, EPUB AHEAD OF PRINT; DOI: 10.1111/tri.13361

5.1 Letter to the Editor

Dear Editors,

Normothermic machine perfusion (NMP) prior to transplantation has gained significant prominence in the recent past, and has been clinically utilized in the setting of liver, heart, lung, and kidney transplantation.[1] Nicholson and Hosgood were the first to report a series of kidney transplants following a brief period of pre-implantation NMP in 18 marginal donors; the success of this initial study and further investigations has led to a multi-center randomized control trial that is currently underway in the UK.[2-4]

One consideration that may impact the subsequent widespread uptake of clinical NMP systems is cost. In particular, the costs of consumables for each individual organ need to be sufficiently low to stimulate further uptake by transplant centers. Consumables must also be sterilizable and provide ease of use for the clinical team.

Our NMP set-up has been described previously.[5] We initially used a custom-designed metal chamber, however this was difficult to clean/re-sterilize, and did not adequately collect and funnel all residual blood into the reservoir. This prompted the design and development of the 3D-printed perfusion chamber (Fig. 1).

The 3D-printed chamber employs gravity drainage of renal venous outflow and any other blood leak (e.g. biopsy site) into a funnel-shaped cavity; only the renal artery is cannulated, allowing open drainage from the renal vein. The chamber is placed above the blood/perfusion fluid reservoir, and therefore blood can drain into the reservoir without necessitating an additional pump mechanism. The need for a separate reservoir may be completely obviated depending on the prime and packed red cell volume used in the circuit.

The kidney itself is placed on a fenestrated ‘mesh’ that can be incorporated into the print; this requires the additional printing of polyvinyl alcohol supports that need to be dissolved in water post-printing. A separate, reusable and sterilizable stainless steel mesh can alternatively be used (Fig. 1D-E), significantly reducing print times.

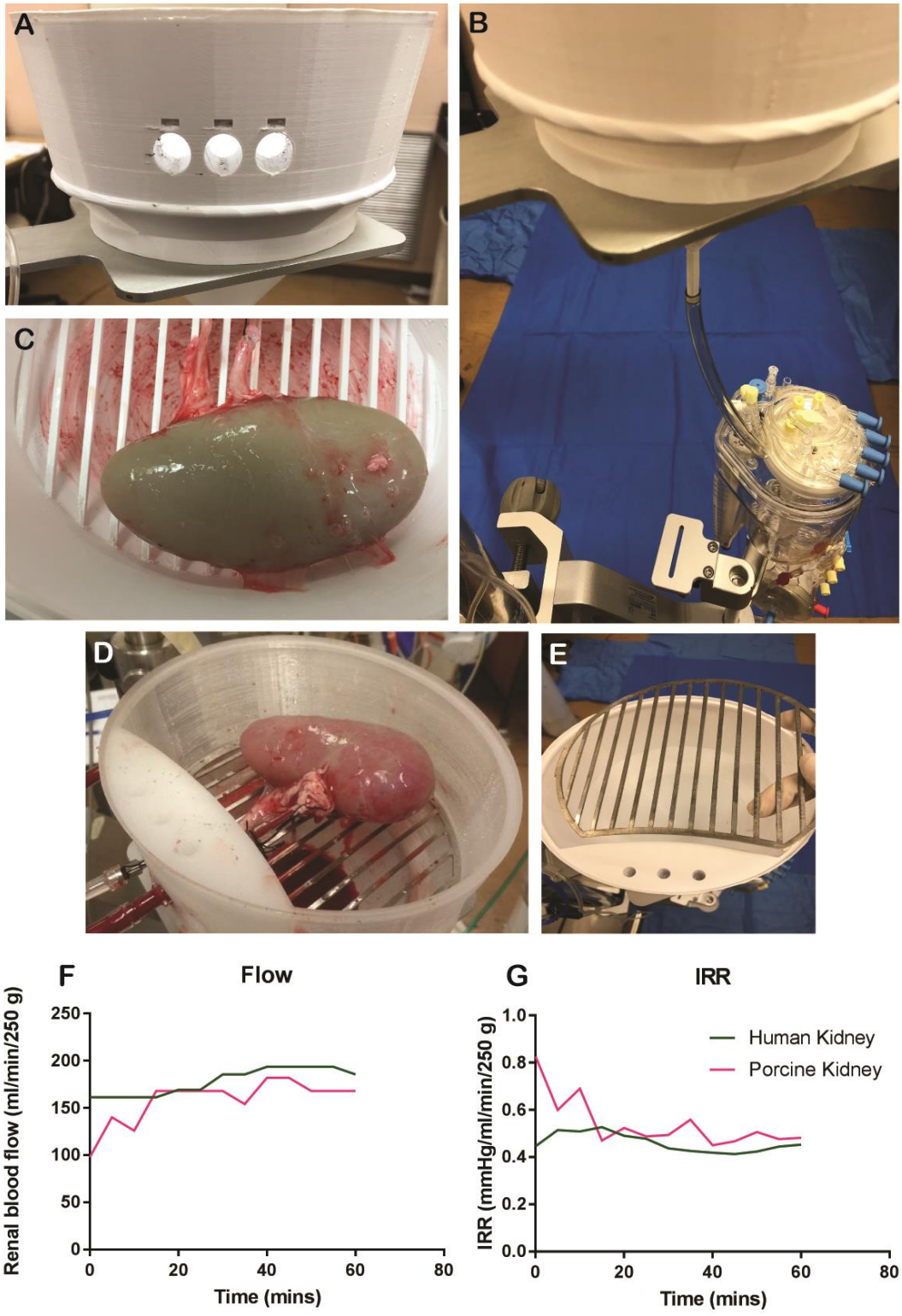


Figure 1. Customized, 3D-printed kidney perfusion chamber. (A) Front aspect, with holes for arterial and ureteric cannulae; (B) Side aspect, showing ¼ inch PVC tubing draining the chamber directly into the venous inflow port of the reservoir; (C) Perfusion chamber with 'mesh' (upon which kidney sits) incorporated into print; (D-E) Perfusion chamber with mesh in this case provided by a reusable, custom-cut stainless steel metal sheet. The perfusion

chamber in (D) was printed using polypropylene (autoclavable), whilst the perfusion chamber in the other images was printed using copolyester. (F-G) Renal blood flow and intra-renal resistance (IRR) in one porcine kidney and one discarded human kidney placed on the 3D-printed perfusion chamber during 1 hour of NMP. The human and porcine kidneys produced 43 ml and 180 ml of urine, respectively.

Use of such a chamber affords the following advantages:

(i) Low cost – the chamber is printed using copolyester (CPE+) or polypropylene (Ultimaker B.V., Geldermalsen, The Netherlands) on an Ultimaker 3 extended 3D printer (Ultimaker B.V., Geldermalsen, The Netherlands). Costs per print are estimated at approximately 15-20 USD.

(ii) Printable at the transplant center on-demand, and readily sterilizable. An ever increasing range of printable materials allows for specific print properties. Advanced printers can print polypropylene, which if used, can be safely autoclaved. If CPE is employed, sterilization can be achieved using ethylene oxide gas or gamma irradiation; in this situation, it is prudent that a relevant number of chambers are pre-printed and made available for use 1-2 weeks prior to any anticipated need. We have successfully printed and used both CPE and polypropylene for the purposes of NMP.

(iii) Its components and dimensions can be readily and easily modified by altering print settings.

(iv) The chamber obviates the need to (a) cannulate the renal vein (and therefore avoids the need to shorten the vein prior to transplantation), and (b) ensure a blood-tight circulation with little to no leak.

(v) The chamber is compatible with perfusion constituents. Albumin, which is an important constituent of the perfusate in some normothermic perfusion setups,[6] is not significantly adsorbed by CPE and therefore remains in the perfusate. An isolated perfusion test was performed using 20% human albumin diluted in 100 ml of 0.9% sodium chloride; this was circulated into and out of the 3D-printed perfusion chamber via ¼ inch PVC tubing using a pump generating a flow rate of 0.5 L/min. There was no albumin adsorption over 1 hour (albumin concentrations at 0, 30, and 60 minutes of perfusion were 99 g/L, 97 g/L, and 102 g/L, respectively).

We have successfully perfused 12 discarded human kidneys and 17 porcine kidneys using this set-up.[7, 8] Each kidney had declining intra-renal resistance (IRR) and increasing flow, in addition to evidence of urine output. Examples of flow and intra-renal resistance parameters in one porcine and human kidney respectively are presented in Figure 1F-G.

Overall, it is hoped that the innovative use of 3D-printing technology can further help facilitate the uptake of normothermic machine perfusion of different organs, including the kidney, by lowering costs and promoting ease of perfusion.

5.2 References

1. Hameed AM, Hawthorne WJ, Pleass HC. Advances in organ preservation for transplantation. *ANZ J Surg.* 2017; 87: 976-80.
2. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant.* 2013; 13: 1246-52.
3. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open.* 2017; 7.
4. Hosgood SA, Nicholson ML. Ex vivo normothermic perfusion of declined human kidneys after inadequate in situ perfusion. *Am J Transplant.* 2014; 14: 490-1.
5. Hameed AM, Miraziz R, Lu DB, et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ J Surg.* 2018; 88: E429-E34.
6. Selzner M, Goldaracena N, Echeverri J, et al. Normothermic ex vivo liver perfusion using steen solution as perfusate for human liver transplantation: First North American results. *Liver Transplant.* 2016; 22: 1501-8.
7. Hameed AM, Lu B, Miraziz R, et al. Intra-renal delivery of drugs targeting ischemia-reperfusion injury of the kidney in a rodent model and porcine model of normothermic machine perfusion. The Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Melbourne Convention Centre, 29th April-1st May, 2018. *Transplant Direct.* 2018; 4: S1.
8. Hameed AM, Rogers N, De Roo R, et al. Normothermic machine perfusion of non-utilized human kidneys – our first two cases. The Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Melbourne Convention Centre, 29th April-1st May, 2018. *Transplant Direct.* 2018; 4: S21.

Chapter 6

CD47-blockade to ameliorate kidney ischemia-reperfusion injury using normothermic machine perfusion – a small and large animal study

Ahmer Hameed
David Lu
Heather Burns
Nicole Byrne
Yi Vee Chew
Sohel Julovi
Kedar Ghimire
Negar Talaei Zanjani
Chow P'ng
Daniel Meijles
Suat Dervish
Ross Matthews
Ray Miraziz
Greg O'Grady
Lawrence Yuen
Henry Pleass
Natasha Rogers
Wayne Hawthorne

As submitted to the Annals of Surgery

6.1 Abstract

Objectives and Summary Background Data: (i) To compare the relative efficacy of CD47-blocking antibody (αCD47Ab), soluble complement receptor 1 (sCR1), and recombinant thrombomodulin (rTM) in a murine model of kidney ischemia-reperfusion injury (IRI), and (ii) investigate direct intra-renal delivery of the most efficacious agent to porcine donation after circulatory death (DCD) kidneys using normothermic machine perfusion (NMP). NMP is an emerging modality for kidney preservation prior to transplantation, and may allow pharmacomodulation of renal IRI without the need for systemic donor/recipient therapies. The aforementioned agents, although proven to be effective in the amelioration of IRI, have not been directly compared, and are not yet in widespread clinical use. NMP may allow the rapid clinical translation of these drugs to allow rejuvenation of damaged donor kidneys prior to transplantation.

Methods: Severe murine kidney IRI was induced; ischemic induction was preceded by intra-venous injection of αCD47Ab, sCR1, rTM, αCD47Ab+sCR1, or vehicle control (n = 7-8/group). Renal function and histopathologic features were compared after 24 hours. Porcine kidneys had 10 min warm ischemia and 6 hrs cold storage, followed by NMP with or without the addition of the porcine-specific version of the most effective murine agent (αCD47Ab) (n = 8-9/group). Feasibility and IRI-related effects of drug delivery were ascertained.

Results: Serum creatinine after 24 hours was significantly reduced in mice treated with CD47, sCR1, or αCD47Ab+sCR1, but not rTM. Histologically-confirmed injury was least severe in the CD47-blockade mice, as was inflammatory leukocyte infiltration, and renal cellular death. αCD47Ab was therefore given via NMP to adult pig kidneys. CD47 receptor blockade was successfully demonstrated by immunofluorescence. Renal perfusion/flow was better when CD47 was blocked, and there was a trend towards improved tubular and glomerular functional parameters. Oxidative stress was significantly reduced in the αCD47Ab-treated kidneys, also with evidence of reduced histologic damage, but not cell-death.

Conclusions: αCD47Ab provides a broad target for the amelioration of IRI. NMP can be successfully utilized for targeted drug delivery to the kidney as a means to ameliorate IRI in the setting of transplantation.

6.2 Introduction

End-stage renal failure (ESRF) has a sizeable global burden of disease, causing at least 1.2 million global annual deaths.[1] Kidney transplantation is the best available treatment for ESRF, conferring a significant survival benefit over dialysis.[2-4] However, there is a perpetual supply-demand gap between patients awaiting transplantation and the availability of deceased donor kidneys. This has necessitated expansion of the donor pool to include more marginal organs, including donation after circulatory death (DCD) kidneys, which are subjected to greater warm ischemia.[5-7] Short-term transplantation outcomes, including delayed graft function (DGF), are inferior in DCD kidneys in comparison to kidneys from brain-dead (DBD) donors with no significant comorbidities.[6] This increased susceptibility to ischemia-reperfusion injury (IRI) and DGF can translate into poorer long-term graft survival.[8]

As such, an improved method of kidney assessment, repair and preservation is required above and beyond the currently accepted gold standard of cold static storage (CS), particularly in this donor kidney subset. Machine perfusion (MP) preservation is an important alternative that has regained prominence.[9] Normothermic MP (NMP) is especially promising, and is now the subject of a multi-center randomized control trial (RCT) comparing it to CS alone in DCD kidneys.[10-13]

Most pharmacotherapeutics shown to ameliorate renal IRI have been unable to bridge the 'valley of death' (translational gap) to the clinic. This is at least partly attributable to the inherent difficulties and ethical considerations associated with the systemic use of such therapies in donors or recipients.[14, 15] NMP can serve as a bridge across this valley by providing a platform for direct, non-systemic drug treatment of the kidney whilst it is undergoing normal metabolic processes.[15, 16] Amongst the multiple anti-IRI agents tested in pre-clinical models, CD47-blocking antibody (αCD47Ab), recombinant thrombomodulin (rTM), and soluble complement receptor 1 (sCR1) are especially translatable as they have been safely employed for other clinical applications.[17-25] However, the comparative efficacy of these agents has not been established. Because IRI is characterized by the activation of multiple intersecting pathways,[26, 27] it is also plausible that synergistic anti-IRI effects may be derived by delivering 2 or more of these agents together.

The primary aims of this study were therefore to directly compare αCD47Ab, sCR1, and rTM in a murine model of renal IRI, and establish the combined efficacy of 2 of the best agents. Secondly,

we aimed to show that direct intra-renal delivery of the chosen drug(s) to porcine DCD kidneys using NMP could enhance renal perfusion parameters and ameliorate IRI.

6.3 Methods

A detailed description of the study methods is provided in Supplemental Digital Content (SDC) 1.

All animal protocols were approved by the Western Sydney Local Health District Animal Ethics Committee, in accordance with the Australian code for the care and use of animals for scientific purposes (8th Ed., 2013), developed by the National Health and Medical Research Council.

6.3.1 PART 1: COMPARISON OF IRI TARGETS – MURINE MODEL

Animals and IRI model

A model of severe unilateral renal IRI was utilized in male C57BL/6 mice (weight 25.3 ± 1.3 g) as follows:

- Right nephrectomy.
- Intra-venous injection of anti-IRI drug(s) diluted in vehicle control, or vehicle control alone (total volume 0.25 ml).
- Left renal ischemia using an arterial microvascular clamp (Roboz Surgical Instrument Co., MD, USA) for 25 mins (mouse temperature maintained at 36°C).
- Mice were euthanized 24 hrs after induction of IRI for collection of blood and renal tissue samples.

Study groups and pharmacotherapeutic agents

Mice were treated with the following agent(s) [n.b. these products are still mainly investigational for the purposes described here]:

1. Group I – 0.9% NaCl (vehicle control) only
2. Group II – rTM (Asahi Kasei Pharma Co., Tokyo, Japan), 1 mg/kg body weight[28]
3. Group III –sCR1 (CDX-1135; Celldex Therapeutics, MA, USA), 25 μg/g body weight[29]
4. Group IV – αCD47Ab (MIAP 301 [sc-12731]; Santa Cruz Biotechnology, TX, USA), 0.8 μg/g body weight[30]

5. Group V – combination of best 2 performing drugs, determined by relative serum creatinine (Cr) decrease compared to vehicle controls – αCD47Ab (0.8 μg/g body weight) and sCR1 (25 μg/g body weight) given as a single combined dose.

Serum samples

Blood (serum) samples were analyzed for urea and Cr levels.

Histology – Hematoxylin and Eosin (H&E)

Renal tubular damage at the corticomedullary junction was scored from 0-5 by 2 blinded renal histopathologists using H&E sections, as described previously.[21]

Immunohistochemistry

Immunohistochemistry was performed for the detection of neutrophil infiltration as described in SDC 1. Positively stained cells were counted from 5 high-power fields (HPF) at the corticomedullary junction in each section.

Reactive oxygen species (ROS) characterization – Cytochrome C and Amplex Red

Superoxide production and hydrogen peroxide-generating activity was calculated in homogenized mouse whole kidney tissue using cytochrome C, and amplex red, respectively. Further details can be found in SDC 1.

Inflammatory markers – pro-inflammatory cytokine/chemokine mRNA expression

Real-time polymerase chain reaction (RT-PCR) was performed using homogenized renal tissue sections for the expression of HPRT1, IL-6, TNF-α, IL-1β, CCL2, and CXCL2, as described in SDC 1. The ΔΔCt method was used to calculate expression fold changes normalized to HPRT1, with the 0.9% NaCl group utilized as the control.

Immunofluorescence

Complement C3 and C9 staining was ascertained using complement C3 (Thermo Fisher Scientific) or C9 (Abcam, Cambridge, UK) polyclonal primary antibodies and a goat anti-rabbit Alexa Fluor 647 secondary antibody (Thermo Fisher Scientific). Staining was visualized using a confocal microscope, and quantified using Image J.

TUNEL staining

Cellular death was ascertained using a commercially available kit (*In Situ* Cell Death Detection Kit, TMR Red; Sigma-Aldrich/Merck, MO, USA), and visualized by confocal microscopy. TUNEL-positive cells were counted from 3-5 HPF in each section.

6.3.2 PART 2: DIRECT INTRA-RENAL DELIVERY OF αCD47AB USING NMP – PORCINE DCD MODEL

Animals and porcine kidney DCD model

Female adult outbred Landrace pigs (70.7 ± 14.2 kg) were utilized for a DCD kidney retrieval model as follows:

- Renal pedicle and aortic dissection and mobilization.
- Cannulation of the infra-renal aorta using a TUR giving set (Baxter Healthcare, IL, USA), through which each pig was exsanguinated for autologous blood collection.
- Clamping of the renal pedicle (simultaneously with exsanguination) for 10 mins to simulate warm ischemia in a DCD setting.
- Nephrectomy, and renal artery and ureteric cannulation.
- Cold perfusion of the kidney after exactly 10 mins (via the renal artery) using 500 ml of University of Wisconsin (UW) solution containing 10,000 IU/L heparin. The 2 experimental groups were – (i) control kidneys (no further additives); (ii) treatment kidneys, which were given the best performing anti-IRI agent from the murine study via the renal artery, immediately after the initial UW flush (i.e. [porcine/human-specific] αCD47Ab – BRIC-126 [sc-59079], Santa Cruz Biotechnology;100 μg diluted in 10 ml UW).
- All kidneys were stored in UW solution prior to NMP (4°C; 6 hrs).

Normothermic machine perfusion

NMP was performed using a modified cardio-pulmonary bypass circuit, as described previously, and outlined in SDC 1.[31] Kidneys were perfused via the renal artery at a mean pressure of 75-85 mmHg and temperature of 37°C (1 hr). The 1 hr time period was chosen as it has been shown to be effective in human kidney transplantation after initial CS, and is now the subject of a multi-center RCT in the UK.[11, 12] The kidney was placed in a customized 3D-printed copolyester perfusion chamber during NMP.[32] Immediately prior to starting NMP in treatment kidneys, 200

μg of αCD47Ab (BRIC-126) was directly injected into the renal arterial line (i.e. ~0.8 μg/g of kidney weight).

Renal tissue, blood, and urine samples

Sequential kidney biopsies, perfusion fluid blood samples (from the arterial limb), and blood gases (from the arterial and venous limbs) were taken for further analyses as described in SDC 1.

Histology

H&E sections were scored from 0-3 (from least to most severe) by a blinded renal histopathologist based on the extent of tubular dilatation, tubular debris, cytoplasmic vacuolation, and inflammatory cell infiltration.[33, 34]

Inflammatory markers – pro-inflammatory cytokine/chemokine mRNA expression

RT-PCR was performed as described using porcine-specific primers for HPRT1, IL-6, TNF-α, IL-1β, and IL-18 (Thermo Fisher Scientific).

Immunofluorescence

αCD47Ab binding to porcine renal tissue was visualized by immunofluorescence using a goat anti-mouse secondary antibody conjugated to Alexa Fluor 647 dye (Thermo Fisher Scientific). Porcine renal tissue oxidative stress was quantified using dihydroethidium (DHE) (Thermo Fisher Scientific), which is indicative of tissue levels of superoxide. TUNEL staining was also performed, as described above. All immunofluorescence sections were co-stained for DAPI to visualize nuclear staining.

Statistical analyses

Data is presented as mean ± standard deviation (SD). Continuous parametric variables were compared using the unpaired student's t-test. In the event that more than 2 groups of parametric variables were to be compared, the ANOVA test was utilized. Area under the curve (AUC) was calculated for renal blood flow (RBF) and intra-renal resistance (IRR) prior to further statistical comparisons. GraphPad Prism v. 7.02 was used for all statistical analyses. A p-value of <0.05 was deemed statistically significant.

6.4 Results

6.4.1 PART 1: MURINE RENAL IRI MODEL (WARM ISCHEMIA)

αCD47Ab results in the greatest protection from injury in a murine model of severe IRI

Severe IRI was evident in vehicle control murine kidneys 24 hours after induction of ischemia, as indicated by serum urea and Cr levels, and the degree of histologic injury seen at the corticomedullary junction (Fig. 1). Treatment with αCD47Ab prior to IRI resulted in a significantly lower serum urea and Cr, and less histologic damage. A significant decrease in serum Cr was also seen in the sCR1 (alone) group, but not the rTM-treated mice. In contrast, rTM-treated mice had significantly less injury evident on histology as compared to controls, but this was not evident in the sCR1 group.

Combination of αCD47Ab and sCR1 does not significantly ameliorate IRI in comparison to CD47 alone

Although mice treated with both αCD47Ab and sCR1 (αCD47Ab+sCR1) showed a significant reduction in serum urea and creatinine in comparison to controls, this decline was not cumulative to that seen with αCD47Ab alone (Fig. 1A). Microscopic (tubular) injury in the αCD47Ab+sCR1 mice was not significantly reduced (Fig. 1B).

Neutrophil influx after IRI is depleted in all treatment groups, in particular αCD47Ab and sCR1 given alone

Leukocytes, especially neutrophils, infiltrate renal tissue after IRI. Extensive neutrophil infiltration was seen in vehicle controls (Fig. 2A). In comparison, all mouse treatment groups showed significantly less neutrophil staining, with the greatest reduction evident in the sCR1 and αCD47Ab groups of mice.

Superoxide but not hydrogen peroxide ROS production is diminished in all treatment groups

Superoxide production was significantly reduced in mice treated with αCD47Ab, sCR1, rTM, or αCD47Ab+sCR1 (Fig. 2B). However, hydrogen peroxide levels did not decrease in any treatment group, and in fact were significantly higher in rTM-treated mice (Fig. 2B).

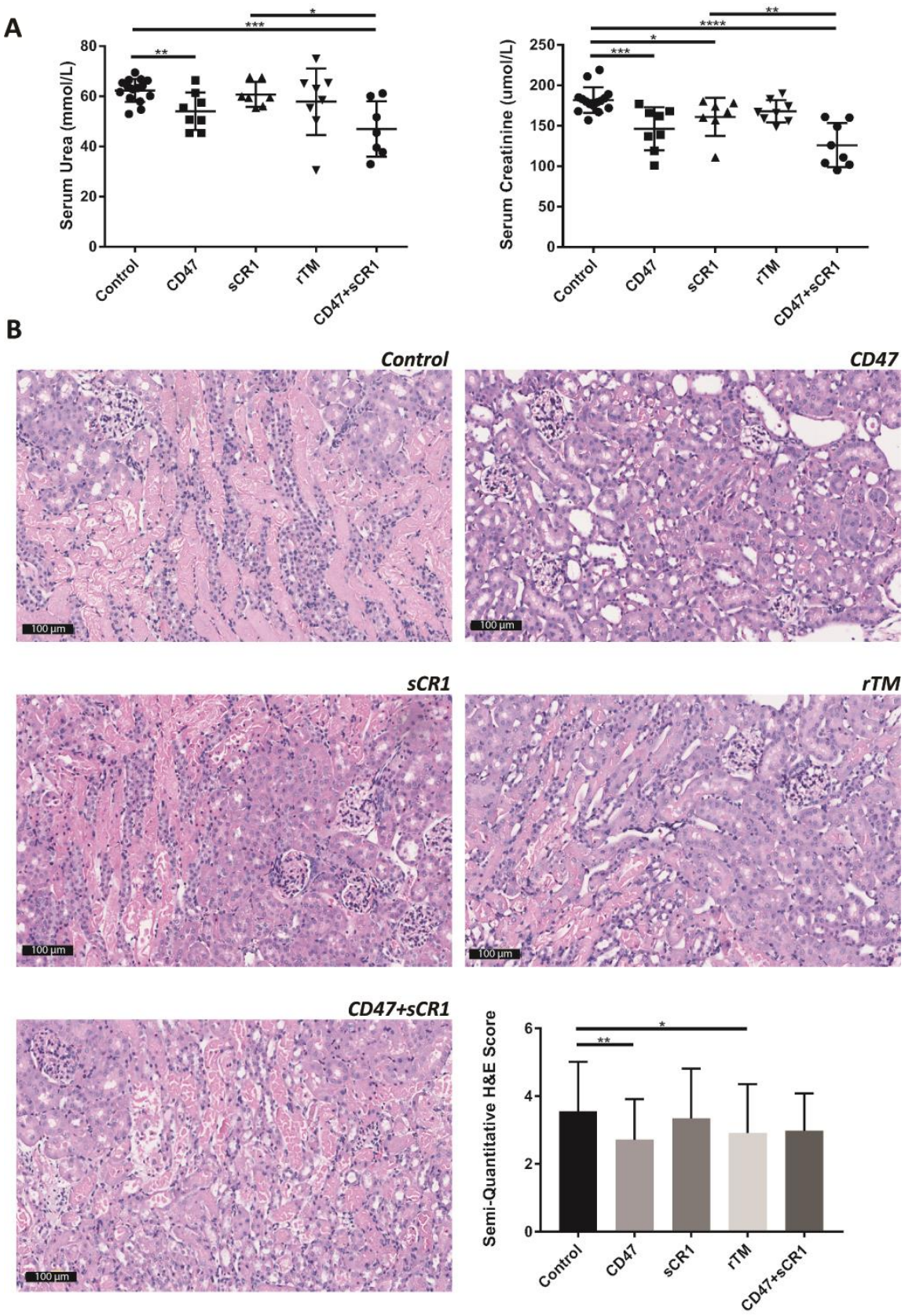


Figure 1. (A) Serum urea and creatinine 24 hours post-IRI in mice treated with 0.9% NaCl (vehicle control), αCD47Ab, sCR1, rTM, or αCD47Ab+sCR1. (B) Representative H&E sections and semi-quantitative renal tubular damage scores from each treatment group 24 hrs after the induction of IRI (20 x). Data shown as mean ± SD; n = 7-12/group. *p<0.05,**p<0.01,***p<0.001,****p<0.0001.

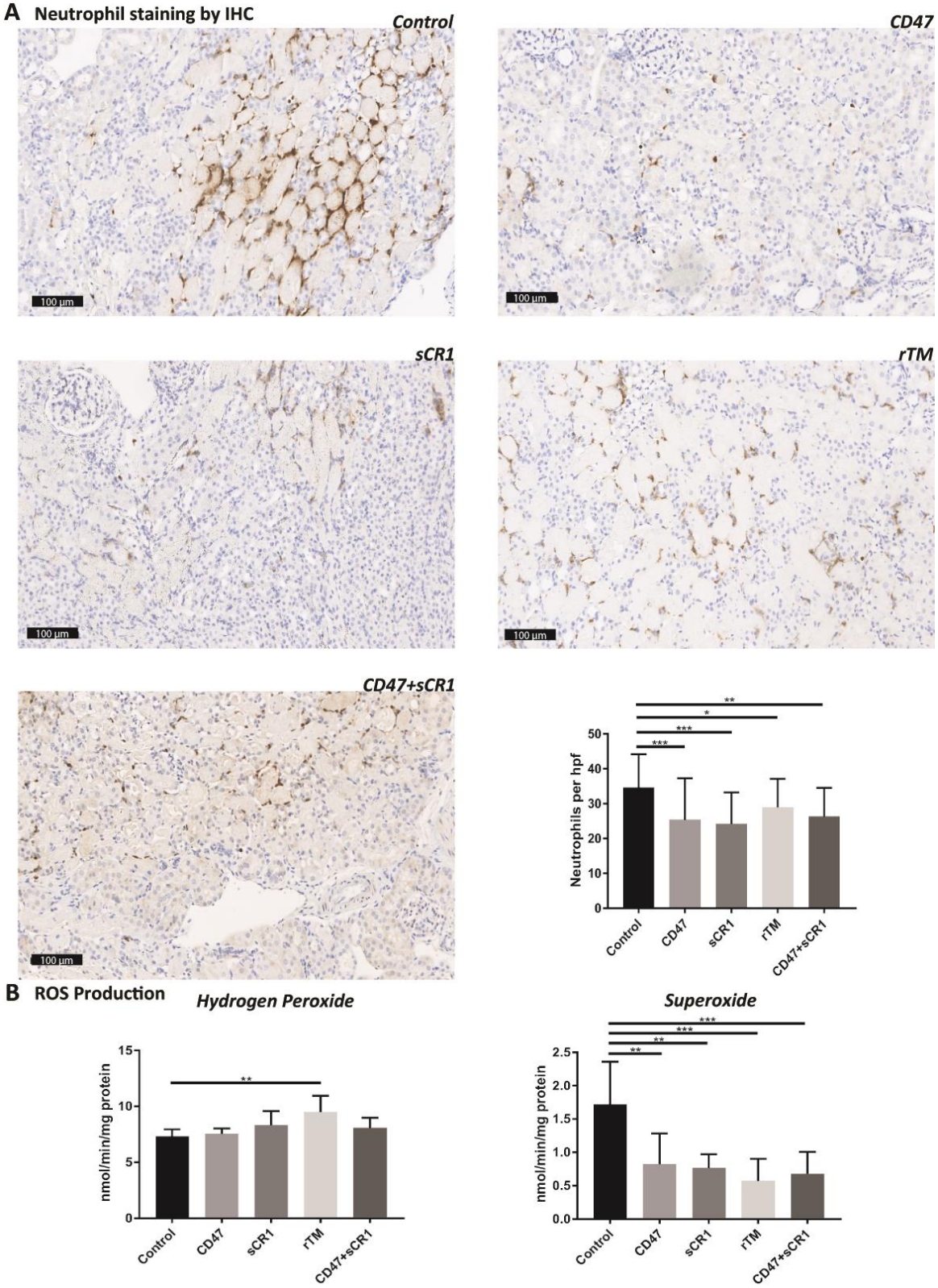


Figure 2. (A) Representative sections and quantitative analyses from each murine treatment group after immunohistochemical staining for neutrophils (number of cells per high power field [HPF]) (20 x). (B) Quantification of reactive oxygen species production (hydrogen peroxide [amplex red] and superoxide [cytochrome C]) in all murine treatment groups. Data shown as mean ± SD; n = 5/group. *p<0.05, **p<0.01,***p<0.001.

Pro-inflammatory cytokine and chemokine mRNA expression is variably modulated in treated mice after IRI

IL-6 levels were significantly lower in all treatment mouse groups at 24 hours in comparison to controls (Fig. 3). However, no significant reductions were seen in the mRNA expression profiles of TNF-α, IL-1β, CCL2, or CXCL2. Interestingly, expression of TNF-α was significantly higher in αCD47Ab-treated mice.

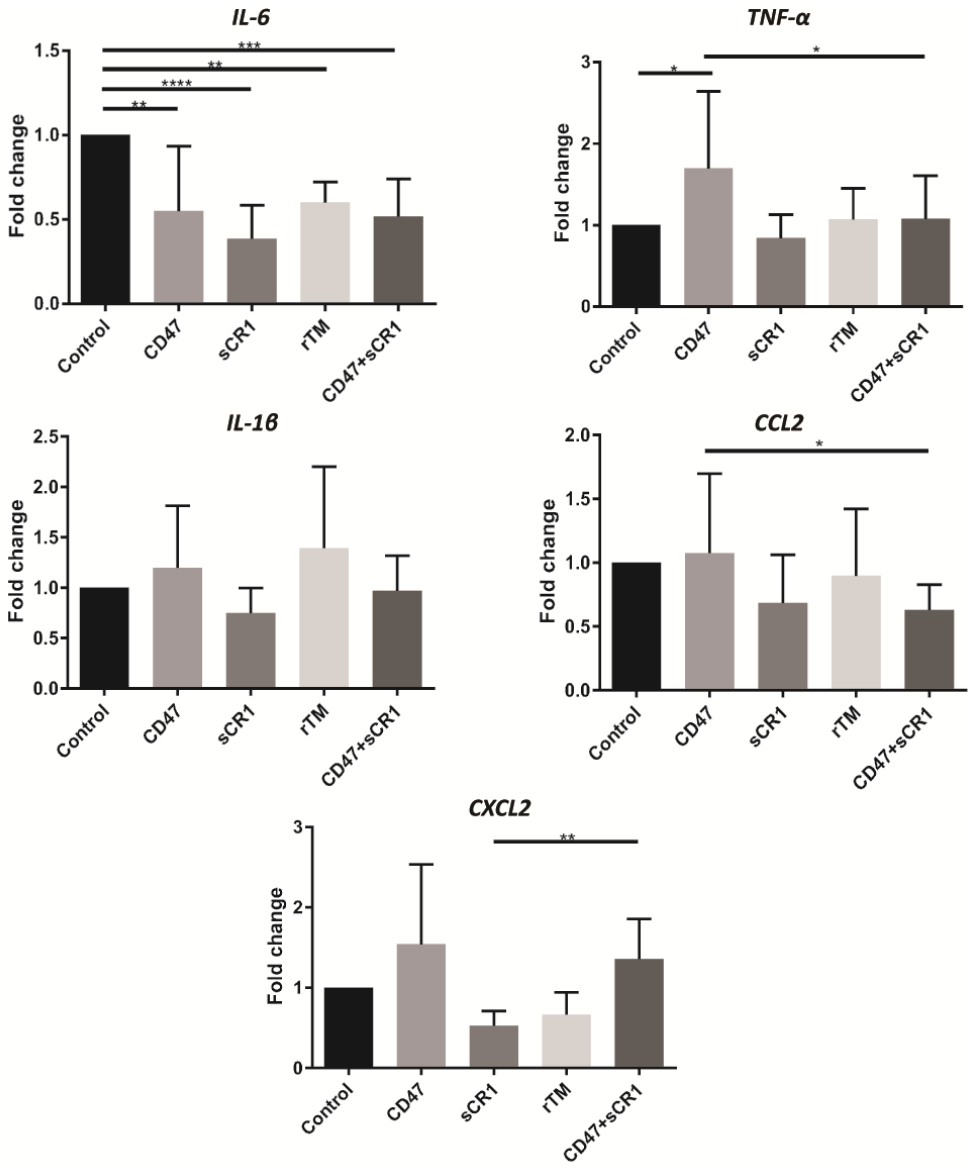


Figure 3. Pro-inflammatory cytokine and chemokine (IL-6, TNF-α, IL-1β, CCL2, and CXCL2) mRNA expression profiles in mouse kidney tissue 24 hrs after the induction of IRI following various drug treatments. Fold change calculated by normalizing to HPRT1, with the 0.9% NaCl (Control) mice used as the reference group. Data shown as mean ± SD; n = 6/group. (B) *p<0.05, **p<0.01,***p<0.001,****p<0.0001.

Complement C3 deposition is reduced by CD47 or sCR1 treatment alone or in combination, but C9 is not affected

All three complement pathways are implicated in IRI, with the activation of C3 and culminating in the formation of the membrane attack complex (C5b-9). No significant differences were seen between any mouse groups with respect to C9 staining (Fig. 4A). C3 deposition was poorly defined, displaying constitutive tubular staining; however, it was significantly reduced in all treatment groups except rTM (see figure, supplemental digital content 2).

Cell death is reduced in all mice treatment groups in comparison to controls

Renal tubular epithelial cells are the primary site of injury following IRI. Cell death quantified by TUNEL staining 24 hours post-IRI induction was most significantly reduced in αCD47Ab+sCR1-treated mice, although the reduction in the combined blockade group was not significantly greater than that achieved by αCD47Ab or sCR1 treatments alone (Fig. 4B).

6.4.2 PART 2: PORCINE RENAL DCD MODEL AND DRUG DELIVERY VIA NMP

As shown in Part 1, αCD47Ab was the most effective IRI treatment in the murine model across multiple comparative domains, and was therefore chosen as the targeted agent for part 2 of the study. DCD porcine kidney NMP was compared with and without αCD47Ab treatment.

αCD47Ab can be directly and effectively delivered to the kidney using NMP

There is no αCD47Ab binding evident in untreated kidneys (Fig. 5A). In the treated kidneys, addition of αCD47Ab to the UW cold flush did not result in binding of the antibody to the kidney (Fig. 5B, 'End CS'). In contrast, direct antibody infusion into the arterial line at the commencement of NMP resulted in widespread αCD47Ab binding along the glomerulus and renal tubular epithelium, which was detectable at the end of NMP (Fig. 5B, 'End NMP').

αCD47Ab treatment during NMP improves renal perfusion parameters

In comparison to untreated kidneys, kidneys receiving αCD47Ab during NMP had a significantly greater RBF and lower IRR (Fig. 5C). There was also a trend towards improved renal oxygen consumption, UO, CrCl, and FeNa in the αCD47Ab-treated kidneys (Fig. 5C).

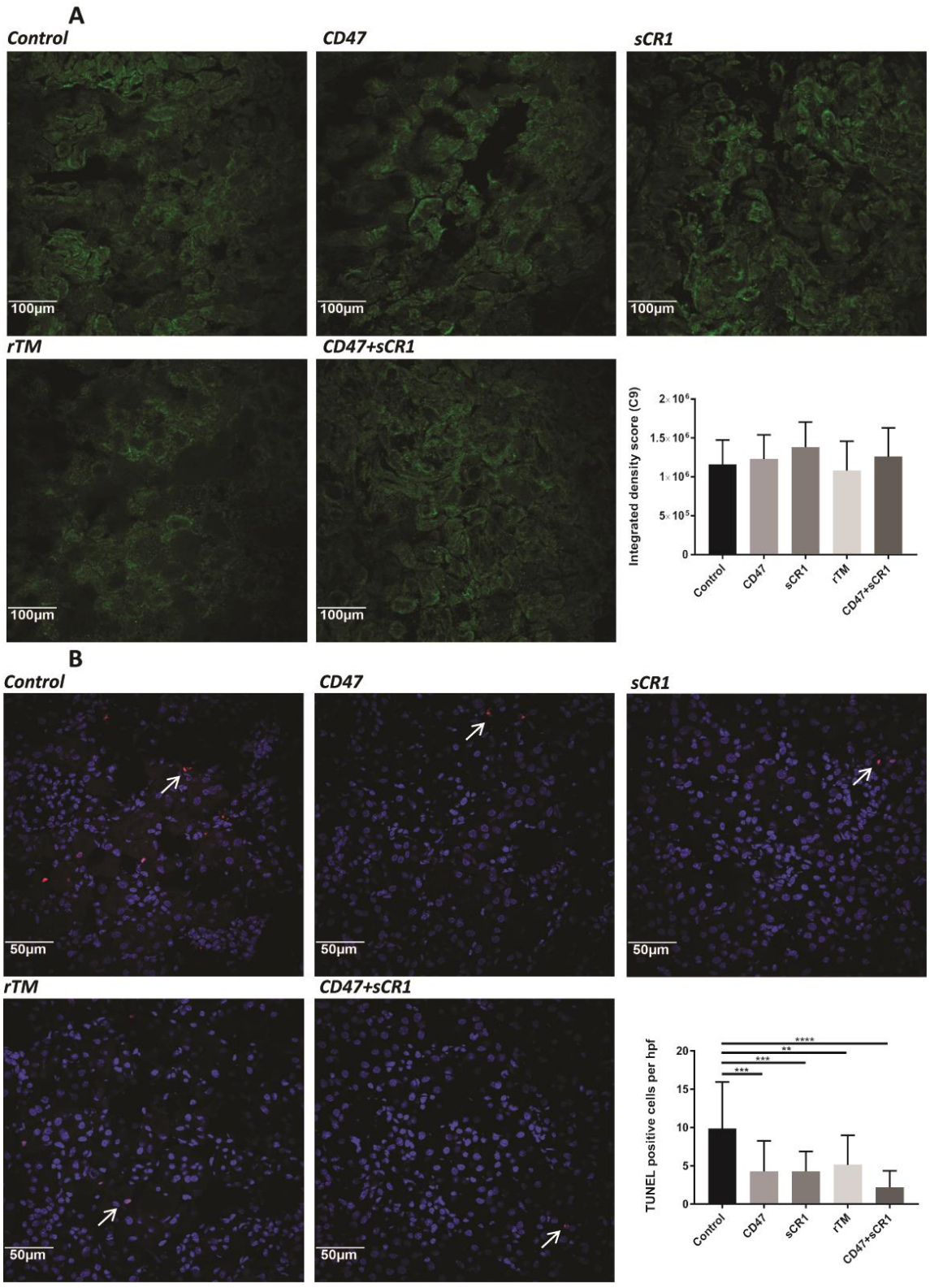


Figure 4. (A) Representative renal tissue photomicrographs (immunofluorescence) and quantitative integrated density scores for complement C9 staining 24 hrs post-IRI in each mouse treatment group (20 x). (B) Quantification of renal cellular death by TUNEL staining, with associated representative photomicrographs (immunofluorescence) (40 x). Data shown as mean ± SD; n = 5-6/group. **p<0.01,***p<0.001, ****p<0.0001.

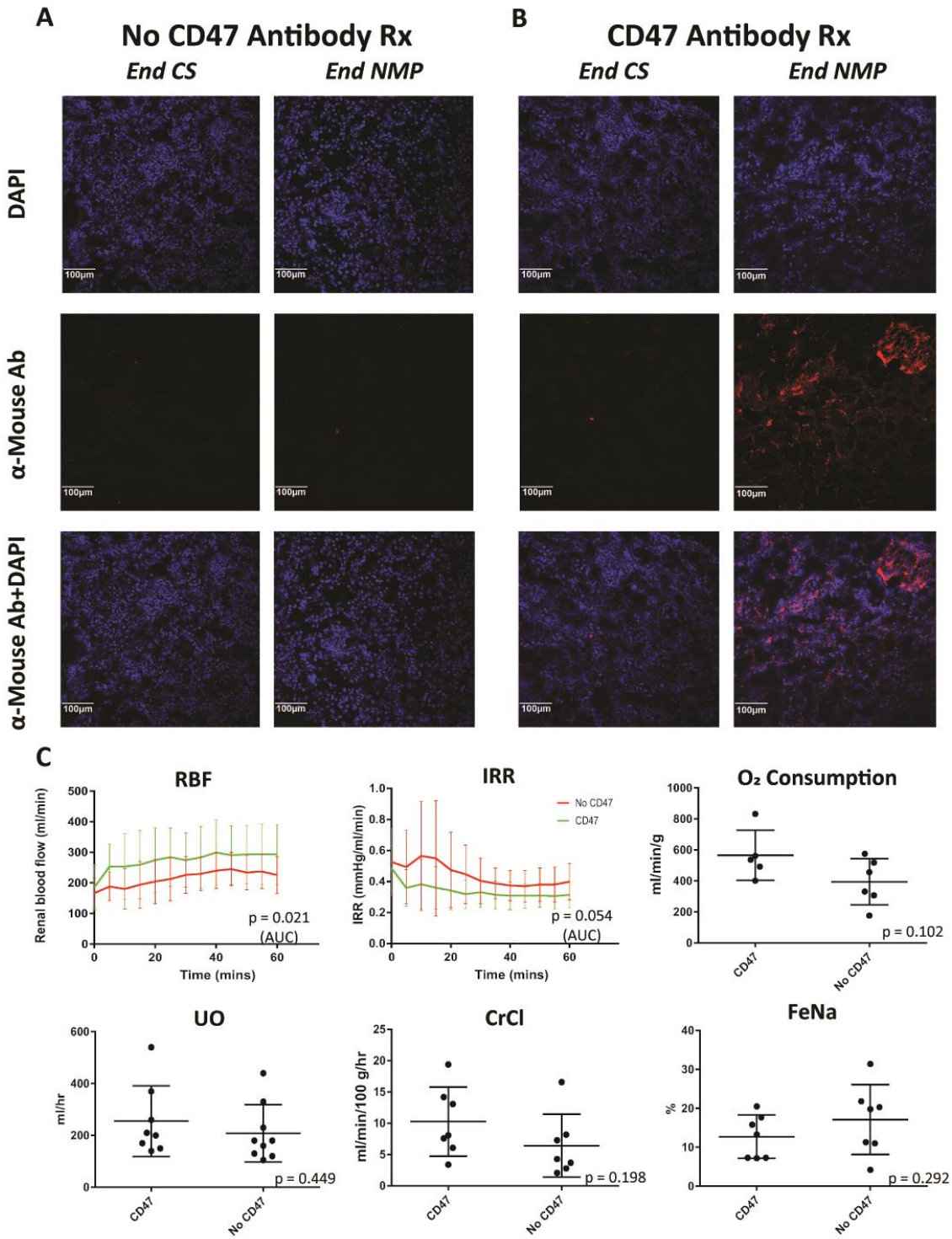


Figure 5. (A-B) αCD47Ab localisation in porcine NMP kidneys by immunofluorescence (20 x). αCD47Ab was given to treatment group kidneys by addition of the drug into the (i) UW cold flush, and (ii) NMP circuit. (A) No antibody binding evident in control kidneys (i.e. untreated kidneys). (B) Faint/minimal antibody binding at end CS (i.e. prior to the commencement of NMP); strong binding is evident in biopsies at the end of NMP, especially in the glomerulus. (C) Flow, IRR, glomerular, and tubular parameters after 1 hr of NMP in porcine kidneys treated with αCD47Ab in comparison to no CD47 treatment. Data presented as mean ± SD; n = 8-9/group. AUC – area under the curve; CrCl – creatinine clearance; CS – cold storage; FeNa – fractional excretion of sodium; NMP – normothermic machine perfusion; UO – urine output; UW – University of Wisconsin solution.

Pro-inflammatory cytokine mRNA expression is increased after NMP

In both treated and untreated kidneys, renal expression of IL-6, TNF-α, IL-1β, and IL-18 increased after NMP in comparison to end CS samples (Fig. 6). Although not significant, the increase in expression of IL-6 and IL-18 was less pronounced in the αCD47Ab group. In congruence with the mouse RT-PCR data, expression levels of TNF-α and IL-1β appeared to be elevated in the αCD47Ab treatment group.

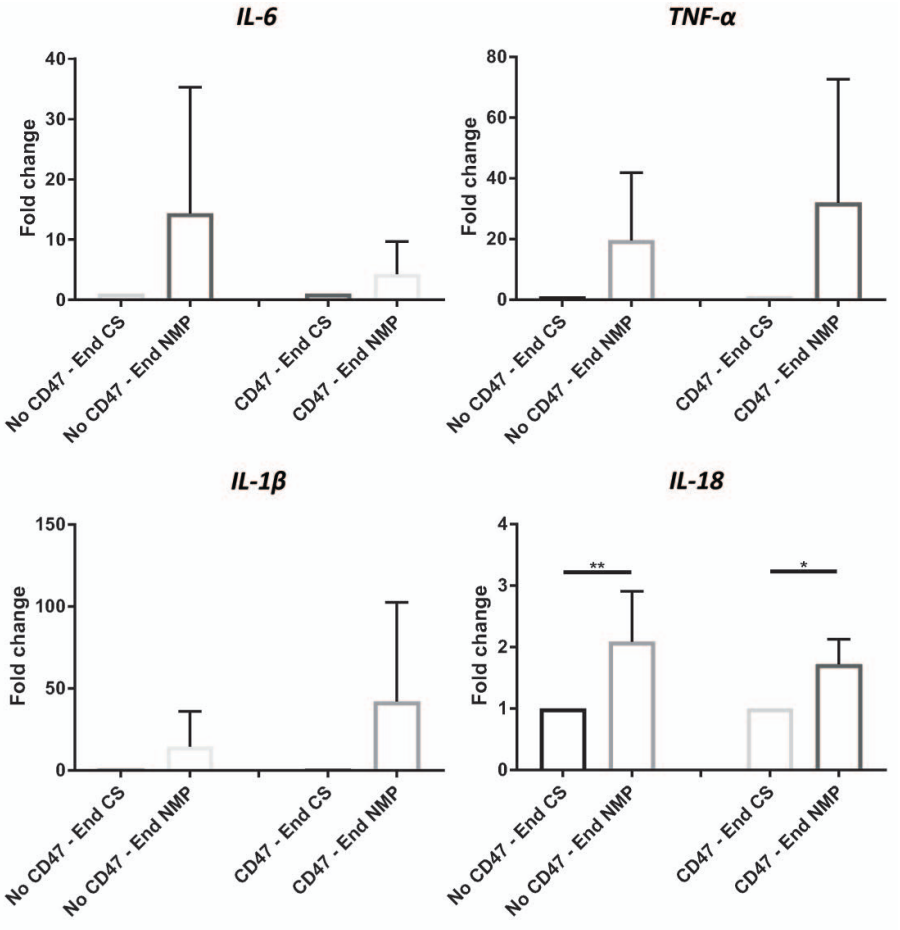


Figure 6. Pro-inflammatory cytokine (IL-6, TNF-α, IL-1β, and IL-18) mRNA expression in porcine tissue from sections taken at the end of NMP in comparison to the end CS reference group. Fold change normalized to HPRT1. Data shown as mean ± SD; n = 7/group. *p<0.05, **p<0.01.

Renal tubular debris is reduced in CD47-treated kidneys after NMP but other histologic parameters remain similar to controls

NMP re-institutes oxygenated blood flow to the kidney after a period of cold ischemia, and as such would be expected to precipitate IRI, albeit at a reduced magnitude due to the leukocyte depletion of the blood. Histologic comparison of the renal tubular condition before and after NMP showed a

significant increase in tubular dilatation and vacuolation in both αCD47Ab-treated and untreated kidneys (Fig. 7A). There was no significant change in inflammatory cell infiltrate in either group. However, there was a significant decrease in tubular debris in the αCD47Ab-treated kidneys after NMP.

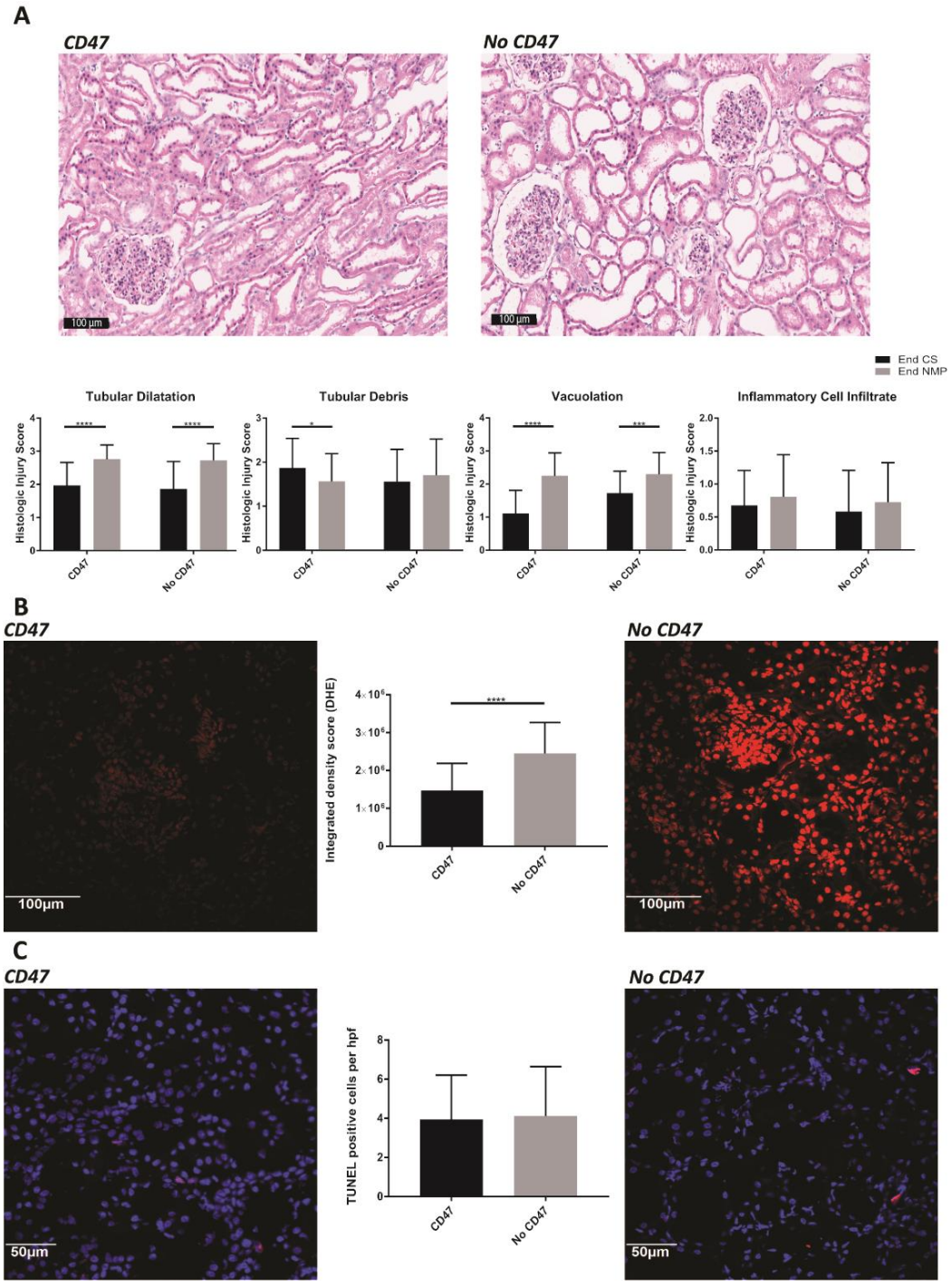


Figure 7. Representative porcine renal tissue photomicrographs and quantitative scoring for (A) tubular injury (H&E staining) (20 x), (B) oxidative stress (DHE staining) (20 x), and (C) renal cellular death (TUNEL staining; 40 x) at the end of NMP. Data shown as mean ± SD; n = 5-7/group. *p<0.05, ***p<0.001, ****p<0.0001.

Renal oxidative stress induced by NMP is reduced by αCD47Ab treatment however renal tubular epithelial cell death remained similar

Oxidative stress quantified by DHE staining was significantly reduced in αCD47Ab-treated kidneys in comparison to controls (Fig. 7B). Renal cellular death however was not significantly different between treated and untreated kidneys (Fig. 7C).

6.5 Discussion

This study provides the first direct *in vivo* comparison of three potent anti-IRI agents, αCD47Ab, sCR1, and rTM, in a murine model of severe renal IRI. We show that αCD47Ab alone provides the greatest level of protection that is not substantially increased by its combination with sCR1. We investigated the feasibility of direct αCD47Ab delivery to the porcine kidney using NMP, showing that it can be given at a dose based on renal weight alone, with evidence of renal tubular and glomerular binding, and subsequent downstream beneficial effects on kidney perfusion, oxidative stress, and tubular and glomerular function.

CD47 provides a plausible target that can be blocked to significantly ameliorate IRI via the modulation of multiple IRI-related pathways. CD47 signaling is important for promoting IRI; injury primarily results from the renal parenchymal cell membrane-associated CD47 binding its ligand thrombospondin-1.[21] Downstream effects include inhibition of nitric oxide and its effects on vascular smooth muscle, exacerbation of oxidative stress, inflammatory cell recruitment, and an impairment of parenchymal cellular repair.[21, 35, 36] As such, receptor blockade should ameliorate IRI by impacting multiple inter-related injurious processes. In our mouse model, we confirmed a significant reduction to renal injury, with better preservation of renal function in the αCD47Ab treated mice, which was superior to that provided by sCR1 or rTM alone. Neutrophil influx was also correspondingly reduced to the largest extent in the αCD47Ab group, in addition to a robust reduction in renal cellular death. Although levels of superoxide also significantly declined in the αCD47Ab group, no reduction was seen in hydrogen peroxide levels. Indeed, substantial fluxes of superoxide may significantly impact the stoichiometry of hydrogen peroxide detection by amplex red, possibly explaining the different quantification trends of superoxide and hydrogen peroxide in the study groups.[37] Interestingly, with the exception of IL-6, there was no reduction in the mRNA expression of TNF-α, IL-1β, CCL2, or CXCL2 in the αCD47Ab-treated mice. A lack of impact on

TNF-α mRNA expression in response to CD47 blockade has also been noted elsewhere, indicating this pathway is not directly involved in CD47-mediated cellular injury during IRI.[21, 38]

In order to investigate the potential synergistic amelioration of IRI by combining different drugs, the 2 most efficacious drugs, αCD47Ab and sCR1, were given as a combined dose. Although serum Cr levels were significantly lower in these mice compared to sCR1 alone, there was no significant difference between the αCD47Ab+sCR1 group and the group of mice treated with αCD47Ab alone. Furthermore, histologic injury, inflammatory cell infiltration, complement deposition, ROS production, and cellular death were not incrementally improved in the combined treatment group compared to αCD47Ab-treated mice. This acts to highlight the relatively broad impact of CD47-blockade on the IRI cascade, therefore serving as a highly effective single agent.
Due to its relative superiority in the mouse renal IRI experiments, αCD47Ab was chosen as the optimal agent to be administered using NMP in a porcine DCD model. Antibody was retained in the renal parenchyma at the end of NMP, ensuring the CD47 receptor remains blocked prior to potential transplantation. The drug was dosed according to kidney weight and not the weight of the donor animal, as NMP affords the opportunity of direct intra-renal delivery. An additional dose of αCD47Ab was given immediately after the induction of cold ischemia to account for potential drug binding/uptake by the CD47 receptor on circulating cells/PRBCs.[39] However, there was no immunofluorescence evidence that this cold perfusion dose caused effective αCD47Ab binding to its receptor. In contrast, Xu *et al*. showed renal binding after pre-implantation delivery of αCD47Ab to porcine kidneys via a direct renal artery cold flush.[38] However, these authors used a dose that was approximately 50 times greater (total 10 mg) than that used in this study, and the αCD47Ab solution used was flushed via the renal artery 5 times.[38] From our current work it can be concluded that NMP facilitates highly specific and targeted delivery of reduced αCD47Ab dose(s) to the kidney that cannot be achieved by adding blocking antibody(s) to the cold flush alone, and this binding is retained over the 1 hr period of NMP.

Addition of αCD47Ab to the NMP perfusion circuit did not induce or worsen renal injury on the machine in comparison to control kidneys. NMP involves reperfusion of the kidney with an oxygenated PRBC-based solution, and as such can be considered as an early induction of IRI after a period of CS. The primary difference between NMP and reperfusion after transplantation is that the latter occurs in a uremic recipient with allogeneic whole blood containing the recipient's

leukocytes, pre-formed antibodies, and complement components. As such, the insult sustained during NMP is unique in its nature. Overall, a pro-inflammatory state is induced.[40, 41] Therefore and unsurprisingly, renal mRNA expression of pro-inflammatory cytokines increased after NMP in this study, albeit to a lesser extent for IL-6 and IL-18 in the αCD47Ab-treated group. Furthermore, there was a mild increase in renal tubular injury parameters as evident by light microscopy post-NMP; these parameters were similar in both groups with the exception of tubular debris, which was significantly reduced in the CD47-blocked group. Importantly, the oxidative stress induced by NMP was significantly less in the αCD47Ab treatment group.

CD47 blockade during NMP enhanced some functional parameters over the course of perfusion. RBF and IRR were significantly better in the treatment group, which may be related to the effects of CD47 binding on the nitric oxide pathway and vascular responsiveness.[35] Encouragingly, renal oxygen consumption, UO, CrCl, and tubular function improved in the αCD47Ab-treated group, although not reaching statistical significance. Additional improvements in these parameters during NMP might require a higher dose of αCD47Ab or the induction of more severe injury through prolongation of ischemic times such that more clear differences may be elucidated. Any ultimate improvement in renal IRI by CD47 blockade needs to be proven after full-scale reperfusion with leukocyte-replete allogeneic blood (i.e. transplantation).

Pharmacomanipulation of the kidney during NMP may also improve the efficacy of the short periods of pre-implantation NMP currently in clinical use.[12] There is some experimental evidence to indicate the longer periods (8 or more hours) of renal NMP are superior to 1 hour of pre-implantation NMP; this is in the setting where no additional anti-IRI drugs are added.[42, 43] Longer periods of NMP are however more labor-intensive, expensive, and likely less readily taken up by transplant centers. Pharmacologic amelioration of IRI during NMP may provide a compromise, allowing shorter pre-implantation NMP.

In conclusion, this paper has shown the feasibility and efficacy of using NMP as a targeted drug delivery system to the kidney as a means to ameliorate IRI. Three proven anti-IRI drugs were compared in a murine kidney model of severe IRI, and αCD47Ab was shown to be most protective. The porcine-specific version of this antibody was tested in a DCD model using NMP, achieving renal binding, and improving some renal perfusion and injury parameters. NMP has a

remarkable potential to not only directly treat and resuscitate donor kidneys prior to implantation, but also to fast-track drug discovery/application from small animal and/or cell culture models into the clinical setting. Its impacts may be significantly amplified through the targeted delivery of anti-IRI drugs to the kidney, which will likely translate into vast future clinical applications.

6.6 References

1. Wang H, Naghavi M, Allen C, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016; 388: 1459-1544.
2. Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med.* 1999; 341: 1725-1730.
3. Oniscu GC, Brown H, Forsythe JLR. How great is the survival advantage of transplantation over dialysis in elderly patients? *Nephrol Dial Transplant*. 2004; 19: 945-951.
4. Sørensen VR, Heaf J, Wehberg S, Sørensen SS. Survival Benefit in Renal Transplantation Despite High Comorbidity. *Transplantation*. 2016; 100: 2160-2167.
5. Chapman JR, Kanellis J. Kidney donation and transplantation in Australia: more than a supply and demand equation. *Med J Aust.* 2018; 209: 242-243.
6. Summers DM, Watson CJ, Pettigrew GJ, et al. Kidney donation after circulatory death (DCD): state of the art. *Kidney Int.* 2015; 88: 241-249.
7. Maggiore U, for the ERAE-DWG, Oberbauer R, et al. Strategies to increase the donor pool and access to kidney transplantation: an international perspective. *Nephrol Dial Transplant*. 2015; 30: 217-222.
8. Lim WH, McDonald SP, Russ GR, et al. Association Between Delayed Graft Function and Graft Loss in Donation After Cardiac Death Kidney Transplants-A Paired Kidney Registry Analysis. *Transplantation*. 2017; 101: 1139-1143.
9. Jochmans I, Akhtar MZ, Nasralla D, et al. Past, Present, and Future of Dynamic Kidney and Liver Preservation and Resuscitation. *Am J Transplant*. 2016; 16: 2545-2555.
10. Hameed AM, Pleass HC, Wong G, Hawthorne WJ. Maximizing kidneys for transplantation using machine perfusion: from the past to the future: A comprehensive systematic review and meta-analysis. *Medicine (Baltimore).* 2016; 95: e5083.
11. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013; 13: 1246-1252.
12. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017; 7.
13. Hosgood SA, Thompson E, Moore T, Wilson CH, Nicholson ML. Normothermic machine perfusion for the assessment and transplantation of declined human kidneys from donation after circulatory death donors. *Br J Surg*. 2018; 105: 388-394.
14. Butler D. Translational research: crossing the valley of death. *Nature*. 2008; 453: 840-842.
15. O'Neill S, Gallagher K, Hughes J, Wigmore SJ, Ross JA, Harrison EM. Challenges in early clinical drug development for ischemia-reperfusion injury in kidney transplantation. *Expert Opin Drug Discov*. 2015; 10: 753-762.
16. Tietjen GT, Hosgood SA, DiRito J, et al. Nanoparticle targeting to the endothelium during normothermic machine perfusion of human kidneys. *Sci Transl Med*. 2017; 9.
17. Sharfuddin AA, Sandoval RM, Berg DT, et al. Soluble thrombomodulin protects ischemic kidneys. *J Am Soc Nephrol*. 2009; 20: 524-534.
18. Kadono K, Uchida Y, Hirao H, et al. Thrombomodulin Attenuates Inflammatory Damage Due to Liver Ischemia and Reperfusion Injury in Mice in Toll-Like Receptor 4–Dependent Manner. *Am J Transplant.* 2017; 17: 69-80.

19. Keshavjee S, Davis RD, Zamora MR, de Perrot M, Patterson GA. A randomized, placebo-controlled trial of complement inhibition in ischemia-reperfusion injury after lung transplantation in human beings. *J Thorac Cardiovasc Surg*. 2005; 129: 423-428.
20. Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore FD. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia-reperfusion in the rat. *J Immunol*. 1992; 149: 1723-1728.
21. Rogers NM, Thomson AW, Isenberg JS. Activation of Parenchymal CD47 Promotes Renal Ischemia-Reperfusion Injury. *J Am Soc Nephrol* . 2012; 23: 1538-1550.
22. Wang X, Xu M, Jia J, et al. CD47 blockade reduces ischemia/reperfusion injury in donation after cardiac death rat kidney transplantation. *Am J Transplant*. 2018; 18: 843-854.
23. Huang Y, Ma Y, Gao P, Yao Z. Targeting CD47: the achievements and concerns of current studies on cancer immunotherapy. *J Thorac Dis*. 2017; 9: E168-E174.
24. Li JS, Jaggers J, Anderson PA. The use of TP10, soluble complement receptor 1, in cardiopulmonary bypass. *Expert Rev Cardiovasc Ther*. 2006; 4: 649-654.
25. Saito H, Maruyama I, Shimazaki S, et al. Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial. *J Thromb Haemost*. 2007; 5: 31-41.
26. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest*. 2011; 121: 4210-4221.
27. Ponticelli C. Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant*. 2014; 29: 1134-1140.
28. Tanemura A, Kuriyama N, Azumi Y, et al. Thrombomodulin administration attenuates ischemia-reperfusion injury of the remnant liver after 70% hepatectomy in rats: simulated model of small-for-size graft in living donor liver transplantation. *Transplant Proc.* 2014; 46: 1107-1111.
29. Pratt JR, Hibbs MJ, Laver AJ, Smith RA, Sacks SH. Effects of complement inhibition with soluble complement receptor-1 on vascular injury and inflammation during renal allograft rejection in the rat. *Am J Pathol*. 1996; 149: 2055-2066.
30. Rogers NM, Zhang ZJ, Wang J-J, Thomson AW, Isenberg JS. CD47 regulates renal tubular epithelial cell self-renewal and proliferation following renal ischemia reperfusion. *Kidney Int.* 2016; 90: 334-347.
31. Hameed AM, Miraziz R, Lu DB, et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ J Surg*. 2018; 88: E429-434.
32. Hameed A, Dervish S, Rogers N, Pleass H, Hawthorne W. A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transpl Int*. 2018. Epub ahead of print; DOI: 10.1111/tri.13361
33. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg.* 2008; 95: 111-118.
34. Hosgood SA, Barlow AD, Yates PJ, Snoeijs MG, van Heurn EL, Nicholson ML. A pilot study assessing the feasibility of a short period of normothermic preservation in an experimental model of non heart beating donor kidneys. *J Surg Res.* 2011; 171: 283-290.
35. Isenberg JS, Ridnour LA, Dimitry J, Frazier WA, Wink DA, Roberts DD. CD47 is necessary for inhibition of nitric oxide-stimulated vascular cell responses by thrombospondin-1. *J Biol Chem*. 2006; 281: 26069-26080.

36. Rogers NM, Zhang ZJ, Wang JJ, Thomson AW, Isenberg JS. CD47 regulates renal tubular epithelial cell self-renewal and proliferation following renal ischemia reperfusion. *Kidney Int.* 2016; 90: 334-347.
37. Dikalov SI, Harrison DG. Methods for detection of mitochondrial and cellular reactive oxygen species. *Antioxid Redox Signal*. 2014; 20: 372-382.
38. Xu M, Wang X, Banan B, et al. Anti-CD47 monoclonal antibody therapy reduces ischemia-reperfusion injury of renal allografts in a porcine model of donation after cardiac death. *Am J Transplant.* 2018; 18: 855-867.
39. Oldenborg P-A, Zheleznyak A, Fang Y-F, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a Marker of Self on Red Blood Cells. *Science*. 2000; 288: 2051-2054.
40. Stone JP, Ball AL, Critchley WR, et al. Ex Vivo Normothermic Perfusion Induces Donor-Derived Leukocyte Mobilization and Removal Prior to Renal Transplantation. *Kidney Int Rep*. 2016; 1: 230-239.
41. Hosgood SA, Moore T, Kleverlaan T, Adams T, Nicholson ML. Haemoadsorption reduces the inflammatory response and improves blood flow during ex vivo renal perfusion in an experimental model. *J Transl Med*. 2017; 15: 216.
42. Kaths JM, Echeverri J, Linares I, et al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant*. 2017; 17: 2580-2590.
43. Kaths JM, Cen JY, Chun YM, et al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. *Am J Transplant*. 2017; 17: 957-969.

Chapter 7

Brief normothermic machine perfusion rejuvenates discarded human kidneys – the potential for discard reduction and improved outcomes

Ahmer Hameed
David Lu
Ellis Patrick
Bo Xu
Min Hu
Yi Vee Chew
Karen Keung
Chow P'ng
Renan Gaspi
Chris Zhang
Paul Robertson
Stephen Alexander
Gordon Thomas
Jerome Laurence
Ronald De Roo
Germaine Wong
Ray Miraziz
Greg O'Grady
Lawrence Yuen
Wayne Hawthorne
Natasha Rogers
Henry Pleass

Manuscript in preparation

7.1 Abstract

Introduction: Normothermic machine perfusion (NMP) is a promising new modality that provides the potential for the resuscitation and improved assessment of kidneys prior to transplantation. Using discarded human kidneys, we aimed to investigate the mechanistic basis and translational potential of NMP as a superior strategy compared to the current gold standard of cold static storage (CS).

Methods: Discarded deceased donor kidneys (n = 15) underwent brief (one hour) NMP after a period of CS during transportation. Renal perfusion, biochemical, and histologic parameters were recorded. Leukocyte efflux from the kidney was measured in selected grafts. NMP was directly compared to CS in paired donor kidneys using simulated transplantation with whole allogeneic blood, followed by assessment of perfusion and functional parameters, markers of ischemia-reperfusion injury (IRI), and RNA sequencing.

Results: All kidneys were successfully perfused, with demonstration of improving renal blood flows and resistance (median 260 ml/min and 0.29 mmHg/ml/min, respectively), and urine output (median 21 ml), in all but one kidney. NMP completely resolved non-perfused regions in discarded DCD kidneys. In paired kidneys, transcriptomic analyses showed induction of stress and inflammatory pathways in NMP kidneys, with upregulation of pathways promoting cell survival and proliferation. Furthermore, the NMP pairs had signifincantly better renal perfusion (1.5-2 fold improvement in flow and resistance) and functional parameters, and amelioration of cell death, oxidative stress, and complement activation.

Conclusions: NMP demonstrated multiple superior outcomes to CS, allowing for the rejuventation of marginal kidneys. NMP has consdiderable potential to enhance early graft function in such kidneys, and also reduce organ discards in order to increase kidney transplantation rates.

7.2 Introduction

Normothermic machine perfusion (NMP) is a recently developed technique that may be applied to deceased donor kidney preservation prior to transplantation. NMP has been shown to have early potential in the enhancement of kidney transplant outcomes.[1-4] It may be performed in conjunction with cold static storage (CS; the current reference standard), and/or as a sole modality prior to transplant, although current human application has only seen employment of the former.

The emergence of NMP is a natural progression from increased prominence of machine perfusion (MP) preservation for organ transplantation.[4, 5] The field of MP in kidney transplantation has largely been dominated by hypothermic MP (HMP).[6] However, this modality has not gained widespread acceptance due to an inability to accurately predict longer-term graft function, in addition to ongoing high rates of delayed graft function (DGF) in donation after circulatory death (DCD) kidneys, and equivocal impacts on graft survival.[6-9] NMP presents a potential solution to these problems, which is required to help close the organ supply-demand gap and improve outcomes from the DCD and expanded criteria (high kidney donor profile index [KDPI]) kidneys that organ transplantation centers are now increasingly reliant upon.[10-13] Not only does NMP have the potential to improve the function of these organs post-transplantation, it may also allow for accurate functional assessment of the graft in a near-physiologic state.[4] Furthermore, NMP enables the directed delivery of therapeutics to the kidney during perfusion while metabolic processes are active.

Although preliminary evidence indicates superiority of NMP over CS alone, and a RCT is currently underway to compare both techniques, many questions remain unanswered prior to the more widespread uptake of NMP worldwide.[1, 14] In particular, little is known about the actual mechanistic changes induced by NMP that may help improve graft outcomes, with the sparse evidence available limited to porcine studies.[15, 16] This is crucial to more clearly inform clinicians regarding how best to utilize NMP, including what type(s) of organs and recipients will benefit the most from this technology. One important area of controversy relates to the thresholds and duration at which NMP will be most beneficial, with current clinical evidence existing only for one hour of pre-implantation NMP.[1, 17, 18] However, experimental (porcine) evidence indicates that longer periods of NMP (> 8 hours) may be more beneficial to subsequent transplant function, whilst others have explored the feasibility of perfusion periods up to 24 hours.[3, 19, 20] Brief (1-3

hour) pre-implantation NMP however continues to remain attractive as it is convenient, and therefore more readily employed, especially considering that current NMP technology is not easily transportable.

Therefore, we aimed to investigate the comparative efficacy of a brief period of NMP following CS, to CS alone, using paired human kidneys, with a particular focus on the mechanistic changes that underlie any potential advantages offered by NMP. We will also examine the following parameters that have not been clearly investigated using human kidneys – (i) biochemical, acid-base, and perfusion-related trends during NMP that may be used to inform decision-making regarding potential transplantation; (ii) passenger leukocyte load of donor kidneys and the use of NMP to induce extravasation of these leukocytes; and (iii) the comparative efficacy of NMP with autologous or banked (allogeneic) blood.

7.3 Methods

7.3.1 ETHICS

Ethics approval for this project was obtained from the Western Sydney Local Health District human research ethics committee. All prospective donors' families were consented for the potential research use of kidneys for research purposes prior to the procurement process. Further project support was obtained from the NSW Organ and Tissue Donation Service (OTDS), and collaboration was also established with the Australian Red Cross Blood Service (ARCBS).

7.3.2 INCLUSION AND EXCLUSION CRITERIA

Kidneys were obtained for the purposes of this research from any deceased donor in the event that – (i) they were deemed unsuitable for transplantation for any reason during or after procurement, or (ii) in the event of a planned liver-only donor whereby the kidneys had been deemed medically unsuitable prior to retrieval. Kidneys were only excluded from subsequent NMP when autologous or allogeneic blood was not available for perfusion.

7.3.3 KIDNEY PROCUREMENT

Retrieval was undertaken in a standard fashion, after aortic cannulation and cold perfusion with Soltran, and in the event of liver or pancreas retrieval, also University of Wisconsin (UW) solution. In the event that autologous blood was to be utilized for subsequent NMP, the inferior

vena cava (IVC) was dissected and immediately accessed using a 28-32 Fr intercostal catheter attached to a TUR giving set at the commencement of cold perfusion. Vented blood was collected into a customized blood bag (LivaNova Australia, Dandenong, Australia) containing anticoagulant-citrate-dextrose solution A (ACD-A) (Aurora Bioscience, Bella Vista, Australia) and saline-adenine-glucose-mannitol solution (SAGM) (Macopharma, Chatswood, Australia), and stored on ice. Kidneys were stored in the final flush solution (University of Wisconsin [UW] solution or Soltran solution), surrounded by 0.9% sodium chloride ice slush, prior to transportation to our center.

Donor and retrieval details that were recorded included age, sex, comorbidities, donation pathway (DBD or DCD), ABO blood group, kidney donor profile index (KDPI), donor cause of death (COD), intended and actual organs retrieved, reason for kidney discard/non-utilization, cross-clamp time, warm ischemic time (WIT), cold ischemic time (CIT), and kidney anatomy. The KDPI estimates the risk of graft failure relative to other donor kidneys and incorporates donor age, history of hypertension or diabetes mellitus, height/weight, COD as stroke, terminal creatinine level, and DCD pathway within overall scoring.[21, 22]

7.3.4 KIDNEY PREPARATION

Kidneys underwent standard back-table preparation. The renal artery was cannulated with heparin tips connected to a ¼ inch luer lock adaptor (Medtronic, MN, USA and LivaNova Australia, Dandenong, Australia), and the cannula was secured with a silk tie. The ureter was cannulated with a shortened heparin tip (Medtronic), which was also secured using a silk tie. Kidneys remained on ice slush until the commencement of NMP.

7.3.5 BLOOD PREPARATION

In the event that autologous blood was used, collected donor whole blood was centrifuged at 3500 RPM for 15 minutes, and the supernatant discarded. The residual packed red blood cell (PRBC) mass was washed with Hartmann's solution, re-centrifuged for 10 minutes, and the supernatant was once again discarded. PRBCs were then passed through a leukocyte filter (Terumo Pty Ltd, Tokyo, Japan) and collected into a new blood bag (total PRBC volume approximately 250 ml).

The ARCBS provided all PRBC units for the NMP cases in which banked blood was utilized (O+ or O- units only; total PRBC volume approximately 250 ml). All subsequent simulated

transplantation experiments were conducted using whole banked blood (O+ or O-), also obtained from the ARCBS. Total volume of each whole blood unit was approximately 500 ml, with 250 ml of this used for each paired kidney (see below).

7.3.6 EX VIVO PERFUSION SET-UP

The NMP system was assembled as previously described.[23] In brief, NMP was undertaken using autologous or banked PRBCs, to which was added Hartmann's solution (150 ml), gelofusine (250 ml), 10% mannitol (50 ml), 10% calcium gluconate (5 ml), 8.4% sodium bicarbonate (15 ml), sterile water for injection (25 ml), and heparin (2000 units). Continuous infusions of nutrient (M199 with ultraglutamine) solution (20 ml/hr), 5% dextrose (5 ml/hr), and verapamil (5 mg in 2 ml, run at 5 ml/hr) were also run during NMP. Creatinine was added to the circuit (700 μmol in 5 ml 0.9% NaCl, to give an approximate concentration of 1000 μmol/L; Merck, Darmstadt, Germany) to enable subsequent quantification of creatinine clearance (CrCl). The kidney was placed in a customized, 3D-printed perfusion chamber.[24] Only the renal artery was cannulated, with the renal vein left open to drain into the reservoir via the perfusion chamber. Urine was collected and output replaced with Hartmann's solution. NMP was undertaken at a temperature of 37°C, with flow rates adjusted to maintain at a mean arterial pressure (MAP) of 75-85 mmHg.

To provide a direct comparison between CS and NMP in the absence of the ability to transplant these kidneys, *ex vivo* reperfusion with whole blood was undertaken in paired kidneys to simulate transplantation. This system utilizes whole blood containing leukocytes, complement, and other inflammatory mediators; furthermore, the protective verapamil infusion was omitted. *Ex vivo* whole blood reperfusion was undertaken at a MAP of 85-95 mmHg (maintained by flow adjustment), at 37°C for 60 minutes, after a simulated second warm ischemic ('anastomotic') time of 30 minutes during which the kidney was left at room temperature. Perfusion parameters (pressure and flow) and urine output (UO) were sequentially recorded during NMP and whole blood reperfusion.

7.3.7 PERFUSION EXPERIMENTS

(i) Single kidneys (n = 7) underwent NMP for 1-3 hours. These kidneys were used to (a) establish NMP system feasibility, functionality, and safety; (b) compare NMP using

autologous and banked blood; and (c) investigate leukocyte extravasation from the graft during NMP.

(ii) Paired kidneys (n = 8, i.e. 4 kidney pairs) were randomly allocated to either the cold static storage ('CS') or 'NMP' groups. 'CS' kidneys underwent standard CS, a subsequent 30 minute simulated SWIT period at room temperature, and then *ex vivo* whole blood reperfusion for 60 minutes to simulate the immediate post-transplant reperfusion period. 'NMP' kidneys underwent CS, followed by one hour of NMP, a simulated SWIT of 30 mins, and finally *ex vivo* whole blood reperfusion for 60 minutes (using the initial NMP circuit set-up).

7.3.8 SAMPLES

Sequential kidney biopsies were taken at the end of CS, after each hour of NMP (if applicable), and at the end of *ex vivo* whole blood reperfusion (if applicable). Biopsy samples were stored in 10% natural buffered formalin, RNALater solution (Ambion/Thermo Fisher Scientific, TX, USA), or snap frozen in dry ice with or without OCT media (Tissue-Tek, ProSciTech, Australia), for subsequent analyses. Blood samples were also taken from the circuit and the start and end of perfusion, as applicable, and sent to the hospital laboratory for quantification of hemoglobin, white cell counts, platelet counts, hematocrit, electrolytes, urea, creatinine, blood sugar level, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), albumin, and osmolality. Arterial and venous blood gas samples were taken during the start and end of perfusion, and analysed for lactate, pH, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2), bicarbonate (HCO_3), and base excess (BE) using the i-STAT Alinity machine (Abbott, IL, USA). Urine samples were taken at the end of perfusion and analysed for electrolyte, creatinine, and protein levels.

7.3.9 MEASUREMENTS AND ANALYSES

Renal blood flow (RBF) and intra-renal resistance (IRR = *MAP/RBF*)[1] was recorded throughout perfusion and normalized to a kidney weight of 250 grams. Urine output (UO) was recorded every hour of perfusion (ml). CrCl (ml/min/100g/hr) during NMP and *ex vivo* whole blood reperfusion was calculated using the following formula – (urine Cr (μmol/L) x urine volume (L))/plasma Cr (μmol/L). Fractional excretion of sodium (FeNa) (%) was calculated as – (100 x plasma Cr (μmol/L) x urine Na (mmol/L))/(plasma Na (mmol/L) x urine Cr (μmol/L)). Renal oxygen

consumption (mmHg*ml/min/g) at end-NMP or *ex vivo* reperfusion was determined using – [RBF (ml/min) x (PaO_2 – PvO_2) (mmHg)]/kidney weight (g).[25]

7.3.10 RENAL HISTOPATHOLOGY

All biopsies underwent Periodic Acid-Schiff (PAS) staining according to standard methods. Each pre- and post-NMP, and post-*ex vivo* whole blood reperfusion was assigned a Remuzzi score by a blinded renal histopathologist.[26] The following parameters were assessed – number of glomeruli; glomerular sclerosis (%); chronic damage (tubular atrophy/interstitial fibrosis; %); arteriolar hyalinosis (0 – absent; 1 – present); intimal elastosis (0 – absent; 1 – less than medial thickness; 2 – more than medial thickness); and extent of acute tubular injury (0 – absent; 1 – loss of tubular cell brush borders/vacuolization; 2 – cell detachment/casts; 3 – coagulation necrosis).

7.3.11 IMMUNOFLUORESCENCE

Renal tubular epithelial cell death was compared between paired kidneys (NMP versus CS pairs, using cryosections cut from samples taken at the end of *ex vivo* whole blood reperfusion) using TUNEL staining. A commercial *in situ* cell death detection kit was utilized for this purpose (Sigma-Aldrich/Merck, MO, USA). Slides were co-stained with DAPI (1:25,000) for 2 minutes. TUNEL staining was quantified using confocal microscopy.

Renal tissue oxidative stress was quantified and compared in paired NMP/CS samples using dihydroethidium (DHE) (Thermo Fisher Scientific), an indicator of tissue superoxide levels. Unfixed cryosections were thawed; DHE (10 μM) was applied to the surface of each section (incubated at 37°C for 22 mins). Slides were co-stained with DAPI as above. Confocal microscopy was utilized for visualization of DHE staining. Integrated densities were quantified using ImageJ software (National Institutes of Health, USA). Each section had 4 images taken, with mean densities for each image calculated from a further 4 regions of interest.

Complement (C9) staining was also performed in paired samples. Cryosections were fixed, blocked, and thence stained with C9 primary antibody raised in rabbits (1:250 dilution; Abcam, Cambridge, UK), and incubated for one hour at room temperature. This was followed by staining with goat anti-rabbit secondary antibody conjugated to Alexa Fluor 647 (1:400 dilution;

Invitrogen, CA, USA) for a further one hour at room temperature. DAPI co-staining was performed. Sections were visualized using confocal microscopy; C9 staining intensity was quantified using ImageJ software, with 4 regions of interest utilized for each section image.

7.3.12 FLOW CYTOMETRY ANALYSIS FOR LEUKOCYTE EFFLUENT FROM THE GRAFT DURING NMP

Blood samples were taken from the circuit at different time points (n = 3 kidneys) to analyze leukocyte extravasation from the graft. Samples were taken from the PRBC blood bag, and then at 'start' NMP (5 minutes after the commencement of NMP), one hour post-commencement of NMP, and 1.5 and 2 hours post-commencement of NMP. Briefly, samples were spun and equivalent amount of "PRBCs" were used for staining. 50 µL of the graft circuiting "PRBCs" and control baseline "PRBCs" were added into a Trucount tube (BD Biosciences) and blocked with pure Fc1.3070 (BD Biosciences), followed by staining with an antibody cocktail and cell lysis/fixation with BD FACS lysing solution (BD Biosciences) according to the manufacturer's instructions and as described previously.[27] Fluorochrome-coupled anti-human antibodies to CD45, CD3, CD11c, CD14, CD16, CD19, CD56, CD123, CD141, HLA-DR, lineage cocktail (CD3, CD14, CD19, CD20, CD56) (BD Biosciences), and CD303 (Miltenyi Biotec) were used. Potential dendritic cell detection was performed using the following markers: HLA-DR+CD3-CD14-CD19-CD20-CD11c+CD141 and/or HLA-DR+CD3-CD14-CD19-CD20-CD11c-CD303+CD123+. Flow cytometric analysis was performed on a BD-LSR Fortessa (BD Biosciences) and Diva software (BD Biosciences) for evaluation of absolute numbers of granulocytes, monocytes, NK cells, B cells, T cells, NKT cells, and dendritic cells. Data was analyzed using FlowJo V10.

7.3.13 RNA EXPRESSION BY NEXT-GENERATION SEQUENCING

Targeted whole transcriptome RNA expression[28, 29] was analyzed using paired kidneys undergoing NMP or CS alone, followed by *ex vivo* whole blood reperfusion. Kidney biopsies from each group were taken at end-CS, end-NMP (if applicable), and end-*ex vivo* reperfusion. RNA extraction was conducted using an ISOLATE II Mini-kit (Bioline Australia). For Ampliseq transcriptome analyses, libraries were prepared using Ion AmpliSeq Transcriptome Human Gene Expression Kit (Thermo Fisher Scientific) following the manufacturer's protocol using 10 ng of total RNA and quantified by qPCR with Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific). Libraries with concentration ranging from 1,515 pM to 6,629 pM were obtained and normalized to

100 pM. Seven to eight normalized libraries were pooled together, templated on the Ion Chef System, then sequenced on Ion 540 Chips using the Ion S5 XL system. Reads were aligned back to the manufacturer's supplied target reference with built in mapping software Tmap. The aligned data was TMM normalized using the edgeR package.[30]

7.3.14 STATISTICAL ANALYSES

Unless otherwise indicated, data is presented in the format mean ± standard deviation (SD). Continuous parametric variables were compared using the unpaired Student's t-test, whilst non-parametric continuous variables have been compared using the Mann-Whitney U test. The paired t-test was used for comparison of baseline and end-NMP data for each individual kidney, or functional data for each paired kidney at the end of *ex vivo* reperfusion. RBF and IRR graphs were compared by first calculating the area under the curve (AUC) for each parameter plotted on the graph. GraphPad Prism v. 7.02 was used for all of these statistical analyses. For all data comparisons, a p-value <0.05 was considered as statistically significant.

Differential expression analysis was performed using voom.[31] For all comparisons, changes in gene expression were deemed significant if they had a Benjamini-Hochberg adjusted p-value < 0.05. Pathway analysis was performed using a hypergeometric test to test if any Gene Ontology or Reactome categories were enriched for differentially expressed genes.[32-34] Wilcoxon-rank-sum tests with directional alternative hypotheses were used on the test statistics to test if any of the pathways were significantly up or downregulated. Further pathway analyses were conducted through the use of Ingenuity Pathway Analysis (IPA) (Qiagen Inc.).[35]

7.4 Results

7.4.1 RENAL HISTOLOGY, HEMODYNAMICS AND URINE OUTPUT DURING NORMOTHERMIC MACHINE PERFUSION (NMP)

Fifteen discarded and/or non-utilized kidneys from 10 human donors were obtained. Donor and perfusion characteristics are summarized in Table 1; also see Supplemental Digital Content (SDC) 1 for images of each kidney before and during perfusion. Eleven kidneys underwent NMP for 1-3 hours as defined in the methods. Four of these 11 kidneys underwent NMP followed by simulated transplantation using *ex vivo* reperfusion with whole blood, whilst their direct pairs had CS alone followed by *ex vivo* whole blood reperfusion. There were no significant changes with respect to the renal tubular pathology when assessed by light microscopy during 1-3 hours of NMP (SDC 2).

Table 1. Donor and perfusion characteristics.

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8	Donor 9	Donor 10
Donor Type	DBD	DCD	DBD	DCD	DCD	DBD	DBD	DBD	DCD	DBD
Study Code	DBD-D1	DCD-D1	DBD-D2	DCD-D2 ('Pair 1')	DCD-D3	DBD-D3 ('Pair 2')	DBD-D4 ('Pair 3')	DBD-D5	DCD-D4	DBD-D6 ('Pair 4')
n, Kidneys	1*	1	2	2	1	2	2	1	1	2
Reason for Discard	SI neuro-endocrine tumor; liver lesions (?cause)**	Poorly perfused at retrieval^^	Liver-only donor^	Declined by all recipient centers (high KDPI)	Declined by all recipient centers (considered unsuitable)***	Liver-only donor^^^^	Ischemic large bowel with contamination of peritoneal cavity (pus)	Physically small kidney in the context of older/ECD donor	Patchy perfusion superior pole left kidney^^	Declined by all recipient centers (high KDPI)
Donor COD	ICH/CVA	ICH/CVA	ICH/CVA	ICH/CVA	Liver failure	ICH/CVA	Cerebral ischemia after VT/VF arrest (?cause)	Cerebral ischemia after exacerbation of asthma & arrest	ICH/CVA	ICH/CVA
KDPI (%)	96	89	52	98	83	98	35	73	64	100
CIT (mean), hr	13.5	7.9	8.6	9.6	26.2	15.1	12.6	60.1^^^	7.5	9.4
WIT, min[φ]	NA	47 (6)	NA	26 (12)	42 (11)	NA	NA	NA	28 (17)	NA
Comparison	NMP only	NMP only	NMP only	NMP vs CS	NMP only	NMP vs CS	NMP vs CS	NMP only	NMP only	NMP vs CS[†]
NMP Duration, hr	3	2	1-2	1	2	1	1	2	1.5	1
Ex Vivo Reperfusion Duration, hr	NA	NA	NA	1.5	NA	1	1	NA	NA	1
Blood Used	Autologous	Autologous	Autologous	Banked (O+)	Banked (O-)	Banked (O+)	Banked (O+)	Banked (O+)	Banked (O-)	Banked (O+)

CIT – cold ischemic time; COD – cause of death; CS – cold static storage; CVA – cerebrovascular accident; DBD – donation after brain death; DCD – donation after circulatory death; Hx – history; HTN – hypertension; ICH – intra-cerebral hemorrhage; KDPI – kidney donor profile index; NA – not applicable; NMP – normothermic machine perfusion; SI – small intestine; VT/VF – ventricular tachycardia/fibrillation; WIT – warm ischemic time

* Both kidneys obtained for research however 2nd kidney not perfused as majority of parenchyma (90%) consisted of cystic tissue

** Frozen section of liver lesions equivocal, however clinically consistent with melanoma liver metastases

*** Declined due to donor hepatorenal syndrome (26 offers made to recipient centers), however contralateral kidney was accepted and transplanted

^ Kidneys considered unsuitable due to elevated donor creatinine (207 µmol/L [2.3 mg/dL]) and proteinuria, although note KDPI was only 52

^^ Contralateral kidney well-perfused and transplanted

^^^ NMP delayed due to logistical reasons

^^^^ Kidneys not considered due to donor comorbidities/KDPI, in addition to low donor eGFR (30-40 ml/min/1.73 m^2)

$^{\varphi}$ Time from extubation/withdrawal of life support to cold perfusion (time from cessation of circulation to cold perfusion in brackets)

† Both kidneys had upper pole arteries; upper pole artery of left ('CS') kidney was divided at retrieval, and as such the upper pole artery of the right kidney ('NMP') was similarly ligated prior to NMP to make both kidneys more comparable

Hemodynamics during NMP generally indicated a rise in renal blood flow (RBF) and decline in intra-renal resistance (IRR) over time, with the exception of one kidney (DCD-D3) (Fig. 1A). Median RBF and IRR after one hour was 260 ml/min/250g (range 172-359) and 0.29 mmHg/ml/min/250g (range 0.23-0.45), respectively. The median hourly urine output (UO) was 21 ml (range 0-46 ml); only one kidney did not produce any urine (DCD-D4) (Fig. 1B; also see explanation in figure caption). Fig. 1B provides a graphical depiction of UO, creatinine clearance (CrCl) and fractional excretion of sodium (FeNa). Visually it can be seen that UO was generally positively correlated with creatinine clearance (CrCl) and inversely related to fractional excretion of sodium (FeNa), although these relationships were not absolute (Fig. 1B).

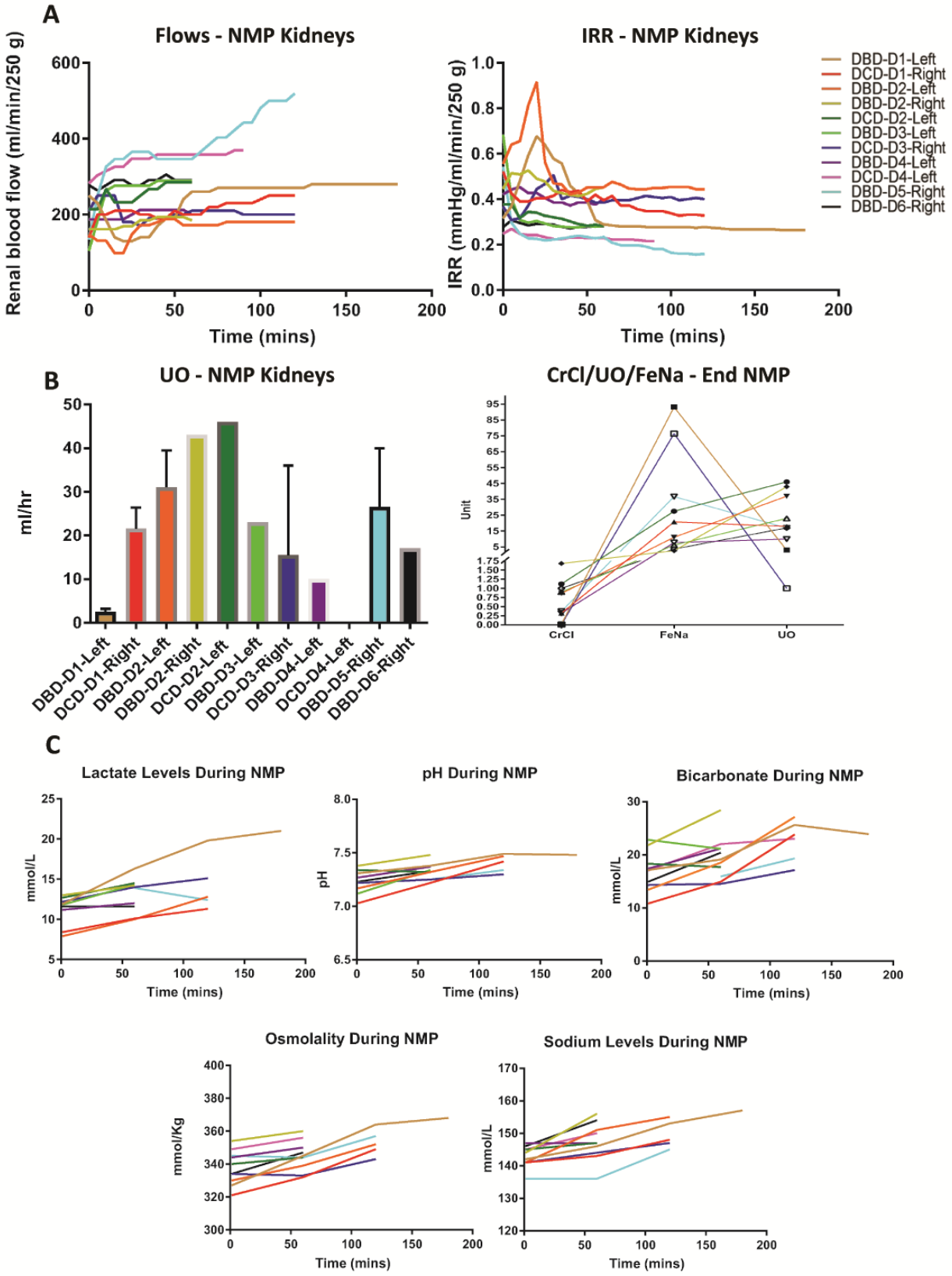


Figure 1. Perfusion, functional, and biochemical parameters and changes during NMP. (A) Renal blood flow and intra-renal resistance (IRR) during NMP, with a MAP maintained between 75-85 mmHg. (B) LEFT PANEL – Urine output (UO) per hour of NMP for each donor kidney. RIGHT PANEL – Relationship between UO, creatinine clearance (CrCl), and fractional excretion of sodium (FeNa) in each donor kidney. (C) Perfusate acid-base balance (pH, lactate,

and bicarbonate) and electrolyte (sodium, and osmolality) concentrations over the course of NMP, plotted for each individual kidney. n.b. Although there was no UO recorded from the DCD-D4 kidney during NMP, perfusate creatinine levels dropped over the course of perfusion (997 µmol/L at baseline → 633 µmol/L after 60 min NMP → 585 µmol/L after 90 min NMP). This indicates either (i) an abnormality related to ureteric function/vermiculation (unlikely as the ureter was not significantly distended during perfusion), or (ii) urine leak into the perfusion chamber from an unidentified site (more likely).

7.4.2 ISCHEMIC TIMES CORRELATE WITH PERFUSION PARAMETERS DURING NMP IN DCD BUT NOT DBD KIDNEYS

NMP is likely to have differential impacts and characteristics in DCD and DBD kidneys depending on the severity of the ischemic insult. In particular, it is useful to gain an understanding of RBF and IRR during NMP and establish potential correlations between perfusion parameters and ischemic times to provide a more objective graft functional assessment prior to transplantation. This also provides an important baseline against which potential therapeutics delivered to the kidney during NMP can be tested. SDC 3 plots each donor kidney's RBF and IRR, split by donor type (DCD or DBD) and arranged according to ischemic time. DCD kidneys with a lower WIT/CIT demonstrated an elevated RBF (median 328 ml/min/250g; range 286-370) and lower IRR (median 0.26 mmHg/ml/min/250g) in comparison to the two kidneys with a higher WIT and CIT (median RBF 205 ml/min/250g [range 200-210] and median IRR 0.41 mmHg/ml/min/250g [range 0.39-0.42]; SDC 3A). In contrast, DBD kidneys showed no obvious correlation between CIT and RBF or IRR; indeed, the two kidneys with the greatest CITs had comparatively better perfusion parameters during NMP (SDC 3B). A correlation between perfusion parameters and KDPI could not be established in either DCD or DBD donor subset.

7.4.3 LACTATE IS NOT CLEARED DURING BRIEF (1-3 HOURS) RENAL NMP

Lactate clearance can be used as a biomarker that defines effective perfusion and/or suitability for transplantation during NMP of other organs such as the liver and heart.[5, 36, 37] Lactate levels did not decline after brief NMP in this series, but in fact increased significantly from baseline (11.2 mmol/L) to 60 minutes (13.1 mmol/L; p = 0.002; Fig. 1C). However, a rising lactate was not indicative of acidemia in the perfusate, and a general uptrend was observed with respect to perfusate pH (7.23-7.33; p = 0.003) and bicarbonate levels (16.8-19.4 mmol/L; p = 0.009; Fig. 1C). Furthermore, there was an observed increase in perfusate sodium levels after 60 minutes of

NMP (143-148 mmol/L; p = 0.007), and a corresponding elevation in osmolality (338-345 mmol/kg; p = 0.004) (Fig. 1C).

7.4.4 LEUKOCYTES ARE IMMEDIATELY MOBILIZED FROM THE GRAFT INTO THE CIRCUIT DURING NMP

Perfusion fluid samples were taken prior to and at defined intervals after the commencement of NMP from donors 8-10, and then analyzed using flow cytometry. A significant efflux of leukocytes (CD45+) was detected in all tested samples within 2-3 minutes of commencement of NMP ("start NMP" samples). Dendritic cells (markers defined in methods) were not detectable by our methods at any time-point. However, large populations of granulocytes ($CD45_{low}SSC_{high}$) were detected, along with smaller populations of monocytes (CD14+), T (CD3+) and B-lymphocytes (CD19+), and NK cells (CD56+) (Fig. 2).

7.4.5 NMP UNDER SPECIAL CIRCUMSTANCES – KIDNEYS WITH MULTIPLE VESSELS, AND KIDNEYS DISCARDED DUE TO POOR IN SITU PERFUSION AT RETRIEVAL

NMP can be safely and effectively performed in kidneys with more than one artery, including more than one artery on a patch and/or separate upper or lower pole arteries (Fig. 3A-B). Back-table arterial reconstruction is not required, and perfusion is facilitated by the use of Y-connectors attached to separate cannulae.

NMP can also be utilized to assess and/or predict adequacy of renal perfusion in kidneys discarded due to poor *in situ* perfusion post-retrieval from the deceased donor (Fig. 3C). Two kidneys in this series (from donor 2 and donor 9) were discarded due to poor perfusion at retrieval in the context of DCD donation. In both cases, NMP 'cleared' the non-perfused region(s) within 10 minutes of commencement, thereby rendering these kidneys potentially transplantable.

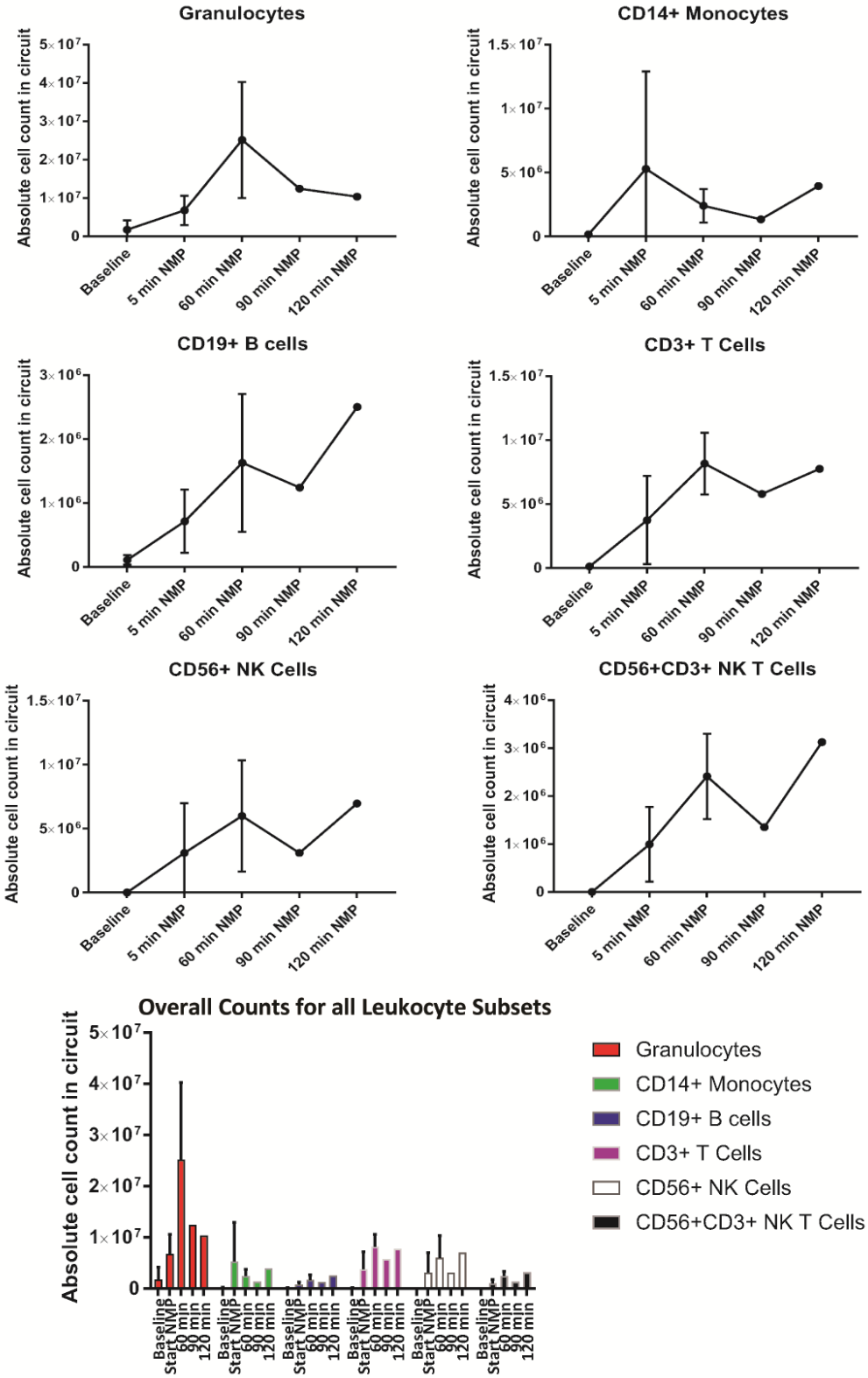


Figure 2. Leukocyte efflux from the donor kidney during NMP. Absolute cell counts for granulocytes, monocytes, NK cells, and lymphocytes were calculated by flow cytometry using the pre-perfusion sample as a baseline, followed by NMP arterial line sampling at selected time points. n = 3 kidneys. n.b. No dendritic cells were detected.

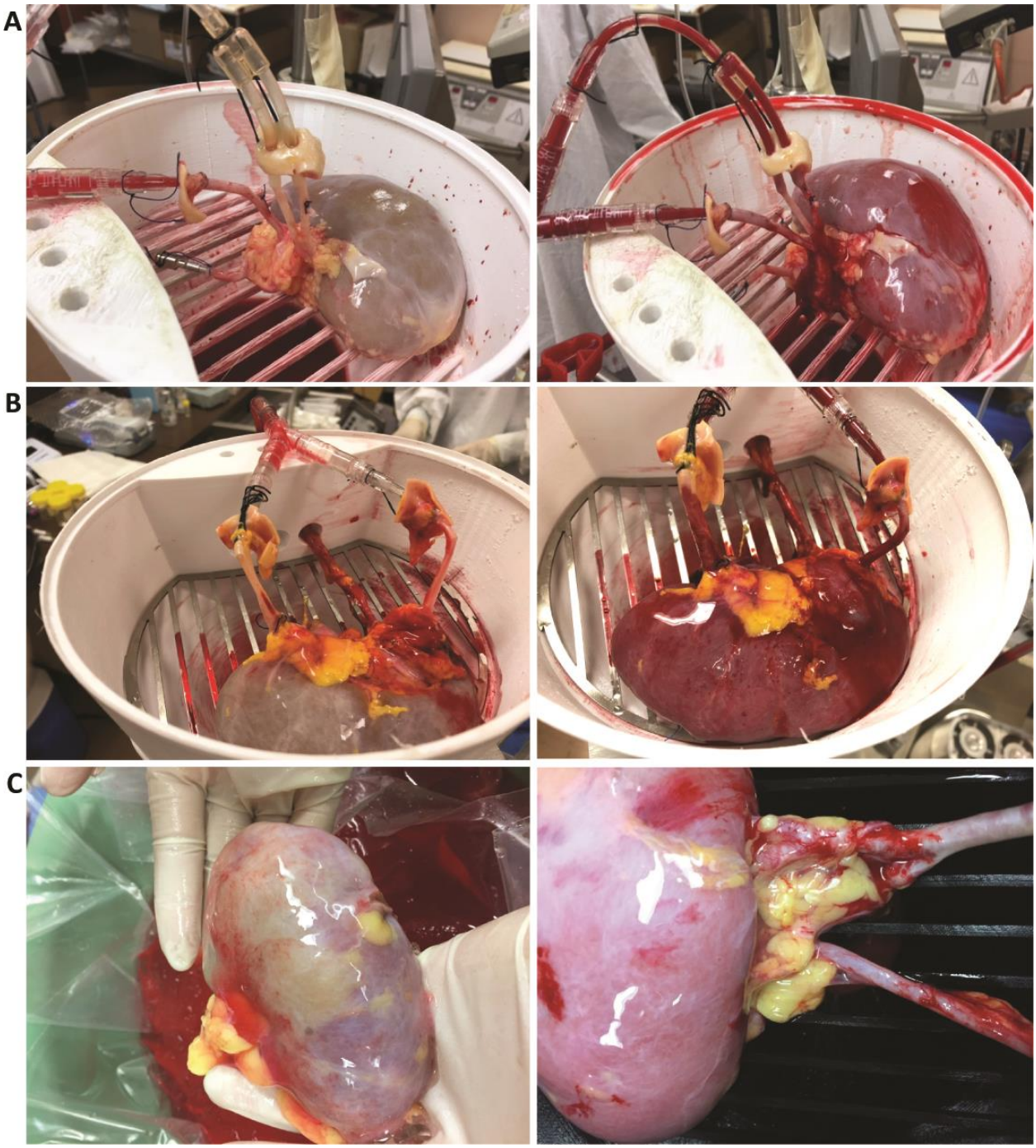


Figure 3. NMP is possible in the presence of multiple arteries, and is a very useful tool in poorly perfused kidneys. (A-B) NMP is feasible and safe for kidneys with multiple renal arteries, achieving good renal blood flows and intra-renal resistance. Kidneys shown are from (A) donor 7 (DBD-D4) and (B) donor 8 (DBD-D5). (C) NMP is ideal for the assessment of kidneys discarded for poor *in situ* perfusion. LEFT PANEL – Kidney (donor 2; DCD-D1) visualized at end-cold storage, discarded due to a non-perfused lower pole and patchy middle region. RIGHT PANEL – The lower pole of the same kidney is pictured 5 minutes after the commencement of NMP, showing complete resolution of the previously non-perfused area.

7.4.6 DONOR AUTOLOGOUS PACKED RED BLOOD CELLS (PRBCS) CAN BE UTILIZED FOR NMP

Banked blood transfusions (allo-) may increase the risk of allosensitization in kidney transplant recipients; the feasibility of using autologous blood for NMP was therefore explored.[38, 39] Autologous whole blood was collected from 3 donors, and PRBCs were subsequently isolated for the perfusion of 4 kidneys; all other kidneys underwent NMP using banked blood group O blood (Table 1). There were no significant baseline differences with respect to KDPI (median 89 versus 83; p = 0.783) and CIT (median 8.9 versus 11.7 hours; p = 0.226) in kidneys perfused with autologous or banked blood, respectively. Baseline blood parameters for both autologous and banked blood are outlined in SDC 4. Hemoglobin levels (43.8 versus 65.3 g/L; p = 0.094) and hematocrit (14.3% versus 20.7%; p = 0.150) were lower in the autologous blood group, and the white cell count was higher (0.15 x 10^9/L versus 0.06 x 10^9/L; p = 0.071), despite using identical volumes of PRBCs, but this did not reach statistical significance; platelet counts were however statistically lower in the banked blood group (45.0 x 10^9/L versus 0.9 x 10^9/L; p < 0.001). Perfusate potassium levels showed a trend towards an increase in the banked blood group (5.4 versus 7.6 mmol/L; p = 0.107); there were no differences in sodium (142.0 versus 143.9 mmol/L; p = 0.404) and bicarbonate (13.0 versus 14.3 mmo/L; p = 0.491) concentrations.

SDC 5 compares NMP parameters between both groups after 60 minutes of NMP. Although AUC for RBF was significantly higher (p = 0.005), and IRR significantly lower (p = 0.001), in the banked blood perfusion group, these differences are unlikely to be clinically meaningful. There were no significant differences with respect to glomerular (CrCl 0.7 vs. 0.5 ml/min/100g/hr; p = 0.610) or tubular function (FeNa 31.9 versus 26.4%; p = 0.806), or renal oxygen consumption (298.0 versus 381.7 ml/min/g; p = 0.330) in the autologous compared to banked blood groups, respectively (SDC 5). Perfusate LDH levels showed a higher trend in the autologous blood group but this was not significant (p = 0.125), while AST levels were significantly lower in the group perfused with banked blood (121.8 versus 49.2 U/L; p = 0.039).

7.4.7 NMP INDUCES GENE EXPRESSION CHANGES INVOLVING INFLAMMATORY, STRESS, CELL DEATH, AND SURVIVAL-RELATED PATHWAYS

Targeted whole transcriptome RNA expression was performed, comparing paired kidneys treated with CS alone or NMP after a period of CS (n = 3 pairs – pair 1-3, identified in Table 1). Samples

were taken at the end of CS ('end-CS'), after NMP (if applicable; 'end-NMP'), and after simulated transplantation ('end-*ex vivo*'). Simulated transplantation allowed for a direct comparison between CS and NMP kidneys, and involved *ex vivo* reperfusion with whole blood at a MAP of 80-90 mmHg, without the addition of any protective mediators. The SWIT ('anastomoses') was approximated by leaving each kidney at room temperature for 30 minutes prior to reperfusion. The principal component analysis (PCA) plot revealed unique population clusters, defined by donor kidney pair (SDC 6). Within each donor kidney pair, end-CS samples were clustered close together, whilst the end-*ex vivo* samples were distinctly different (SDC 6). Importantly, at baseline (i.e. end-CS) within the kidney pairs there were no statistically significant differentially expressed genes between the NMP and CS groups (data not shown).

One hour of NMP induced multiple gene expression changes. In comparison to biopsies taken at the end of CS, NMP in the same kidneys modified expression of 200 genes (n = 196 were significantly upregulated, and n = 4 were down-regulated) (Fig. 4A). A total of 115 pathways were significantly enriched for differentially expressed genes. The most differentially up and down-regulated genes and pathways are also indicated in Fig. 4A, whilst SDC 7 outlines expression patterns for all genes and pathways. *Ex vivo* whole blood reperfusion (simulated transplantation on the circuit using whole blood) produced distinctly different gene expression changes (65 genes) in comparison to those induced by NMP (Fig. 4B, and SDC 8). This therefore indicates the technique is a valid simulation for transplantation that does not merely recapitulate changes induced by the NMP process. These gene expression changes were not evident in the CS group of kidneys after *ex vivo* whole blood reperfusion, as shown in the scatter plots displayed in Figs. 4-5.

After simulated transplantation, paired kidneys subjected to either NMP or CS alone displayed highly disparate gene signatures characterized by the differential expression of 495 genes (435 up- and 60 down-regulated, respectively) (Fig. 5A). These are indicated in the scatter plot displayed in Fig. 5A. A full list characterizing gene expression and pathway changes is provided in SDC 9. The top 20 (plausible) pathways that were significantly impacted by NMP as determined by Ingenuity Pathway Analysis (IPA) are summarized in Fig. 5B, ordered based on the –log(p-value). Diseases/functions activated or repressed by NMP in comparison to CS alone, as predicted by IPA based on differential gene expression profiles, are outlined in SDC 10-11. Overall, the signatures

revealed were strongly consistent with a decrease in cell death and apoptosis in NMP kidneys, with a corresponding increase in cell survival, viability, and proliferative functions.

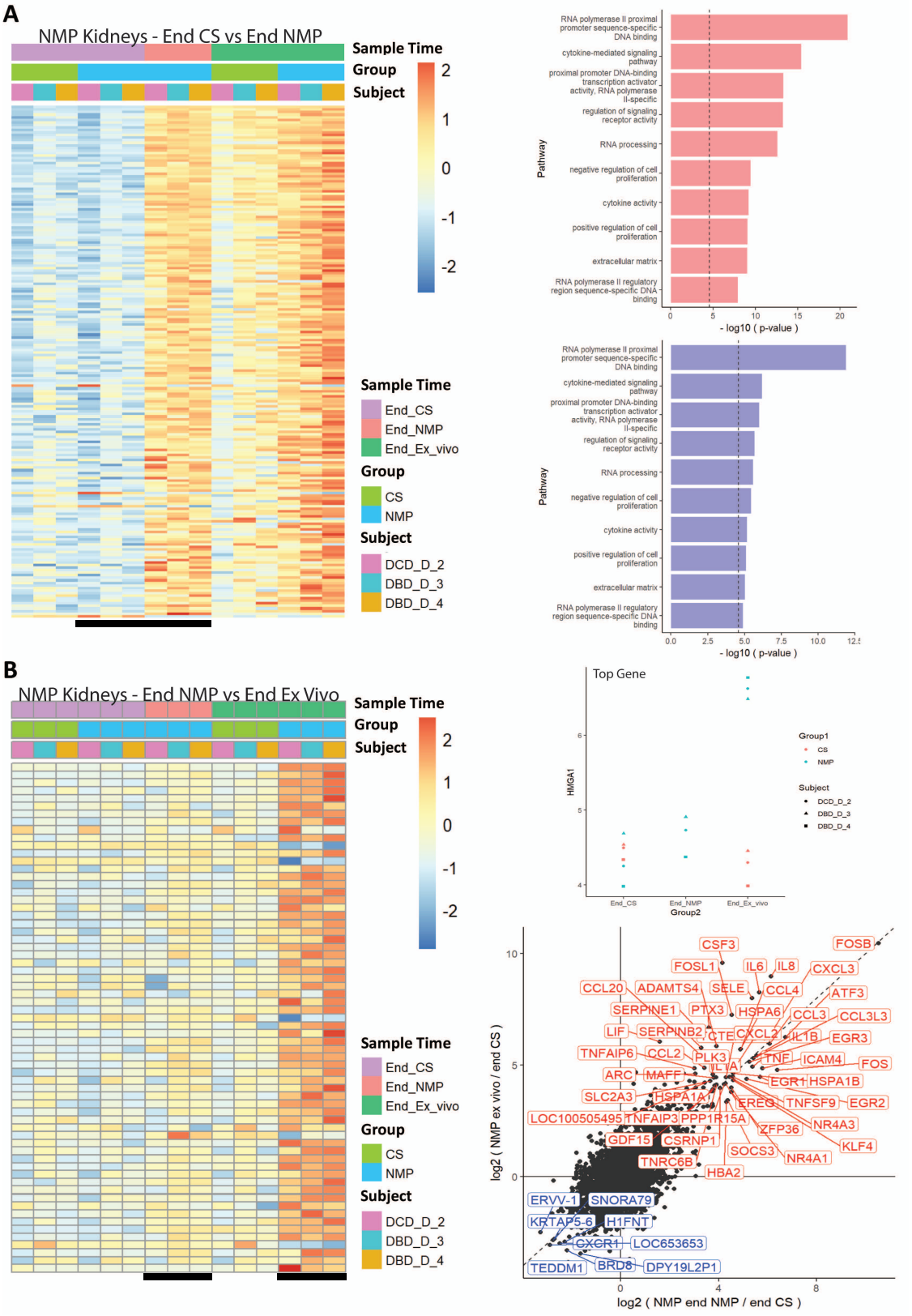


Figure 4. Whole transcriptome RNA sequencing in kidneys that underwent NMP. Sequential biopsies were taken immediately prior to the commencement of NMP (end-CS), after one hour of NMP (end-NMP), and after one hour of simulated transplantation (end-*ex vivo*). (A) LEFT PANEL – Gene expression changes (heatmap) after NMP in comparison to the end-CS period (for the same kidneys). RIGHT PANELS – Pathway analyses, displaying the most up- (TOP) and down-regulated (BOTTOM) pathways after NMP. (B) LEFT PANEL – Heatmap showing differentially expressed genes in the NMP group of kidneys after *ex vivo* whole blood reperfusion (in comparison to the end-NMP samples from the same kidneys). RIGHT PANELS – (TOP) HMGA1 is the most differentially expressed gene between end-NMP and end-*ex vivo* samples. (BOTTOM) Scatter plot showing the most up- and down-regulated genes after *ex vivo* reperfusion (simulated transplantation) in comparison to end-NMP samples from the same kidneys. Relevant comparator columns are indicated by the dark black lines.

7.4.8 ONE HOUR OF NMP ENHANCES RENAL HEMODYNAMICS AND FUNCTION, AND AMELIORATES ISCHEMIA-REPERFUSION INJURY (IRI) IN COMPARISON TO CS KIDNEYS

Over the period of *ex vivo* whole blood reperfusion, RBF was greater, and IRR was lower, at most time points in NMP compared to paired CS kidneys (Fig. 6A). RBF and IRR at the one hour time point after *ex vivo* reperfusion in the NMP and CS pairs respectively was 250.3 ± 79.7 ml/min/250g versus 152.1 ± 138.8 ml/min/250g ($p = 0.175$), and 0.4 ± 0.1 mmHg/ml/min/250g versus 0.9 ± 0.6 mmHg/ml/min/250g ($p = 0.137$). Aggregated (AUC) RBF was significantly higher ($p = 0.023$) and IRR was significantly lower in the NMP-'treated' kidneys ($p = 0.009$).

Paired comparisons of other functional parameters and injury markers were also performed (Fig. 6B), and showed a significantly better (lower) FeNa and perfusate AST in the NMP group ($p = 0.034$ and $p = 0.043$, respectively). There were strong trends favoring NMP over CS kidneys with respect to CrCl, oxygen consumption, and UO, although these did not reach statistical significance.

Furthermore, renal tubular epithelial cell death, as measured by TUNEL staining, was significantly ameliorated in the NMP-treated kidneys after simulated transplantation (5.9 versus 9.6 TUNEL-positive cells/HPF; $p < 0.001$); Fig. 7A). Similar significant trends were seen with respect to oxidative stress (quantified using integrated density of DHE staining; $p = 0.022$; Fig. 7B), and complement activation (measured by integrated density of complement C9 staining; $p = 0.002$; Fig. 7C). Comparative histologic sections (PAS stain) from one donor pair (DBD-D3 – 'Pair 2') are shown in Fig. 7D. Overall, there were no significant differences with respect to acute tubular injury following *ex vivo* whole blood reperfusion, regardless of the initial treatment (SDC 12).

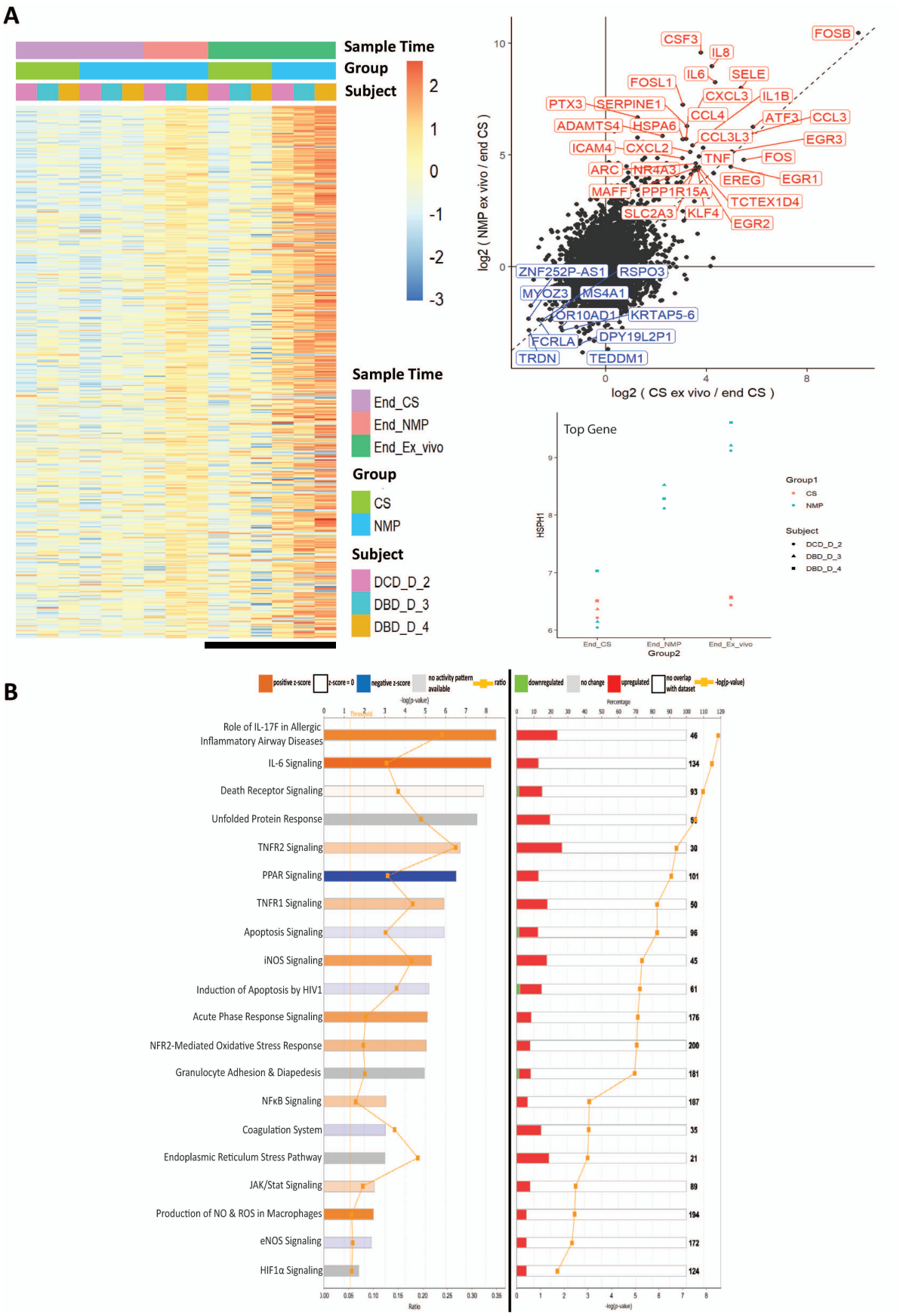


Figure 5. Whole transcriptome RNA sequencing in kidney pairs that underwent one hour of NMP following CS in comparison to CS alone. Comparisons were conducted after a simulated second warm ischemic period of 30 mins

and then reperfusion of each kidney with whole allogeneic blood at a MAP of 85 mmHg and temperature of 37°C. (A) LEFT PANEL – Gene expression heatmap comparing paired kidneys having CS or NMP, after *ex vivo* whole blood reperfusion. RIGHT PANELS – (TOP) Scatter plot outlining the most significantly up- and down-regulated genes in the NMP group in comparison to CS paired kidneys (relevant columns indicated by dark black lines). (BOTTOM) Most differentially expressed gene between NMP and CS kidneys (HSPH1), with an obvious difference in expression at the end-*ex vivo* time point for all 3 kidney pairs. (B) Ingenuity pathway analyses (IPA) showing top canonical pathways significantly up- or down-regulated in the NMP group of kidneys in comparison to kidneys having CS alone. Pathways are ordered by magnitude of –log(p-value). LEFT PANEL – Indication of pathway activation or repression based on the z-score, which gives an indication of the non-randomness of pathway directionality. A positive z-score suggests pathway induction, and a negative z-score denotes pathway suppression. RIGHT PANEL – Percentage (indicated by colored bars) of total number of genes (indicated by numbers to right of bars) in a specific pathway that are differentially expressed in NMP compared to CS kidneys.

7.5 Discussion

Normothermic machine perfusion prior to kidney transplantation presents a paradigm shift in the preservation of deceased donor grafts, with the potential to simultaneously recondition and objectively assess the donor kidney. We present the largest series of discarded human kidney NMP outside of the UK, and demonstrate many novel findings that should help motivate translation of this technique to clinical transplantation. In particular, using a paired kidney design and simulated transplantation, we show that brief (one hour) NMP is superior to CS alone, as evidenced by enhanced early perfusion parameters, glomerular and tubular function, and amelioration of IRI. Transcriptome-wide sequencing demonstrated activation of protective stress-related responses, together with promotion of cell survival and proliferation. The existing potential of NMP to objectively assess renal allografts and reduce discard rates through assessment perfusion-related parameters was confirmed. We also demonstrated the feasibility of using autologous (donor) blood during NMP compared to the use of 3rd party (banked) blood. Finally, we showed a massive efflux of passenger leukocytes from the donor kidney into the NMP circuit during perfusion, which may be targeted to modulate the acute rejection response in the recipient.

The attractiveness of brief pre-implantation NMP lies in its simplicity and the logistical advantages this method affords above continuous methods of NMP, which require perfusion during the whole transportation period. However, this technique remains experimental other than a single UK trial. One important reason for a reluctance of uptake by centers is a lack of understanding regarding the mechanistic benefits, if any, that can be offered by only one hour of

NMP. This is notwithstanding the clear benefits of reducing graft discard rates offered by brief NMP, especially in the setting of poorly perfused DCD kidneys, which was shown by Hosgood *et al*. and supported by the findings here.[18] Our unique study design comparing paired kidneys has allowed us to confidently explore the impacts of NMP without requiring large patient numbers. In particular, this design removes the confounding influences of different donor and recipient parameters, which contribute to variability in transplantation outcomes.

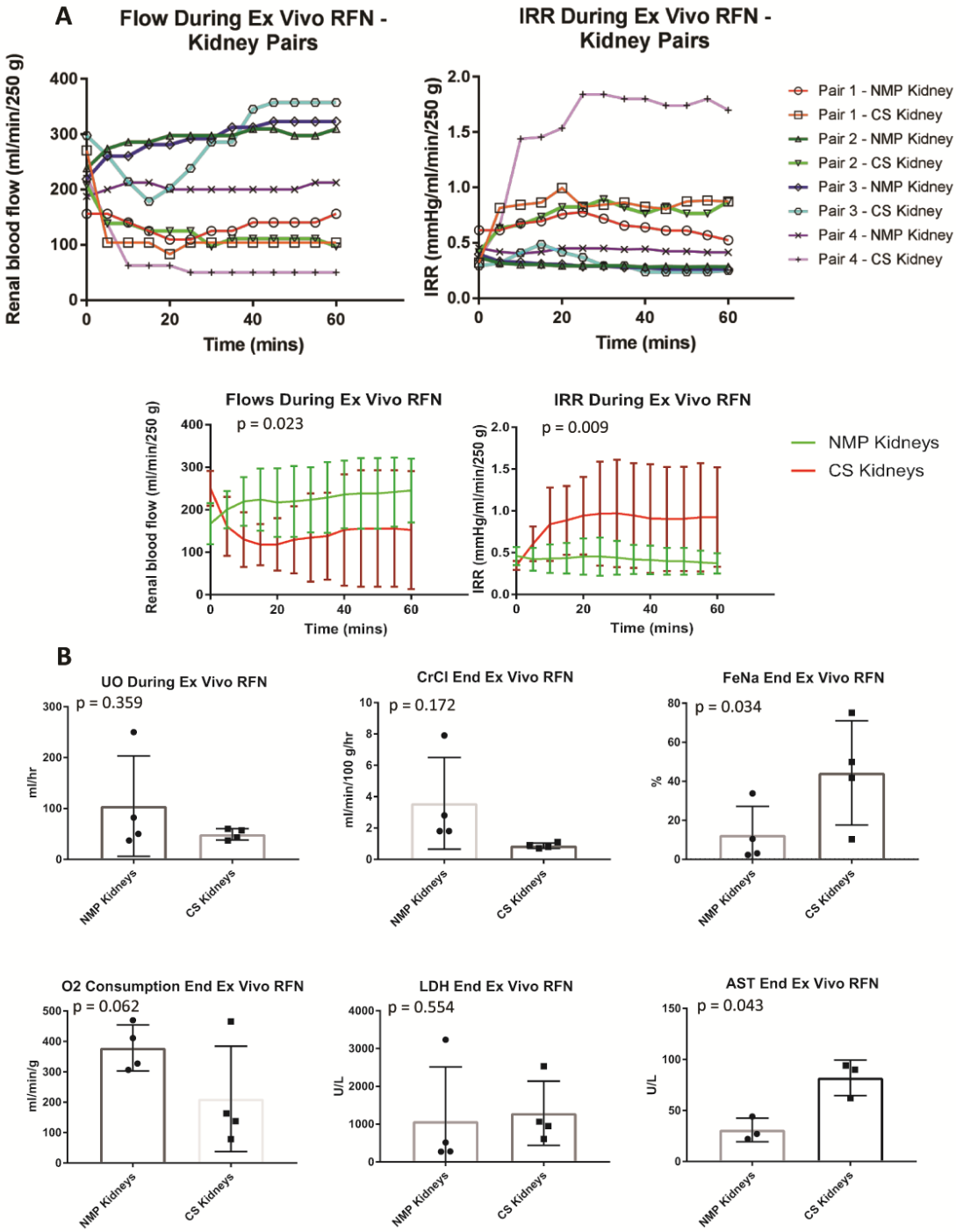


Figure 6. Perfusion and functional parameters (after simulated transplantation [whole blood reperfusion (RFN)]) in kidney pairs from the same donor, with 1 kidney having CS alone and the contralateral kidney undergoing CS followed by one hour of NMP. (A) UPPER PANELS – Renal blood flow and intra-renal resistance (IRR) graphed for each individual donor kidney. LOWER PANELS – Cumulative flow and IRR for the kidneys stored by CS alone in comparison to contralateral kidneys having CS followed by NMP. (B) Comparison of renal functional parameters between the two study groups after simulated transplantation – urine output (UO), creatinine clearance (CrCl), fractional excretion of sodium (FeNa), oxygen consumption, and perfusion fluid levels of lactate dehydrogenase (LDH) and aspartate aminotransferase (AST).

NMP kidneys displayed better perfusion and functional parameters in comparison to the CS pairs after simulated transplantation. Whole transcriptome sequencing demonstrated that a large number of genes were differentially expressed in kidney after NMP in comparison to CS controls. Important gene signatures included the significant upregulation of pro-inflammatory cytokines (including IL-6), chemokines, and heat shock proteins (HSPs). Interestingly, porcine studies by Nicholson and Hosgood in the UK have demonstrated increased expression of HSP-70 and IL-6 in kidney tissue after NMP, and Stone *et al*. demonstrated a general pro-inflammatory response during NMP.[15, 40, 41] NMP likely rejuvenates and/or conditions the kidney through induction of HSPs, in an ischemic preconditioning (IPC)-like response.[42-45] Additional pathway analyses were dominated by differential impacts of NMP on unfolded protein signaling responses (which is largely HSP-dependent), cell death/apoptosis-related cascades, and cell survival. These factors were demonstrated not only in pathway predictions, but confirmed by TUNEL, DHE, and complement staining, which were all significantly improved in NMP kidneys. Overall, the combination of gene expression data, pathway analyses, tissue staining, and finally *in vivo* renal function, provides a convincing picture of the beneficial impacts that may be attributed to brief pre-implantation NMP.

By itself, brief pre-implantation NMP is protective and beneficial to the graft, even after a period of CS. However, the potential capabilities of NMP extend far beyond this conditioning effect. Owing to the nature of NMP, the kidney is functional at a normal metabolic rate in oxygenated and normothermic conditions. This provides a unique opportunity to objectively assess the graft before implantation, and previous studies have shown a correlation between 12-month kidney transplant function and macroscopic kidney perfusion during NMP, in addition to total urine output, and renal blood flows achieved.[17, 18] Additional work has demonstrated a correlation between IRR

during NMP and transplant kidney function in porcine studies, and further attributed predictive value to perfusate pH, bicarbonate, AST, and lactate levels.[46] In contrast, lactate was not a good predictive factor in our study. Our study has also verified the relationship between UO, CrCl, and FeNa during NMP, and importantly demonstrates that ischemic time crucially impacts upon RBF and IRR in DCD kidneys.

Passenger leukocytes play a role in the initiation and regulation of the alloimmune response directed against the transplanted organ.[47, 48] Depletion of these leukocytes requires whole body/organ irradiation, which has variable success and is not feasible in the transplant setting.[49] Stone *et al*. demonstrated efflux of passenger leukocytes during NMP of both the porcine kidney and lungs.[50 41] We now demonstrate the efflux of substantial numbers of passenger leukocytes from human donor kidneys into the perfusion circuit during NMP, providing obvious therapeutic potential in an attempt to modulate rejection in the recipient. Leukocyte filters have been incorporated into lung perfusion systems to capture circulating leukocytes, but have uncertain efficacy, likely due to saturation of the filter.[51] Nevertheless, NMP provides the unique opportunity to deliver directed therapeutic targets to the kidney, which may include targeting of such leukocytes. Delivery of other agents that specifically target endothelial cells, ameliorate IRI, and/or attempt to modulate endothelial cell MHC antigen expression using gene therapies, have also been demonstrated by groups including our own.[52-54]

Existing renal NMP devices have differed in perfusion settings and constituents.[1, 3, 19, 20, 23] Therefore, parameters such as RBF, IRR, and UO may not be readily compared between different studies in terms of significance and predictive potential. Nevertheless, our use of NMP in discarded human kidneys enhanced RBF and IRR in all but one kidney, providing good predictive value for subsequent transplant graft function.[17] Furthermore, DCD kidneys used here that were discarded due to poor *in situ* perfusion after retrieval were homogenously and effectively perfused during NMP. Overall, NMP has a remarkable potential to reduce kidney discards and increase utilization rates, and this was also recently reflected in a liver NMP RCT.[5]

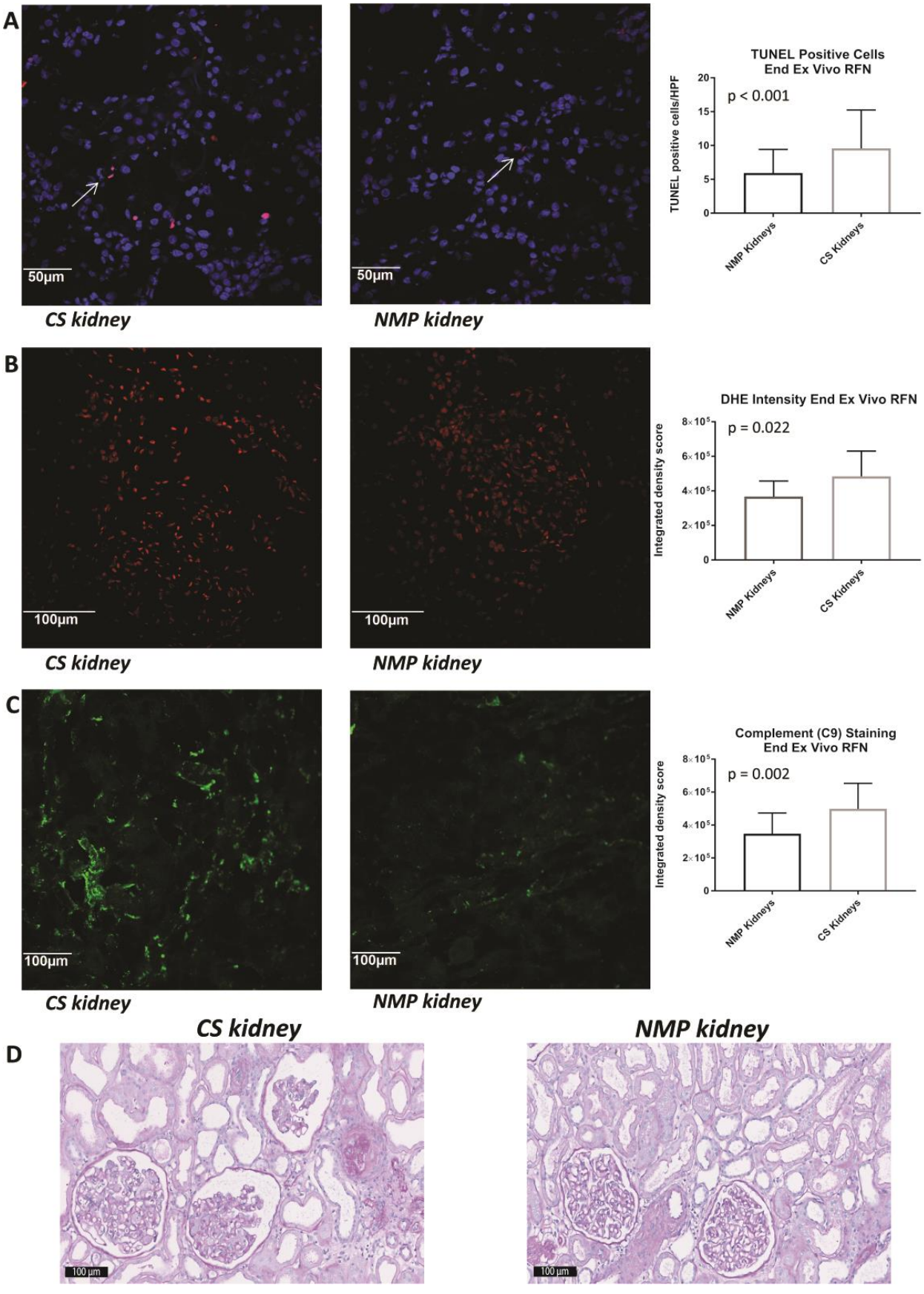

A
50µm
50µm
CS kidney
NMP kidney
TUNEL Positive Cells
End Ex Vivo RFN
p < 0.001
TUNEL positive cells/HPF
20
15
10
5
0
NMP Kidneys
CS Kidneys
B
100µm
100µm
CS kidney
NMP kidney
DHE Intensity End Ex Vivo RFN
p = 0.022
Integrated density score
8×10⁵
6×10⁵
4×10⁵
2×10⁵
0
NMP Kidneys
CS Kidneys
C
100µm
100µm
CS kidney
NMP kidney
Complement (C9) Staining
End Ex Vivo RFN
p = 0.002
Integrated density score
8×10⁵
6×10⁵
4×10⁵
2×10⁵
0
NMP Kidneys
CS Kidneys
CS kidney
NMP kidney
D
100 µm
100 µm

Figure 7. Histopathology and ischemia-reperfusion injury in kidney pairs having NMP or CS followed by simulated transplantation. (A) Representative photomicrograph (pair 2; DBD-D3) and cumulative comparison of renal cell death/apoptosis in both study groups as determined by TUNEL staining (40 x). Similar immunofluorescence-based comparisons of (B) oxidative stress (using DHE staining) (pair 3; DBD-D4), and (C) complement C9 staining (pair 2; DBD-D3) after *ex vivo* whole blood reperfusion (20 x). (D) Representative photomicrograph of a kidney pair (pair 2; DBD-D3) after simulated transplantation following either CS or NMP; periodic acid-schiff stain (20 x).

This study was wholly reliant upon the provision of discarded and/or non-utilized deceased donor human kidneys, and as such all study variables could not be controlled. In particular, depending on resource and staffing availability, not all factors (e.g. leukocyte efflux) could be tested for all kidneys. Although kidney numbers are relatively small (n = 15), we included more kidneys than other recent published discarded human NMP series.[20, 55] More importantly, direct comparisons of CS and NMP using paired kidneys from the same donor have added greater reliability to our results. Although final result validation requires kidney transplantation, *ex vivo* perfusion as a simulation of transplantation is an acceptable alternative when transplantation is not possible.[16, 25, 56, 57]

In summary, this study has utilized brief NMP of discarded human kidneys to provide the clearest insight to date with respect to the mechanistic basis and superiority of NMP to CS alone. Strength has been added to the notion that NMP can reduce kidney discard rates and therefore increase organ utilization in recipients.

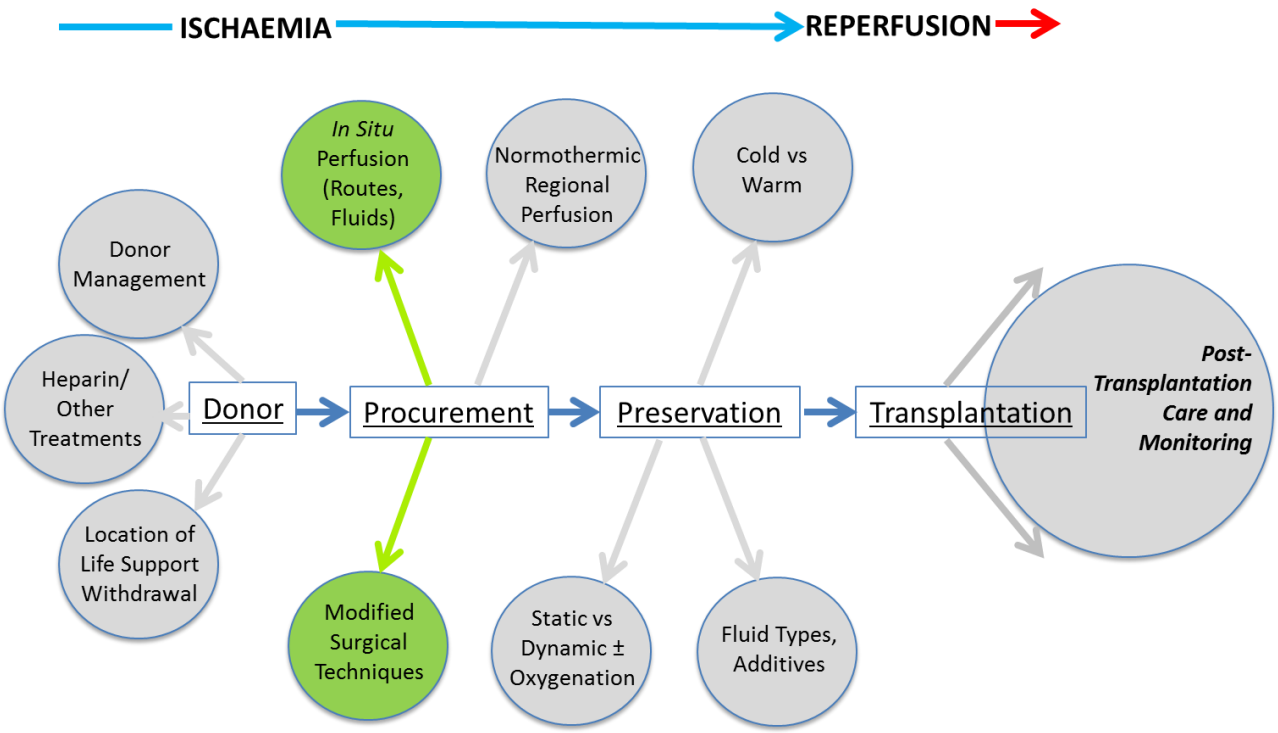
7.6 References

1. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013; 13: 1246-1252.
2. Hosgood SA, Thompson E, Moore T, Wilson CH, Nicholson ML. Normothermic machine perfusion for the assessment and transplantation of declined human kidneys from donation after circulatory death donors. *Br J Surg.* 2018; 105: 388-394.
3. Kaths JM, Echeverri J, Linares I, et al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant*. 2017; 17: 2580-2590.
4. Jochmans I, Nicholson ML, Hosgood SA. Kidney perfusion: some like it hot others prefer to keep it cool. *Curr Opin Organ Transplant*. 2017; 22: 260-266.
5. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018; 557: 50-56.
6. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2009; 360: 7-19.
7. Watson CJ, Wells AC, Roberts RJ, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant*. 2010; 10: 1991-1999.
8. Moers C, Varnav OC, van Heurn E, et al. The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. *Transplantation*. 2010; 90: 966-973.
9. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg*. 2010; 252: 756-764.
10. Summers DM, Watson CJ, Pettigrew GJ, et al. Kidney donation after circulatory death (DCD): state of the art. *Kidney Int.* 2015; 88: 241-249.
11. Mirshekar-Syahkal B, Summers D, Bradbury LL, et al. Local Expansion of Donation After Circulatory Death Kidney Transplant Activity Improves Waitlisted Outcomes and Addresses Inequities of Access to Transplantation. *Am J Transplant*. 2017; 17: 390-400.
12. Rege A, Irish B, Castleberry A, et al. Trends in Usage and Outcomes for Expanded Criteria Donor Kidney Transplantation in the United States Characterized by Kidney Donor Profile Index. *Cureus*. 2016; 8: e887-e887.
13. Garcia GG, Harden P, Chapman J. The global role of kidney transplantation. *Lancet*. 2012; 379: e36-e38.
14. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017; 7.
15. Hosgood SA, Patel M, Nicholson ML. The conditioning effect of ex vivo normothermic perfusion in an experimental kidney model. *J Surg Res.* 2013; 182: 153-160.
16. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg.* 2008; 95: 111-118.
17. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. *Br J Surg.* 2015; 102: 1433-1440.
18. Hosgood SA, Thompson E, Moore T, Wilson CH, Nicholson ML. Normothermic machine perfusion for the assessment and transplantation of declined human kidneys from donation after circulatory death donors. *Br J Surg*. 2018; 105: 388-394.

19. Kaths JM, Cen JY, Chun YM, et al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. *Am J Transplant*. 2017; 17: 957-969.
20. Weissenbacher A, Lo Faro L, Boubriak O, et al. Twenty-four-hour normothermic perfusion of discarded human kidneys with urine recirculation. *Am J Transplant*. 2018. *Epub ahead of print*; DOI: 10.1111/ajt.14932
21. TSANZ. A Guide to the Australian Kidney Donor Profile Index (KDPI) 2016. Available at: https://www.tsanz.com.au/standalonepages/documents/AustralianKDPIINFOv1.0.pdf. Accessed March, 2017.
22. OPTN. A Guide to Calculating and Interpreting the Kidney Donor Profile Index (KDPI). USA: The Organ Procurement and Transplantation Network, 2012.
23. Hameed AM, Miraziz R, Lu DB, et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ journal of surgery*. 2018; 88: E429-434.
24. Hameed A, Dervish S, Rogers N, Pleass H, Hawthorne W. A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transpl Int*. 2018. *Epub ahead of print*; DOI: 10.1111/tri.13361
25. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct*. 2017; 3: e140.
26. Remuzzi G, Cravedi P, Perna A, et al. Long-term outcome of renal transplantation from older donors. *N Engl J Med.* 2006; 354: 343-352.
27. Hu M, Wang C, Zhang GY, et al. Infiltrating Foxp3(+) regulatory T cells from spontaneously tolerant kidney allografts demonstrate donor-specific tolerance. *Am J Transplant*. 2013; 13: 2819-2830.
28. Perico L, Morigi M, Rota C, et al. Human mesenchymal stromal cells transplanted into mice stimulate renal tubular cells and enhance mitochondrial function. *Nat Commun*. 2017; 8: 983.
29. Goel S, DeCristo MJ, Watt AC, et al. CDK4/6 inhibition triggers anti-tumor immunity. *Nature*. 2017; 548: 471-475.
30. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010; 26: 139-140.
31. Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014; 15: R29.
32. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genet*. 2000; 25: 25-29.
33. GO Consortium. Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Res*. 2017; 45: D331-D338.
34. Fabregat A, Jupe S, Matthews L, et al. The Reactome Pathway Knowledgebase. *Nucleic Acids Res*. 2018; 46: D649-D655.
35. Kramer A, Green J, Pollard J, Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics*. 2014; 30: 523-530.
36. Watson CJE, Kosmoliaptsis V, Randle LV, et al. Normothermic Perfusion in the Assessment and Preservation of Declined Livers Before Transplantation: Hyperoxia and Vasoplegia—Important Lessons From the First 12 Cases. *Transplantation*. 2017; 101: 1084-1098.
37. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet*. 2015; 385: 2585-2591.

38. Obrador GT, Macdougall IC. Effect of Red Cell Transfusions on Future Kidney Transplantation. *Clin J Am Soc Nephrol*. 2013; 8: 852-860.
39. Leffell MS, Kim D, Vega RM, et al. Red Blood Cell Transfusions and the Risk of Allosensitization in Patients Awaiting Primary Kidney Transplantation. *Transplantation*. 2014; 97: 525-533.
40. Yang B, Hosgood SA, Bagul A, Waller HL, Nicholson ML. Erythropoietin regulates apoptosis, inflammation and tissue remodelling via caspase-3 and IL-1beta in isolated hemoperfused kidneys. *Eur J Pharmacol.* 2011; 660: 420-430.
41. Stone JP, Ball AL, Critchley WR, et al. Ex Vivo Normothermic Perfusion Induces Donor-Derived Leukocyte Mobilization and Removal Prior to Renal Transplantation. *KI Reports*. 2016; 1: 230-239.
42. Kume M, Yamamoto Y, Saad S, et al. Ischemic preconditioning of the liver in rats: Implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. *J Lab Clin Med.* 1996; 128: 251-258.
43. Konstantinov IE, Arab S, Li J, et al. The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. *J Thorac Cardiovasc Surg*. 2005; 130: 1326-1332.
44. Gassanov N, Nia AM, Caglayan E, Er F. Remote Ischemic Preconditioning and Renoprotection: From Myth to a Novel Therapeutic Option? *J Am Soc Nephrol.* 2014; 25: 216-224.
45. Das DK, Maulik N. Cardiac genomic response following preconditioning stimulus. *Cardiovasc Res.* 2006; 70: 254-263.
46. Kaths JM, Hamar M, Echeverri J, et al. Normothermic ex vivo kidney perfusion for graft quality assessment prior to transplantation. *Am J Transplant*. 2018; 18: 580-589.
47. Harper IG, Ali JM, Harper SJF, et al. Augmentation of Recipient Adaptive Alloimmunity by Donor Passenger Lymphocytes within the Transplant. *Cell Rep*. 2016; 15: 1214-1227.
48. Oberhuber R, Heinbokel T, Cetina Biefer HR, et al. CD11c+ Dendritic Cells Accelerate the Rejection of Older Cardiac Transplants via Interleukin-17A. *Circulation*. 2015; 132: 122-131.
49. Tai H-C, Zhu X, Lin YJ, et al. Attempted Depletion of Passenger Leukocytes by Irradiation in Pigs. *J Transplant*. 2011; 2011: 9.
50. Stone JP, Critchley WR, Major T, et al. Altered Immunogenicity of Donor Lungs via Removal of Passenger Leukocytes Using Ex Vivo Lung Perfusion. *Am J Transplant*. 2016; 16: 33-43.
51. Luc JGY, Aboelnazar NS, Himmat S, et al. A Leukocyte Filter Does Not Provide Further Benefit During Ex Vivo Lung Perfusion. *ASAIO J*. 2017; 63: 672-678.
52. Tietjen GT, Hosgood SA, DiRito J, et al. Nanoparticle targeting to the endothelium during normothermic machine perfusion of human kidneys. *Sci Transl Med*. 2017; 9.
53. Hameed A, Rogers N, Pleass H, Lu B, Miraziz R, Hawthorne W. Intra-Renal Delivery of Drugs Targeting Ischemia-Reperfusion Injury of the Kidney using Normothermic Machine Perfusion. *Transplantation*. 2018; 102: S700.
54. Figueiredo C, Carvalho Oliveira M, Chen-Wacker C, et al. Immunoengineering of the Vascular Endothelium to Silence MHC Expression During Normothermic Ex Vivo Lung Perfusion. *Hum Gene Ther*. 2018. *Epub ahead of print*; DOI: 10.1089/hum.2018.117
55. Kabagambe SK, Palma IP, Smolin Y, et al. Combined Ex Vivo Hypothermic and Normothermic Perfusion for Assessment of High-Risk Deceased Donor Human Kidneys for Transplantation. *Transplantation*. 2018. *Epub ahead of print*; DOI: 10.1097/TP.0000000000002299

56. Schopp I, Reissberg E, Luer B, Efferz P, Minor T. Controlled Rewarming after Hypothermia: Adding a New Principle to Renal Preservation. *Clin Transl Sci*. 2015; 8: 475-478.
57. von Horn C, Minor T. Improved approach for normothermic machine perfusion of cold stored kidney grafts. *Am J Transl Res*. 2018; 10: 1921-1929.



PART 3 – THE LIVER AND PANCREAS

Chapter 8

Use of the harmonic scalpel in cold phase recovery of the pancreas for transplantation: the Westmead technique

Ahmer Hameed
Teresa Yu
Lawrence Yuen
Vincent Lam
Brendan Ryan
Richard Allen
Jerome Laurence
Wayne Hawthorne
Henry Pleass

As published in the Transplant International 2016, 29(5): 636-38; DOI: 10.1111/tri.12777

8.1 Letter to the Editor

Dear Editors,

Pancreatic transplantation for the treatment of type I diabetes offers the current gold standard treatment for a previously incurable disease.[1] During our extensive experience with *en bloc* liver and pancreas recoveries, we noted the time-consuming nature of individually dividing vessels along the greater curvature of the stomach, in addition to dissection of the superior mesenteric pedicle close to the root of the small bowel mesentery. Additionally, small vessels around the pancreatic graft borders are often missed during cold phase dissection, and are thus likely sources of blood loss during organ reperfusion in the recipient.[2]

The ultrasonically activated Harmonic Scalpel (Smithfield, RI, USA) uses high frequency ultrasound vibrations to cut and coagulate tissue.[3] The mechanical energy at the tip of the shear results in the denaturation of proteins, which then form a coagulum to produce haemostasis.[3] Direct comparisons between the Harmonic Scalpel (HS) and electrocautery have shown that the HS is associated with reduced operative time and bleeding.[4, 5]

Herein, we describe easily adaptable modifications to the *en bloc* technique incorporating pancreas recovery by using the HS that allows for more timely and effective procurement of the organ; to our knowledge the use of the HS has not yet been described for this procedure.

The standard technique for procurement of the pancreas for transplantation has been described in detail previously.[6-8] Our HS modification (the modified (Westmead) technique) to the standard recovery technique can be divided into an *in situ* and *ex situ* phase.

In situ, the instrument is used for dissection around the greater curvature of the stomach, including division of the short gastric vessels. The HS is further utilized in mobilizing the splenic flexure of the colon, which is often surrounded by diffuse fatty and vascular tissue. This enables almost bloodless dissection down onto the pancreas and lower pole of the spleen, and facilitates rapid skeletonization of the pancreas to allow its mobilization to the midline.

Following perfusion within the cold phase of dissection, the HS allows the sealing of small jejunal

branches, facilitating the rapid and safe creation of a more defined superior mesenteric artery (SMA) and vein (SMV) pedicle inferior to the pancreatic head (Fig. 1a). This pedicle can then be easily and safely ligated with the single deployment of a vascular stapler, whilst ensuring minimal vessel leakage in the recipient. Complete *en bloc* removal of the liver-pancreas block then proceeds in a standard fashion.

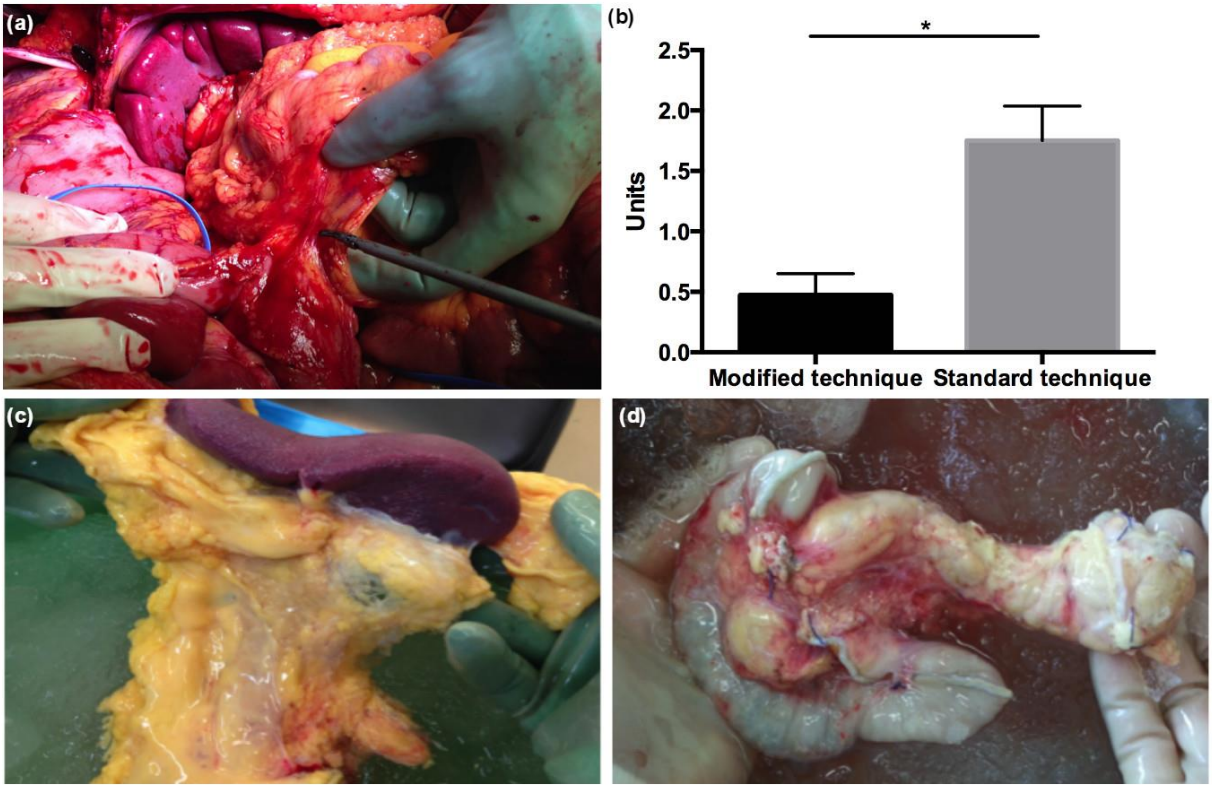


Figure 1. (a) Creation of a more defined SMA/SMV pedicle with the Harmonic Scalpel prior to stapling. (b) PRBC requirement in pancreas recipients by use of Harmonic Scalpel (modified (Westmead) technique) in the donor (n = 19 for Westmead technique, n = 36 for standard technique group) [* $p < 0.01$, Mann Whitney test]. (c) Final back table specimen after use of standard techniques, and (d) after use of Harmonic Scalpel (Westmead technique).

Ex situ the HS can also effectively be employed on the back-table for further clearing of extraneous tissues from the pancreas. We first use the device to separate the pancreas from the spleen via division of the splenorenal ligament. The splenic artery and vein are individually ligated with sutures, having skeletonized the vessels using the HS technique. It is then utilized for the removal of any remaining/excess fatty tissue around the body and tail of the pancreas, such that there is no further adherent tissue requiring removal at the recipient center. We believe that

the quality of the final retrieved organ is significantly superior compared to cases when the HS is not employed (Fig. 1c, 1d), thereby facilitating a more timely implantation process at the recipient center as little further dissection of the specimen is required.

In the period 2011-2015, there were 21 recipients of pancreas transplants where the donation surgery was performed using the Westmead technique (WT); 20 of these were simultaneous pancreas-kidney (SPK) transplants. One of 20 (5%) SPK transplant patients in the WT group underwent graft pancreatectomy due to graft vascular thrombosis compared to 6 of 102 (5.8%) in the standard technique group ($p = 0.68$), and it is thereby as safe from this perspective.

Blood loss and PRBC requirement in recipients of SPK transplants retrieved using the WT ($n = 19$) was significantly less when compared to a random subset of SPK recipients of organs where the standard technique was used ($n = 36$). PRBC requirement was 1.8 units (95% CI 1.2-2.3) in the standard technique group compared to 0.5 units (95% CI 0.1-0.9) in the WT group ($p < 0.01$) (Fig. 1b). Mean blood loss in standard group was 928 ml (95% CI 533-1322), compared to 488 ml (95% CI 324-652 ml) in the WT group ($p = 0.14$).

It is unlikely that other confounding variables are responsible for the lower blood product requirement in the WT group as only SPK transplants were compared that were performed within the same unit by experienced surgeons with similar surgical techniques, with exclusion of patients on significant anti-coagulation or anti-platelet therapy. Regardless, a difference in surgical technique may have partly contributed to the final result; a prospective, randomized trial would be able to definitively answer this. Blood product requirements in the standard technique group are comparable to the few reports in the literature regarding transfusions in pancreas transplant recipients.[9, 10]

Overall, the use of the HS is a modification that is technically safe and simple, yet allows rapid dissection of the pancreas with a subsequent reduction in blood loss upon reperfusion, especially from small peri-pancreatic vessels. Propagation of this method will likely improve recipient outcomes, or at a minimum stimulate interest in alternative technique(s) for pancreatic procurement. Further prospective, randomized comparative data is required to prove the

effectiveness of the Westmead technique over more conventional strategies for organ recovery, especially with regards to back-table dissection and longer-term recipient outcomes.

8.2 References

1. Thwaites SE, Gurung B, Yao J, Kable K, Robertson P, Ryan BJ *et al*. Excellent outcomes of simultaneous pancreas kidney transplantation in patients from rural and urban Australia: a national service experience. *Transplantation.* 2012; 94: 1230.
2. Mizrahi SS, Jones JW, Bentley FR. Preparing for Pancreas Transplantation: Donor Selection, Retrieval Technique, Preservation, and Back-Table Preparation. *Transplant Rev.* 1996; 10: 1.
3. Lee SJ, Park KH. Ultrasonic energy in endoscopic surgery. *Yonsei Med J.* 1999; 40: 545.
4. Huang J, Yu Y, Wei C, Qin Q, Mo Q, Yang W. Harmonic Scalpel versus Electrocautery Dissection in Modified Radical Mastectomy for Breast Cancer: A Meta-Analysis. *PLoS ONE.* 2015; 10: e0142271.
5. Swanstrom LL, Pennings JL. Laparoscopic control of short gastric vessels. *J Am Coll Surg.* 1995; 181: 347.
6. Fridell JA, Powelson JA, Sanders CE, Ciancio G, Burke GW, 3rd, Stratta RJ. Preparation of the pancreas allograft for transplantation. *Clin Transplant.* 2011; 25: E103.
7. Dodson F, Pinna A, Jabbour N, Casavilla A, Khan F, Corry R. Advantages of the rapid en bloc technique for pancreas/liver recovery. *Transplant Proc.* 1995; 27: 3050.
8. Oniscu G, Forsythe J, Fung J. Abdominal Organ Retrieval and Transplantation Bench Surgery 2013. Hoboken: Wiley.
9. Halpern H, Miyoshi E, Kataoka LM, Khouri Fo RA, Miranda SB, Marumo CK, *et al*. Anesthesia for pancreas transplantation alone or simultaneous with kidney. *Transplant Proc.* 2004; 36: 3105.
10. Sparkes T. Blood transfusion in simultaneous pancreas and kidney transplant and pancreas alone transplant procedures: P706. 16th Congress of the European Society for Organ Transplantation; Vienna: *Transplant Int.* 2013; p. 340-61.

Chapter 9

A systematic review and meta-analysis of cold *in situ* perfusion and preservation for pancreas transplantation

Ahmer Hameed
Germaine Wong
Jerome Laurence
Vincent Lam
Henry Pleass
Wayne Hawthorne

As published in HPB 2017, 19(11): 933-43; DOI: 10.1016/j.hpb.2017.07.012

9.1 Abstract

Background: This study aimed to synthesize evidence regarding the most effective solution for *in situ* perfusion and preservation of the pancreas in donation after brain death donors, and to identify the optimal *in situ* flush volume(s) and route(s) during pancreas procurement.

Methods: The Embase, Medline and Cochrane databases were searched (1980-2017). Articles comparing pancreas graft outcomes between two or more different perfusion/preservation fluids (University of Wisconsin (UW), histidine-tryptophan-ketoglutarate (HTK) and/or Celsior) were included, and comparisons were estimated using random effects models.

Results: Thirteen articles were included (939 pancreas transplants). Overall, confidence in the available evidence was low. A higher serum peak lipase (standardized mean difference 0.47, 95% CI 0.23-0.71, $I^2 = 0$) was observed in pancreatic grafts perfused/preserved with HTK compared to UW, but no differences in short-term (one-month) pancreas allograft survivals or early thrombotic graft loss rates between UW and HTK solutions were observed. Similarly, there were no significant differences in the rates of graft pancreatitis, thrombosis and graft survival between UW and Celsior solutions, and between aortic-only and dual aorto-portal perfusion. Perfusion volumes could not be analyzed due to a lack of comparative data.

Discussion: The use of UW cold perfusion may reduce the peak serum lipase, but there is no quality evidence to suggest UW cold perfusion improves graft survival and reduces thrombosis rate, especially in younger donors or with shorter ischemic times. Further research is needed to establish longer-term graft outcomes using the different perfusion/preservation solutions, the comparative efficacy of Celsior, and ideal perfusion volumes.

9.2 Introduction

Hypothermia has long been the dominant paradigm in organ preservation, and is most effectively initiated by the cold vascular *in situ* flush.[1-3] Subsequently, organs are retrieved and immersed in the same preservation fluid as is used for the flush for cold static storage (CS) and transportation prior to transplantation.

Multiple types of perfusion/preservation fluids have been investigated in abdominal organ procurement, with various combinations and volumes of perfusion.[2-6] However, there is no universal consensus regarding the optimal perfusion/preservation fluid, nor the route(s) or ideal volume of flush. There are considerable variations in recommendations in different jurisdictions.[1, 7, 8] UK guidelines recommend 50-70 ml/kg of UW solution for aortic perfusion in the retrieval of the pancreas from donation after brain death (DBD) donors, with or without UW portal perfusion *in situ* or on the back-table, and no pre-flush.[7] Australian recommendations in DBD donors suggest the use of either low-viscosity solution alone, such as HTK, or low-viscosity pre-flush followed by 1.5-2 L of UW flush; centers are given leniency with regards to aortic-only or dual perfusion.[8] There are no clear guidelines from the American Society of Transplant Surgeons regarding DBD organ procurement. Eurotransplant advocates for HTK or UW aortic only perfusion, without a pre-flush; the option of portal perfusion is provided if the pancreas is not procured.[1]

Clinical evidence regarding perfusion/preservation fluids is not unequivocally in favor of one solution over another for pancreas preservation, although a single registry analysis suggests a higher incidence of graft loss with HTK compared to UW solution for preservation of the pancreas.[9, 10]

The relative efficacy of the various preservation solutions for the pancreas, in the context of *in situ* perfusion volume and route, has not been systematically explored. Therefore, the aims of this systematic review and meta-analysis were to synthesize the existing evidence regarding effective solution for *in situ* perfusion and subsequent CS of the DBD pancreas, and to identify the optimal *in situ* flush volume(s) and route(s) during pancreas procurement.

9.3 Methods

The protocol for this systematic review was prospectively registered with PROSPERO (registration number – CRD42016038993).[11] The review was undertaken with adherence to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[12]

9.3.1 STUDY SELECTION AND ELIGIBILITY

Randomized controlled trials (RCT; or quasi-RCTs) and/or observational articles were deemed eligible for this review, without language restriction. An article was only included if it presented data for a minimum of at least 10 patients/transplants per study group, and included information regarding perfusion fluid route(s), flush volume(s), back-table perfusion and final preservation of the pancreas. Paediatric studies, animal experiments, articles without a control group, and studies exploring machine perfusion, were excluded from the analysis. Conference abstracts were also excluded due to insufficient perfusion data and/or quality. Only data from DBD donors was included; if mixed DCD and DBD donor data was presented in an article, this study was excluded from further analysis if the DBD patient data could not be extracted.

9.3.2 LITERATURE SEARCH STRATEGY

Literature searching was conducted by two independent researchers, and encompassed the Embase, Medline and Cochrane databases, and the Cochrane Register of Controlled Trials (1980 to January 2017). The full search strategy is outlined in Supplemental Digital Content (SDC) 1 (Table). A manual search of relevant full-text article reference lists was conducted to identify further potential eligible articles.

9.3.3 DATA EXTRACTION

Two independent reviewers extracted study data into a pre-determined template for the following parameters:

<u>Baseline Characteristics and Study Demographics</u>

Author(s), study date and period, center(s); donor patients/transplants, type of pancreas transplant; donor cardiac arrest and vasopressor/inotrope requirements, donor and recipient age, donor intensive care unit (ICU) stay, donor body mass index (BMI); aortic or dual perfusion (flush), use of pre-flush and type (a pre-flush is defined as the removal of static blood from organs using a solution that is different to the final flush and preservation solution), use of back-table perfusion

and its type and route, perfusion volume(s), perfusion (preservation) solution(s) used, procurement technique; cold ischemic time (CIT) and warm ischemic time (WIT).

Recipient Outcomes

Primary study outcomes included peak amylase and lipase in the first week post-transplantation, the number of pancreatitis episodes, and thrombotic graft loss. Other secondary outcomes of interest included C-peptide and HbA1C at last follow-up, acute rejection rates, graft survival (one, six & 12-month – survivals beyond this reported only sporadically), hospital length-of-stay (LOS), and surgical complications (e.g. exocrine pancreatic leak). Graft pancreatitis was variably defined in the included studies. The study definition was accepted in this analysis. The definitions included a serum amylase levels > 2.5 times the upper limit of normal (ULN) from post-operative day two onwards,[13] surgical appearance on reperfusion,[14] amylase levels > 2.5 times the ULN with associated pain,[15] pancreatic enzyme derangement with increased insulin requirements,[16] or amylase > 2 times ULN with associated clinical or radiologic features of pancreatitis.[17-19]

9.3.4 DATA ANALYSIS

Median ischemic times, donor/recipient ages, perfusion volumes, and graft survival were calculated (to allow a comparison between Celsior and UW or HTK) based on the number of patients in each study group. If necessary prior to meta-analysis, continuous variables initially underwent standardized mean difference (SMD) calculations between study groups using the Practical Meta-analysis Effect Size Calculator.[20]

Meta-analyses were conducted using studies with directly comparable groups, as determined by the nature of perfusion solution used, perfusion route(s), and graft ischemic times. Only observational studies were included in meta-analyses as there were insufficient RCTs with comparable groups eligible for meta-analysis. Risk ratios (RR) and SMD between two comparable groups were estimated using Dersimonian Laird random effects models. Publication bias was assessed using funnel plots. Heterogeneity was evaluated using the I^2 statistic, and considered the I^2 thresholds of < 25%, 25-49%, 50-75% and > 75% to represent low, moderate, high and very high heterogeneity. Subgroup analyses/meta-regression to further define sources of heterogeneity could not be conducted due to insufficient data. Meta-analyses were conducted, where applicable, using Comprehensive Meta-Analysis Version 2.2 (Biostat, Inc., Englewood, New Jersey, USA).

9.3.5 RISK OF BIAS

The Cochrane Collaboration's bias assessment tool was utilized to formally assess RCTs, and includes the domains of random sequence generation, allocation concealment, blinding, incomplete outcome data and selective reporting.[21] Cohort studies undergoing meta-analysis were screened for bias through the utilization of the Newcastle-Ottawa scale; this incorporates in its assessment of bias the domains of representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, comparability of cohorts, assessment of outcomes and follow-up timing and attrition.[22] Publication bias was determined by examining funnel plots for each meta-analysis parameter analyzed.

9.3.6 QUALITY OF EVIDENCE

The overall quality of evidence and thus confidence that may be derived from the summary estimates derived from meta-analyses was assessed utilizing the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guidelines.[23]

9.4 Results

9.4.1 OVERALL STUDY SELECTION AND CATEGORIES

Articles comparing different perfusion/preservation solutions and techniques for pancreas transplantation were analyzed. The study selection process is summarized in Fig. 1. A total of 805 records were identified. Following screening, 10 data-sets (incorporating 13 studies with overlapping data) were included in qualitative analyses, out of which only four cohort studies had sufficient data and were eligible for meta-analyses.[13-19, 24-29] Seven study data-sets were observational in nature, and three were RCTs.

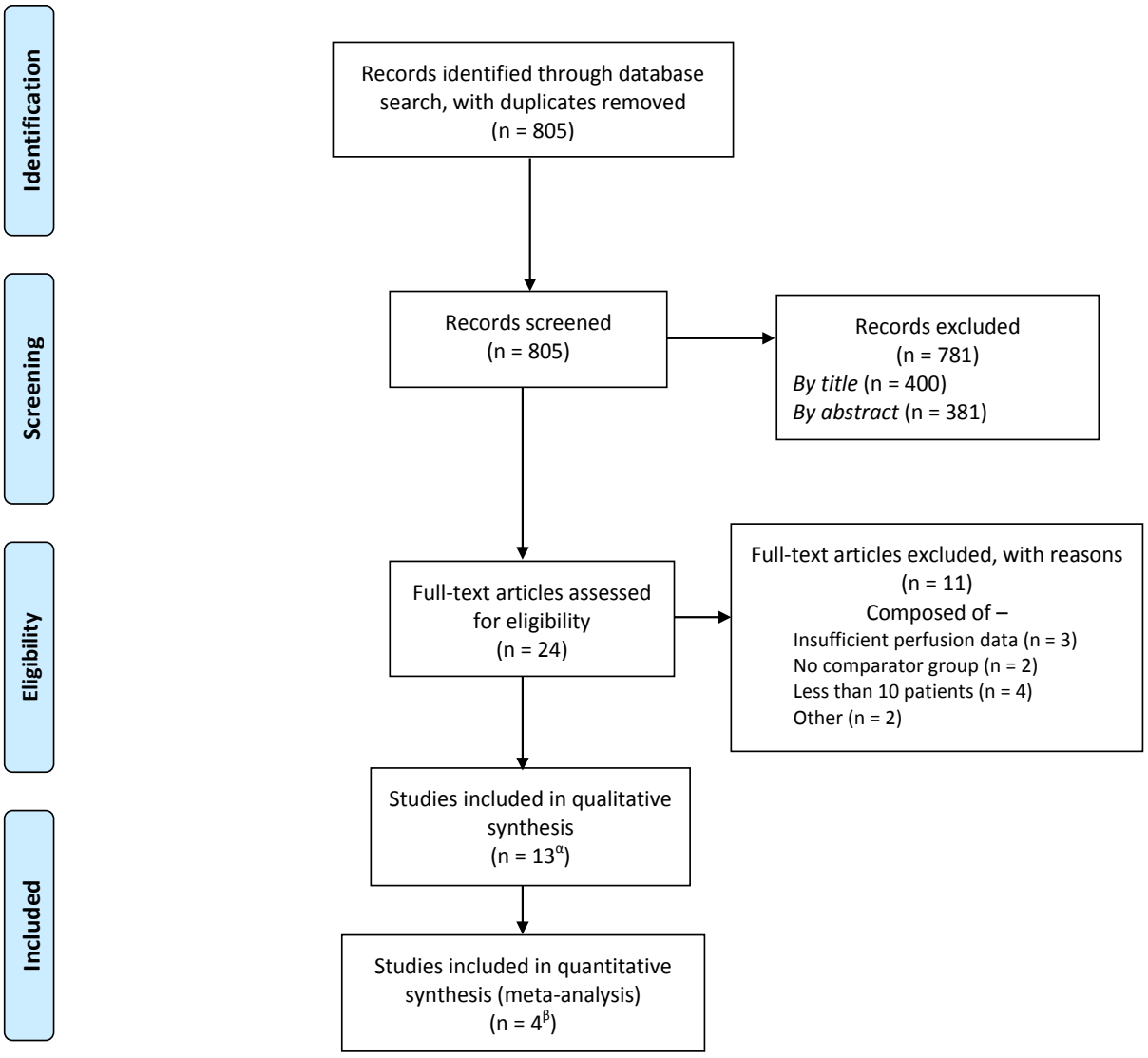


Figure 1. Study selection flow diagram.

α Includes articles with overlapping results that were analysed together

β Parameters analysed: peak amylase & lipase, graft pancreatitis, thrombotic graft loss, hospital length of stay, and one-month graft survival

9.4.2 RISK OF BIAS ASSESSMENT

The overall risk of bias for observational studies was considered high. A summary of bias assessment using the Newcastle-Ottawa scale is provided in SDC 2 (Table). All studies provided a representative cohort of pancreas donors and recipients, and a clear description of the exposure/intervention chosen. Comparability of study cohorts, as determined by similar donor/recipient ages and/or ischemic times, was demonstrated in 62.5% of cohort studies included

in meta-analyses. A majority of studies failed to specify whether the pancreas was retrieved *en bloc* with the liver, and whether a rapid retrieval technique was utilized.

The overall risk of bias for RCTs was largely indeterminate due to the difficulty to assess a majority of domains (Table, SDC 3). Risk of bias with respect to random sequence generation and blinding was difficult to ascertain/unclear in two of the three studies (Table, SDC 3). All studies had a low risk of bias with respect to incomplete outcome data. Both allocation concealment and selective reporting could not be assessed from available data in any of the included RCTs. Funnel plots were generated to assess publication bias, but were uninformative owing to only three or four studies being included in each comparison (Graph, SDC 4).

Overall quality of study evidence is summarized utilizing the GRADE evidence profile (Table, SDC 5). Quality of evidence is either low or very low for all outcome measures investigated. Overall study evidence was downgraded due to the observational nature of studies included in meta-analyses, and small sample sizes and/or wide confidence intervals (imprecision).

9.4.3 BASELINE CHARACTERISTICS OF INCLUDED STUDIES

Whole pancreas perfusion study characteristics, including comparator groups, donor and recipient ages and ischemic times, are summarized in Table 1. Six whole pancreas studies compared UW to HTK perfusion; eight of the studies overall specified the utilization of aortic-only pancreas perfusion. A total of 939 pancreatic transplants were included in the analysis; these comprised, where specified, 664 simultaneous pancreas-kidney transplants, 90 pancreas transplants alone, and 144 pancreas-after-kidney transplants. Median CIT was 10.1 hours, and median donor and recipient ages were 26.2 and 41.9 years, respectively. A rapid procurement technique was utilized in four articles;[30] retrieval type was not clearly specified in the other studies. All studies investigated *in situ* perfusion with subsequent CS in DBD donors.

Pancreas retrieval was performed *en bloc* with the liver, with separation of the organs on the back-table, in three of the included study series. The remaining studies did not specify what organ(s) were procured in addition to the pancreas, or the order in which they were removed.

Table 1. Baseline characteristics of the included studies.

PANCREAS Studies	Study Type	Study Year	Organs Retrieved[^]	Total Transplants	Comparator/ Intervention Groups	*n* per group	*n*, SPK/PTA/PAK[ε]	Donor Age (Mean)	Recipient Age (Mean)	CIT (hrs) (Mean)	Aortic or Dual Perfusion	Total Perfusion (Flush) Volume (L)
Alonso et al.[15]	Cohort	2008	NR	97	UW perfusion **&** CS	81	52/5/24	26.0	42.3	15.4	Aortic	2.6
					HTK perfusion **&** CS	16	12/1/13	27.3	41.7	13.9	Aortic	4.9
Becker et al.[24]	Cohort	2007	NR	95	UW perfusion **&** CS	47	47/0/0	34.6**	43.3	12.0	Aortic	4.8
					HTK perfusion **&** CS	48	48/0/0	29.7**	39.5	10.1	Aortic	9.7
Boggi et al.[17, 18]	RCT	2004	Liver (*en bloc*)	112	UW perfusion **&** CS	56	NR	29.3	39.3	10.1	Aortic	5.6
					Celsior perfusion **&** CS	56	NR	31.0	38.7	10.8	Aortic	7.9
Englesbe et al.[25]	Cohort	2006	NR	77	UW perfusion **&** CS	41	24/3/14	24.6	37.8	7.7	Aortic	3
					HTK perfusion **&** CS	36	22/1/13	25.2	37.5	9.5	Aortic	5
Fridell et al.; Agarwal et al.[26-28]	Cohort	2010	Liver (*en bloc*)	308	UW perfusion **&** CS	50	11/22/17	27.2	41.9	9.3*	Dual[α]	3.3
					HTK perfusion **&** CS	258	160/39/57	26.2	42.8	8.3*	Dual[α]	3.9
Gonzalez et al.[13]	Cohort	2005	Liver (*en bloc*)	46	UW perfusion **&** CS	30	30/0/0	NR	35.4	13.5	Aortic	2
					EC **pre-flush** + UW perfusion **&** CS	16	16/0/0	NR	35.4	13.7	Aortic	2[β]
Manrique et al.[19]	Cohort	2006	NR	72	UW perfusion **&** CS	44	NR[γ]	27.1	36.2**	8.3	Dual	2.4
					Celsior perfusion **&** CS	28	NR[γ]	25.3	41.0**	8.7	Dual	2.4
Nicoluzzi *et al*.[29]	RCT	2008	NR	31	UW perfusion **&** CS	15	15/0/0	30	33	15	Aortic	0.8
					Celsior perfusion **&** CS	16	15/0/0	28	33	16	Aortic	0.8
Potdar et al.[14]	Cohort	2004	NR	33	UW perfusion **&** CS	17	10/4/3	29.5**	36.9	15.1	Aortic	3.5
					HTK perfusion **&** CS	16	6/6/4	21.9**	41.3	14.0	Aortic	9
Schneeberger et al.[16]	RCT	2009	NR	68	UW perfusion **&** CS	41	NR[γ]	NR	44.2	11.8	Aortic	3
					HTK perfusion **&** CS	27	NR[γ]	NR	43.0	10.8	Aortic	6.5
Summary Data	R – 7 studies RCT – 3 studies	Range – 1995-2009	Liver (*en bloc*) – 3 studies	Total – 939	NA	939	SPK – 664 PTA – 90 PAK – 144	Median – 26.2 Range – 21.9-34.6	Median – 41.9 Range – 33.0-44.2	Median – 10.1 Range – 7.7-16.0	NA	NA

CIT – cold ischemic time; CS – cold storage; EC – Euro-Collins; HTK – histidine-tryptophan-ketoglutarate; NA – not applicable; NR – not recorded; P – prospective; PAK – pancreas after kidney transplant; PTA – pancreas transplant alone; R – retrospective; RCT – randomized control trial; SPK – simultaneous pancreas kidney transplant; UW – University of Wisconsin; WIT – warm ischemic time

* Total ischemic time

** Statistically significant difference between the two study groups (i.e. $p < 0.05$)

[α] Dual perfusion indicates aortic + portal perfusion; in the Fridell et al. data-set,[26-28, 31] the portal circulation was slowly perfused with plasmalyte, and was accessed through the inferior mesenteric vein

[β] One liter EC pre-flush + one liter formal UW flush

[γ] Not recorded by perfusion fluid; for Manrique et al.,[19] in total there were 67 SPKs and 5 PAKs, whilst for Schneeberger et al.,[16] there were 65 SPKs, 2 PTAs, and 1 PAK

[Φ] The majority of studies included

[^] In addition to the pancreas

[ε] By each group as specified in Table 1

9.4.4 PERFUSION AND PRESERVATION CHARACTERISTICS

Table 1 outlines the perfusion and preservation fluids utilized in each study group, in addition to the routes and volumes of *in situ* perfusion. A 'pre-flush' to remove static blood was only utilized in one included article.[13] Aortic-only perfusion was most prevalent in the pancreas studies, with UW being the most popular perfusion solution and was used at lower volumes than HTK (3 L [range 0.88-5.6 L] compared to 6.5 L [range 4.9-9.7 L], respectively). Back-table perfusion with UW was used in two studies (1 L, volume only recorded in one study),[14, 28] and HTK in two studies (1 L, volume only recorded in one study).[14, 28] This back-table flush was given via the splenic artery and superior mesenteric artery (SMA)/coeliac axis. Two studies explicitly specified not using back-table flush.[17, 29] In one of two pancreas back-table flush studies,[14] only *in situ* aortic perfusion was performed, whilst dual perfusion was utilized in the other article due to combined liver-pancreas procurement.[28, 31]

9.4.5 TRANSPLANT OUTCOMES

<u>Peak serum amylase/lipase and graft pancreatitis rates</u>

Of the seven studies that included peak serum amylase and/or lipase as outcomes, only four (57.1%) provided sufficient data for meta-analyses. Pancreatic allografts being perfused with and

subsequently preserved in UW had a lower serum peak lipase compared to those preserved in HTK solution (SMD 0.42, 95% CI 0.14-0.69; p = 0.003; I^2 = 0; n = 205 patients; Fig. 2). However, the difference in peak amylase did not reach statistical significance (SMD 0.32, 95% CI -0.13-0.76; p = 0.159; I^2 = 67.0; n = 302 patients; Fig. 2).

In pancreatic allografts perfused and subsequently preserved in UW compared to HTK, via the aortic-only route, graft pancreatitis rates were considerably higher in the HTK group in Alonso *et al*.'s study (9 of 16 [56.3%] HTK patients versus 19 of 81 [23.5%] UW patients; p = 0.01).[15] There was no statistical difference in pancreatitis rates between UW and HTK in the study by Potdar *et al*., as defined by pancreatic appearance upon reperfusion (5 of 16 [31.3%] HTK patients compared to 4 of 17 [23.5%] UW patients; p = 0.62).[14]

Of the three UW versus Celsior studies, including two studies with aortic-only perfusion and one study utilizing dual perfusion, there were no significant differences in peak amylase, lipase or graft pancreatitis rates.[17-19, 29]

Thrombotic graft loss rates

Of the eight studies that reported thrombotic graft loss rates, only three (37.5%) provided sufficient data for meta-analyses. There were no significant differences between thrombotic graft loss rates between pancreata perfused via the aorta using UW or HTK (time period not recorded in most studies; n = 269 patients; Fig. 2).

Thrombotic graft loss rates were also no different in the articles comparing UW and Celsior *in situ* pancreas perfusion and preservation.[17-19, 29]

Hospital length-of-stay

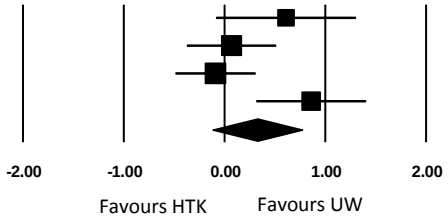
Hospital length-of-stay (LOS) was reported in three articles, all of which compared UW and HTK, and were also eligible for meta-analysis. Mean difference between hospital LOS in the HTK and UW groups was 2.91 days (95% CI -0.04-5.87; p = 0.053; I^2 = 0; n = 174 patients; Fig. 2).

A

Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Potdar et al., 2004	Peak amylase	0.610	0.356	0.127	-0.088	1.308	1.712	0.087
Englesbe et al., 2006	Peak amylase	0.070	0.228	0.052	-0.378	0.518	0.306	0.759
Becker et al., 2007	Peak amylase	-0.090	0.205	0.042	-0.492	0.312	-0.438	0.661
Alonso et al., 2008	Peak amylase	0.860	0.280	0.079	0.310	1.410	3.066	0.002
		0.319	0.226	0.051	-0.125	0.763	1.410	0.159

n = 302 patients

$I^2 = 67.0$

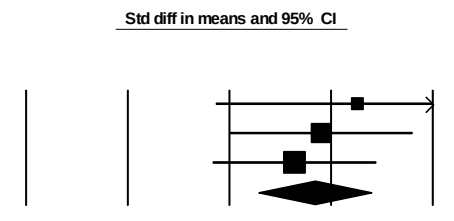


B

Study name	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Potdar et al., 2004	Peak lipase	0.630	0.357	0.127	-0.069	1.329	1.765	0.077
Englesbe et al., 2006	Peak lipase	0.450	0.231	0.053	-0.003	0.903	1.946	0.052
Becker et al., 2007	Peak lipase	0.320	0.207	0.043	-0.085	0.725	1.550	0.121
		0.417	0.141	0.020	0.140	0.694	2.951	0.003

n = 205 patients

$I^2 = 0$

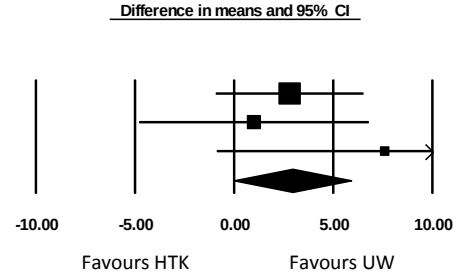


C

Study name	Outcome	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Englesbe et al., 2006a	Hospital LOS	2.800	1.914	3.663	-0.951	6.551	1.463	0.143
Englesbe et al., 2006b	Hospital LOS	1.000	2.967	8.803	-4.815	6.815	0.337	0.736
Alonso et al., 2008	Hospital LOS	7.600	4.333	18.773	-0.892	16.092	1.754	0.079
		2.916	1.508	2.273	-0.039	5.872	1.934	0.053

n = 174 patients

$I^2 = 0$

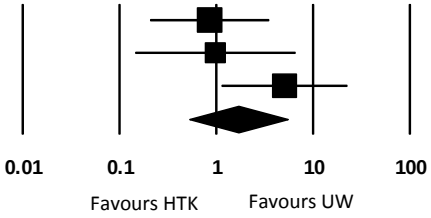


D

Study name	Outcome	Risk ratio	Lower limit	Upper limit	Z-Value	p-Value
Englesbe et al., 2006	Thrombotic graft loss	0.854	0.205	3.564	-0.216	0.829
Becker et al., 2007	Thrombotic graft loss	0.979	0.144	6.667	-0.022	0.983
Alonso et al., 2008	Thrombotic graft loss	5.063	1.121	22.866	2.108	0.035
		1.673	0.518	5.400	0.860	0.390

n = 269 patients

$I^2 = 37.9$



E

Study name	Outcome	MH risk ratio	Lower limit	Upper limit	Z-Value	p-Value
Potdar et al., 2004	1-month graft survival	3.176	0.139	72.747	0.723	0.469
Englesbe et al., 2006	1-month graft survival	1.898	0.487	7.392	0.924	0.356
Alonso et al., 2008	1-month graft survival	2.500	0.500	12.510	1.115	0.265
Becker et al., 2007	1-month graft survival	0.979	0.340	2.820	-0.039	0.969
		1.515	0.737	3.117	1.130	0.259

n = 302 patients

$I^2 = 0$

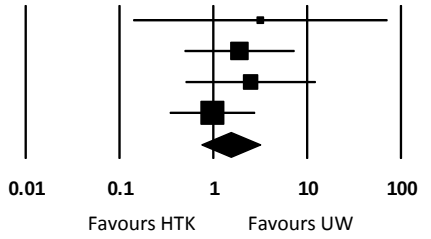


Figure 2. Forest plots for (A) peak amylase, (B) peak lipase, (C) hospital length-of-stay, (D) thrombotic graft loss rates, and (E) one-month graft survival after *in situ* aortic perfusion and preservation of the pancreas with UW or HTK.

Exocrine pancreatic leak and fistula formation

Pancreatic leakage, as evidenced by a peri-pancreatic fluid collection, abscess and/or fistula formation, was not uniformly or consistently reported and hence could not be statistically analyzed. The leak rate for UW *in situ* perfusion/preservation groups was reported in six studies, with a median of 10.0% (range 0-13.3%). Median leak rates were similar in both HTK and Celsior perfusion/preservation groups, at 11.1% (range 11.1-31.3%, n = 2 studies) and 10.0% (10.1-17.9%, n = 3 studies), respectively.

Graft survival

One-month graft survival was reported in five studies, out of which four (80%) were eligible for meta-analysis. There was no significant difference in one-month pancreatic graft survivals subsequent to UW or HTK *in situ* aortic perfusion and preservation, although there was a trend favoring UW (n = 302 patients; Fig. 2). Twelve-month graft survival data for this comparator group was available for only two studies,[15, 25] and as such formal meta-analyses were not conducted. In the study by Alonso *et al*., pancreatic graft survival at 12 months after UW and HTK perfusion/preservation was 90% and 81%, respectively ($p = 0.09$); corresponding levels in Englesbe *et al*.'s article were 89% and 72.5%, respectively ($p > 0.05$).[15, 25]

To allow for survival comparisons between Celsior perfusion/preservation and UW or HTK, one, six, and 12-month pancreas graft survivals were collated. Survival data is presented in Table 2. Survival data for pancreas procurement after dual perfusion was only available from one study[28] and thus no meaningful comparisons could be made. More data were available for the assessment of aortic-only perfusion; aortic perfusion using UW provided a median 12-month graft survival of 90%, compared to 81% for HTK-perfused grafts. Only one Celsior aortic-only perfusion article was available (from a single center in Pisa, Italy), with 12-month pancreas allograft survival of 95.9%.[18]

Other perfusion/preservation group comparisons

Fridell *et al*. compared UW and HTK dual perfusion and preservation; there were no significant differences in peak amylase or lipase, whilst pancreatitis and thrombotic graft loss rates were not recorded.[28] The one study that employed an Euro-Collins pre-flush followed by a formal UW flush

found no differences with the UW-only perfusion/storage group in terms of graft pancreatitis and thrombotic graft loss rates.[13]

Table 2. Comparison of median one, six and twelve-month graft survivals in pancreatic grafts obtained after UW, HTK or Celsior perfusion. Data presented as median (range).**

PANCREAS	***UW (Aortic Perfusion)***	***UW (Dual Perfusion)***	***HTK (Aortic Perfusion)***	***HTK (Dual Perfusion)***	***Celsior (Aortic Perfusion)***	***Celsior (Dual Perfusion)***
1-month survival, % (range; n *studies)*	95 (87.0-100; 5)	94 (NA*; 1)	87.5 (85-93.8; 4)	95 (NA; 1)	NA	NA
6-month survival, % (range; n *studies)*	90 (80.0-95.8; 6)	NA	85.4 (81-86.1; 4)	NA	95.9 (67-95.9; 2)	NA
12-month survival, % (range; n *studies)*	90 (82.6- 95.8; 4)	86 (NA; 1)	81 (72.5-85.4; 3)	92 (NA; 1)	95.9 (NA; 1)	NA

HTK – histidine-tryptophan-ketoglutarate; NA – not applicable; UW – University of Wisconsin

* NA here indicates that there was no extractable study data available

** Median overall survivals weighted by total patient numbers in each study group

9.5 Discussion and Conclusions

This systematic review has compared the various different DBD pancreas perfusion and preservation conditions, and analyzed their potential for impacting graft outcomes in the recipient. Overall the quality of evidence was poor, with wide confidence intervals for effect estimates and only a small number of studies. Furthermore, the majority of included data was from younger donors, with relatively short CITs. At best, UW *in situ* perfusion and preservation results in less biochemical pancreatic enzyme release in comparison to HTK, and may manifest in lower graft pancreatitis rates, although definitions for this vary between studies. There were no clear differences between UW and HTK for other short-term graft parameters, including thrombotic graft loss. HTK-preserved pancreata tended to have lower graft survivals in comparison to UW. Despite meta-analyses not being possible in the comparison between UW and Celsior, there was no evidence of deleterious consequences in the short and longer term when Celsior was utilized. Study heterogeneity and limited data precluded any conclusions being drawn regarding ideal

perfusion volumes or routes, although aortic-only perfusion with lower volumes of UW was the most common occurrence.

An important consideration in the interpretation of data from this study is the separate but also likely synergistic impact on the pancreatic allograft of *in situ* perfusion during procurement, and also subsequent CS preservation in the same perfusion fluid. As such, it is very difficult to tease out the individual effects of the initial flush and then subsequent preservation on graft outcomes. This suggests that both factors must be considered before analyzing the efficacy of a CS preservation fluid, and as such, only articles including both procurement and preservation data were included in this study.

A number of abdominal organ perfusion fluids exist, which vary in constituents/composition and viscosities. The three most commonly employed solutions for the pancreas, which also tend to be the same for all abdominal organ procurement, are UW, HTK and Celsior. These all contain impermeants designed to counteract cellular edema, buffers to counteract ischemic acidosis, and energy substrates to encourage ATP formation upon reperfusion.[32] UW differs further in that it is an 'intra-cellular' type solution that is of higher viscosity due to the presence of hydroxyethyl starch and as such its flow rates during organ flushing are lower.[32, 33] In contrast, higher flush volumes are recommended in particular for HTK to allow for equilibration of the fluid's electrolyte content with the graft extracellular space, although this has been challenged by others.[14, 28, 34, 35]

UW compared to HTK pancreas perfusion and preservation resulted in a reduction of recipient peak lipase, which may translate to lower graft pancreatitis rates. A formal comparison of graft pancreatitis was precluded not only by insufficient studies reporting this parameter, but more importantly by the significant variability in how graft pancreatitis was defined.[36] Clinical acute graft pancreatitis must be distinguished from histologic pancreatitis and definitions incorporating clinical signs, biochemical parameters and/or imaging findings should be preferred over the utilization of individual parameters.[36, 37] If indeed UW is superior to HTK with respect to recipient graft pancreatitis, this may at least partially be related to the 'low-flow' nature of the pancreas being better-suited to the more viscous UW solution compared to faster flush rates achieved with HTK and the potential for hyper-perfusion.[38]

The impact of perfusion/preservation fluid on graft outcomes may also be modified by the duration of cold ischaemia. In the study by Englesbe *et al*., where both study groups had a CIT of less than 10 hours, UW was not clearly advantageous in comparison to HTK.[25] In contrast, CITs of more than 12 hours were seen in Alonso *et al*.'s article, with superior outcomes in the UW group, possibly suggesting that UW is a better preservative in the event of longer ischemic times.[15] Although pancreas articles could not be meta-analyzed for differences between UW and Celsior, this comparison was made in three different studies, including two studies with CITs of 12 hours or less, and showed no significant outcome disparities between either perfusion solution.[17-19, 29] Overall, especially when attempts are made to minimize pancreas CIT, it is possible that the choice of preservation solution may not significantly impact subsequent transplantation outcomes.

Another important consideration is the quality of the donor pancreas, as determined by factors such as donor age. Median donor age for all included studies in this systematic review was 26.2 years. Current evidence indicates a decline in pancreas transplantation rates, in part related to donor factors, and therefore the future may see the increased utilization of so-called expanded criteria donors, including DCD and older DBD donors.[39-41] There is conflicting evidence regarding post-transplantation outcomes when older and/or DCD pancreata are utilized, however.[41-44] Although one strategy in the expanded criteria donor cohort could include the minimization of CITs through local allocation alone, optimal and novel donor management and preservation strategies will likely need to be employed to further enhance recipient outcomes.[39, 40, 43, 45]

Pancreas retrieval is almost always undertaken in a multi-organ retrieval setting, where the liver and kidneys are also often procured. As such, high quality perfusion and preservation of the pancreatic allograft needs to be undertaken without compromising the quality and outcomes of other retrieved organs, in particular the liver. Only three of the studies included here specified liver procurement in addition to the pancreas, but hepatic allograft outcomes were not discussed.[13, 18, 28] A systematic review and meta-analysis by O'Callaghan *et al*. however did not show any significant differences in liver transplantation outcomes when UW, Celsior or HTK solutions were utilized.[6] In contrast, a recent European registry analysis suggested a higher risk of liver allograft loss when HTK solution was employed, which was in fact also shown in a pancreas registry analysis.[9, 46] A further confounding factor not considered by these studies is the effect of the route

of *in situ* perfusion, namely aortic-only in comparison to dual perfusion. Few comments can be made regarding pancreas retrieval after dual perfusion from this present article, due to the lack of included studies investigating this technique. Nevertheless, pancreatic procurement after dual perfusion is discouraged due to possible risks of increased graft injury stemming from venous congestion and graft edema.[2, 47] Significantly, dual *in situ* perfusion does not seem to provide clear benefits for liver transplantation outcomes, and as such its routine use must be questioned, especially in a multi-organ retrieval setting.[48, 49] We are currently in the process of formally investigating dual compared to aortic-only *in situ* perfusion for liver retrieval in a further systematic review.

Procurement teams have the option of employing a 'pre-flush' prior to the final *in situ* organ flush. A pre-flush is advocated by relatively few authors as a means to improve final preservation fluid distribution within the organ, especially prior to the use of UW flush due to its high viscosity and its possible tendency to aggregate with red blood cells.[33] Pre-flush employment may also decrease the total volume of UW required, thereby reducing preservation costs due to the significantly greater expense of UW in comparison to fluids such as HTK.[13, 27] Gonzalez *et al.*'s study was the only article included here that utilized a pre-flush.[13] These authors compared Euro-Collins pre-flush followed by UW aortic flush with UW aortic flush alone for pancreas procurement, and showed no significant post-transplantation outcome differences between both over a three-month time period.[13] It is clear that most major retrieval units do not utilize or report on a pre-flush technique, however, and if it continues to be utilized by some units it would be worth a larger prospective trial to ensure its value and ensure it is not in fact detrimental.

Certain biases and disadvantages must be considered in the interpretation of findings from this review. Firm conclusions could not be made regarding longer-term graft outcomes and ideal perfusion routes and volumes, owing to a paucity of available data. Furthermore, the fact that most included articles were retrospective in nature introduced confounding and heterogeneity to the cumulative data; this was reflected by low or very low quality of evidence as determined by the GRADE assessment. Despite our attempts to minimize biases and account for study heterogeneity by only meta-analyzing comparable study cohorts, and using a random effects model in all cases, the cumulative evidence presented here must be interpreted with caution.

In summary, this is the first review to systematically investigate DBD donor pancreas *in situ* perfusion and preservation prior to transplantation. Although cumulative evidence suggests that UW may reduce ischemia-reperfusion injury of the pancreas, as manifested by a lower peak lipase, longer-term outcomes, the comparative efficacy of UW and Celsior, and ideal perfusion volumes remain uncertain. The development of uniform pancreas procurement and preservation guidelines will require additional studies that are prospective in nature and higher-powered, although this may be difficult owing to declining pancreas transplantation activity in some centres. Currently, it can only be concluded that pancreas procurement after *in situ* aortic perfusion and subsequent cold static storage using UW solution remains safe and is the most commonly reported option.

9.6 References

1. Eurotransplant Foundation. Eurotransplant Manual. Leiden: Netherlands: Eurotransplant 2016.
2. Brockmann JG, Vaidya A, Reddy S, Friend PJ. Retrieval of abdominal organs for transplantation. *Br J Surg.* 2006; 93: 133-46.
3. Oniscu GC, Forsythe JLR, Fung JJ. *Abdominal organ retrieval and transplantation bench surgery*. John Wiley & Sons: Chichester, West Sussex, 2013.
4. Feng L, Zhao N, Yao X, Sun X, Du L, Diao X et al. Histidine-tryptophan-ketoglutarate solution vs. University of Wisconsin solution for liver transplantation: a systematic review. *Liver Transpl.* 2007; 13: 1125-36.
5. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. Preservation solutions for static cold storage of kidney allografts: a systematic review and meta-analysis. *Am J Transplant.* 2012; 12: 896-906.
6. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. The effect of preservation solutions for storage of liver allografts on transplant outcomes: a systematic review and meta-analysis. *Ann Surg.* 2014; 260: 46-55.
7. Zalewska K, Ploeg R. National Standards for Organ Retrieval from Deceased Donors (NORS Retrieval Standards). Bristol, UK; 2014.
8. TSANZ. Guidance Document - Surgical Technique for Deceased Donor Abdominal Organ Procurement (ATCA-TSANZ Guidelines G003/2015). Sydney, Australia: TSANZ; 2015.
9. Stewart ZA, Cameron AM, Singer AL, Dagher NN, Montgomery RA, Segev DL. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation. *Am J Transplant.* 2009; 9: 217-21.
10. Parsons RF, Guarrera JV. Preservation solutions for static cold storage of abdominal allografts: which is best? *Curr Opin Organ Transplant.* 2014; 19: 100-7.
11. Hawthorne W, Hameed A, Pleass H. Organ perfusion and preservation: current methods to provide optimal organ preservation and best transplantation outcomes. PROSPERO 2016: CRD42016038993.http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016038993 [2016 December].
12. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* 2000; 283: 2008-12.
13. Gonzalez AM, Filho GJ, Pestana JO, Linhares MM, Silva MH, Moura RM et al. Effects of Eurocollins solution as aortic flush for the procurement of human pancreas. *Transplantation.* 2005;80: 1269-74.
14. Potdar S, Malek S, Eghtesad B, Shapiro R, Basu A, Patel K et al. Initial experience using histidine-tryptophan-ketoglutarate solution in clinical pancreas transplantation. *Clin Transplant.* 2004;18: 661-5.
15. Alonso D, Dunn TB, Rigley T, Skorupa JY, Schriner ME, Wrenshall LE et al. Increased pancreatitis in allografts flushed with histidine-tryptophan-ketoglutarate solution: a cautionary tale. *Am J Transplant.* 2008;8: 1942-5.
16. Schneeberger S, Biebl M, Steurer W, Hesse UJ, Troisi R, Langrehr JM et al. A prospective randomized multicenter trial comparing histidine-tryptophane-ketoglutarate versus University of Wisconsin perfusion solution in clinical pancreas transplantation. *Transpl Int.* 2009;22: 217-24.
17. Boggi U, Coletti L, Vistoli F, Del Chiaro M, Signori S, Croce C et al. Pancreas preservation with University of Wisconsin and Celsior solutions. *Transplant Proc.* 2004;36: 563-5.

18. Boggi U, Vistoli F, Del Chiaro M, Signori S, Croce C, Pietrabissa A et al. Pancreas preservation with University of Wisconsin and Celsior solutions: a single-center, prospective, randomized pilot study. *Transplantation.* 2004;77: 1186-90.
19. Manrique A, Jimenez C, Herrero ML, Meneu JC, Abradelo M, Moreno A et al. Pancreas preservation with the University of Wisconsin versus Celsior solutions. *Transplant Proc.* 2006;38: 2582-4.
20. Wilson D. Practical Meta-Analysis Effect Size Calculator. http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php [2016 November].
21. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* 2011; 343.
22. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [2016 June].
23. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 2011; 64: 383-94.
24. Becker T, Ringe B, Nyibata M, Meyer zu Vilsendorf A, Schrem H, Luck R et al. Pancreas transplantation with histidine-tryptophan-ketoglutarate (HTK) solution and University of Wisconsin (UW) solution: is there a difference? *J Pancreas.* 2007; 8: 304-11.
25. Englesbe MJ, Moyer A, Kim DY, Granger DK, Pietroski R, Yoshida A et al. Early pancreas transplant outcomes with histidine-tryptophan-ketoglutarate preservation: a multicenter study. *Transplantation.* 2006; 82: 136-9.
26. Agarwal A, Powelson JA, Goggins WC, Milgrom ML, Fridell JA. Organ preservation with histidine-tryptophan ketogluatarate solution in clinical pancreas transplantation: an update of the indiana university experience. *Transplant Proc.* 2008; 40: 498-501.
27. Fridell JA, Agarwal A, Milgrom ML, Goggins WC, Murdock P, Pescovitz MD. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution for organ preservation in clinical pancreas transplantation. *Transplantation.* 2004; 77: 1304-6.
28. Fridell JA, Mangus RS, Powelson JA. Histidine-tryptophan-ketoglutarate for pancreas allograft preservation: the Indiana University experience. *Am J Transplant.* 2010; 10: 1284-9.
29. Nicoluzzi J, Macri M, Fukushima J, Pereira A. Celsior versus Wisconsin solution in pancreas transplantation. *Transplant Proc.* 2008; 40: 3305-7.
30. Boggi U, Vistoli F, Chiaro MD, Signori S, Pietrabissa A, Costa A et al. A simplified technique for the en bloc procurement of abdominal organs that is suitable for pancreas and small-bowel transplantation. *Surgery.* 2004; 135: 629-41.
31. Imagawa DK, Olthoff KM, Yersiz H, Shackleton CR, Colquhoun SD, Shaked A et al. Rapid en bloc technique for pancreas-liver procurement. Improved early liver function. *Transplantation.* 1996; 61: 1605-9.
32. Bon D, Chatauret N, Giraud S, Thuillier R, Favreau F, Hauet T. New strategies to optimize kidney recovery and preservation in transplantation. *Nat Rev Nephrol.* 2012; 8: 339-47.
33. van der Plaats A, t Hart NA, Morariu AM, Verkerke GJ, Leuvenink HG, Ploeg RJ et al. Effect of University of Wisconsin organ-preservation solution on haemorheology. *Transpl Int.* 2004; 17: 227-33.
34. Blech M, Hummel G, Kallerhoff M, Ringert RH. Electrolyte equilibration of human kidneys during perfusion with HTK-solution according to Bretschneider. *Urol Res.* 1997; 25: 331-5.

35. Troisi R, Meester D, Regaert B, Jacobs B, Van den Broucke C, Cuvelier C et al. Physiologic and metabolic results of pancreatic cold storage with Histidine-Tryptophan-Ketoglutarate-HTK solution (Custodiol) in the porcine autotransplantation model. *Transpl Int.* 2000; 13: 98-105.
36. Nadalin S, Girotti P, Konigsrainer A. Risk factors for and management of graft pancreatitis. *Curr Opin Organ Transplant.* 2013; 18: 89-96.
37. Small RM, Shetzigovski I, Blachar A, Sosna J, Klausner JM, Nakache R et al. Redefining late acute graft pancreatitis: clinical presentation, radiologic findings, principles of management, and prognosis. *Ann Surg.* 2008; 247: 1058-63.
38. Squifflet JP, LeDinh H, de Roover A, Meurisse M. Pancreas Preservation for Pancreas and Islet Transplantation: A Minireview. *Transplant Proc* 2011; 43: 3398-401.
39. Barlow AD, Hosgood SA, Nicholson ML. Current state of pancreas preservation and implications for DCD pancreas transplantation. *Transplantation.* 2013; 95: 1419-24.
40. Stratta RJ, Gruessner AC, Odorico JS, Fridell JA, Gruessner RWG. Pancreas Transplantation: An Alarming Crisis in Confidence. *Am J Transplant.* 2016; 16: 2556-62.
41. Shahrestani S, Webster AC, Lam VW, Yuen L, Ryan B, Pleass HC et al. Outcomes From Pancreatic Transplantation in Donation After Cardiac Death: A Systematic Review and Meta-Analysis. *Transplantation.* 2017; 101: 122-30.
42. Boggi U, Del Chiaro M, Signori S, Vistoli F, Amorese G, Croce C et al. Pancreas transplants from donors aged 45 years or older. *Transplant Proc.* 2005; 37: 1265-7.
43. Proneth A, Schnitzbauer A, Viebahn R, Schenker P, Arbogast H, Manekeller S et al. Extended pancreas donor program - the EXPAND study: a prospective multicenter trial testing the use of pancreas donors over age 50. *Transpl Int.* 2016; 29: 50.
44. Kayler LK, Wen X, Zachariah M, Casey M, Schold J, Magliocca J. Outcomes and survival analysis of old-to-old simultaneous pancreas and kidney transplantation. *Transpl Int.* 2013; 26: 963-72.
45. Proneth A, Schnitzbauer AA, Zeman F, Foerster JR, Holub I, Arbogast H et al. Extended pancreas donor program – the EXPAND study rationale and study protocol. *Transplant Res.* 2013; 2: 12.
46. Adam R, Delvart V, Karam V, Ducerf C, Navarro F, Letoublon C et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant.* 2015; 15: 395-406.
47. Nghiem DD, Cottington EM. Pancreatic flush injury in combined pancreas-liver recovery. *Transpl Int.* 1992; 5: 19-22.
48. Anthuber M, Zuelke C, Forst H, Welte M, Groh J, Maag K et al. Experiences with a simplified liver harvesting technique--single aorta in situ flush followed by portal back table flush. *Transplant Proc.* 1993; 25: 3154-5.
49. de Ville de Goyet J, Hausleithner V, Malaise J, Reding R, Lerut J, Jamart J et al. Liver procurement without in situ portal perfusion. A safe procedure for more flexible multiple organ harvesting. *Transplantation.* 1994; 57: 1328-32.

Chapter 10

A systematic review and meta-analysis of cold *in situ* perfusion and preservation of the hepatic allograft: working towards a unified approach

Ahmer Hameed
Jerome Laurence
Vincent Lam
Henry Pleass
Wayne Hawthorne

As published in the Liver Transplantation 2017, 23(12): 1615-27; DOI: 10.1002/lt.24829

10.1 Abstract

Background: The efficacy of cold *in situ* perfusion and static storage of the liver is one possible determinant of transplantation outcomes. The aim of this study was to determine whether there is evidence to substantiate a preference for a particular perfusion route (aortic or dual) or perfusion/preservation solution in donation after brain-death (DBD) liver transplantation.

Methods: The Embase, Medline and Cochrane databases were utilized (1980-2017). Random effects modeling was used to estimate effects on transplantation outcomes based upon (i) aortic or dual *in situ* perfusion, and (ii) the use of University of Wisconsin (UW), histidine-tryptophan-ketoglutarate (HTK), Celsior and/or Institut Georges Lopez-1 (IGL-1) for perfusion/preservation.

Results: Twenty-two articles were included (2294 liver transplants). The quality of evidence ranged from very low to moderate (GRADE score). Meta-analyses were conducted for 14 eligible studies. Whilst there was no difference in the primary non-function (PNF) rate, a higher peak alanine aminotransferase (ALT) was recorded in dual compared to aortic-only UW-perfused livers (Standardized Mean Difference 0.24; 95% CI 0.01-0.47); a back-table portal venous flush was undertaken in the majority of aortic-only perfused livers. There were no relevant differences in peak enzymes, PNF, thrombotic graft loss, biliary complications or one-year graft survival in comparisons between dual-perfused livers using UW, HTK, Celsior or IGL-1.

Conclusion: There is no significant evidence that aortic-only perfusion of the DBD liver compromises transplantation outcomes, and may be favored owing to its simplicity. However, there is currently insufficient evidence to advocate for the use of any particular perfusion/preservation fluid over the others.

10.2 Introduction

Cold *in situ* perfusion and subsequent cold static storage (CS) of the liver is the most commonly pursued approach prior to transplantation. Across different jurisdictions internationally, there are many differences in protocols for the composition and route of administration of perfusion/preservation fluid.[1-3] Perfusion fluid(s) utilized in this process vary by composition, viscosity, and volumes administered; most commonly, University of Wisconsin (UW) or histidine-tryptophan-ketoglutarate (HTK) solutions are used.[4-6] *In situ* perfusion can be instituted via cannulation of the aorta alone, with or without additional access to the portal venous system to achieve 'dual' perfusion. A back-table flush is then often performed via the portal vein and/or hepatic artery in the donor center before the liver is stored in the same solution for transportation.

One reason for inconsistency between guidelines is the conflicting evidence with respect to perfusion fluid composition. Analysis of European and American registry data suggests an association between the use of HTK and hepatic allograft loss.[7-8] However, a systematic review and meta-analysis by O'Callaghan *et al*. found no significant outcome differences between UW, Celsior or HTK.[9] Moreover, there is a paucity of data regarding the route or volume of *in situ* perfusion, in particular aortic-only compared to dual perfusion. Indeed, an important unknown is whether both *in situ* perfusion and subsequent CS preservation impact transplantation outcomes, rather than just the preservation fluid itself during transportation.

In this systematic review and meta-analysis we analyzed published data pertaining to outcomes of liver transplantation after procurement from donation after brain death (DBD) donors, with the aim of identifying evidence supporting a specific perfusion route, volume(s) and/or fluid(s).

10.3 Methods

The protocol for this systematic review was prospectively registered with PROSPERO (registration number – CRD42016038993).[10] The review was undertaken with adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRIMSA) statement and Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[11, 12]

10.3.1 STUDY SELECTION AND ELIGIBILITY

Both English and non-English language randomized control trials (RCTs) and observational studies were included. Study inclusion mandated information with respect to *in situ* perfusion route(s) and volume(s), with at least 10 transplants in each study group. UW, HTK, Celsior or Institut Georges Lopez-1 (IGL-1) solution(s) must have been utilized as the final perfusion/CS solution in included articles, with comparisons either between these perfusion solutions, or between aortic and dual perfusion, pre-flush versus no pre-flush, or variable perfusion volumes. All pediatric and experimental studies were excluded, in addition to studies utilizing machine perfusion preservation of the liver. Live donor data was not included in analyses. A uniform lack of perfusion data and poor study quality necessitated the exclusion of conference abstracts/proceedings. Only DBD donor data was included and analyzed here as it became apparent after an extensive literature search that there was insufficient published literature comparing *in situ* perfusion solution(s) and/or route(s)for donation after circulatory death (DCD) hepatic allografts.

10.3.2 LITERATURE SEARCH STRATEGY

Two independent researches reviewed (A.H. & W.H.) the Embase, Medline and Cochrane databases, including in-process and Epub ahead of print citations (January 1980 to February 2017). Supplemental Digital Content (SDC) 1 outlines the search strategy. Reference lists from full-text articles of relevance were subsequently manually searched to help include all available studies.

10.3.3 DATA EXTRACTION

A template was derived prior to the extraction of study data by two independent reviewers for the following parameters:

<u>Baseline data</u>

Author(s); study date and period; center(s); donor patients/transplants; donor cardiac arrest and vasopressor/inotrope requirements; donor intensive care unit (ICU) stay; donor liver function tests, cause of death, split liver utilization and allocation region;[13] donor and recipient age; recipient model for end-stage liver disease (MELD) or Child-Pugh score at transplant; procurement technique (classic or rapid);[14, 15] cold ischemic time (CIT) and warm ischemic time (WIT); aortic or dual perfusion (flush); use of pre-flush (defined as an *in situ* perfusion fluid used prior to the

final perfusion fluid) and type; use of back-table perfusion and its type and route; perfusion volume(s); and perfusion (preservation) solution(s) used.

Outcome data

Primary study outcomes extracted included: peak post-transplant aspartate aminotransferase (AST) and alanine aminotransferase (ALT), graft loss post-arterial thrombosis, and graft primary non-function (PNF).

Secondary study outcomes included: ischemic biliary complications, and graft survival (one-year). Ischemic biliary complications were defined as biliary strictures/stenosis in the absence of graft vessel thrombosis and/or rejection.[16] Initial poor function, a commonly used definition for which is provided by Ploeg *et al*.,[17] was not considered in the analysis due to insufficient data and variable definitions amongst the different studies.

10.3.4 DATA SYNTHESIS AND STATISTICS

Meta-analyses for risk ratios (RR), mean difference (MD) or standardized MD (SMD), where applicable, were calculated using a random effects model in all cases. If necessary prior to meta-analysis, continuous variables initially underwent standardized mean difference (SMD) calculations between study groups using an online calculator.[18] Meta-analyses were conducted using Comprehensive Meta-Analysis Version 2.2 (Biostat, Inc., Englewood, New Jersey, USA). Funnel plots were created for assessment of publication bias, where appropriate. Heterogeneity was estimated using the I^2 statistic, with a value $\geq 50\%$ representing a high level of heterogeneity.

10.3.5 RISK OF BIAS ASSESSMENT

RCTs included in meta-analyses were assessed for bias by utilizing the Cochrane Collaboration's assessment tool, whilst cohort/observational studies were subjected to the Newcastle-Ottawa scale.[19, 20]

10.3.6 QUALITY OF EVIDENCE

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guidelines were utilized to derive overall evidence quality for meta-analyses.[21]

10.4 Results

10.4.1 STUDY SELECTION

Figure 1 outlines the study selection process. There were 22 articles included in the systematic review, which were combined into 19 data-sets after accounting for overlapping data. RCTs or quasi-RCTs accounted for nine data-sets, whilst six and four data-sets were from retrospective and prospective cohort studies, respectively.[16, 22-41] Fourteen articles were eligible for meta-analyses.

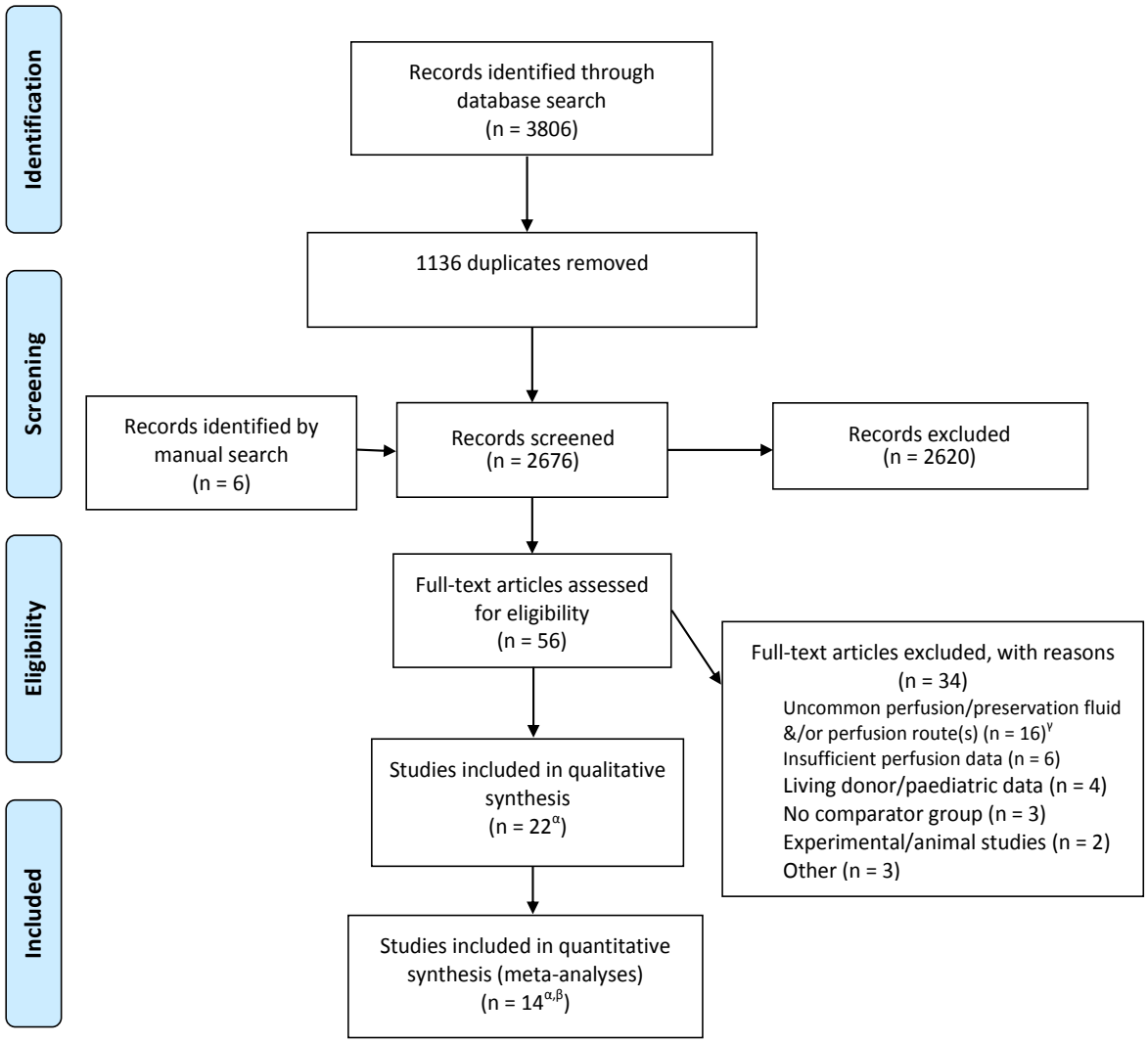


Figure 1. Study selection flow diagram.

α Includes articles with overlapping results that were analyzed together

β Parameters meta-analysed: peak aspartate aminotransferase & alanine aminotransferase, primary non-function, thrombotic graft loss, 12-month graft survival

γ Therefore unable to perform collective analysis of data

10.4.2 BIAS ASSESSMENT

The Cochrane Collaboration's tool was utilized for bias assessment of RCTs. Overall, selection bias and attrition bias were minimal, as evidenced by a low risk of bias for a majority of studies with regards to random sequence generation and incomplete outcome data presentation, respectively. There was a high risk of performance bias as it is extremely difficult if not impossible to blind surgical/perfusion staff. The remaining domains presented a mixed bias risk and/or were difficult to assess due to a lack of appropriate information (see Table, SDC 2).

Cohort study bias assessment is presented in SDC 3. Study cohort comparability was established in 78.6% of studies, especially with regards to organ CITs and donor and recipient ages. Less than 60% of the articles had adequate follow-up. The nature of outcome assessment by study personnel (i.e. independent blind assessment and/or record linkage) was not specified in 57.1% of cases.

There were too few studies within each parameter analysis to enable the appropriate interpretation of any funnel plots.

10.4.3 BASELINE STUDY CHARACTERISTICS

Summary information regarding liver perfusion articles is provided in Table 1. Overall, there were 2294 liver transplants, with a median CIT of 8.2 hours. The comparison between UW and HTK was the most common (six studies), followed by UW and Celsior (four studies). The majority of article data-sets utilized dual perfusion alone (12 of 19, 63.2%). Where specified, a rapid retrieval technique was explicitly employed in seven studies,(15) whilst a mixture of rapid and classic procurement techniques were specified in five studies.(14) The different study comparator groups are also compared with respect to other donor and recipient characteristics, such as cause of death, graft steatosis, graft peak transaminases, split liver utilization, and recipient sex and hepatitis virus status (SDC 4). Where reported, the vast majority of donor deaths were secondary to trauma or a cerebrovascular accident; in general, whole livers with mild steatosis or less were employed, with normal donor transaminases (donor AST and/or ALT was reported in seven studies, out of which it was only elevated in 10% of patients from one study[30]).

Table 1. Summary liver perfusion and preservation study characteristics.

LIVER Studies	Study Type	Study Period	Comparator Groups	*n* per group	Donor Age	Recipient Age	Recipient MELD (mean) or Child Score	CIT (hrs)	Aortic or Dual Perfusion	Total Perfusion (Flush) Volume (L)[Ω]	Back-table Flush HA/PV (L)
Anthuber et al., 1993[22]	R	1989-1993	UW perfusion/CS	74	31.1	48.6	NR	8.9	Aortic	NR	0/1
			UW perfusion/CS	57	33.7	47.5	NR	8.7	Dual	4	NR
Avolio et al., 2006[23]	R	NR	UW perfusion/CS	22	NR	52	Child C – 31.8%	9.8	Aortic	5.5	0/1
			HTK perfusion/CS	17	NR	44	Child C – 29.4%	10.7	Aortic	9	0/1
Boillot et al., 1993[42]	R	1990-1992	UW perfusion/CS	33	29.3	39.0	NR	11.0	Aortic	NR[†]	NR
			UW perfusion/CS	28	30.8	39.5	NR	10.8	Dual	NR[†]	NR
Cavallari et al., 2003[24]	RCT	1999-2001	UW perfusion/CS	90	49	49	Child C – 68.3%	7.3	Dual	4.2**	0.3/0.7
			Celsior perfusion/CS	83	45	50	Child C – 66.7%	7.4	Dual	6.3**	0.3/0.7
Chui et al., 1998[25]	RCT	1994-1995	Marshall (Ross) pre-flush + UW perfusion/CS[κ]	20	36.6	46.7[Φ]	NR	9.7[δ]	Aortic	6	0/0.2
			Marshall (Ross) pre-flush + UW perfusion/CS[κ]	20	35.1	38.2[Φ]	NR	8.9[δ]	Dual	6	0/0.2
De Goyet et al., 1994[26]	R	1990-1991	UW perfusion/CS	76	22	23	NR	13.3[δ]	Aortic	3	0/0.25
			UW perfusion/CS	64	22	24	NR	12.9[δ]	Dual	3.3	0/0
Dondero et al., 2010[39]	RCT	2007-2009	UW perfusion/CS	92	54	52	MELD – 15	< 8	Dual	4	0/0.75
			IGL-1 perfusion/CS	48	59	51	MELD – 17	< 8	Dual	4	0/0.75
Erhard et al., 1994[27]	RCT	1990-1992	UW perfusion/CS	30	31.4[γ]	41.4	NR	9.4	Dual	4	0/0.25
			HTK perfusion/CS	30	37.5[γ]	43.5	NR	9.7	Dual	20	0/0.5
Gabel et al., 2001[41]	R	NR	UW perfusion/CS	22	51[Φ]	51	NR	NR[ξ]	Aortic	3	NR
			UW perfusion/CS	22	41[Φ]	52	NR	NR[ξ]	Dual	4	NR
Garcia-Gil et al. 2006; Garcia-Gil et al., 2011[28, 29]	RCT	2001-2003	UW perfusion/CS	51	50.7	52.5	MELD – 15.7	6.6	Dual	5	0/1
			Celsior perfusion/CS	51	47.7	53.4	MELD – 15.3	6.4	Dual	6	0/1
Hatano et al., 1997[30]	P	NR	UW perfusion/CS	18	42.1	44.3	NR	12	Dual	4	NR
			HTK perfusion/CS	30	39.2	44.9	NR	10.2	Dual	20	NR

Lopez-Andujar et al., 2009[31]	Quasi-RCT*	2003-2005	UW perfusion/CS	104	51.4	52.9	Child C – 51.9%	6	Dual	4.4	0/0
			Celsior perfusion/CS	92	54.3	53.1	Child C – 48.9%	5.4	Dual	4.5	0/0
Mangus et al., 2006; Mangus et al., 2008[35, 36]	P	2001-2006	UW perfusion/CS	98	38	49	MELD – 17	8[γ]	Aortic	3.2***	NR
			HTK perfusion/CS	111	38	51	MELD – 18	6[γ]	Aortic	3.8***	NR
Meine et al., 2006[32]	RCT	2003-2004	UW perfusion/CS	65	38.1[γ]	49.9	Child C – 44.4%	9.7	Dual	3	0.5/0.5
			HTK perfusion/CS	37	44.6[γ]	51.4	Child C – 44.4%	8.8	Dual	5	0.5/0.5
Meine et al., 2015[37]	P	2009-2014	HTK perfusion/CS	65	45.4	53.3[ξ]	26	8.2	Dual	6	NR
			IGL-1 perfusion/CS	113	44.6	64.1[ξ]	22	8.2	Dual	4	NR
Moench et al., 2006[16]	P	1997-2005	UW perfusion/CS	268	47.3	50.9	NR	9.7[γ]	Dual	5	0.3/0[ϖ]
			HTK perfusion/CS	32	51.8	50.3	NR	11.0[γ]	Dual	12.5	NR
Nardo et al., 2001[34, 40]	RCT	NR	UW perfusion/CS	60	52.9	51.0	Child C – 53.3%	7.3	Dual	4.5	NR
			Celsior perfusion/CS	53	51.0	50.0	Child C – 43.4%	7.0	Dual	6.5	NR
Nardo et al., 2005[33]	RCT	NR	HTK perfusion/CS	20	57.9	51.3	Child C – 60%	7.5	Dual	10.5[€]	NR
			Celsior perfusion/CS	20	64.0	55.1	Child C – 70%	7.6	Dual	6.3[€]	NR
Wiederkehr et al., 2014[38]	R	2008-2013	HTK perfusion/CS	125	43.4[γ]	54.9[γ]	MELD – 17.5	7.4[γ]	Dual	3	0.5/0.5
			IGL-1 perfusion/CS	53	35.4[γ]	51[γ]	MELD – 19.9	5.4[γ]	Dual	3	0.5/0.5
Summary Data	P – 4 R – 6 RCT – 9	Range – 1989-2014	*UW vs HTK* – 6 *UW vs Celsior* – 4 *HTK vs IGL-1* – 2 *Other solution comparisons* – 7 *Use of pre-flush* – 1	Total – 2294	Median – 45 Range – 22-64	Median – 50.9 Range – 23-64.1	NA	Median – 8.2 Range – 5.4-13.3	*Aortic perfusion alone* – 2 *Dual perfusion alone* – 12 *Aortic vs dual perfusion* – 5	NA	NA

CIT – cold ischemic time; CS – cold storage; HA – hepatic artery; HTK – histidine-tryptophan-ketoglutarate; IGL-1 – Institut Georges Lopez; L – liters; MELD – model for end-stage liver disease score; NR – not recorded; P – prospective; PV – portal vein; R – retrospective; RCT – randomized control trial; UW – University of Wisconsin

No significant differences between parameters in comparator groups unless otherwise indicated

* Pseudo-randomized

** Estimate based on 60 ml/kg for UW, and 90 ml/kg for Celsior

*** Includes back-table flush volume, given via portal vein

[¥] Only standard criteria donor data from Mangus et al., 2008 included;[35] perfusion details utilized from Mangus et al., 2006 study[36]

[Φ] Significance not specified

[δ] Total ischemic time

[γ] $p < 0.05$

[€] Estimate based on 150 ml/kg for HTK, and 90 ml/kg for Celsior

[ξ] Article states no statistically significant difference between each group

[†] *In situ* perfusion ceased when "liver was palpably cold and free of blood"

[κ] Four liters of Ross pre-flush was given, followed by 2 L of UW flush, in both study groups

[ϖ] Given in 74 patients

[Ω] Does not include back-table flush volume, unless otherwise indicated

10.4.4 PERFUSION CHARACTERISTICS

UW solution was the most commonly employed perfusion and preservation solution. None of the included studies described the use of one fluid for perfusion and another for CS. Pre-flush was only utilized in one study.[25] UW dual perfusion was undertaken at lower volumes (median 4.4 L, range 3.0-5.0 L; n = 12 studies) compared to HTK (median 6 L, range 3.0-20.0 L; n = 7 studies) and Celsior (median 6.3 L, range 4.5-6.3 L; n = 5 studies), but not IGL-1 (median 4.0 L, range 3.0-4.0 L; n = 3 studies). Median volumes for aortic-only UW and HTK perfusion were 3.2 L (range 3.0-5.5 L; n = 4 studies) and 3.8 L (range 3.8-9.0 L; n = 2 studies), respectively.

A median of 1.0 L of perfusion fluid was utilized on the back-table for each of the UW (range 0.25-1.0; n = 10 studies), HTK (range 0.5-1.0; n = 5 studies), Celsior (n = 2 studies) and IGL-1 (n = 2 studies) groups. When the back-table perfusion route is stratified by perfusion fluid, the portal vein was solely utilized in 5 of 10 studies employing UW, compared to one study that only utilized the hepatic artery and three studies that undertook back-table perfusion via the portal vein and

hepatic artery or bile duct. Three HTK studies (out of 5) utilized only the portal vein on the back-table, whilst mixed porto-arterial perfusion was pursued in a further two studies. One study each using Celsior employed solely the portal vein or both the portal vein and hepatic artery, whilst both IGL-1 articles utilized mixed back-table perfusion. Importantly, all studies that employed aortic-only *in situ* perfusion did so in conjunction with back-table portal perfusion, with the exception of one article in which the utilization of back-table perfusion was not specified.[41]

10.4.5 META-ANALYSES

Aortic versus Dual perfusion (UW)

Overall study quality was very low (see Table, SDC 5). Two parameters were eligible for meta-analysis – peak ALT and graft PNF. There were no significant differences between aortic or dual UW perfusion with respect to PNF rates (Figure 2). Peak ALT post-transplantation was however significantly lower in the aortic-only perfusion group (SMD 0.24; 95% CI 0.01-0.47; p = 0.04).

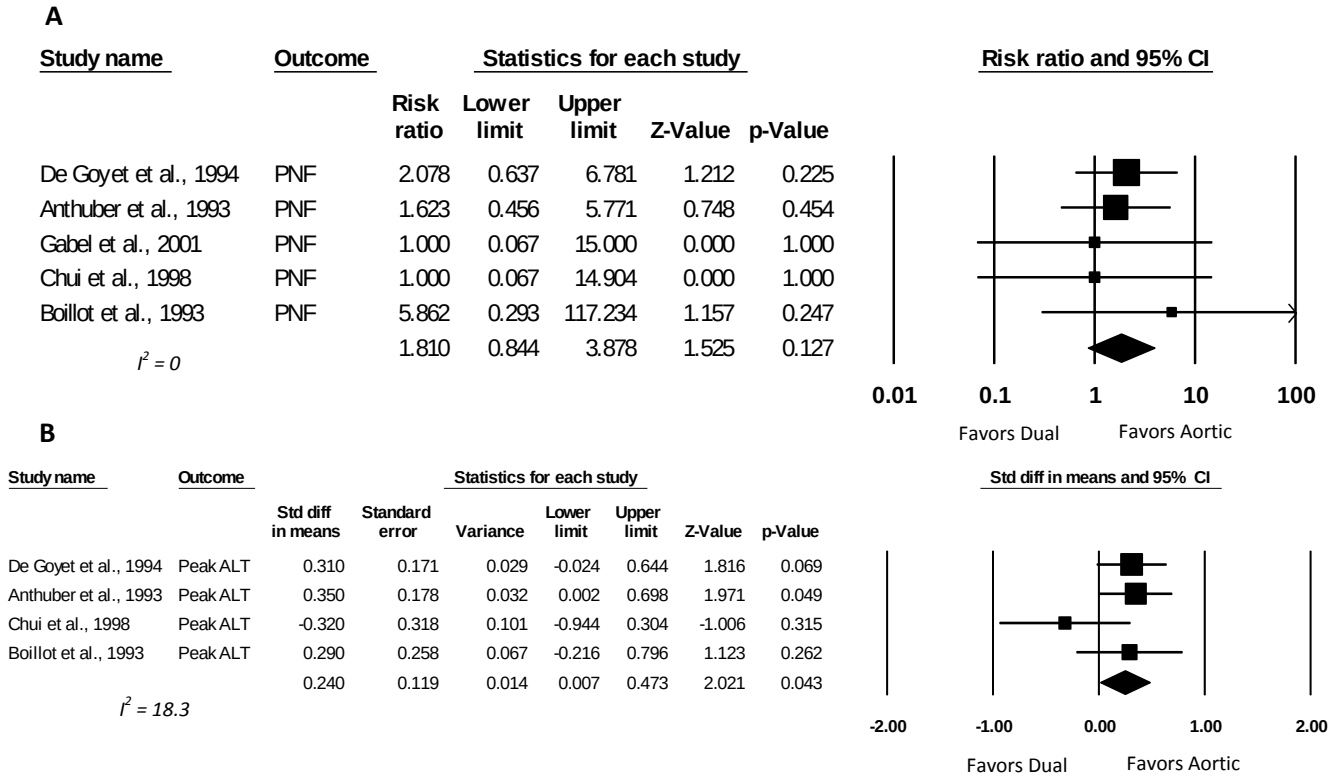


Figure 2. Forest plots for (A) PNF, and (B) peak ALT after *in situ* aortic or dual UW perfusion and preservation of the liver.

UW versus HTK dual perfusion

Study quality, as derived using the GRADE guidelines, was once again very low (SDC 5). There were no significant differences in peak post-transplantation ALT or AST upon UW or HTK dual perfusion and preservation (Figure 3).

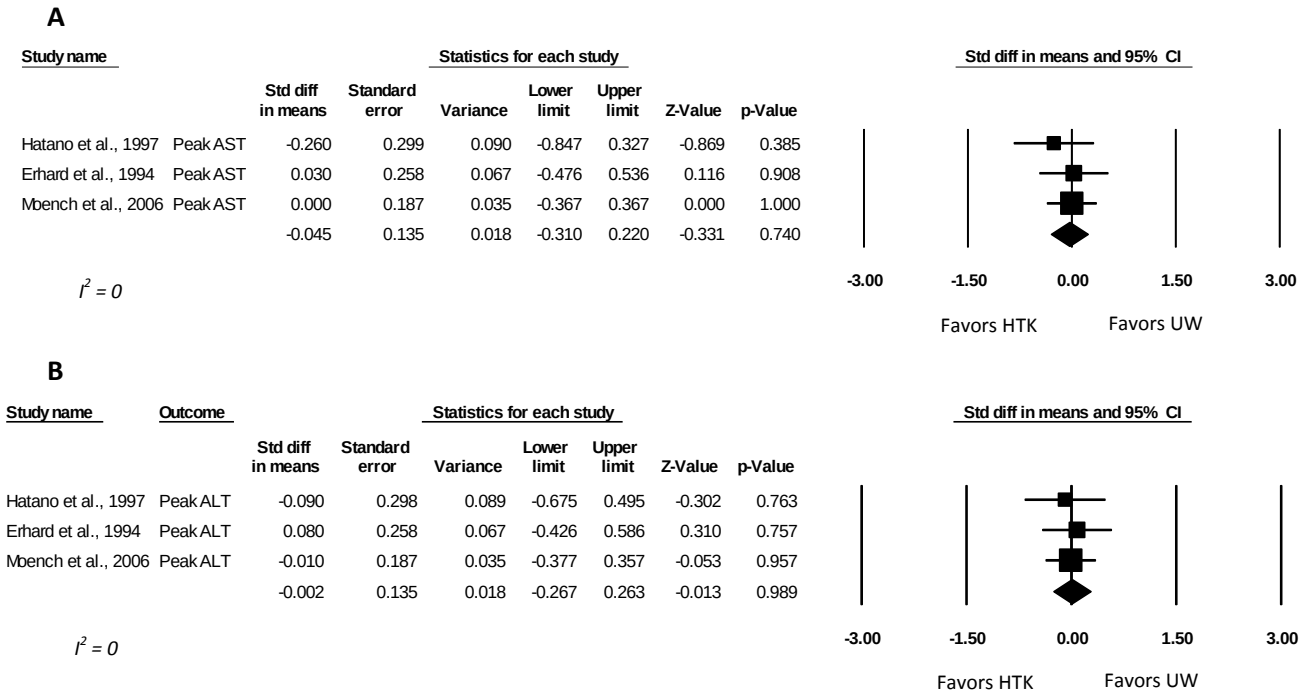


Figure 3. Forest plots for (A) peak AST, and (B) peak ALT after *in situ* dual perfusion and preservation of the liver with UW or HTK.

UW versus Celsior dual perfusion

Study quality, based on the GRADE guidelines, was moderate (SDC 5). Thrombotic graft loss/re-transplantation and PNF rates, in addition to 1-year graft survival, were not significantly different for either perfusion/preservation solution (Figure 4).

10.4.6 OTHER COMPARISONS

Thrombotic graft loss

Three studies compared graft loss secondary to hepatic artery thrombosis after UW aortic-only versus dual perfusion.[26, 41, 42] In the aortic-only perfusion groups, rates were 3.9% (three patients)[26], 0% [42] and 4.5% (one patient)[41], whilst in the respective dual-perfused groups, thrombotic graft loss occurred in 6.3% (four patients)[26], 0 patients[42] and 0 patients[41] ($p > 0.05$).

Graft loss secondary to hepatic arterial thrombosis in the various study groups was generally sparsely reported. For studies employing aortic-only *in situ* perfusion, data was available only for UW perfusion/CS (median 3.9%; range 0-4.5%; n = 131 patients, 3 studies). In the dual-perfused groups, UW-perfused/CS livers had a median hepatic arterial thrombotic graft loss rate of 1.0% (range 0-6.3%; n = 359, 6 studies), compared to 3.1% (range 0-3.1%; n = 85, 2 studies), 2.0% (range 0-2.4%; n = 246, 4 studies) and 0.9% (n = 113, 1 study) for HTK, Celsior and IGL-1, respectively.

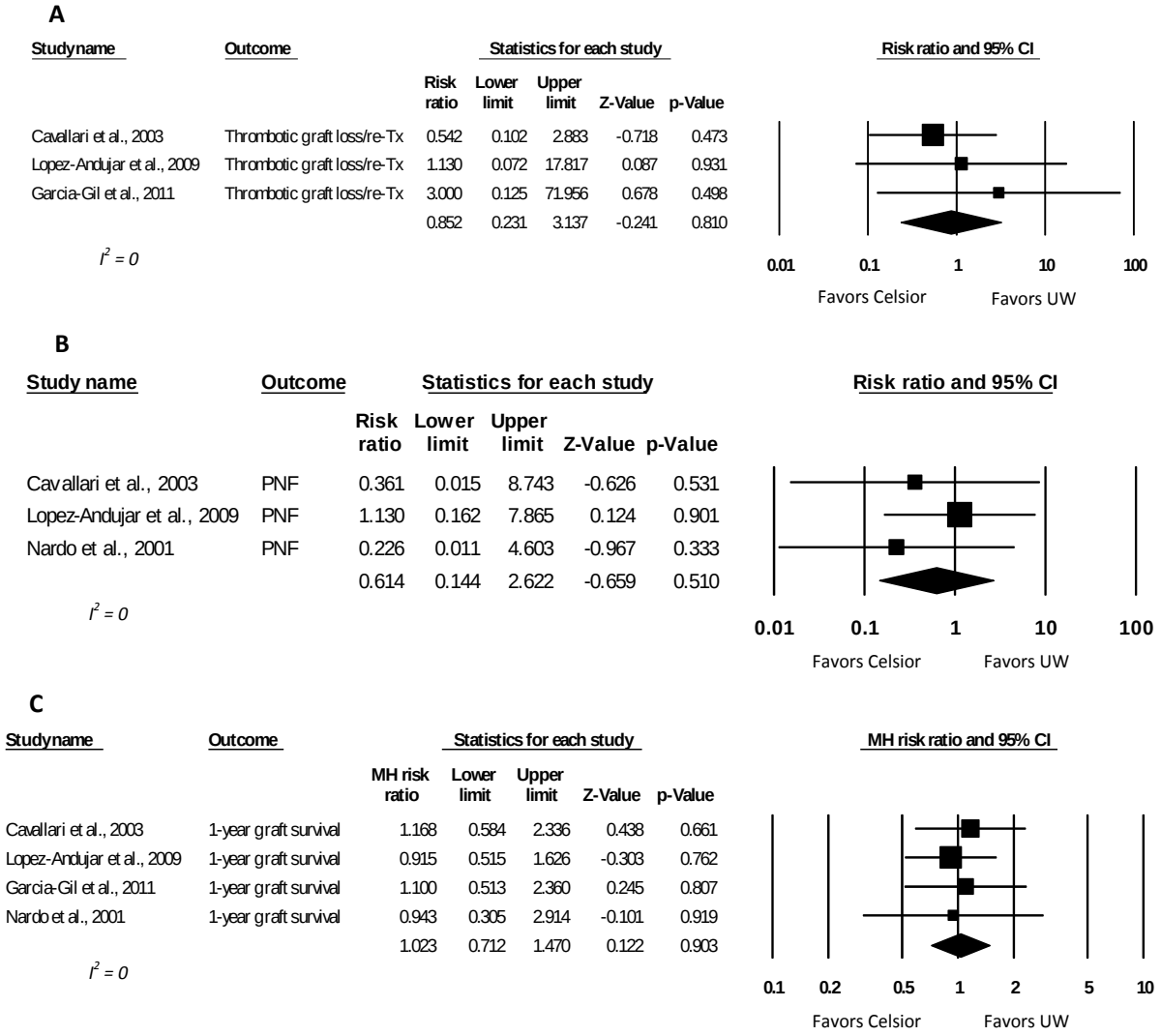


Figure 4. Forest plots for (A) thrombotic graft loss/re-transplantation, (B) PNF, and (C) 1-year graft survival after *in situ* dual perfusion and preservation of the liver with UW or Celsior.

Ischemic anastomotic and non-anastomotic biliary complications (Ischemic-type biliary lesions [ITBL])

One article reported biliary stenosis/ITBL after utilization of aortic-only perfusion and hepatic preservation.[23] Multiple intra-hepatic stenosis occurred in none of the patients receiving a graft perfused with UW compared to one (5.9%) from the HTK-perfused recipient cohort, with up to six months follow-up ($p > 0.05$). All patients in this study underwent portal vein back-table perfusion at the donor center, but not hepatic artery back-table perfusion.

Biliary complication rates after *in situ* liver dual perfusion/ CS using UW were available from five articles. Comparative anastomotic and/or non-anastomotic stricture rates between UW and Celsior in Lopez-Andujar *et al*.'s study were 3.9% (4/103) versus 2.2% (2/92), respectively, and 11.8% (6/51) versus 15.7% (8/51) in another study ($p > 0.05$ for both studies).[29, 31] Dondero *et al*. compared UW and IGL-1, with non-anastomotic stricture rates of 3.3% (3/92) versus 2.1% (1/48), respectively ($p > 0.05$).[39] Hepatic arterial back-table perfusion was not utilized in any of these studies. UW was compared to HTK by both Moench *et al*. and Meine *et al*., with no significant differences in ischemic biliary complications between both perfusion/preservation fluids in either study.[16, 32] Notably, Moench *et al*. suggested that ITBL rates were significantly lower in UW-perfused and preserved livers that underwent high-pressure arterial back-table perfusion compared to UW perfusion without this (2.7% compared to 21.1%, $p < 0.001$).[16]

Graft survivals

Meta-analyses were not possible for graft survival comparisons in a majority of cases, with the exception of UW versus Celsior dual perfusion (Figure 4). There was no one-year graft survival data for aortic or dual perfusion using IGL-1, aortic-only perfusion utilizing Celsior, or aortic versus dual UW perfusion/CS. Aortic compared to dual UW perfusion/CS survivals were however available after 20 months in one study – 72.9% (62/85 Patients) versus 61.5% (48/78), respectively ($p > 0.05$).[26]

One-year graft survivals were available from one study for UW (n = 98 patients) compared to HTK aortic-only (n = 98 patients) liver perfusion; respective survivals were 83.7% and 86.5% ($p > 0.05$).[35] UW dual perfusion yielded a median one-year graft survival of 85.0% (range 80.0-93.8%, n = 370 patients, 5 studies), compared to 83.0% for Celsior dual perfusion (range 78.4-90.6%, n = 299, 5 studies). One-year graft survival after HTK dual perfusion was 94.0% (range 75.0-94.0%, n = 2 studies), although this analysis only included data from a total of 57 patients.

10.5 Discussion and Conclusions

This systematic review has attempted to analyze the data in the literature regarding the ideal perfusion route (aortic-only or dual), volume(s) and solution(s) for DBD liver transplantation. *In situ* liver perfusion utilizing UW is the most common occurrence in the literature. UW appears to be perfused via the aortic and portal routes in a majority of studies, and at lower volumes compared to HTK and Celsior. Although the overall quality of included articles was either low or moderate, the most important finding of this study is the lack of a significant beneficial effect to the use of dual perfusion over aortic-only perfusion with respect to early and one-year graft outcomes. Furthermore, after stratifying by *in situ* perfusion routes, we were unable to show significant differences in post-transplantation outcomes including thrombotic graft loss, graft survival and ITBL for grafts that underwent UW, HTK, Celsior or IGL-1 perfusion and subsequent CS. This latter observation should however be interpreted in the context of insufficient study data for these parameters in the majority of perfusion fluid and/or route comparisons, thereby preventing further statistical analyses.

Dual perfusion during procurement entails cannulation and fluid perfusion via both the aorta and portal vein, and necessarily requires more preparation time and dissection in comparison to aortic-only perfusion. Furthermore, dual perfusion poses added potential risks when the pancreas is to be retrieved, due to potential blockage of pancreas perfusate outflow and subsequent pancreatic congestion.[43-45] Although the dual perfusion technique should theoretically achieve more comprehensive liver perfusion and cooling, at a faster rate, final liver temperature appears to be very similar to that achieved via aortic-only cooling.[25] Perhaps of more significance than the rate at which an organ is cooled is its rate of rewarming, which may partially explain the advantages of controlled rewarming and/or subnormothermic machine perfusion.[46, 47] Furthermore, aortic-only cooling also indirectly provides a portal flush through the mesenteric venous outflow.[25] An additional consideration that possibly explains the equivalence of the two techniques is the use of a portal venous back-table flush in at least five of seven articles utilizing aortic-only *in situ* perfusion. Meta-analyses in this study showed a lower graft peak ALT but not PNF after aortic-only versus dual perfusion, and there was no evidence of impaired graft survival. The impact of possible confounding factors such as donor liver steatosis, elevated donor enzymes and split liver utilization could not be reliably assessed due to insufficient available data (SDC 4). Nevertheless, the overall outcome data from this systematic review and meta-analysis does not support the

additional time and complexity of establishing dual perfusion *in situ* compared to aortic-only perfusion.

The only objective evidence in favor of dual perfusion in the literature to our knowledge is provided by D'Amico *et al*., who compared aortic to dual perfusion using Celsior for "suboptimal" liver procurement, without associated pancreas retrieval.[48] This study was excluded from our analyses as it employed a modified portal perfusion technique using Celsior with simultaneous tourniquet clamping of splenomesenteric inflow, and focused on suboptimal grafts. D'Amico *et al*. included data from a total of 35 patients, and although not statistically significant, the aortic-flush group here had a trend towards greater CITs and donor hemodynamic compromise, and a higher proportion of recipients with hepatitis C as the reason for transplantation. Use of dual perfusion in suboptimal/expanded criteria livers in preference to aortic-only perfusion is not supported by other major studies, and as such this remains an area for further investigation. Moreover, some authors also recommend dual perfusion during DCD liver retrieval.[49] Similarly, this recommendation is not supported by any significant evidence in the literature and requires additional research.

Multiple abdominal organ perfusion and preservation fluids are available, with differing viscosities, electrolyte compositions, and other mediators. Although previous systematic reviews have attempted to compare hepatic allograft outcomes stratified by preservation fluid, the *in situ* perfusion routes were altogether ignored; it is highly likely that final graft outcomes are related not only to organ preservation during transportation per se, but also to the period of *in situ* perfusion.[9, 50] From our findings, there appears to be no difference in at least short-term liver transplant outcomes when DBD grafts are perfused and subsequently stored in UW, HTK, Celsior or IGL-1. Survival data was limited and far from conclusive for one fluid over another. However, in a recent multi-center European database analysis, Adam *et al*. suggested lower three-year graft survivals in HTK-preserved grafts, including split livers, in comparison to UW, IGL-1 and Celsior.[8] The possible deleterious effect of HTK may be related to CITs and donor status, with Stewart *et al*. showing a further increase in graft loss for HTK livers compared to UW when DCD livers and/or livers with CITs more than eight hours were transplanted.[7]

ITBL present a significant complication of liver transplantation that can potentially be targeted by alterations in perfusion fluids and techniques. Indeed, Eurotransplant guidelines recommend high-

pressure arterial perfusion of the hepatic graft on the back-table to prevent ITBL based on the work of Moench *et al*.[3, 51] The theoretical basis for this is provided by the apparent impairment in perfusion of small vessels supplying the biliary tree if higher viscosity fluids such as UW are employed; this may be negated by high pressure perfusion via the aorta or on the back-table via hepatic artery.[16, 51, 52] The corollary of this is that the use of HTK itself may reduce intra-hepatic biliary strictures when compared to UW, especially in DCD donors, due to its lower viscosity.[35, 53, 54] Data from the studies included in this review does not appear to support these assertions,[16, 32], although this may have been impacted by the fact that only DBD donor data was included. Furthermore, back-table hepatic arterial perfusion was not utilized in multiple studies, seemingly without deleterious consequences to biliary luminal integrity.

Procurement costs are an important consideration in most parts of the world, and have in some cases driven research into alternative flush and perfusion strategies. The majority of articles comparing perfusion economics analyze alternatives to the relatively higher cost UW solution. One liter of UW costs $300 to $500 US dollars.[55-57] Adam *et al.* in France substituted UW dual liver perfusion with Euro-Collins aortic perfusion/ UW portal perfusion, demonstrating savings of $750 per case, and perhaps even improved immediate graft parameters.[58] A potential area of cost-saving may also be provided by switching from dual to aortic-only UW perfusion, with lower UW volumes used in aortic-only perfusion, although this remains to be formally proven. Considering that cumulative evidence does not seem to support dual liver perfusion, a cost advantage here may provide further impetus to utilize the single route.

Results presented in this systematic review and meta-analysis must be interpreted cautiously. In particular, overall study quality, as determined by the GRADE assessment, was mostly very low, and at best moderate (SDC 5). Selection bias also needs to be considered as much of the study data is derived from recipient liver transplantation outcomes, and as such is confounded by the omission of grafts that may have been discarded. Heterogeneity, small study sample sizes, inadequate patient follow-up in some studies, and a significant proportion of observational studies all introduced further biases to overall effect estimates, necessitating the use of random effects models in all meta-analyses. With respect to the RCTs alone, blinding of research personnel was of concern, although this is to be expected in studies of this nature; furthermore, a significant proportion of domains could not be assessed due to a lack of appropriate information. In addition,

we could not formulate conclusions regarding optimal volumes of preservation solution during *in situ* perfusion due to a paucity of relevant data.

Overall, we have attempted to correlate liver transplantation outcomes with the initial route of *in situ* cold perfusion, in addition to the preservation solution used for this perfusion and subsequently also for static cold storage. Because it is extremely difficult, if not impossible, to tease out the individual effects of *in situ* perfusion and then later cold static storage/preservation, study groups have been analyzed with both factors in mind.

We have shown that despite the ubiquity of dual perfusion in the literature and guidelines, its utilization has not been supported by better outcomes in comparison to aortic-only perfusion for DBD liver transplantation. It should however be noted that aortic-only perfusion is usually accompanied by a portal venous back-table flush. There is insufficient data to draw robust conclusions about the outcome associated with the use of different perfusion/preservation fluids, especially with regards to graft survivals, ITBL rates, and thrombotic graft loss rates. Outcome data is also lacking regarding the utilization of an *in situ* pre-flush, optimal perfusion volumes, perfusion in DCD donors, appropriate protocols for back-table perfusion, and the use of dual perfusion in suboptimal donors. Additional appropriately powered RCTs focusing on these specific issues are required to resolve these questions. If aortic-only perfusion is indeed proven to be cheaper and not deleterious in comparison to dual perfusion, including in the DCD and expanded criteria donor setting, this may influence procurement surgeons towards the utilization of a more unified retrieval approach.

10.6 References

1. Zalewska K, Ploeg R. National Standards for Organ Retrieval from Deceased Donors (NORS Retrieval Standards). Bristol, UK; 2014.
2. TSANZ. Guidance Document - Surgical Technique for Deceased Donor Abdominal Organ Procurement (ATCA-TSANZ Guidelines G003/2015). Sydney, Australia: TSANZ; 2015.
3. Eurotransplant Foundation. Eurotransplant Manual. Leiden: Netherlands: Eurotransplant 2016.
4. Lema Zuluaga GL, Serna Agudelo RE, Zuleta Tobon JJ. Preservation solutions for liver transplantation in adults: celsior versus custodiol: a systematic review and meta-analysis with an indirect comparison of randomized trials. *Transplant Proc*. 2013; 45: 25-32.
5. Voigt MR, DeLario GT. Perspectives on abdominal organ preservation solutions: a comparative literature review. *Prog Transplant*. 2013; 23: 383-391.
6. Latchana N, Peck JR, Whitson BA, Henry ML, Elkhammas EA, Black SM. Preservation solutions used during abdominal transplantation: Current status and outcomes. *World J Transplant*. 2015; 5: 154-164.
7. Stewart ZA, Cameron AM, Singer AL, Montgomery RA, Segev DL. Histidine-Tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. *Am J Transplant.* 2009; 9: 286-293.
8. Adam R, Delvart V, Karam V, Ducerf C, Navarro F, Letoublon C, et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant*. 2015; 15: 395-406.
9. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. The effect of preservation solutions for storage of liver allografts on transplant outcomes: a systematic review and meta-analysis. *Ann Surg*. 2014; 260: 46-55.
10. Hawthorne W, Hameed A, Pleass H. Organ perfusion and preservation: current methods to provide optimal organ preservation and best transplantation outcomes. PROSPERO 2016: CRD42016038993. http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016038993. Accessed December 2016.
11. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009; 339.
12. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000; 283: 2008-2012.
13. Braat AE, Blok JJ, Putter H, Adam R, Burroughs AK, Rahmel AO, et al. The Eurotransplant Donor Risk Index in Liver Transplantation: ET-DRI. *Am J Transplant*. 2012; 12: 2789-2796.
14. Starzl TE, Hakala TR, Shaw BW, Jr., Hardesty RL, Rosenthal TJ, Griffith BP, et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet*. 1984; 158: 223-230.
15. Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. *Surg Gynecol Obstet*. 1987; 165: 343-348.
16. Moench C, Otto G. Ischemic type biliary lesions in histidine-tryptophan-ketoglutarate (HTK) preserved liver grafts. *Int J Artif Organs.* 2006; 29: 329-334.
17. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, et al. Risk factors for primary dysfunction after liver transplantation--a multivariate analysis. *Transplantation*. 1993; 55: 807-813.

18. Wilson D. Practical Meta-Analysis Effect Size Calculator. http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php. Accessed November 2016.
19. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011; 343: d5928.
20. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed June 2016.
21. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011; 64: 383-394.
22. Anthuber M, Zuelke C, Forst H, Welte M, Groh J, Maag K, et al. Experiences with a simplified liver harvesting technique--single aorta in situ flush followed by portal back table flush. *Transplant Proc*. 1993; 25: 3154-3155.
23. Avolio AW, Agnes S, Nure E, Maria G, Barbarino R, Pepe G, et al. Comparative evaluation of two perfusion solutions for liver preservation and transplantation. *Transplant Proc*. 2006; 38: 1066-1067.
24. Cavallari A, Cillo U, Nardo B, Filipponi F, Gringeri E, Montalti R, et al. A multicenter pilot prospective study comparing Celsior and University of Wisconsin preserving solutions for use in liver transplantation. *Liver Transplant.* 2003; 9: 814-821.
25. Chui AK, Thompson JF, Lam D, Koutalistras N, Wang L, Verran DJ, et al. Cadaveric liver procurement using aortic perfusion only. *ANZ J Surg*. 1998; 68: 275-277.
26. de Ville de Goyet J, Hausleithner V, Malaise J, Reding R, Lerut J, Jamart J, et al. Liver procurement without in situ portal perfusion. A safe procedure for more flexible multiple organ harvesting. *Transplantation*. 1994; 57: 1328-1332.
27. Erhard J, Lange R, Scherer R, Kox WJ, Bretschneider HJ, Gebhard MM, et al. Comparison of histidine-tryptophan-ketoglutarate (HTK) solution versus University of Wisconsin (UW) solution for organ preservation in human liver transplantation. A prospective, randomized study. *Transpl Int*. 1994; 7: 177-181.
28. Garcia-Gil FA, Arenas J, Guemes A, Esteban E, Tome-Zelaya E, Lamata F, et al. Preservation of the liver graft with Celsior solution. *Transplant Proc.* 2006; 38: 2385-2388.
29. Garcia-Gil FA, Serrano MT, Fuentes-Broto L, Arenas J, Garcia JJ, Guemes A, et al. Celsior versus University of Wisconsin preserving solutions for liver transplantation: postreperfusion syndrome and outcome of a 5-year prospective randomized controlled study. *World J Surg*. 2011; 35: 1598-1607.
30. Hatano E, Kiuchi T, Tanaka A, Shinohara H, Kitai T, Satoh S, et al. Hepatic preservation with histidine-tryptophan-ketoglutarate solution in living-related and cadaveric liver transplantation. *Clinical Sci (Lond)*. 1997; 93: 81-88.
31. Lopez-Andujar R, Deusa S, Montalva E, San Juan F, Moya A, Pareja E, et al. Comparative prospective study of two liver graft preservation solutions: University of Wisconsin and Celsior. *Liver Transplant*. 2009; 15: 1709-1717.
32. Meine MH, Zanotelli ML, Neumann J, Kiss G, de Jesus Grezzana T, Leipnitz I, et al. Randomized clinical assay for hepatic grafts preservation with University of Wisconsin or histidine-tryptophan-ketoglutarate solutions in liver transplantation. *Transplant Proc*. 2006; 38: 1872-1875.
33. Nardo B, Bertelli R, Montalti R, Beltempo P, Puviani L, Pacile V, et al. Preliminary results of a clinical randomized study comparing Celsior and HTK solutions in liver preservation for transplantation. *Transplant Proc*. 2005; 37: 320-322.

34. Nardo B, Catena F, Cavallari G, Montalti R, Di Naro A, Faenza A, et al. Randomized clinical study comparing UW and Celsior solution in liver preservation for transplantation: preliminary results. *Transplant Proc*. 2001; 33: 870-872.
35. Mangus RS, Fridell JA, Vianna RM, Milgrom MA, Chestovich P, Chihara RK, et al. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution in extended criteria liver donors. *Liver Transplant*. 2008; 14: 365-373.
36. Mangus RS, Tector AJ, Agarwal A, Vianna R, Murdock P, Fridell JA. Comparison of histidine-tryptophan-ketoglutarate solution (HTK) and University of Wisconsin solution (UW) in adult liver transplantation. *Liver Transplant*. 2006; 12: 226-230.
37. Meine MH, Leipnitz I, Zanotelli ML, Schlindwein ES, Kiss G, Martini J, et al. Comparison Between IGL-1 and HTK Preservation Solutions in Deceased Donor Liver Transplantation. *Transplant Proc*. 2015; 47: 888-893.
38. Wiederkehr JC, Igreja MR, Nogara MS, Goncalves N, Montemezzo GP, Wiederkehr HA, et al. Use of IGL-1 preservation solution in liver transplantation. *Transplant Proc*. 2014; 46: 1809-1811.
39. Dondero F, Paugam-Burtz C, Danjou F, Stocco J, Durand F, Belghiti J. A randomized study comparing IGL-1 to the University of Wisconsin preservation solution in liver transplantation. *Ann Transplant*. 2010; 15: 7-14.
40. Nardo B, Beltempo P, Bertelli R, Montalti R, Vivarelli M, Urbani L, et al. Comparison of Celsior and university of Wisconsin solutions in cold preservation of liver from octogenarian donors. *Transplant Proc*. 2004; 36: 523-524.
41. Gabel M, Liden H, Norrby J, Friman S, Wolfbrandt A, Olausson M. Early function of liver grafts preserved with or without portal perfusion. *Transplant Proc*. 2001; 33: 2527-2528.
42. Boillot O, Benchetrit S, Dawahra M, Porcheron J, Martin X, Fontaumard E. Early graft function in liver transplantation: comparison of two techniques of graft procurement. *Transplant Proc*. 1993; 25: 2626-2627.
43. Nghiem DD, Cottington EM. Pancreatic flush injury in combined pancreas-liver recovery. *Transpl Int*. 1992; 5: 19-22.
44. Brockmann JG, Vaidya A, Reddy S, Friend PJ. Retrieval of abdominal organs for transplantation. *Br J Surg*. 2006; 93: 133-146.
45. Sollinger HW, Vernon WB, D'Alessandro AM, Kalayoglu M, Stratta RJ, Belzer FO. Combined liver and pancreas procurement with Belzer-UW solution. *Surgery*. 1989; 106: 685-691.
46. Bruinsma BG, Yeh H, Özer S, Martins PN, Farmer A, Wu W, et al. Subnormothermic Machine Perfusion for ex vivo Preservation and Recovery of the Human Liver for Transplantation. *Am J Transplant*. 2014; 14: 1400-1409.
47. Minor T, Efferz P, Fox M, Wohlschlaeger J, Luer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am J Transplant*. 2013; 13: 1450-1460.
48. D'Amico F, Vitale A, Gringeri E, Valmasoni M, Carraro A, Brolese A, et al. Liver transplantation using suboptimal grafts: impact of donor harvesting technique. *Liver Transplant*. 2007; 13: 1444-1450.
49. Oniscu GC, Forsythe JLR, Fung JJ. Abdominal organ retrieval and transplantation bench surgery. Chichester, West Sussex: John Wiley & Sons; 2013.
50. Feng L, Zhao N, Yao X, Sun X, Du L, Diao X, et al. Histidine-tryptophan-ketoglutarate solution vs. University of Wisconsin solution for liver transplantation: a systematic review. *Liver Transplant*. 2007; 13: 1125-1136.
51. Moench C, Moench K, Lohse AW, Thies J, Otto G. Prevention of ischemic-type biliary lesions by arterial back-table pressure perfusion. *Liver Transplant*. 2003; 9: 285-289.

52. Langrehr JM, Schneller A, Neuhaus R, Vogl T, Hintze R, Neuhaus P. [Etiologic factors and incidence of ischemic type biliary lesions (ITBL) after liver transplantation]. *Langenbecks Arch Chir Suppl Kongressbd*. 1998; 115: 1560-1562.
53. Mangus R, Fridell J, Kubal C, Chihara R, Marshall W, Tector A. A comparison of liver transplant biliary complications for deceased donor drafts preserved with histidine-tryptophan-ketoglutarate and University of Wisconsin solutions. *Transplantation*. 2016; 100: S17.
54. Heidenhain C, Pratschke J, Puhl G, Neumann U, Pascher A, Veltzke-Schlieker W, et al. Incidence of and risk factors for ischemic-type biliary lesions following orthotopic liver transplantation. *Transpl Int*. 2010; 23: 14-22.
55. Gonzalez AM, Filho GJ, Pestana JO, Linhares MM, Silva MH, Moura RM, et al. Effects of Eurocollins solution as aortic flush for the procurement of human pancreas. *Transplantation*. 2005; 80: 1269-1274.
56. Alonso D, Dunn TB, Rigley T, Skorupa JY, Schriner ME, Wrenshall LE, et al. Increased pancreatitis in allografts flushed with histidine-tryptophan-ketoglutarate solution: a cautionary tale. *Am J Transplant*. 2008; 8: 1942-1945.
57. Englesbe MJ, Heidt D, Sung R, Pietroski R. Does using HTK solution for cold perfusion of cadaveric kidneys save money? *Transplantation*. 2006; 81: 1750.
58. Adam R, Astarcioglu I, Raccuia JS, Ducot B, Reynes M, Bismuth H. Beneficial effects of Eurocollins as aortic flush for the procurement of human livers. *Transplantation*. 1996; 61: 705-709.

Chapter 11

Reply to Letter "Liver preservation solutions: Absence of proof is not proof of absence"

Ahmer Hameed
Jerome Laurence
Vincent Lam
Henry Pleass
Wayne Hawthorne

As published in Liver Transplantation 2018, 24(8): 1144-46; DOI: 10.1002/lt.25195

11.1 Letter to the Editor

Dear Editor,

We thank Adam and colleagues for their interest in our recent article.[1] In particular, they are concerned, and we are very sympathetic to their view, that histidine-tryptophan-ketoglutarate (HTK) is not being presented as a cause of graft loss due to insufficient power. We are also well aware of the large registry analyses referenced to by Adam *et al*, two of which are also specifically referenced in the Discussion of our own article and therefore not missed.[2, 3]

However, if our paper is read in detail, it will be evident that the conclusions stem from the lack of strong evidence within the constraints of strict inclusion/exclusion criteria as specified by the systematic review's inclusion/exclusion criteria. The low quality of evidence, small patient numbers, and limits of article selection are all outlined at-length in our article. The primary reason that Adam *et al*. and Stewart *et al.*'s studies were not included in our systematic review/meta-analysis is that they did not include relevant retrieval details, such as perfusion route(s), volume(s), and back-table perfusion methods, and were hence not applicable to the aims of our study. Furthermore, our emphasis was on shorter-term graft outcomes, as longer-term graft survival was not expounded upon in a majority of studies – as such, only meta-analyses comparing peak AST and ALT after University of Wisconsin (UW) or HTK perfusion/preservation were possible. It should also be noted that both older and more recent systematic reviews/meta-analyses comparing HTK to other preservation fluids have also not shown significantly deleterious effects upon the use of HTK.[4-8] Similarly, they were unable to explore longer-term graft survival and could not include the analyses by Stewart *et al*. and Adam *et al*. in the formulation of their final conclusions.

There are many proponents and opponents of big data within the field of transplantation, and it is not our intention to take a 'side' in this debate here. Nevertheless, major issues with registry data still need to be considered.[9-11]

These studies cannot always be considered to provide the final word especially when it is technically and ethically feasible to conduct multi-center randomized control trials, in association with a common event rate (graft loss). We also note the concerns expressed by Nashan *et al*. regarding the original analyses by Adam and colleagues, and applaud the further analyses addressing some of the expressed concerns, including a propensity-based analysis.[11-13]

However, another potential confounder not considered by these analyses is the use of aortic-only or dual perfusion, which may significantly impact graft function – once again, this was not clearly shown by our systematic review, but is nonetheless still controversial and has spurred us to look at Australian liver transplantation outcomes over the preceding 10 years (Paper Submitted for Publication).

In summary, and specifically in response to Adam *et al*.'s comments:

1. "*Was this meta-analysis able to obtain a good scientific comparison of preservation solutions*?" The meta-analyses were conducted in a scientifically sound manner, using strict inclusion/exclusion criteria, and in association with risk of bias assessments. Statistical comparisons could only be made depending on the availability of appropriate data-points within each included study.
2. "*What is the clinical relevance of the results of this meta-analysis*?" This systematic review/meta-analysis aimed to approach the broader issue of retrieval technique, and perfusion practices, in the context of disparate practices and guidelines throughout the transplant world. It should also be interpreted in conjunction with a parallel systematic review/meta-analysis for pancreas transplantation.[14] We highlight that many individual issues related to retrieval practice, in particular the choice between aortic-only and dual perfusion, need to be definitively resolved, such that retrieval and perfusion can be unified worldwide.
3. "*How to integrate this "lack of evidence" in the knowledge of significant difference demonstrated by large patient cohort studies*?" In no way did we attempt to hide the results from the aforementioned large cohort studies, far the opposite we indeed discussed these in our paper. Even within the Eurotransplant region, HTK is not specifically precluded for the purposes of liver preservation, despite the conclusions of these registry studies.[15]

Overall, it is expected that readers and in particular expert policy makers should be able to synthesize all available evidence to come to an informed decision regarding the ultimate choice of perfusion/preservation fluid. We are in no way promoting HTK, and indeed utilize UW for our own liver and multiorgan retrievals. However it is imperative that each individual study only makes conclusions applicable to the data at hand – national/international guidelines should be able to synthesize the best recommended practice and also drive any further research

that may be required. With the increasing interest in organ preservation and many disparate views regarding the optimal technique for liver preservation, as evidenced by the letter by Adam *et al.*, the transplant community needs to instigate further multi-center RCTs across the deceased organ donor sector.

11.2 References

1. Hameed AM, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: Working toward a unified approach. *Liver Transpl*. 2017; 23: 1615-1627.
2. Adam R, Delvart V, Karam V, Ducerf C, Navarro F, Letoublon C, et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant*. 2015; 15: 395-406.
3. Stewart ZA, Cameron AM, Singer AL, Montgomery RA, Segev DL. Histidine-Tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. *Am J Transplant*. 2009; 9: 286-293.
4. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. The effect of preservation solutions for storage of liver allografts on transplant outcomes: a systematic review and meta-analysis. *Ann Surg*. 2014; 260: 46-55.
5. Rao F, Yang J, Gong C, Huang R, Wang Q, Shen J. Systematic review of preservation solutions for allografts for liver transplantation based on a network meta-analysis. *Int J Surg*. 2018; 54: 1-6.
6. Lema Zuluaga GL, Serna Agudelo RE, Zuleta Tobon JJ. Preservation solutions for liver transplantation in adults: celsior versus custodiol: a systematic review and meta-analysis with an indirect comparison of randomized trials. *Transplant Proc*. 2013; 45: 25-32.
7. Szilágyi A, Mátrai P, Hegyi P, Tuboly E, Pécz D, Garami A, et al. Compared efficacy of preservation solutions on the outcome of liver transplantation: Meta-analysis. *World J Gastroenterol*. 2018; 24: 1812-1824.
8. Feng L, Zhao N, Yao X, Sun X, Du L, Diao X, et al. Histidine-tryptophan-ketoglutarate solution vs. University of Wisconsin solution for liver transplantation: a systematic review. *Liver Transpl*. 2007; 13: 1125-1136.
9. Kaplan RM, Chambers DA, Glasgow RE. Big data and large sample size: a cautionary note on the potential for bias. *Clin Transl Sci*. 2014; 7: 342-346.
10. Wadström J, Ericzon B-G, Halloran PF, Bechstein WO, Opelz G, Serón D, et al. Advancing Transplantation: New Questions, New Possibilities in Kidney and Liver Transplantation. *Transplantation*. 2017; 101: S1-S42.
11. Nashan B, Spetzler V, Schemmer P, Kirste G, Rahmel A. Regarding "Compared Efficacy of Preservation Solutions in Liver Transplantation: A Long-Term Graft Outcome Study From the European Liver Transplant Registry". *Am J Transplant*. 2015; 15: 3272-3273.
12. Adam R, Delvart V, Karam V. Reply to Letter Regarding "Compared Efficacy of Preservation Solutions in Liver Transplantation: A Long-Term Graft Outcome Study From the European Liver Transplant Registry". *Am J Transplant*. 2015; 15: 3274-3275.
13. Adam R, Cailliez V, Karam V. Evaluation of HTK Preservation Solutions in Liver Transplantation: A Long-Term Propensity-Based Analysis of Outcome From the European Liver Transplant Registry. *Am J Transplant*. 2017; 17: 585-586.
14. Hameed AM, Wong G, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. *HPB (Oxford)*. 2017; 19: 933-943.
15. Eurotransplant. Eurotransplant Manual - Chapter 9: The Donor. Leiden: The Netherlands; 2017.

Chapter 12

Aortic versus dual perfusion for retrieval of the liver after brain death: a national registry analysis

Ahmer Hameed
Tony Pang
Peter Yoon
Glenda Balderson
Ronald De Roo
Lawrence Yuen
Vincent Lam
Jerome Laurence
Michael Crawford
Richard Allen
Wayne Hawthorne
Henry Pleass

As published in Liver Transplantation 2018 SEPT 7, EPUB AHEAD OF PRINT; DOI: 10.1002/lt.25331

12.1 Abstract

There is lack of consensus in the literature regarding the comparative efficacy of *in situ* aortic-only compared to dual (aortic and portal venous) perfusion for retrieval and transplantation of the liver. Recipient outcomes from the Australia/New Zealand Liver Transplant Registry (2007-16), including patient and graft survival, and causes of graft loss, were stratified by perfusion route. Subgroup analyses were conducted for higher risk donors. A total of 1382 liver transplant recipients were analyzed (957 aortic-only; 425 dual perfusion). There were no significant differences in five-year graft and patient survivals between the aortic-only and dual cohorts (80.1 versus 84.6%, and 82.6 versus 87.8%, respectively), or in the odds ratios of primary non-function, thrombotic graft loss, or graft loss secondary to biliary complications or acute rejection. When analyzing only higher-risk donors (n = 369), multivariate graft survival was significantly less in the aortic-only cohort (HR 0.52, 95% CI 0.27-0.98). Overall, there was a trend towards improved outcomes when dual perfusion was utilized, which became significant when considering higher-risk donors alone. Inferences into the ideal perfusion technique in multi-organ procurement will require further investigation by way of a RCT, and outcomes after the transplantation of other organs will also need to be considered.

12.2 Introduction

Liver perfusion during deceased donor organ retrieval can be conducted by cannulation of the aorta and portal vein (dual perfusion), or via the abdominal aorta alone. Intuitively, aortic perfusion alone is simpler and faster to achieve, as it involves one less step during retrieval, and will not obstruct pancreatic venous outflow unlike dual perfusion achieved via an inferior or superior mesenteric venous cannula.[1, 2]

There is controversy in the literature regarding the utility of each approach in comparison to the other. In a recent systematic review and meta-analysis, we showed that aortic and dual perfusion likely achieve equivalent outcomes for DBD, standard criteria liver recipients; however, studies included in this comparison all had small sample sizes, and maximum recipient follow-up was 20 months.(3-8) In contrast, D'Amico *et al.* compared the two techniques in 35 "suboptimal" grafts, and showed significantly poorer outcomes in aortic-only perfused livers.(9) Overall, due to disparate results from existing studies, small patient numbers, and relatively short patient follow-up, retrieval guidelines with respect to the utilization of aortic-only or dual perfusion significantly vary between and within different jurisdictions.[8, 10-12]

We therefore aimed to analyze the efficacy of aortic and dual perfusion using a larger national cohort with a prolonged period of follow-up. This cohort was analyzed as a whole, with further subgroup analyses conducted for higher risk donor grafts. Recipient outcomes including graft and patient survival, and causes of graft loss, were stratified by perfusion route.

12.3 Methods

12.3.1 DATA COLLECTION AND DATA-POINTS

The Australia and New Zealand Liver Transplant Registry (ANZLTR) and the Australia and New Zealand Organ Donation (ANZOD) Registry were utilized for the collection of relevant study data-points. Both donor and recipient parameters were obtained. Donor characteristics were as follows – preservation fluid type(s), donor age, sex, cause of death (COD), liver enzymes, pressor requirements, state (region) of retrieval, and body mass index (BMI). Recipient characteristics obtained included – recipient age, sex, primary liver diagnosis, Model for End-Stage Liver Disease (MELD) score, graft number for the recipient, and recipient transplant center. Transplantation parameters recorded were – cold ischemia time (CIT), secondary warm ischemia time (SWIT), graft utilization locally or interstate ("shipped"), graft and patient survival, reason for graft loss (primary non-function [PNF], hepatic artery thrombosis [HAT], portal vein thrombosis [PVT], biliary complications, acute rejection), and the need for re-transplantation. PNF was defined as the need for re-transplantation and/or patient death within 7 days due to graft non-function. Each Australian state has a dedicated liver transplantation unit, and distinct liver retrieval team(s). Graft retrieval technique (i.e. aortic or dual perfusion), and back-table retrieval practices are not recorded in either database and were therefore obtained by surveying senior surgeons from each unit that performed the retrieval. Retrieval team practices with regards to aortic or dual perfusion have remained consistent over the study period. Ethics approval for this project was obtained from the local institutional review board. No organs from executed prisoners were used.

12.3.2 STUDY INCLUSION AND EXCLUSION CRITERIA

Adult (≥ 16 years), Australian liver DBD donor and corresponding recipient data was analyzed from the period 2007-2016, inclusive. Partial liver donors (split or reduced-size grafts) were excluded from analyses, as were donors in whom University of Wisconsin (UW) solution was not utilized as the final perfusion and preservation fluid, and patients who had a previous liver transplant. DCD donors could not be included as dual perfusion was not commonly employed in this donor subset.

12.3.3 RETRIEVAL TECHNIQUE

All units employed a pre-flush, consisting of 2-4 L of either Hartmann's solution, 0.9% NaCl or Ross/Marshall's Hyperosmolar Citrate Solution (Soltran, Baxter Healthcare, UK), given via the

aorta, and in the cases of dual perfusion, also via the portal vein. This was followed by a formal UW flush of 2-6 L, again via the aorta, and in the cases of dual perfusion, also via the portal vein. Retrieval teams undertaking dual perfusion accessed the portal vein via a cannula inserted into the inferior mesenteric vein, unless the pancreas was also retrieved, in which case the portal vein was usually transected just proximal to the pancreas and accessed directly. The decision to undertaken aortic or dual perfusion was specific to each retrieval unit, and not impacted by consideration of donor or recipient factors. All retrieval teams gave an additional back-table portal venous flush.

12.3.4 PATIENT OUTCOMES AND STATISTICAL ANALYSES

Recipient data was stratified by the *in situ* perfusion route utilized, i.e. aortic or dual perfusion. Final outcomes of interest were graft survival (all-cause), patient survival, and cause of graft loss (PNF, HAT, PVT, acute rejection, or biliary complications). All statistical analyses were undertaken using IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY, USA), and Stata, Version 14.0 (College Station, Texas, USA).

Baseline patient data was compared using the student's t-test or Mann Whitney U test, and chi-square test or Fisher's exact test, as appropriate. Survival data between study groups was compared using Kaplan-Meier curves, with statistical significance obtained using the log-rank test. Cox regression models were constructed for graft and patient survival data, stratifying for aortic and dual perfusion, and other relevant donor or recipient factors (donor/recipient gender, donor/recipient age, donor COD or recipient cause of liver failure, donor BMI, recipient MELD, CIT, SWIT, recipient transplant center, and graft shipping versus local utilization). Multivariate Cox regression models comparing aortic and dual perfusion survival outcomes were then constructed using a backward stepwise approach and included all univariate factors with a p-value < 0.2 and/or baseline characteristics that were significantly different between both study cohorts. Model diagnostics were performed using the global proportional hazards test and Cox-Snell residuals. The level of data missingness was $< 5\%$ for all variables used in Cox regression models, with the exception of MELD, CIT, and SWIT, which were missing in 11.1%, 18.9%, and 20.4% of cases, respectively. The technique of multiple imputations was employed to account for any missing data, using chained equations; 20 imputed data-sets were created. Causes of graft loss were compared using Fisher's exact test, and univariate and multivariate logistic regression.

12.3.5 SUBGROUP ANALYSIS

The Donor Risk Index (DRI) (USA) characterizes risk of liver graft failure based on the presence of donor age > 40, and especially for donors > 60 years, DCD donors, partial/split grafts, lower height, African-American race, and cerebrovascular accident (CVA) or "other" COD for the donor.[13] Additional factors incorporated in the risk model are a CIT above 8 hours, and regional or national shipping of organs. The Eurotransplant DRI (ET-DRI) utilizes the existing DRI score, with the omission of donor race and height, and further incorporates donor gamma-glutamyl transferase (GGT) above 50 and rescue liver offers as risk factors.[14] These indices have not yet been validated in the Australian setting. We therefore only undertook subgroup graft survival and cause of graft loss analyses for the highest risk donors in our cohort, defined by age > 60 years, COD "other" (i.e. death unrelated to CVA, trauma, and/or anoxia), and/or with a CIT ≥ 12 hrs. Graft shipping was not considered as it produced better outcomes than locally procured grafts (see Results), whilst donor height had no association with graft survival, donor race was not available, and donor GGT data was missing for a large proportion of patients.

12.4 Results

12.4.1 BASELINE PARAMETERS

Over the study period, a total of 1382 liver transplant recipients were included as they fulfilled the inclusion criteria. In total, 957 transplant livers were procured using aortic-only *in situ* perfusion, in comparison to 425 livers in which dual perfusion was employed. Baseline study characteristics are summarized in Table 1. There were no significant differences between aortic and dual groups, with the exception of CIT, SWIT, and recipient MELD (7.0 versus 6.3 hours; 45.4 versus 37.8 minutes; and 18 versus 14, respectively; $p < 0.001$).

12.4.2 ALL-CAUSE GRAFT LOSS (WHOLE COHORT)

Fig. 1A shows the unadjusted Kaplan-Meier curve comparing aortic-only and dual perfusion. Actuarial 5-year graft survival rates were 80.1% for the aortic-only group, compared to 84.6% for the dual group; overall, there were no significant differences in graft survival ($p = 0.066$). Table 2 shows results univariate and multivariate Cox regression analyses of covariates potentially associated with graft survival. Aortic and dual perfusion did not differ in both analysis types. Interestingly, shipped grafts had better outcomes than unshipped grafts, even after adjustment for

confounders (HR 0.62, 95% CI 0.42-0.94, p = 0.022). Baseline characteristics stratified by graft shipping are shown in Supplemental Digital Content 1.

Table 1. Baseline liver transplant donor and recipient characteristics. Data presented as mean (SD), or median (IQR).

WHOLE COHORT				***HIGHER RISK DONORS***		
	Aortic	**Dual**	*p-value*	**Aortic**	**Dual**	*p-value*
Transplants, *n* (%)	957 (69.2)	425 (30.8)	NA	278 (75.3)	91 (24.7)	NA
Donor Age (SD)	42.7 (18.3)	42.1 (17.4)	0.591	55.1 (20.1)	58.6 (17.0)	0.103
Donor Sex (%)						
• Male	507 (53.0)	241 (56.7)	0.219	131 (47.1)	49 (53.8)	0.279
• Female	450 (47.0)	184 (43.3)		147 (52.9)	42 (46.2)	
Donor BMI (SD)	25.8 (5.1)	25.7 (5.2)	0.832			
Donor COD (%)						
• Trauma	222 (23.2)	110 (25.9)	0.185	26 (9.4)	8 (8.8)	0.750
• CVA/ICH	463 (48.4)	207 (48.7)		149 (53.6)	55 (60.4)	
• Anoxia	193 (20.2)	86 (20.2)		24 (8.6)	6 (6.6)	
• Other	79 (8.3)	22 (5.2)		79 (28.4)	22 (24.2)	
CIT (hr) (SD)	7.0 (2.5)	6.3 (2.5)	**< *0.001***	7.6 (2.9)	6.7 (2.8)	***0.040***
SWIT (min) (SD)	45.4 (14.2)	37.8 (16.9)	**< *0.001***	45.8 (16.1)	38.2 (17.5)	***0.001***
Graft Disposition (%)						
• Unshipped*	768 (80.3)	357 (84.0)	0.115	225 (80.9)	76 (83.5)	0.643
• Shipped*	188 (19.6)	68 (16.0)		53 (19.1)	15 (16.5)	
Recipient Age (SD)	52.0 (11.1)	51.2 (11.3)	0.231	51.1 (11.4)	52.1 (10.7)	0.460
Recipient Sex (%)						
• Male	666 (69.6)	314 (73.9)	0.109	194 (69.8)	62 (68.1)	0.794
• Female	291 (30.4)	111 (26.1)		84 (30.2)	29 (31.9)	
Recipient Primary Diagnosis (%)						
• Fulminant/Subacute Hepatic Failure	92 (9.6)	35 (8.2)	0.462	30 (10.8)	10 (11.0)	0.928
• Cholestatic Cirrhosis	132 (13.8)	54 (12.7)		29 (10.4)	12 (13.2)	
• HBV/HCV-related Cirrhosis	251 (26.2)	132 (31.1)		76 (27.3)	22 (24.2)	
• Alcoholic Cirrhosis	139 (14.5)	68 (16.0)		42 (15.1)	17 (18.7)	
• HCC	157 (16.4)	59 (13.9)		37 (13.3)	13 (14.3)	
• NAFLD/NASH-related Cirrhosis	66 (6.9)	31 (7.3)		26 (9.4)	7 (7.7)	
• Other	120 (12.5)	46 (10.8)		38 (13.7)	10 (11.0)	
Recipient MELD (IQR)	18 (13-26)	14 (10-20)	**< *0.001***	19 (13-27)	14 (9-21)	**< *0.001***

BMI – body mass index; CIT – cold ischemic time; COD – cause of death; CVA – cerebrovascular accident; HBV/HCV – hepatitis B or C virus; HCC – hepatocellular carcinoma; ICH – intracerebral hemorrhage; IQR – inter-quartile range; MELD – model for end-stage liver disease score; NA – not applicable; NAFLD – non-alcoholic fatty liver disease; NASH – non-alcoholic steato-hepatitis; SD – standard deviation; SWIT – second warm ischemic time

* "Unshipped" denotes graft utilized in same state it was procured

12.4.3 PATIENT SURVIVAL (WHOLE COHORT)

Fig. 1B shows the unadjusted Kaplan-Meier curve comparing aortic-only and dual perfusion, with respective actuarial 5-year patient survival rates of 82.6% and 87.8%. Overall patient survival was significantly lower in the aortic-only cohort (Fig. 1B; p = 0.026); after adjustment for confounders, there were no differences in patient survival between both perfusion groups (HR 0.75, 95% CI 0.53-1.06, p = 0.103) (Table 2).

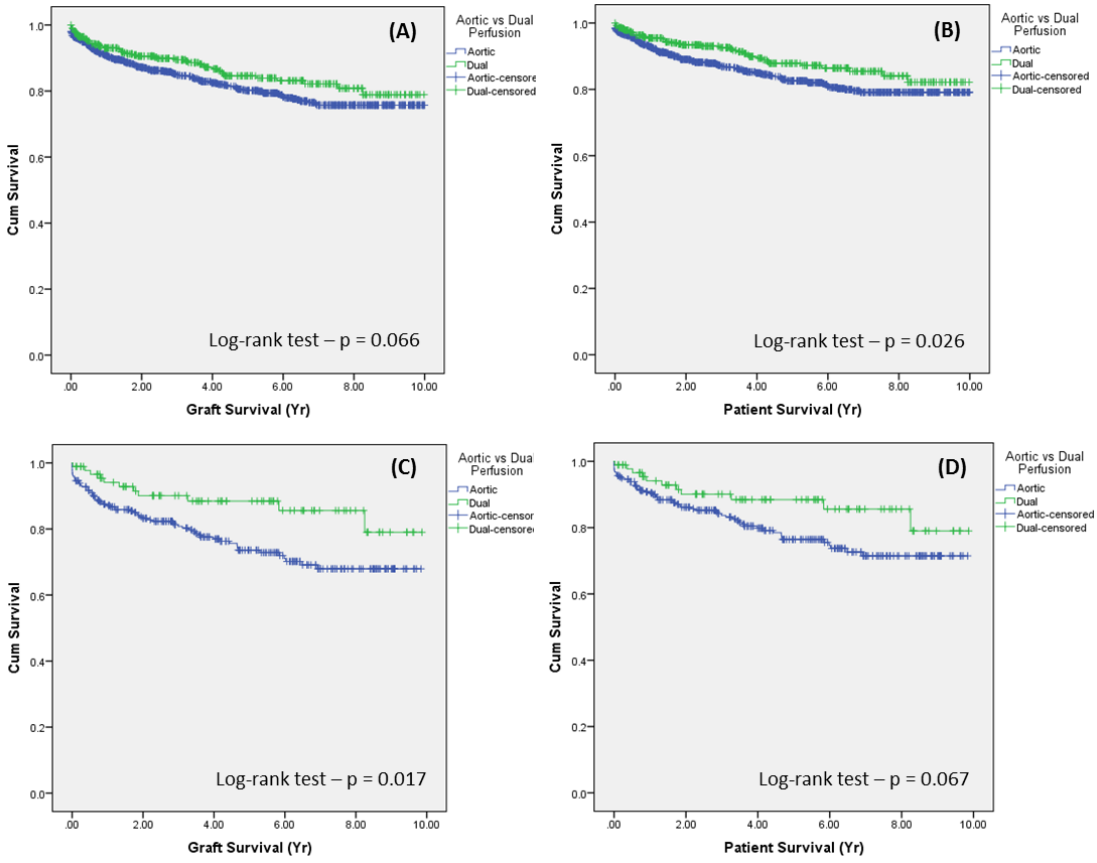


Figure 1. Unadjusted Kaplan-Meier curves comparing (a) all-cause graft loss (survival), and (b) patient survival after aortic-only or dual in situ liver perfusion for the complete patient cohort. (c-d) Kaplan-Meier curves for graft and patient survival, respectively, in the subgroup of transplants performed from donors > 60, and/or CIT ≥ 12 hours, and/or if donor COD was "other."

Table 2. Univariate and (adjusted) multivariate hazard ratios for graft and overall patient (recipient) survival, when stratified by various donor, transplant, and recipient characteristics – whole patient cohort.

GRAFT LOSS			***OVERALL RECIPIENT DEATH***		
Covariate	*HR, 95% CI*	*p-value*	*Covariate*	*HR, 95% CI*	*p-value*
UNIVARIATE ANALYSES			*UNIVARIATE ANALYSES*		
Dual vs. Aortic	0.81 (0.59-1.11)	0.184	**Dual vs. Aortic**	0.74 (0.52-1.04)	0.084
Recipient Sex (female vs. male)	1.18 (0.88-1.58)	0.260	**Recipient Sex (female vs. male)**	1.29 (0.94-1.77)	0.110
Recipient Age	1.01 (1.00-1.03)	***0.034***	**Recipient Age**	1.01 (0.998-1.02)	0.144
Reason for Liver Tx – fulminant vs. not	1.01 (0.61-1.66)	0.979	**Reason for Liver Tx – fulminant vs. not**	0.99 (0.62-1.57)	0.967
Recipient MELD	1.01 (0.998-1.02)	0.092	**Recipient MELD**	1.01 (0.996-1.03)	0.157
CIT	1.04 (0.99-1.11)	0.145	**CIT**	1.04 (0.97-1.10)	0.256
CIT, categorized (vs. 0-6 hr)	• 6-12 hr – 1.38 (1.01-1.89) • > 12 hr – 1.43 (0.62-3.33)	• 6-12 hr – ***0.043*** • > 12 hr – 0.403	**CIT, categorized (vs. 0-6 hr)**	• 6-12 hr – 1.34 (0.95-1.89) • > 12 hr – 1.60 (0.69-3.74)	• 6-12 hr – 0.097 • > 12 hr – 0.277
SWIT	1.01 (0.995-1.01)	0.325	**SWIT**	1.01 (0.995-1.02)	0.346
Donor Age	1.00 (0.996-1.01)	0.302	**Donor Age**	1.01 (0.998-1.02)	0.144
Donor Age, categorized (vs. < 40)	• 40-60 yr – 0.94 (0.69-1.28) • 60-70 yr – 1.23 (0.84-1.81) • > 70 yr – 1.60 (0.98-2.63)	• 40-60 yr – 0.689 • 60-70 yr – 0.293 • > 70 yr – ***0.062***	**Donor Age, categorized (vs. < 40)**	• 40-60 yr – 0.96 (0.68-1.35) • 60-70 yr – 1.36 (0.89-2.05) • > 70 yr – 1.76 (1.04-2.98)	• 40-60 yr – 0.805 • 60-70 yr – 0.152 • > 70 yr – ***0.036***
Donor Sex	1.12 (0.85-1.47)	0.428	**Donor Sex**	1.06 (0.79-1.43)	0.696
Donor BMI	0.99 (0.97-1.02)	0.528	**Donor BMI**	0.996 (0.97-1.03)	0.785
Donor COD (vs. trauma)	• CVA/ICH – 1.06 (0.74-1.51) • Anoxia – 1.22 (0.81-1.85) • Other – 1.49 (0.87-2.56)	• CVA/ICH – 0.768 • Anoxia – 0.347 • Other – 0.146	**Donor COD (vs. trauma)**	• CVA/ICH – 1.03 (0.70-1.52) • Anoxia – 1.14 (0.72-1.79) • Other – 1.36 (0.75-2.48)	• CVA/ICH – 0.871 • Anoxia – 0.586 • Other – 0.312
Shipped vs. Unshipped	0.63 (0.42-0.94)	***0.022***	**Shipped vs. Unshipped**	0.66 (0.43-1.01)	0.056
Transplant Center	• 2 – 0.63 (0.23-1.72) • 3 – 0.76 (0.49-1.16) • 4 – 0.68 (0.43-1.09) • 5 – 0.86 (0.60-1.23) • 6 – 0.90 (0.58-1.39)	• 2 – 0.369 • 3 – 0.202 • 4 – 0.107 • 5 – 0.396 • 6 – 0.637	**Transplant Center**	• 2 – 0.54 (0.17-1.71) • 3 – 0.61 (0.38-1.00) • 4 – 0.61 (0.36-1.02) • 5 – 0.81 (0.55-1.19) • 6 – 0.88 (0.55-1.40)	• 2 – 0.291 • 3 – 0.051 • 4 – 0.060 • 5 – 0.287 • 6 – 0.577
MULTIVARIATE ANALYSES			*MULTIVARIATE ANALYSES*		
Recipient Age	1.01 (1.00-1.03)	***0.037***	**Recipient Age**	1.03 (1.01-1.05)	***< 0.001***
Shipped vs. Unshipped	0.62 (0.42-0.93)	***0.019***	**Shipped vs. Unshipped**	0.65 (0.42-0.999)	***0.050***
Dual vs. Aortic	0.81 (0.60-1.11)	0.188	**Dual vs. Aortic**	0.75 (0.53-1.06)	0.103
			Recipient Sex (female vs. male)	1.46 (1.06-2.01)	***0.020***

BMI – body mass index; CI – confidence interval; CIT – cold ischemic time; COD – cause of death; HR – hazard ratio; MELD – model for end-stage liver disease; SWIT – secondary warm ischemic time; Tx – transplant

12.4.4 CAUSES OF GRAFT LOSS (WHOLE COHORT)

Different causes of graft loss were compared in liver transplant recipients after aortic versus dual *in situ* perfusion, including PNF, HAT, PVT, biliary complications, and acute rejection (Table 3). There were no significant differences in unadjusted odds ratios (OR) between recipients after aortic-only or dual perfusion in donors. Subsequent re-transplantation rates amongst the two groups did not differ (OR 1.05, 95% CI 0.55-2.01, p = 0.876).

Table 3. Unadjusted odds ratios for different causes of graft loss after dual in situ perfusion in comparison to aortic-only perfusion – whole patient cohort.

Cause of Graft Loss	*OR, 95% CI*	*p-value*
PNF	0.25 (0.03-1.97)	0.187
HAT	1.23 (0.45-3.35)	0.684
PVT	4.52 (0.41-49.99)	0.219
Biliary Complications	0.32 (0.02-6.22)	0.452
Acute Rejection	1.50 (0.25-9.03)	0.656

HAT – hepatic artery thrombosis; PNF – primary non-function; PVT – portal vein thrombosis

12.4.5 SUBGROUP ANALYSES OF HIGHER RISK DONORS

Baseline characteristics of the higher risk donor subgroup are also shown in Table 1, whilst Table 4 and 5 analyze graft/recipient survival, and causes of graft loss, respectively. When analyzing only cases in which donors were > 60 years, donor COD was "other," and/or CIT was ≥ 12 hours there were 278 recipients in the aortic-only cohort, and 91 recipients in the dual perfusion cohort.

Graft survival was significantly lower in the aortic-only cohort in comparison to dual perfusion using univariate Cox regression (HR 0.52, 95% CI 0.27-0.98; p = 0.044). This was also reflected in the unadjusted Kaplan-Meier curve (Fig. 1C). After multivariate Cox regression, dual perfusion remained protective over aortic-only perfusion with respect to graft loss (HR 0.48, 95% CI 0.26-0.92, p = 0.028). Overall patient survival was not significantly different between either group upon univariate (Fig. 1D) and multivariate analyses (HR 0.58, 95% CI 0.30-1.11, p = 0.098). There were no significant differences between groups with respect to causes of graft loss (Table 5).

Table 4. Univariate and (adjusted) multivariate hazard ratios for graft and overall patient (recipient) survival, when stratified by various donor, transplant, and recipient characteristics – higher risk donors only.

GRAFT LOSS			***OVERALL RECIPIENT DEATH***		
Covariate	*HR, 95% CI*	*p-value*	*Covariate*	*HR, 95% CI*	*p-value*
UNIVARIATE ANALYSES			*UNIVARIATE ANALYSES*		
Dual vs. Aortic	0.52 (0.27-0.98)	***0.044***	**Dual vs. Aortic**	0.62 (0.32-1.18)	0.145
Recipient Sex (female vs. male)	1.51 (0.94-2.43)	0.088	**Recipient Sex (female vs. male)**	1.45 (0.87-2.41)	0.157
Recipient Age	1.02 (0.996-1.04)	0.107	**Recipient Age**	1.03 (1.00-1.05)	***0.049***
Reason for Liver Tx – fulminant vs. not	0.72 (0.31-1.65)	0.434	**Reason for Liver Tx – fulminant vs. not**	0.67 (0.27-1.66)	0.382
Recipient MELD	1.02 (0.99-1.04)	0.173	**Recipient MELD**	1.01 (0.99-1.04)	0.339
CIT	0.998 (0.91-1.09)	0.958	**CIT**	0.98 (0.89-1.08)	0.695
SWIT	1.01 (0.99-1.02)	0.480	**SWIT**	1.00 (0.99-1.02)	0.682
Donor Age	1.00 (0.99-1.01)	0.877	**Donor Age**	1.00 (0.99-1.02)	0.653
Donor Sex	0.96 (0.61-1.53)	0.867	**Donor Sex**	0.88 (0.54-1.44)	0.602
Donor COD (vs. trauma)	• CVA/ICH – 2.70 (0.72-10.2) • Anoxia – 2.88 (0.64-12.9) • Other – 2.77 (0.70-11.0)	• CVA/ICH – 0.141 • Anoxia – 0.166 • Other – 0.146	**Donor COD (vs. trauma)**	• CVA/ICH – 2.78 (0.68-11.3) • Anoxia – 2.78 (0.55-14.0) • Other – 2.45 (0.57-10.6)	• CVA/ICH – 0.154 • Anoxia – 0.215 • Other – 0.230
Shipped vs. Unshipped	1.00 (0.56-1.80)	0.993	**Shipped vs. Unshipped**	1.10 (0.60-2.02)	0.772
Transplant Center	• 2 – 0.31 (0.04-2.30) • 3 – 0.42 (0.17-1.09) • 4 – 0.68 (0.32-1.42) • 5 – 0.78 (0.42-1.44) • 6 – 0.97 (0.48-1.96)	• 2 – 0.254 • 3 – 0.074 • 4 – 0.300 • 5 – 0.426 • 6 – 0.925	**Transplant Center**	• 2 – 0.36 (0.49-2.62) • 3 – 0.47 (0.18-1.23) • 4 – 0.57 (0.25-1.31) • 5 – 0.68 (0.35-1.34) • 6 – 0.96 (0.46-2.03)	• 2 – 0.310 • 3 – 0.124 • 4 – 0.187 • 5 – 0.266 • 6 – 0.914
MULTIVARIATE ANALYSES			*MULTIVARIATE ANALYSES*		
Recipient Age	1.03 (1.00-1.05)	***0.037***	**Recipient Age**	1.03 (1.01-1.06)	***0.019***
Recipient Sex (female vs. male)	1.73 (1.07-2.81)	***0.026***	**Recipient Sex (female vs. male)**	1.67 (0.997-2.81)	0.051
Dual vs. Aortic	0.49 (0.26-0.92)	***0.028***	**Dual vs. Aortic**	0.58 (0.30-1.11)	0.098

BMI – body mass index; CI – confidence interval; CIT – cold ischemic time; COD – cause of death; HR – hazard ratio; MELD – model for end-stage liver disease; SWIT – secondary warm ischemic time; Tx – transplant

In this subset of patients, grafts that were transplanted within the same state did not have different outcomes to shipped grafts (Graft survival: HR 1.00, 95% CI 0.56-1.80, p = 0.993; Patient survival: HR 1.10, 95% CI 0.60-2.02, p = 0.772). Furthermore, this parameter was not included in final graft and patient survival models after multivariate analyses as it did not have a significant effect on final model parameters (data not shown).

Table 5. Different causes of graft loss after dual *in situ* perfusion in comparison to aortic-only perfusion, expressed as a proportion of total patients in each cohort – higher risk donors only.

Cause of Graft Loss	*n, Aortic (%)*	*n, Dual (%)*	*p-value*
PNF	3 (1.1)	0 (0)	1.000
HAT	4 (1.4)	0 (0)	0.576
PVT	0 (0)	1 (1.1)	0.247
Biliary Complications	2 (0.7)	0 (0)	1.000
Acute Rejection	1 (0.4)	0 (0)	1.000

12.5 Discussion

This paper has compared aortic-only *in situ* perfusion during DBD whole liver retrieval to dual aorto-portal perfusion with respect to recipient outcomes using a national registry over a 10-year period. When both standard and expanded-risk grafts are analyzed together, there are no significant differences in graft and patient survival, or in causes of graft loss, between either *in situ* perfusion technique. However, dual perfusion is superior when utilized in higher risk donors defined by advanced age (> 60 years), and/or COD "other," and/or with a prolonged cold ischemic time (≥ 12 hours).

The primary aim of *in situ* liver perfusion is to achieve rapid graft cooling and commence 'preservation' by the expulsion of residual blood and exposing the graft parenchyma to cold preservation fluid. Despite the liver's dual circulation, aortic-only perfusion should theoretically be able to simultaneously achieve portal perfusion via the mesenteric venous drainage, albeit in a slightly delayed fashion.[6] Although appropriate liver perfusion takes longer when aortic-only perfusion is utilized, the final liver temperature achieved by either modality does not significantly

differ (12.5 ± 3.4°C versus 11 ± 3°C, for the aortic-only and dual-perfused livers, respectively; $p > 0.05$).[6]

Accordingly, the short-term equivalence of both perfusion techniques with respect to graft outcomes (PNF and peak alanine aminotransferase [ALT]) has been shown previously in our meta-analysis.[8] However, only standard criteria donor livers were considered, and there was insufficient data available for comparison of longer-term outcomes.[8] In concordance with these results from the meta-analysis, D'Amico *et al*. in their small cohort of patients showed 100% six-month graft survival in optimal livers utilizing either aortic or dual perfusion; however, there were significantly superior results in 'expanded criteria' grafts after dual perfusion, and the trial was terminated early.[9] These authors conducted portal perfusion via a cannula inserted in the inferior mesenteric vein, additionally minimized/reduced mesenteric venous return by tightening a tourniquet across the distal portal vein.[9, 15] Expanded criteria donors were defined by the presence of at least one feature of donor age > 60 years, hepatic steatosis > 20%, and/or total ischemia time > 10 hours, or two out of other lesser arbitrary criteria.[9]

Interestingly and perhaps surprisingly, when comparing the higher-risk donor subgroup within each perfusion cohort, we have also now shown superior graft survival outcomes in the higher-risk dual perfusion group, despite the utilization of a back-table portal venous flush in all cases. Our definition of higher risk donors incorporated factors from the DRI/ET-DRI, albeit without the inclusion of some factors such as graft shipping that did not fit the data-set utilized. Recipient parameters were not considered in the subgroup analyses for the same reason they are not included in risk scores such as the DRI, as the idea is to facilitate an appropriate donor-recipient 'match' based on the characteristics of the donor organ.

Unexpectedly, we also found that shipped grafts had better outcomes in comparison to unshipped grafts, even after accounting for multiple confounders. The only significant difference between both groups was mean donor age (40.2 and 43.1 years for shipped and unshipped grafts, respectively; $p = 0.030$), whilst CIT, SWIT, donor COD, perfusion route, and recipient characteristics did not significantly differ. Interestingly, shipping did not significantly impact outcomes in the higher risk donor cohort. This finding is difficult to explain, and warrants further investigation, although it may relate to center-level transplantation practices and patient selection,

which could not be fully accounted for in our models, and altered retrieval-related practices in anticipation of shipping.

The influence of either perfusion method on pancreas transplantation outcomes in the context of multi-organ procurement is yet to be definitively ascertained. Dual perfusion adds theoretical risks when the pancreas is to be retrieved, due to pancreatic congestion from the potential blockage of pancreas perfusate outflow secondary to the portal venous catheter.[1, 2] This can be avoided by transecting the portal vein immediately proximal to the pancreas and inserting the cannula directly into the proximal portal vein.[16] The ideal perfusion approach during combined liver and pancreas retrieval must account for the impact on both organs, and the relative risks and benefits weighed especially against the life-saving nature of liver transplantation.

Data-base analyses have inherent disadvantages that must be acknowledged. Missing data, inconsistent recording, and loss to follow-up patients are some clear limitations. Furthermore, each center had slightly different perfusion protocols within the aortic-only and dual perfusion groups, which may have made a small impact on results. Most importantly, each state in Australia has one liver transplantation unit that may vary with respect to donor and recipient selection. As aortic and dual perfusion practices tend to split by individual units, our results will at least somewhat reflect differing unit patient selection bias. However, as the majority of livers retrieved within a state are also transplanted in the same state, we incorporated the transplant center in multivariate analyses to help account for any confounding introduced by this factor. The perfusion/preservation fluid utilized can impact graft outcomes, and as such only grafts that were given a UW final flush and preservation were included.[17, 18] The exclusion of partial liver transplants and patients undergoing repeat transplantation slightly narrows the generalizability of this analysis, however this was deemed necessary due to a likely significant confounding of results.[13, 19, 20]

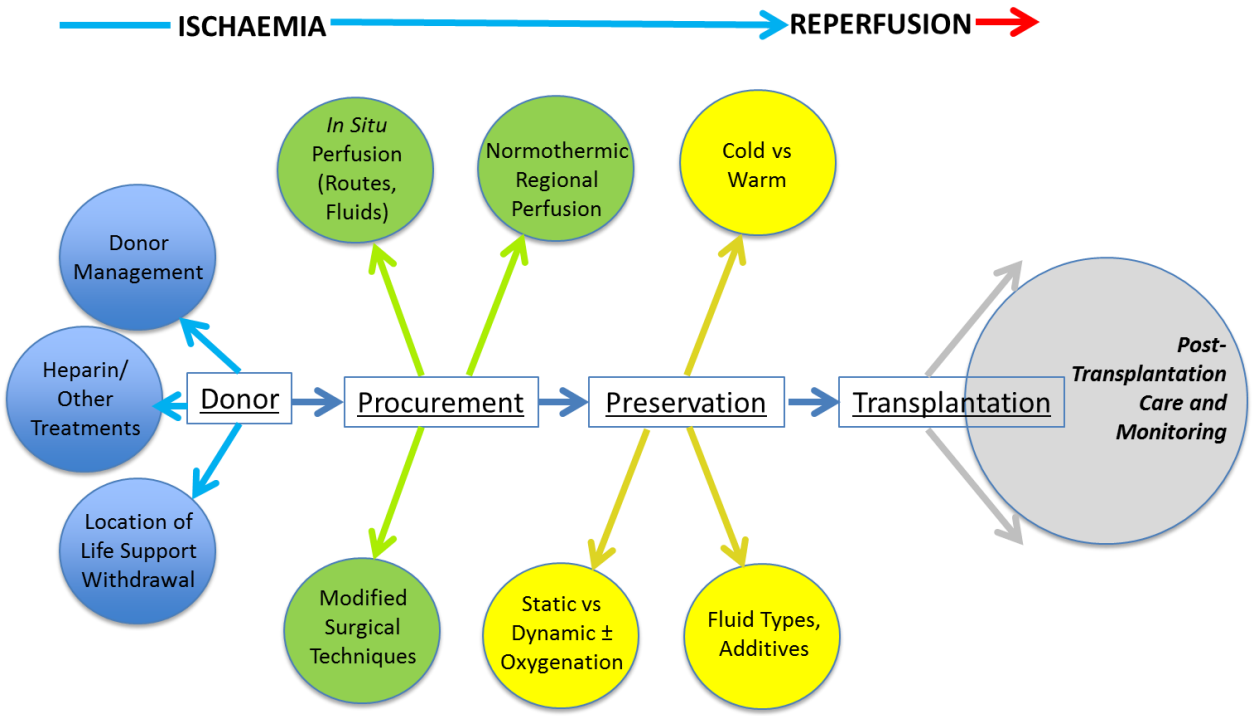
At the least, results from this review warrant further confirmation and investigation in the form of a multi-center trial with prolonged recipient follow-up. Any such trial should also analyze pancreas transplantation outcomes in recipients from the same donor. Another future consideration is any potential impact of hypo- and/or normothermic perfusion of the liver, which has not yet been clinically implemented in Australasia but is gaining significant prominence in the global setting.[21, 22]

The ultimate goal of organ retrieval in the age of multi-organ procurement should be a unified approach amongst all retrieval surgeons and units that maximizes organ yield and transplantation outcomes from the different organs retrieved. Transplant centers should endeavor to collaboratively investigate and discuss this issue, and organize further studies, such that uniform global guidelines can be developed.

12.6 References

1. Nghiem DD, Cottington EM. Pancreatic flush injury in combined pancreas-liver recovery. *Transpl Int*. 1992; 5: 19-22.
2. Brockmann JG, Vaidya A, Reddy S, Friend PJ. Retrieval of abdominal organs for transplantation. *Br J Surg*. 2006; 93: 133-146.
3. de Ville de Goyet J, Hausleithner V, Malaise J, Reding R, Lerut J, Jamart J, et al. Liver procurement without in situ portal perfusion. A safe procedure for more flexible multiple organ harvesting. *Transplantation*. 1994; 57: 1328-1332.
4. Anthuber M, Zuelke C, Forst H, Welte M, Groh J, Maag K, et al. Experiences with a simplified liver harvesting technique--single aorta in situ flush followed by portal back table flush. *Transplant Proc*. 1993; 25: 3154-3155.
5. Gabel M, Liden H, Norrby J, Friman S, Wolfbrandt A, Olausson M. Early function of liver grafts preserved with or without portal perfusion. *Transplant Proc*. 2001; 33: 2527-2528.
6. Chui AK, Thompson JF, Lam D, Koutalistras N, Wang L, Verran DJ, et al. Cadaveric liver procurement using aortic perfusion only. *ANZ J Surg*. 1998; 68: 275-277.
7. Boillot O, Benchetrit S, Dawahra M, Porcheron J, Martin X, Fontaumard E. Early graft function in liver transplantation: comparison of two techniques of graft procurement. *Transplant Proc*. 1993; 25: 2626-2627.
8. Hameed AM, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: Working toward a unified approach. *Liver Transpl*. 2017; 23: 1615-1627.
9. D'Amico F, Vitale A, Gringeri E, Valmasoni M, Carraro A, Brolese A, et al. Liver transplantation using suboptimal grafts: impact of donor harvesting technique. *Liver Transpl*. 2007; 13: 1444-1450.
10. Zalewska K, Ploeg R. National Standards for Organ Retrieval from Deceased Donors (NORS Retrieval Standards). Bristol, UK; 2014.
11. TSANZ. Guidance Document - Surgical Technique for Deceased Donor Abdominal Organ Procurement (ATCA-TSANZ Guidelines G003/2015). Sydney, Australia: TSANZ; 2015.
12. Eurotransplant Foundation. Eurotransplant Manual. Leiden: Netherlands: Eurotransplant 2016.
13. Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, et al. Characteristics Associated with Liver Graft Failure: The Concept of a Donor Risk Index. *Am J Transplant.* 2006; 6: 783-790.
14. Braat AE, Blok JJ, Putter H, Adam R, Burroughs AK, Rahmel AO, et al. The Eurotransplant donor risk index in liver transplantation: ET-DRI. *Am J Transplant*. 2012; 12: 2789-2796.
15. Starzl TE, Hakala TR, Shaw BW, Jr., Hardesty RL, Rosenthal TJ, Griffith BP, et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet*. 1984; 158: 223-230.
16. Sollinger HW, Vernon WB, D'Alessandro AM, Kalayoglu M, Stratta RJ, Belzer FO. Combined liver and pancreas procurement with Belzer-UW solution. *Surgery*. 1989; 106: 685-691.
17. Adam R, Delvart V, Karam V, Ducerf C, Navarro F, Letoublon C, et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant*. 2015; 15: 395-406.
18. Mangus RS, Fridell JA, Vianna RM, Milgrom MA, Chestovich P, Chihara RK, et al. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution in extended criteria liver donors. *Liver Transpl*. 2008; 14: 365-373.

19. Marudanayagam R, Shanmugam V, Sandhu B, Gunson BK, Mirza DF, Mayer D, et al. Liver retransplantation in adults: a single-centre, 25-year experience. *HPB*. 2010; 12: 217-224.
20. Ghabril M, Dickson R, Wiesner R. Improving outcomes of liver retransplantation: an analysis of trends and the impact of Hepatitis C infection. *Am J Transplant*. 2008; 8: 404-411.
21. Ceresa CDL, Nasralla D, Coussios CC, Friend PJ. The case for normothermic machine perfusion in liver transplantation. *Liver Transpl*. 2018; 24: 269-275.
22. Schlegel A, Muller X, Dutkowski P. Hypothermic liver perfusion. *Curr Opin Organ Transplant*. 2017; 22: 563-570.



PART 4 – GENERAL DISCUSSION

Chapter 13 – Discussion

This thesis has explored selected aspects of abdominal organ procurement and preservation as targets for improvement and modification with the aims of enhancing donor organ availability and recipient transplantation outcomes. Abdominal organs are retrieved by the same underpinning processes, including similar surgical techniques, perfusion procedures, and perfusion fluids. As such, the breadth of topics covered in this thesis encompasses kidney, liver, and pancreas *in situ* perfusion and subsequent static or dynamic preservation, and in particular their impacts on organ function and damage secondary to ischaemia-reperfusion injury (IRI).

Over the course of this chapter, each aspect of organ procurement and preservation explored in this thesis will be addressed. The order of topics pursued, in addition to models and methods utilised will be justified. Results will be placed in the context of the existing literature, and clinical implications and future directions will then be explored.

13.1 Machine perfusion and renal IRI – more organs, better outcomes

13.1.1 JUSTIFICATION OF SCIENTIFIC METHODS AND MODELS USED

Part 1 of this dissertation commences with a systematic review of the literature, with identification of knowledge gaps and fruitful targets for further research. These then form the basis for the development and optimization of pre-clinical models for IRI and MP. The ultimate aim is to provide better options and/or evidence for application to clinical practice.

The directions pursued stemmed from the following considerations:

(i) Primary basis of work – as described over the course of this dissertation, better methods of deceased donor kidney resuscitation, preservation, and/or repair are required to minimize or reverse the comparatively greater deleterious impacts of IRI on DCD and higher KDPI kidneys.

(ii) MP provides a real, viable, and potentially implementable solution to this problem. However, MP is not a uniform procedure, incorporating the utilisation of multiple types of machines/devices, and can be modified in many different ways including temperature, oxygenation, and perfusion fluid constituents (Fig. 1).

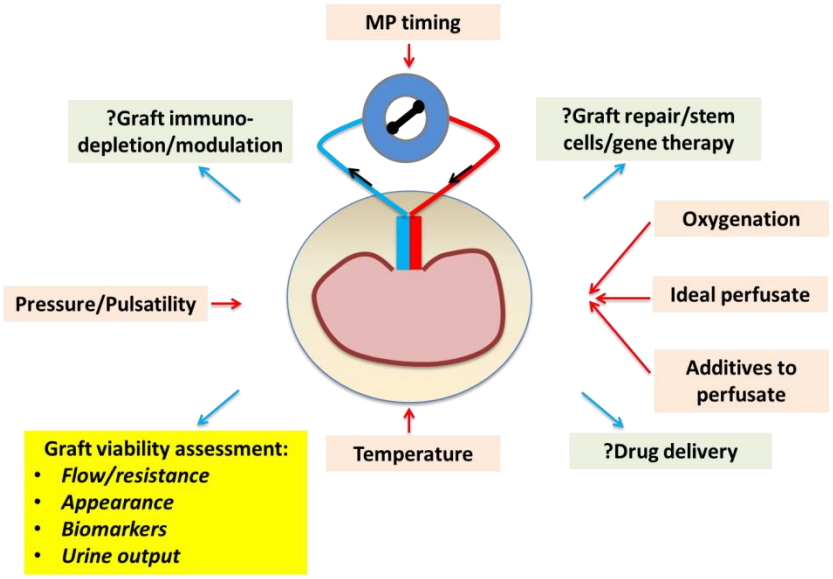


Figure 1. MP preservation entails kidney perfusion via the renal artery using a mechanical pump-based mechanism. Many options are available with respect to how MP parameters are set. Alterations may be made to perfusion temperature (normothermic, hypothermic, or subnormothermic), timing (pre-implantation, continuous, or some other combination with CS), pressure/pulsatility (pulsatile, or non-pulsatile), and fluid constituents (blood, or non-

blood-based; oxygenated, or non-oxygenated, and so on). Further heterogeneity is potentially introduced to the process when drug therapy(s) are added to the circuit, or other modifications are made. Depending on the exact methods and constituents used, graft viability assessment parameters and the significance of different biomarkers will vary.

(iii) A multi-centre RCT in Europe compared continuous HMP to CS, showing a significant reduction in DGF rates, and a significant improvement in one-year graft survival, in favour of the HMP group.[1] Three-year data from this trial showed an ongoing survival benefit in the MP kidneys. Subgroup analyses showed that this benefit was present in DBD and DBD-ECD kidneys, but not DCD kidneys, and the impacts of DGF were significantly more pronounced in DBD kidneys.[2] A DCD-specific publication related to this study demonstrated a significant reduction in DGF rates, although the DGF rate for MP kidneys was still high at 53.7%, and there were no impacts upon graft survival.[3] A later UK-specific RCT comparing HMP and CS in the DCD setting contradicted the Moers *et al*. trial, with no reduction in DGF rates in HMP-treated kidneys.[4]

(iv) As a result of conflicting data, and the equivocal impacts of HMP especially in DCD kidneys, uptake from the Moers *et al*. trial has not been as widespread as anticipated. Furthermore, other pre-clinical and clinical studies had been published regarding the potential utilisation of MP with modified settings, including the use of oxygenated HMP, subnormothermic MP (SNMP), NMP, and perfusion fluid modification using different IRI-targeting therapies.[5-9]

(v) Previous systematic reviews had been published, amalgamating cumulative evidence comparing the clinical outcomes of HMP in comparison to CS.[10-14] However, the role of the aforementioned modifications, including the use of oxygenation, temperature manipulation, and/or the addition of drugs, had not been considered in these analyses.

Due to the ongoing clinical equipoise with respect to implementation of MP practices, and significant variations in approach available to transplant centres, we performed an updated and wide-ranging systematic review and meta-analysis that incorporated both clinical and pre-clinical data.[15] I was able to present the findings and major conclusions from this review, in addition to a update of all relevant renal MP literature, at a specially-convened Transplantation Society of Australia and New Zealand (TSANZ) MP workshop.[16]

Factors considered by the renal committee at this meeting included the equivocal impacts of MP on graft survival, especially in the DCD setting, and the emergence of other MP modalities that may ultimately prove to be superior. In particular, the possible advantages of NMP were noted, in particular the opportunity to more objectively assess the graft during its normal metabolic processes. Evidence considering the efficacy of only one hour of pre-implantation NMP in comparison to the longer times required for (non-oxygenated) HMP increased enthusiasm for the NMP approach.[1, 5, 17] Furthermore, the applicability of the Moers *et al*. clinical trial to the Australian setting was questioned. This was largely attributed the longer ischaemic times seen in Europe in comparison to Australia, in addition to logistical difficulties associated with transporting perfusion machines on the small private jets commonly used for the transport of organ procurement teams in Australia.

We therefore decided to undertake local development of a NMP system that could provide a platform for testing porcine kidneys, then progressing to testing on discarded human kidneys. The main clinical and scientific problems we wished to tackle included:

(i) Could we develop a working, optimized NMP system that would provide realistic and invaluable exposure to the NMP process, prior to potential implementation in the clinical transplantation setting?
(ii) Is there a way to use NMP to circumvent systemic donor and/or recipient treatment to ameliorate renal IRI, especially in the DCD setting, using therapies that have been extensively tested in the pre-clinical setting? This is essential in the Australian donor setting, and also relevant to other places such as the UK, as ante-mortem interventions are not allowed in the DCD setting as discussed in the Chapter 1.
(iii) Could we provide further evidence for the superiority of one hour of NMP in comparison to CS alone, and if yes then what is the mechanistic basis for this? This is especially important to understand prior to clinical implementation, as pre-clinical evidence from Toronto suggests that longer periods of kidney NMP (> 8 hours) are superior to one hour of pre-implantation NMP, which the suggestion that the one hour period may in fact be damaging.[18, 19]

<u>NMP model</u>

The main barrier to undertaking this work was the lack of an accessible commercial device at its commencement. As such, I had to develop my own NMP set-up. The basis of this system was a

pump allowing the circulation of perfusion fluid through an arterial filter, oxygenator, and heat-exchanger, prior to achieving renal perfusion via the renal artery. Components were purchased, borrowed, or self-designed, in consultation with cardiac perfusionists, and optimized or modified over a period of months. Eventually I was able to modify an existing cardio-pulmonary bypass (CPB) set-up to allow NMP of porcine kidneys, adding/replacing components as appropriate to accommodate renal perfusion. Our initial system was based upon the Leicester/Cambridge circuit, with potential system alterations further informed by the work of the Toronto NMP group.[5, 19, 20] Modifications tested included the addition of a colloid component to the perfusion circuit, the use of carbogen instead of 100% oxygen, pressure versus flow-controlled perfusion, and the utilisation of a vasodilator infusion in the circuit.[21-23] Verapamil was employed as the vasodilator instead of prostacyclin, which is used by the Cambridge group, as verapamil is more cost-effective, easy to obtain, and no less effective with respect to its pharmacodynamics.[21]

A roller pump was used instead of a centrifugal pump due to availability and funding restrictions. However, there was insignificant pump-related haemolysis during perfusion, and cardiac studies also show that pump-related haemolysis can be minimized to a similar level to centrifugal pumps by adjusting occlusion settings.[23, 24] Support was obtained from a cardiac perfusionist team during the NMP set-up and optimization period, with advice obtained about features such as tubing configuration, the ideal oxygenator/reservoir/heat-exchanger, safe use of the pump and contingencies such as a recirculation line, appropriate roller pump occlusion settings, and accurate pressure monitoring.

A significant issue that was encountered was the inability to acquire a commercial perfusion chamber that was appropriate for the kidney. As such, a perfusion chamber was designed and 3D-printed to allow for an appropriate support structure for kidneys whilst simultaneously facilitating free venous drainage during NMP.[25] Unlike both the Cambridge and Toronto set-ups, the renal vein was not cannulated. The two primary reasons for this approach were as follows: (i) During our initial porcine kidney NMP experiments, it was noted that the venous cannula was obstructing renal outflow and causing a significant rise in circuit mean arterial pressure. Although the effects of this could have been dampened by utilizing a larger cannula, we opted for a system that allowed the vein to remain open because (ii) Avoidance of a cannula/ligature of the renal vein would mean the vein does not have to be shortened prior to potential transplantation. The perfusion chamber

also facilitated perfusate salvage, such that the NMP circuit could be maintained, without loss of blood from the circuit.

Overall, the NMP set-up developed in our lab using porcine test kidneys was safe, consistent, and reproducible in its function. This allowed us to proceed to further porcine and discarded human kidney NMP experiments, and provided a platform through which one or more agents could be given to further repair the kidney. Indeed, our published systematic review analyzing MP in kidney transplantation showed significant untapped potential with respect to the use of MP as a mechanism for therapeutic drug delivery.[15] NMP in particular is a promising modality for pharmaco-manipulation of the kidney owing to the near-physiologic temperature and pH achieved, which is ideal for drug(s) to exert their therapeutic effects.[26] As cells will have normal metabolic processes during NMP, pathways that are dysregulated by ischaemic injury can be more easily modified by pharmacologic intervention. Therefore one of the primary aims of this thesis was to employ the developed NMP set-up as a direct renal drug delivery modality, to allow targeting of the IRI process and modify perfusion characteristics. Prior to proceeding with this work, a candidate drug for delivery using NMP was identified and tested in a small animal model.

IRI model in mice

In order to be able to utilise NMP as a means to provide direct anti-IRI drug therapy to high KDPI and/or DCD kidneys, we needed to investigate what types of pharmacologic agent(s) would be of benefit, confirm optimal dose rates and timing, and ascertain whether combining one or more drugs would be synergistic or antagonistic. We utilised a rodent warm IRI model for approximation of the DCD setting, as a quicker, less laborious, and less expensive means to help answer some of these questions. Furthermore, we opted for a relatively simple IRI model instead of a rodent kidney transplant model due to time limitations, and the technical difficulties associated with this procedure (n.b. surgeries were primarily conducted by myself).[27]

The three agents tested in the rodent model were CD47-blocking antibody (αCD47Ab), recombinant thrombomodulin (rTM), and soluble complement receptor 1 (sCR1). Out of many possible agents that have been tested in the IRI setting and could have been used here, these three agents were utilised owing to their (i) clear demonstrated benefit in IRI, (ii) ease of availability,

and (iii) potential for clinical translation as each agent has been administered in the clinical trial setting (not necessarily for the amelioration of IRI).

IRI experiments were initially conducted in rats due to a larger vascular caliber, as an attempt was made to simulate direct intra-renal perfusion of drug(s) by injection via the infra-renal aorta (Fig. 2). However, rats had a high post-procedural mortality and/or needed to be euthanized due to hind limb paralysis (data not shown).

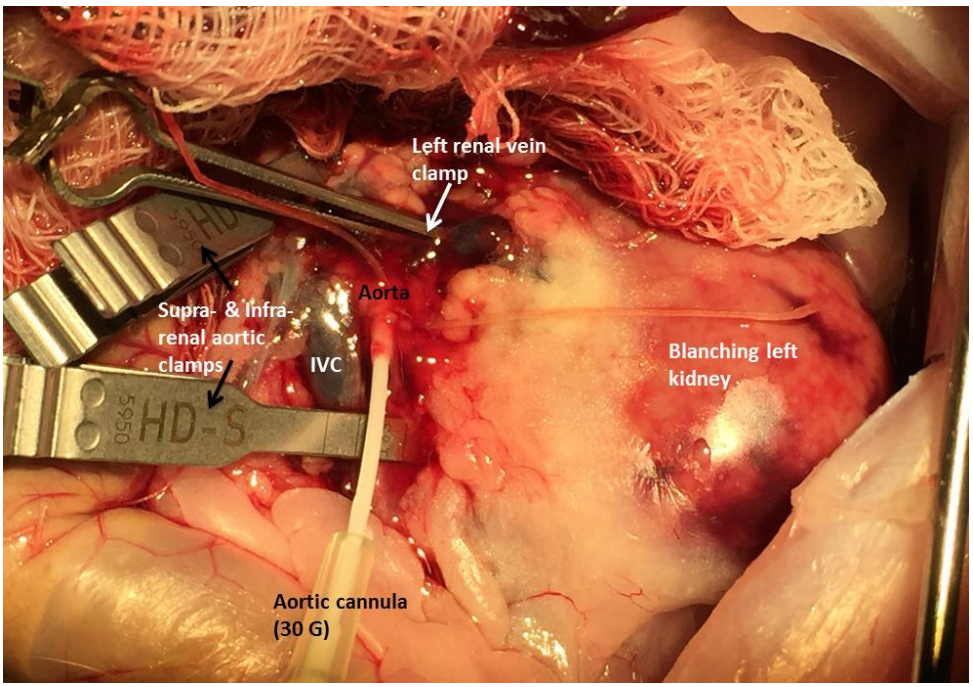


Figure 2. Direct intra-renal perfusion of drug(s) in a rat model. The aorta is clamped above and below the left renal artery, and accessed using a 30 gauge (G) cannula. The distal aspect of the left renal vein is also clamped, and a venotomy is made distal to this. The kidney is perfused via the aortic cannula, with any renal effluent expelled via the venotomy site on the renal vein.

As such, we changed our protocols to instead use a mouse model with intra-venous drug injection. C57BL/6 mice were used due to our broader research group's extensive experience with these mice in the context of IRI models.

Drug delivery using NMP

We were then able to proceed to the testing of the feasibility and efficacy of intra-renal αCD47Ab (porcine-specific) administration to porcine DCD kidneys using NMP. The other drugs tested in

the murine model, sCR1 or rTM, were not utilised due to the superiority of αCD47Ab in affecting a wide range of pathophysiological pathways changed by IRI. The combination of αCD47Ab and sCR1 was not used for the NMP model, as this combination did not provide significant synergy in comparison to the use of αCD47Ab in the murine renal IRI experiments. However, in the future it is clear that these alternate drugs, combinations, or indeed any other anti-IRI drug, can be conveniently administered and tested using NMP, either in a large animal model or using discarded human kidneys. The αCD47Ab was given in 2 phases: (i) immediately after the cold UW flush (to account for potential antibody uptake by PRBCs in the circuit), and (ii) into the arterial line immediately preceding the commencement of NMP (such that the majority of the drug dose passes through the kidney, before encountering the rest of the PRBC mass). The drug was dosed based on renal weight, and not the pig's whole weight. The fact that clear drug binding was evident in the renal parenchyma at the end of NMP, in addition to the αCD47Ab having a beneficial effects on renal flow/resistance parameters, and some features of IRI, indicated that NMP can successfully facilitate intra-renal drug delivery at a significantly reduced dose in comparison to systemic administration.

Human kidney NMP

Discarded human kidneys present a precious resource, and must therefore be used in a manner that is respectful, appropriate, and maximizes their scientific utility. As such, the discarded human kidney work was performed in a staggered manner after gaining sufficient porcine NMP experience. The human discarded kidney NMP project was performed for the following reasons: (i) to gain experience with human kidney perfusion as a prelude to a clinical trial/application in transplantation; (ii) to identify a reasonable baseline regarding perfusion parameters, urine output, and biochemical changes in the perfusate; (iii) to compare the use of allogeneic (banked) PRBCs with autologous (donor) PRBCs during NMP; (iv) to elucidate the passenger leukocyte load of deceased donor kidneys; and (v) to compare the mechanistic basis of any potential superiority of brief pre-implantation NMP in comparison to CS alone, especially with respect to IRI and gene expression changes.

Aim (v) was especially pertinent as we approached the decision to undertake a local clinical trial using NMP faced with uncertainty of the NMP duration that should be utilised. Nicholson and Hosgood have successfully employed one hour of pre-implantation NMP and shown superiority in

comparison to CS alone in ECD and DCD kidneys.[5] These results have formed the basis for their ongoing UK multi-centre RCT.[28] In contrast, Selzner's group in Toronto has suggested that one hour of NMP is inferior to NMP undertaken for 8 or more hours, albeit only in porcine auto-transplantation studies.[29, 30] The one hour time period was chosen for our discarded human studies as we felt this is much more readily implementable locally. As such, further mechanistic evidence supporting the efficacy of this perfusion time would give our own centre additional impetus for the clinical implementation of NMP. Our unique study design, which allowed for the comparison of CS alone with CS and one hour of NMP, involved the use of kidney pairs from the same donor, thereby eliminating the influence of any donor-derived variability. In the absence of the ability to transplant these kidneys, *ex vivo* allogeneic whole blood reperfusion was used after a simulated SWIT of 30 minutes to simulate transplantation. Such a technique has been used by Nicholson's group in animal studies amongst others, and provides an acceptable compromise.[31-34]

13.1.2 RENAL MACHINE PERFUSION WORK UNDERTAKEN IN THE CONTEXT OF WIDER PERFUSION-RELATED RESEARCH – BACK TO THE FUTURE

MP preservation of the kidney has seen emerging popularity over the past 10-20 years, however this era does not represent the first use of this technology in the sphere of transplantation. Indeed, HMP-based set-ups were commonly utilised in the 1970s and early 1980s, only to be supplanted by CS.[35] The decline of MP was related to the development of better cold preservation solutions, more common utilisation of DBD donors, and evidence suggesting that MP performed no better than CS.[35, 36] When considering the MP literature, it is clear that its impacts are disparate based on the perfusion settings used, and on the type of deceased donor, i.e. DCD or DBD, and much current work is focusing on its role in the DCD and/or ECD (high KDPI) setting. Prior to fitting the NMP-related work presented in this thesis into the general storyline of renal MP research and utilisation, current evidence for other MP modalities first needs to be discussed. Only then can an accurate comparison be made that informs future decisions regarding their comparative utility(s). This section will focus on renal MP research, and will be supplemented in particular from liver MP evidence where the clinical renal experience is sparse or non-existent.

HMP

The resurgence of MP utilisation is related to the increasing use of marginal and/or DCD donors, which require improved methods of organ preservation and assessment, in addition to the

development of better, more portable machines and perfusion solutions.[35] HMP has been the dominant modality in the recent peak of MP use in the sphere of kidney transplantation. Postulated mechanisms of action for HMP include better protection and preservation of endothelial integrity and by extension subsequent tubular and glomerular function, in addition to a possible amelioration of IRI by reducing pro-inflammatory cytokine and adhesion molecule expression.[15, 37] Another important consideration is that organs preserved and stored by simple CS are likely to have minimal remaining intravascular preservation solution due to gravity-related vascular collapse.[38] This means that minimal preservation fluid is at the organ/vessel interface during transportation, and ischaemic end-products are allowed to accumulate within the organ.[38] HMP in contrast likely ensures a homogenous and continuous distribution of cold preservation fluid within the organ during the perfusion period, improving the efficacy of the fluid used.[39]

It is unlikely that HMP alone will have an uptake that is significantly greater than its current utilisation world-wide, and modifications to the HMP process and/or alternative forms of MP will most likely come into greater prominence in order to help further close the organ supply-demand gap by using more marginal organs. Indeed, in the Australian setting only one kidney transplant centre uses HMP, and largely on an *ad hoc* basis. Along the spectrum of dynamic preservation approaches, the current viable options are indicated in Fig. 3.

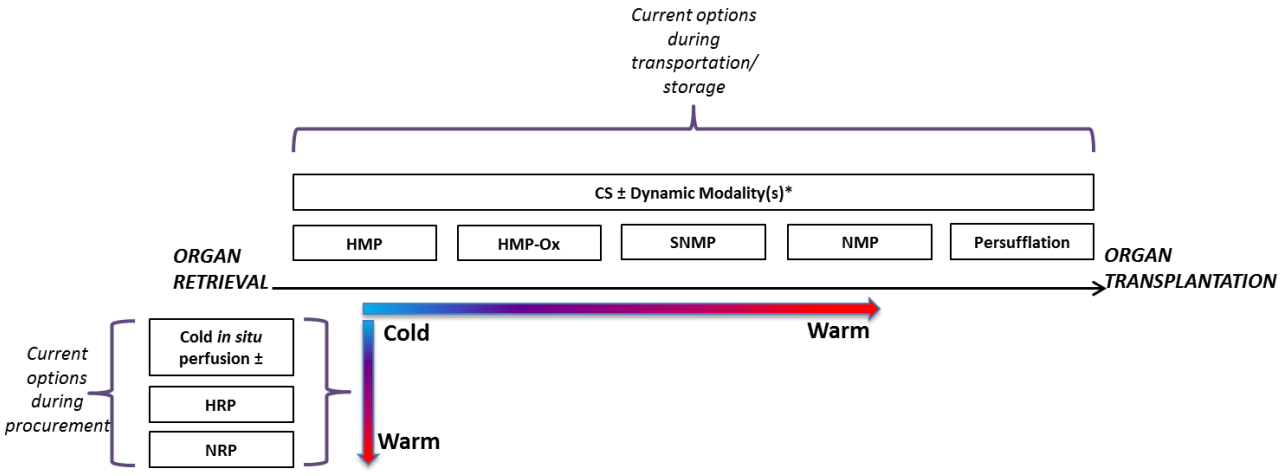


Figure 3. Dynamic preservation options currently available and/or tested during organ procurement and/or transportation. A spectrum exists from cold (hypothermic) methods to warm (normothermic) methods. Hypothermic temperatures generally range from 0-10 ^{o}C, in comparison to 20-34^{o}C for subnormothermia, and 34-37^{o}C for normothermia. HRP and NRP are the hypothermic and normothermic versions of abdominal regional perfusion

(ARP). SNMP may also incorporate the concept of controlled oxygenated rewarming (COR). Persufflation entails gaseous perfusion of an organ with oxygen or an oxygen-mixture, either in an antegrade fashion via its arterial inflow, or through the vein in a retrograde manner. HMP – hypothermic MP; HMP-Ox – oxygenated HMP; HRP – hypothermic regional perfusion; MP – machine perfusion; NMP – normothermic machine perfusion; NRP – normothermic regional perfusion; SNMP – subnormothermic MP. * Dynamic modalities can be used continuously, or in conjunction with CS (i.e. before or after CS).

A technically minor but potentially crucial modification of HMP involves surface oxygenation of the perfusion solution, i.e. HMP-Ox. The efficacy and mechanistic effects of HMP-Ox in comparison to HMP alone are not well-elucidated, especially in clinical studies. It is important to note that kidneys retain ongoing metabolism during hypothermic storage, albeit at a significantly reduced rate. Our systematic review/meta-analysis attempted to compare oxygenated and non-oxygenated HMP in pre-clinical studies.[15] Although only a limited number of studies were eligible for inclusion, there was some indication that HMP-Ox results in better tubular preservation and subsequent function.[15]

HMP-Ox for deceased donor liver preservation perhaps gives a better indication of the potential benefits of this technique in kidney transplantation. Pre-implantation liver HMP-Ox, or "Hypothermic Oxygenated Perfusion" (HOPE), has shown equivalent short-to-medium-term transplantation results for DCD livers in comparison to DBD livers.[40, 41] Its effect is believed to be dependent on oxygenation in combination with hypothermia, and does not seem to be related to the HMP component alone.[42, 43] During ischaemia, and in the absence of oxygen, there is mitochondrial succinate accumulation; after reperfusion, this results in a reversal of electron transport, mitochondrial dysfunction and significant ROS formation, which may be exacerbated by normothermic perfusion systems.[43-45] HOPE is purported to mitigate against these effects by allowing a replenishment of ATP in hypothermic conditions in which minimal energy is required, reduce succinate accumulation, and prevent significant ROS generation upon transplantation.[43, 45] There are 2 major European trials investigating the utility of HMP-Ox in kidney transplantation are nearing completion and publication, and will better delineate the future role of such a technology: (i) 'COPE-POMP' – pre-implantation HMP-Ox versus CS in the ECD donors, and (ii) 'COPE-COMPARE' – continuous HMP-Ox versus continuous HMP in DCD III donors aged ≥ 50 years.[46, 47]

SNMP and COR

Subnormothermic machine perfusion (SNMP) presents another dynamic kidney preservation option that lies further to the right of the temperature spectrum, and can also incorporate the concept of controlled oxygenated rewarming (COR). However, these approaches have not yet been utilised in the context of clinical kidney transplantation, and evidence is limited to pre-clinical studies. The concept underlying SNMP is the achievement of an elevated organ temperature such that direct hypothermia-related injury is avoided. If temperatures of 20-25 °C are employed then an oxygen carrier is generally not required in the perfusate, in contrast to near-physiologic temperatures up to 34 °C which will require an oxygen-carrier.[46, 48] Hoyer *et al*. compared continuous SNMP (20 °C) (including surface oxygenation to achieve a venous oxygen pressure/tension [P_vO_2] that is greater than 150 mmHg [20 kPa]) to continuous HMP-Ox or CS in a porcine DCD model, showing significantly better perfusion and functional characteristics in the SNMP group after simulated transplantation.[8] Similarly, better perfusion characteristics were also demonstrated in the SNMP group in comparison to HMP by Gage *et al*.[49] A recent study compared SNMP using Steen solution to NMP using whole blood alone or whole blood and Steen solution, again demonstrating improved resistance/flows in the SNMP group in comparison to NMP with whole blood but not NMP with Steen and whole blood.[50] However, potential therapeutic mechanisms of action promoting the use of SNMP in these studies are not clear, and no animal let alone human transplantation studies have been performed to strengthen any notion of efficacy for SNMP in kidney transplantation.

COR in contrast currently has a better-defined underlying mechanistic basis, although it has also not yet been employed in the context of clinical kidney transplantation. As its name implies, COR entails staged rewarming of the graft in an oxygenated environment, with the express aim of avoiding the more sudden temperature flux and the associated mitochondrial dysfunction inherent with reperfusion during transplantation after removal from a CS environment.[46, 48, 51] The potential merit of this concept was first demonstrated by Minor and colleagues using porcine livers that were subjected to CS followed by COR, SNMP, or HMP-Ox, and then *ex vivo* whole blood reperfusion.[52] Both COR and SNMP reinstituted ATP levels prior to reperfusion, whilst after simulated transplantation the COR group had significantly reduced hepatocellular biochemical injury and apoptosis, and enhanced bile production and flow parameters, in comparison to the

other treatment groups.[52] Schopp *et al*. later utilised COR in porcine kidneys, demonstrating improved renal function, reduced injury, and enhanced mitochondrial recovery in comparison to continuous HMP or pre-'implantation' HMP-Ox.[33] A more recent porcine kidney study has further shown the beneficial effects related to mitochondrial recovery imparted by COR.[34]

Another possibility within the realm of SNMP is the performance of SNMP at a near-physiologic temperature (~30-34 °C). However, once again human transplantation studies investigating this approach are still lacking.[48] Brasile and colleagues from the Netherlands are perhaps the greatest proponents of this approach, employing the use of an exsanguinous metabolic support (EMS) media during SNMP, which contains an acellular tissue-culture medium-like perfusate that is supplemented with bovine haemoglobin.[53-56] Their work provides some support for the notion that DCD kidneys in particular do not tolerate cold ischaemic damage specifically, and require *ex vivo* perfusion at a near-physiologic temperature during the preservation period and prior to transplantation.[53]

<u>MP in the context of other approaches – ARP (NRP/HRP) and Persufflation</u>

The exact role of MP with respect to other technologies such as ARP and persufflation has not yet been clearly defined. The concept of 'continuous' NMP in the DCD setting, commenced during retrieval by the institution of NRP, and continued *ex vivo* using NMP, is potentially attractive. Preliminary porcine liver transplantation evidence demonstrated the superiority of this approach over CS alone, or NRP alone.[57] More recently, a Chinese group demonstrated a case of "ischaemia-free" transplantation of the liver by instituting liver NMP *in vivo* in the donor (as opposed to NRP of all abdominal organs), which was continued *ex vivo* and then *in vivo* in the recipient whilst the graft was being implanted.[58] Data with regards to the use of such approaches in kidney transplantation is currently lacking.

Persufflation as a technology has extremely limited clinical transplantation evidence, and there is no human evidence comparing its efficacy to MP.[59] Few porcine studies demonstrate potential advantages over MP with respect to IRI and oxidative stress, although the technique has so far only been compared to HMP.[60-62] The sparse human studies that have utilised renal persufflation in the setting of transplantation have at the least demonstrated that this technique is feasible, although

its use has not taken off and significant further work is required to define its role in deceased donor kidney preservation.[63]

NMP

The NMP literature is slightly more complicated to interpret in the context of all other MP work as NMP can potentially fulfill multiple purposes, including:

(i) Reconditioning and/or rejuvenation of a high-risk graft;
(ii) Simulation and/or provision of an objective assessment of graft function;
(iii) Acting as a vehicle for the delivery of drugs/therapies to the kidney; and/or
(iv) In some pre-clinical studies NMP is employed as a simulated model for transplantation to test the effects of new therapies in an isolated organ perfusion set-up.

A significant proportion of the current evidence and studies exploring the utility of renal NMP has been outlined in other sections of this dissertation and discussion. However as is the case with other MP modalities, many questions still need to be answered such that consensus recommendations and guidelines can be developed informing routine clinical use.

Utilisation for functional graft assessment

When considering the use of NMP in the context of all MP modalities, perhaps it is best to consider what NMP can offer that is unique compared to the other technologies. By virtue of re-instituting normothermic, sanguinous, and oxygenated perfusion, NMP is arguably the best modality to achieve an objective assessment of graft function whilst its metabolic machinery is fully switched on. Therefore more informed decisions regarding graft utilisation or discard can be made. NMP of the liver has reached significant prominence in the recent past, and serves as a good case-in-point for the utility of NMP with respect to graft functional assessment, whereby great importance is placed upon such factors as bile production and bile pH.[64, 65]

Although there is less clinical experience with renal NMP in comparison to the liver, NMP of the kidney can similarly objectively simulate graft function through measurement of parameters such as urine output, creatinine clearance, tubular function, and renal oxygen consumption.[31, 66, 67] Nicholson and Hosgood developed a renal allograft assessment score incorporating macroscopic appearance (score 1-3, from best to worst), RBF (score 1 if < 50 ml/min/100 g), and urine output

(score 1 if < 43 ml in 60 mins), however this is currently only able to predict graft function up until 12 months post-transplantation.[66] Other potential markers that can be utilised to strengthen the confidence of this assessment includes the measurement of urinary neutrophil gelatinase-associated lipocalcin (NGAL) and endothelin-1 (ET-1), which correlate with perfusion parameters and the donor's terminal creatinine level.[68] Selzner's group in Toronto also demonstrated that perfusion parameters (specifically IRR) correlate with post-transplant renal function, albeit in pigs.[69] In addition, perfusate acid-base parameters (pH, bicarbonate, and base excess) and levels of AST and lactate from hour 1-4 of NMP negatively correlated with post-transplant creatinine levels.[69]

Poor macroscopic *in situ* perfusion and clearance of blood from the kidney during DCD retrieval is an important cause for renal discard prior to transplantation.[67, 70] An objective assessment of macroscopic perfusion appearance during renal NMP in particular is an immediate advantage provided over HMP that can be readily used to reduce graft discard rates. This was shown by Nicholson and Hosgood, who demonstrated that out of 10 kidneys that were declined by all transplant centres and subsequently assessed using NMP, eight were declined due to poor macroscopic perfusion (after *in situ* and back-table perfusion), and five of these were successfully transplanted post-NMP.[67] Similarly in our discarded human kidney NMP series, we obtained two DCD kidneys that were discarded due to poor *in situ* perfusion after retrieval. However, both developed excellent global macroscopic perfusion during NMP (within the first 5-10 minutes) and were therefore potentially transplantable.

In our own discarded human kidney series, a combination of WIT and CIT clearly correlated with RBF and IRR during NMP in DCD but not DBD kidneys, suggesting that other markers may be more relevant in the DBD setting. Furthermore, we demonstrated that urine output is a relatively good indicator of glomerular and tubular function, but confidence in this assessment should incorporate measurement of creatinine clearance and/or fractional excretion of sodium. It is also essential that urine output is interpreted in the context of the exact perfusion circuit and constituents utilised, as the outputs achieved in all major published NMP series including our own are grossly different.[18, 23, 66, 71] In concordance with the discarded kidney series from the Oxford study, we were unable to demonstrate lactate clearance during brief NMP, and question the value of this marker in kidney NMP.[71] Our findings in combination with those from other groups

suggest that obvious improvements in tubular condition (light microscopy) should also not be expected during NMP, and likely cannot be used as a marker for effective or successful perfusion.[18, 71-75]

Overall, NMP biomarkers with sufficient predictive value for long-term graft function remain lacking. Any scoring system or biomarker measurement will still need to be supplemented with relevant donor and recipient parameters to inform decisions regarding transplantation until more NMP clinical transplantation experience is gained.[67]

A novel modality for the delivery of therapeutic agents

Another distinct advantage of NMP is that it is likely to be more useful than hypothermic modalities as a direct delivery mechanism for intra-renal therapeutics such as anti-IRI drugs/agents, gene therapies, and mesenchymal stem cells or other reparative agents. The primary reason for this is that the kidney retains close to physiologic metabolism during NMP, providing an ideal environment for such agents to function optimally.[26, 76, 77] In comparison, if given during HMP, drug/therapeutic agent uptake is altered, and downstream cascades and effects of the therapy are sub-functional at lower temperatures.[77] Interestingly however, in the setting of a randomized trial Guarrera and colleagues demonstrated improved renal function after renal HMP using Vasosol solution in comparison to HMP with Belzer MP solution.[9] Vasosol solution contains additional protective mediators, including N-acetylcysteine (antioxidant function), nitroglycerin and prostaglandin E1 (vasodilatory function), and L-arginine (nitric oxide precursor).[9] These agents would not be reliant upon binding to the kidney to exert their effects however, and it is more plausible that any beneficial effects would have been exerted during reperfusion in the recipient in the context of residual retention of such agents in the renal vasculature.

Our porcine work exploring the feasibility and efficacy of CD47-blocking antibody therapy to the kidney during NMP quite clearly demonstrates the utility of NMP as a drug-delivery modality. Not only is effective antibody binding to the kidney achieved and retained at the end of NMP, but blockade of the CD47 receptor was shown to have beneficial effects during NMP itself with respect to perfusion and some IRI-related parameters. This study also demonstrated how renal NMP can be used to potentially rapidly translate murine IRI agents into a more clinically

meaningful setting, rather than languishing in the pre-clinical phase for an indefinite period due to constraints related to donor and/or recipient systemic therapies. Drug delivery during NMP can potentially be made even more targeted by such approaches as conjugating nanoparticles to anti-CD31 antibody (against endothelial cells), achieving more prolonged and specific drug accumulation in the renal vasculature.[74] Other possible therapies that have been tested in the pre-clinical NMP setting with beneficial effects include vasodilatory nitric oxide and carbon monoxide-releasing agents, erythropoietin, and cobalt protoporphyrin as a means to induce increased expression of protective heme-oxygenase 1 (HMOX-1).[55, 78, 79]

The potential of NMP to treat and enhance the kidney will only increase in the future, as other therapies such as stem cells, growth factors, and gene-altering technology is explored and perfected. Brasile *et al*. demonstrated that the addition of fibroblast growth factors to their EMS media and normothermic perfusion for 24 hours enhanced the cytoskeletal integrity and synthetic function of canine kidneys with severe warm ischemic damage.[56] The reparative effects of mesenchymal stem cells delivered via NMP are also currently being studied and hold considerable promise.[80] Furthermore, the use of gene therapies, either vector-based or through RNA interference approaches, may be potentially safer and more effective if delivered during NMP, although all of these techniques still remain experimental.[77] If NMP is undertaken with such a reparative aim in mind, it is likely that it needs to be performed over a longer period such that gene or stem-cell treatments have enough time to exert their relevant effects. Achievement of efficacy during brief pre-implantation NMP will rely on effective retention of the specific agent in appropriate areas/cell types in the kidney, such that they can fulfil their functions after transplantation.[77] Another possible crucial role for NMP in the future is the modulation of the recipient's alloimmune response to the donor allograft by depleting passenger leukocytes from the graft, which we showed exist in large numbers in human kidneys and migrate into the NMP circuit. Other groups have also started investigating other methods for immunomodulation during NMP, such as the use of RNA interference methods to silence MHC expression on the vascular endothelium prior to implantation.[81]

System physiology and individual components

Although some physiologic conditions are established and maintained during NMP, it is false to claim that the entirety of the kidney's *in vivo* homeostatic function is replicated on the circuit. As

an example, the kidney is an organ that is exquisitely reliant upon neuro-hormonal feedback, including the renin-angiotensin-aldosterone system (RAAS).[82] The involvement of the RAAS system during *ex vivo* NMP has not been extensively investigated. We attempted to measure aldosterone and renin levels during porcine perfusion; although aldosterone was detectable, no renin was detected.[23] The implications of this need to be characterized in significantly more detail in the future. Artificial conditions created during NMP readily explain the fact that prolonged or indefinite periods of organ NMP are very difficult to maintain. Our human perfusion data displayed significant changes in serum electrolyte and acid-base content during the course of perfusion, with similar changes shown by other groups.[69, 71] Weissenbacher *et al*. suggested recirculation of urine within the circuit as a potential solution to allow for the maintenance of perfusion fluid homeostasis, which readily allowed 24 hours of discarded human kidney NMP.[71] The exact mechanisms of this however remain to be elucidated.

An appropriate oxygen-carrying source is required to support tissue metabolism under normothermic conditions. Blood (PRBCs) represents the most obvious modality, and in general can be allogeneic (banked) or autologous. Allogeneic blood in particular may confer disadvantages with respect to potentially stimulating an immune response, resource utilisation considerations, and additional logistical considerations with respect to cross-matching and sourcing.[83] Most clinical NMP set-ups utilize allogeneic blood, including for the liver and kidney studies that have already been undertaken.[5, 65] Alternatively, the St Vincent's team's DCD cardiac NMP set-up employs autologous whole blood collected immediately prior to cold *in situ* perfusion during retrieval.[84] For the first time, we showed in our discarded human kidney NMP series that autologous PRBCs isolated from the donor blood simultaneous to cold perfusion can also be effectively used for renal NMP. Potassium-rich perfusion fluid was centrifuged and removed from the PRBCs, and NMP was performed without overt deleterious effects in comparison to banked blood. A mean haemoglobin (Hb) level of 43.8 g/L was achieved using autologous blood in our circuit; this is above the 30 g/L threshold established in porcine liver NMP studies that is required to sustain adequate oxygenation.[85] Non-blood based oxygen-carrying sources have also been explored in the context of kidney NMP (pre-clinical) with variable success, and are summarized elsewhere; the clinical utility of these remains to be seen.[77] However, the clinical use of one such agent (HBOC-201) has recently been described in a series of six machine-perfused livers, with subsequent successful transplantation.[86]

NMP duration

It is important to emphasize that although the aforementioned studies in Toronto suggested superior results after 8-16 hours of renal NMP in comparison to one hour of pre-implantation NMP (a) these were porcine and not human studies, and pigs were all sacrificed at 8 days post-operatively, and (b) by post-operative days 7-8, serum creatinine results in study groups having 1, 8, or 16 hours of pre-implantation NMP all converged to very similar levels without intervening dialysis.[18, 19] Furthermore, no true mechanistic basis was provided conferring an advantage to prolonged NMP. No true clinical comparisons exist between brief and prolonged NMP of the kidney, and at this stage a truly informed recommendation cannot be made for the clinical setting. Prolonged renal NMP is however feasible and safe, and can safely extend the preservation period of the kidney.[18, 71, 87]

Mechanistic basis for NMP

The mechanistic basis for any therapeutic efficacy attributable to renal NMP is not yet clearly defined. It is difficult to amalgamate evidence from different NMP groups in this regard as only the Cambridge group has used this technology clinically, and other groups that have performed pre-clinical renal NMP have done so for variable time periods with/without different perfusion settings and fluid constituents. Furthermore, (i) analyses comparing kidney pairs from the same donor with or without NMP have so far been lacking, and (ii) exploration of gene expression changes have largely been performed after NMP, rather than also investigating the ultimate alterations induced after transplantation.

Published evidence clearly displays that renal NMP is associated with a significant pro-inflammatory state. Stone *et al*. showed using porcine kidneys an NMP-induced pro-inflammatory response characterized by increasing perfusate concentrations of interferon-γ, IL-1β, IL-6, IL-18, and CXCL-8, amongst others, and a large efflux of passenger leukocytes.[88] There was a corresponding increase in cell-free DNA, indicative of cell death, which may either indicate cell damage on the circuit, or the clearance of cells already irreversibly damaged by the preceding ischaemic state and/or damage to the circulating leukocytes.[88, 89] However, this inflammatory state did not compromise kidney perfusion, which displayed favorable flow characteristics, urine

output, and oxygen consumption over six hours of NMP.[88] Hosgood *et al*. had also shown increases in pro-inflammatory cytokines during porcine kidney NMP, including levels of IL-6.[90, 91]

Our discarded human kidney NMP study was very unique in comparison to previously published projects in that for the first time, in this study we attempted to gain a better understanding of the functional and mechanistic alterations induced by NMP using paired human kidney discards. This analysis was made all the more powerful by gaining sequential samples at the end of CS, after one hour of NMP, and also after simulated transplantation with whole allogeneic blood. After NMP, the same kidneys had a significant inflammatory signature as determined by mRNA expression, and in particular induction of cytokine-mediated signaling. This pro-inflammatory state cytokine and chemokine-state remained, and was significantly increased in comparison to CS counterparts even after simulated transplantation. However, amongst paired kidneys in our analyses, after simulated transplantation the NMP kidneys had better RBF and IRR, glomerular and tubular functional parameters, and less IRI as characterized by TUNEL staining, oxidative stress, and complement activation. Brief NMP after CS has proven to have a conditioning (beneficial) effect in other pre-clinical studies, and after clinical transplantation.[5, 32, 77, 91] Furthermore, porcine kidneys exposed to 30 minutes of warm ischemia have been shown to have better function after eight hours of NMP and auto-transplantation in comparison to immediate transplantation without any intervening storage period or NMP.[87] Taken together, all of these results indicate that NMP has a conditioning and/or reparative effect in kidneys damaged by warm ischaemia.

From the summation of existing studies and our data, there is strong evidence for the induction of a pro-inflammatory state by NMP, which exceeds the inflammatory response in kidneys that have undergone CS and subsequent whole blood reperfusion. However, there is also very convincing evidence for a NMP-mediated protection with respect to renal flows, functional parameters, and IRI-related damage. The question that then follows is how can these two apparent contradictions be reconciled to explain the possible (beneficial) mechanistic basis for NMP?

The first hypothesis is that this inflammatory response may in fact be beneficial rather than damaging. Indeed, pathway analyses outlined in our study indicated the promotion of cell survival and proliferation, with a reduction in cellular death and apoptosis. In our paired human data, there was no elevation of mRNA expression of traditional ‘anti-inflammatory’ cytokines such as IL-4,

IL-10, IL-13, interferon-α (IFN-α), and transforming growth receptor-β (TGF-β). However, it is well-known that many cytokines can fulfil both pro- and anti-inflammatory functions, depending on timing, the cellular environment, and type(s) of target cells.[92, 93] IL-6, which has well-known pro-inflammatory functions, also has proven anti-inflammatory effects, both of which are mediated by different signaling pathways (classical IL-6 signaling versus *trans* signaling).[94] Interestingly, in the context of acute kidney injury (AKI), IL-6 signaling via these different pathways can simultaneously promote or ameliorate renal injury, and effects differ based on whether the pathway is stimulated before or after the injurious stimulus.[95] Furthermore, induction of renal protection seems to be mediated by a reduction in oxidative stress.[95] In our RNA expression data, pathway analyses showed up-regulation of the positive regulation of tyrosine phosphorylation of the STAT protein pathway ($p = 0.008$); this pathway is induced by IL-6 signaling. Furthermore, IL-6 expression was significantly enhanced in the NMP kidneys compared to CS controls (log-fold change 3.8, $p < 0.01$). Hosgood *et al*. have also shown significant IL-6 increases (actual levels as measured by ELISA) in NMP kidneys.[91] Therefore the elevation of IL-6 after NMP and subsequent whole blood reperfusion may be postulated to be protective. However, further investigation will be required to more clearly prove this hypothesis.

An important alternative consideration for the increased inflammatory response one hour after whole blood reperfusion of NMP kidneys is that it is occurring to the same degree in both NMP and CS kidneys after reperfusion, but peaks after the 1-3 hour time period. As such, it is possible that a similar increase may be expected in the CS kidneys if simulated transplantation was allowed to run for longer. Indeed, NMP kidneys were overall exposed to two hours of reperfusion (one hour of NMP followed by one hour of whole blood reperfusion [simulated transplantation]), in comparison to just one hour for their CS counterparts. Cytokine levels increase in time-dependent manner during reperfusion, with Stone *et al*. showing significantly higher inflammatory cytokine levels in the NMP circuit after 6 hours of NMP in comparison to the first hour.[88] Therefore, the reduced cytokine/chemokine mRNA expression in the CS may merely reflect that levels were measured prior to a peak in these factors. This question would be answered by allowing simulated transplantation to run for a significantly longer period, and re-measuring cytokine expression profiles. Furthermore, cytokine levels should be measured in the circuit and correlated with RNA expression levels. Additional consideration should also be given to the massive leukocyte efflux/mobilization induced by NMP, and the possible contribution of these mobilized and/or

dying leukocytes to the enhanced pro-inflammatory response post-whole blood reperfusion. A similar signature has been observed in NMP of the lungs.[89]

Another putative mechanism that is likely to explain some of the protection offered by NMP in comparison to CS is the induction of heat shock proteins (HSPs). Nicholson and Hosgood's group have demonstrated significantly elevated HSP-70 expression in the NMP kidneys compared to CS after simulated transplantation (i.e. whole blood reperfusion).[79, 91] Our human RNA expression data also shows significant elevations of RNA expression for a multitude of different types of HSP in the NMP kidneys, including HMOX-1 and genes encoding HSP-70. HSP-related pathways were also upregulated, including the HSP binding protein pathway ($p = 0.004$) and the response to unfolded proteins ($p < 0.001$). HSPs play a cytoprotective role in response to numerous injurious stimuli, including IRI, with a protective effect largely mediated through their repair and/or removal of damaged proteins, and interference of other apoptotic and inflammatory pathways.[96-99] HSPs are a critical component of ischemic preconditioning (IPC), and indeed NMP may function via similar methods as IPC.[100-103]

There are many other possible mechanisms that may play a part after the performance of NMP. Brief NMP increases the ATP-to-ADP ratio at the end of the preservation period in comparison to CS kidneys.[32] An improved maintenance of aerobic metabolism may enhance mitochondrial recovery and/or reduce mitochondrial damage; this has not been investigated in renal NMP, but has however been shown in livers preserved by NMP.[104] HSPs, including HSP 70, also mediate mitochondrial protection, which is another possible mechanism for the beneficial effects of NMP.[105, 106] In addition, kidneys undergoing longer periods of NMP display significantly elevated parenchymal cell proliferation and repair (as indicated by the Ki-67 index) after transplantation in comparison to CS kidneys.[87] Indeed, cell proliferation pathways were significantly altered in our human kidney series in NMP compared to CS kidney pairs.

Importantly, NMP induces a unique form of ischaemia-reperfusion that occurs in the absence of damaging leukocytes, platelets, or complement. Notably, leukocyte-depletion of the blood used during NMP has significant beneficial effects.[107, 108] Leukocytes, complement, and platelets are all essential contributors to IRI in transplant allografts, and their absence during the NMP process

may allow graft reconditioning and restoration of energy stores outside of a damaging environment, with a subsequent reduction in IRI in the recipient.[87]

Overall, it can be seen that there are many potential mechanisms and pathways induced and/or altered by the NMP process, which are summarized in Fig. 4.

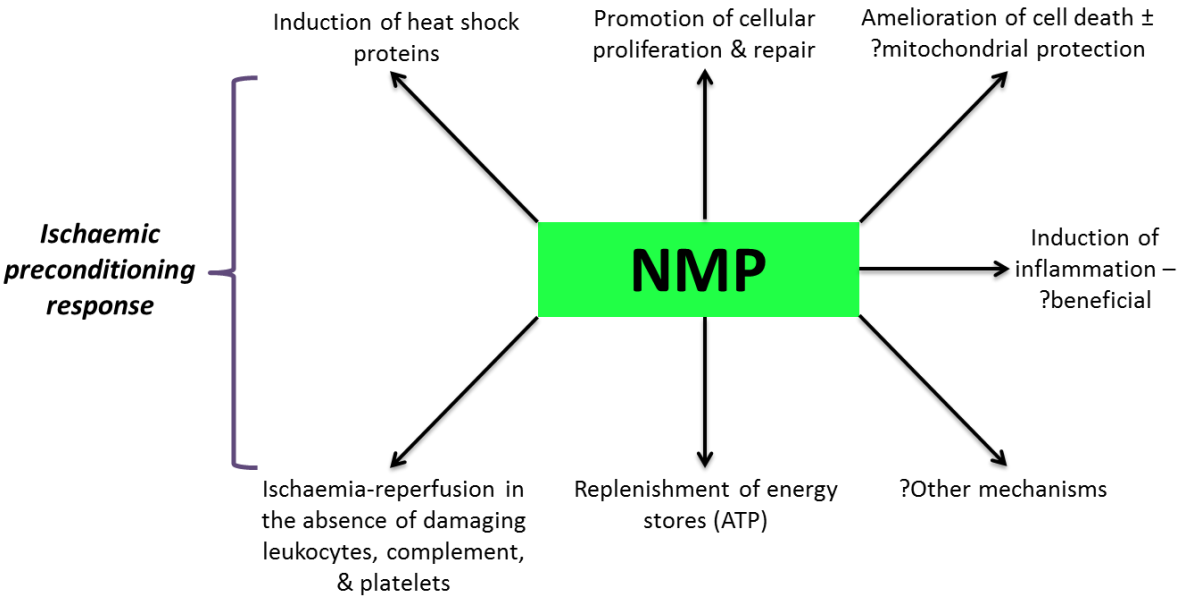


Figure 4. Possible mechanisms of action contributing to the likely beneficial effects of NMP in donor allografts.

NMP in the context of other types of MP

Beyond the likely advantages of NMP in graft assessment and the delivery of therapeutic agents/substances, the exact comparative role of NMP with respect to other MP modalities needs to be more clearly defined. A large part of the problem faced when deciding between different MP approaches lies in the lack of head-to-head comparisons between each technique in the context of clinical kidney transplantation. One of the most important reasons these comparisons do not exist is that the individual utility of each approach with respect to the current gold standard of CS has still not been defined, mainly due to high levels of variability associated with each method. As an example, although renal HMP has been utilised in clinical transplantation for a considerable period of time, there is little long-term efficacy data, and trials exploring modifications to the process such as oxygenation are still being conducted, as discussed above. This again leads us to the question of when and how we should utilize NMP for deceased donor kidneys. This question will

require further insights into the mechanisms lying at the heart of NMP, in particular after different time points during NMP and post-transplantation, and will be further informed upon completion of the renal NMP trial conducted by Nicholson and Hosgood in the UK.[109] Overall, the complexities of MP decision-making and utilisation are outlined in Fig. 5.

Perhaps in the future we will be using a combination of approaches in the same kidney. A particularly appealing combination may consist of initial COR, taking advantage of the mitochondrial recovery associated with this technique, followed by NMP for objective graft assessment, conditioning, and also possible resuscitation using therapeutic agents. Indeed, Porte's group in The Netherlands has demonstrated the feasibility of combining different MP settings in the context of clinical liver transplantation, with grafts initially undergoing HMP, followed by COR, and finally NMP (Netherlands Trial Register Number NTR5972).[86]

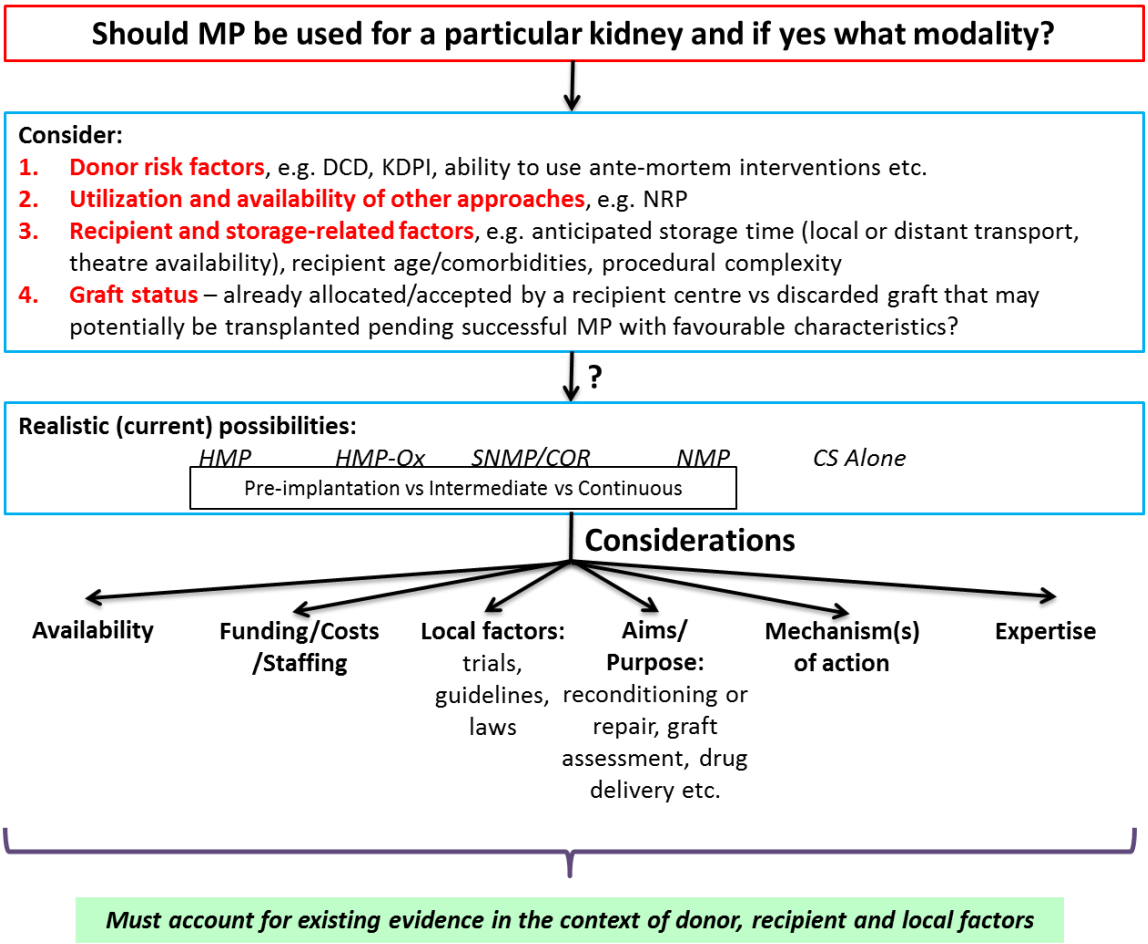


Figure 5. Possible decision tree and relevant factors to consider when considering the use of different MP modalities. 'Intermediate' MP implies MP wedged between 2 significant periods of CS, e.g. when a graft is transported using CS to a central MP laboratory, after which it is sent to the recipient centre once again via CS. Different dynamic techniques may also be used in combination. CS – cold static storage; DCD – donation after circulatory death; HMP – hypothermic MP; HMP-Ox – oxygenated HMP; KDPI – kidney donor profile index; MP – machine perfusion; NMP – normothermic machine perfusion; NRP – normothermic regional perfusion; SNMP – subnormothermic MP.

13.2 Abdominal organ procurement, *in situ* perfusion, and subsequent cold preservation – back to basics

Cold *in situ* perfusion and subsequent CS is an effective and time-honoured approach to the preservation of deceased donor organs prior to transplantation. However even the basic principles upon which this is based are complicated by the existence of varying techniques, perfusion/preservation fluids, and surgical preferences, and an optimal approach is not defined.

If we are truly going to be able to compare studies between CS and MP then surely standardized principals must be applied in the undertaking of CS. For this reason, epidemiologic techniques were applied to try and enhance our understanding of optimal procurement, perfusion, and static preservation in the context of liver and pancreas transplantation. A primary stimulus for this work was the lack of consistency in guidelines and practices between units/jurisdictions regarding the procurement of these organs. This is especially problematic as MP continues to gain increasing prominence, especially in the sphere of liver transplantation,[65] and trial outcomes and interpretation may be confounded by variable *in situ* perfusion practices prior MP commencement. Furthermore, we felt that there was considerable scope to try and improve procurement and CS practices with subsequent positive impacts on liver and pancreas transplantation. This is essential in resource-limited settings where MP would be difficult to institute on a wider scale. It is also very important for institutions where MP use is being explored and/or utilised more commonly, as CS is generally the comparator against which the efficacy of MP is ascertained.

13.2.1 IN SITU PERFUSION AND PRESERVATION FLUIDS

The pancreas is often procured together with the liver and kidneys, and therefore multi-organ procurement and perfusion/preservation techniques account for the outcomes of all of these organs. As outlined in the Introduction (section 1.2.4), kidney transplant outcomes are not grossly

influenced by the type of perfusion/preservation fluid used, and this consideration is more important for the pancreas and liver. UW is the dominant perfusion fluid used, and we showed that its use resulted in biochemically superior results after pancreas transplantation, without a significant impact on other outcomes.[110] A corresponding analysis in liver transplantation revealed no obvious superiority of UW over other fluids, in particular HTK, which contradicted some large registry analyses.[111-113] A more recent large registry analysis of the Eurotransplant region however once again failed to demonstrate a difference between UW and HTK in liver transplantation after adjusting for all relevant risk factors and accounting for clustering of fluid use by geographic region/retrieval units.[114] However overall, owing to the possible benefits of UW use for the pancreas, especially for more prolonged preservation periods, it is advisable to continue to use UW in the multi-organ retrieval setting. Well-conducted prospective studies with long-term outcome data are lacking, and need to be pursued in the future, especially if we ever hope to be able to pursue direct head-to-head comparisons between CS and MP of any type.

13.2.2 IN SITU PERFUSION ROUTES

Liver perfusion during procurement can be conducted using the aortic-only route, or via the aorta and portal vein. Data from our systematic review and meta-analysis indicated no difference between these routes in standard DBD liver transplantation.[111] However, only short-term outcomes could be analyzed, and most existing studies had small numbers with insufficient follow-up. Furthermore, one paper indicated significantly inferior graft survival outcomes after aortic-only perfusion in expanded criteria/higher risk grafts.[115] This catalyzed our analysis of the Australia and New Zealand Liver Transplant Registry, which allowed for comparison of prospectively collected graft and patient survivals over a prolonged period, with a larger patient subset.[116] Although we once again demonstrated the absence of significant outcome differences between aortic or dual perfusion after transplantation of standard criteria DBD livers, a significant difference became apparent in higher risk donors, despite accounting for relevant confounders.[116] Interestingly, a very recent publication from Italy also aortic and dual perfusion with respect to the risk of developing ischemic-type biliary lesions (ITBL), and showed a significantly greater risk in the aortic-only group for donors 80 years or older.[117] Taken together, these results suggest the significant role perfusion route can play in liver transplantation outcomes, especially as we continue to use higher risk/marginal donor livers to meet organ demand. A multi-centre RCT is warranted comparing aortic-only and dual perfusion in liver transplantation, especially focusing on suboptimal donors.

The contribution of each technique to DCD liver transplant outcomes also needs to be more clearly defined. Only then can a unified approach that optimizes CS of the liver be advocated, against which newer technologies such as MP will need to be compared.

13.3 Closing remarks

We are currently at an exciting juncture in the history of transplantation, especially with regards to the availability of newer technologies designed to enhance deceased donor organ preservation, increase organ utilisation, and optimize subsequent transplantation outcomes. As the demand for organs continues to rise, it is imperative that the transplantation community continues to improve organ preservation methods, presents a more unified approach, and utilises new technologies in the fight to increase organ availability and further enhance transplantation outcomes. These aims must be targeted at multiple fronts, and must begin with appropriate management of the donor. Organ retrieval must be undertaken in a meticulous manner, and may be enhanced by the use of such approaches as abdominal regional perfusion. In most settings, such a technique is still not available or logistically feasible, and cold *in situ* perfusion remains a cornerstone. Subsequent to organ retrieval, a significant number of preservation options have become available, and range from the existing gold standard of cold static storage, to dynamic methods such as machine perfusion. Furthermore, a combination of static and dynamic techniques can also be employed, especially if this will help enhance the feasibility of utilizing beneficial dynamic approaches. There is still significant scope to improve cold static storage, especially for the liver and pancreas, which is imperative if any true incremental benefit of machine perfusion is to be calculated. However perhaps the most exciting aspect of machine perfusion is that there is potentially no limit to the advantages afforded by this technique, if not now, then in the future. There are many opportunities related to the use of machine perfusion, not least with respect to graft conditioning, but also graft assessment and repair, which may be further improved by the routine use of therapeutic agents directly delivered to organs during *ex vivo* perfusion.

Indeed, it is clear that cold static storage will always be limited in what it can achieve, and it is unlikely that significant further advances will be made in this regard (Fig. 6). Currently, we ask the question of how we can improve organ procurement and preservation to slow the rate of organ decline prior to transplantation. Perhaps we should now be asking how we can enhance graft capability and function above that demonstrated in the organ donor. Therefore, we will be able to

resuscitate and use organs that previously never would have been deemed suitable for transplantation, expanding the donor pool whilst simultaneously improving transplantation outcomes.

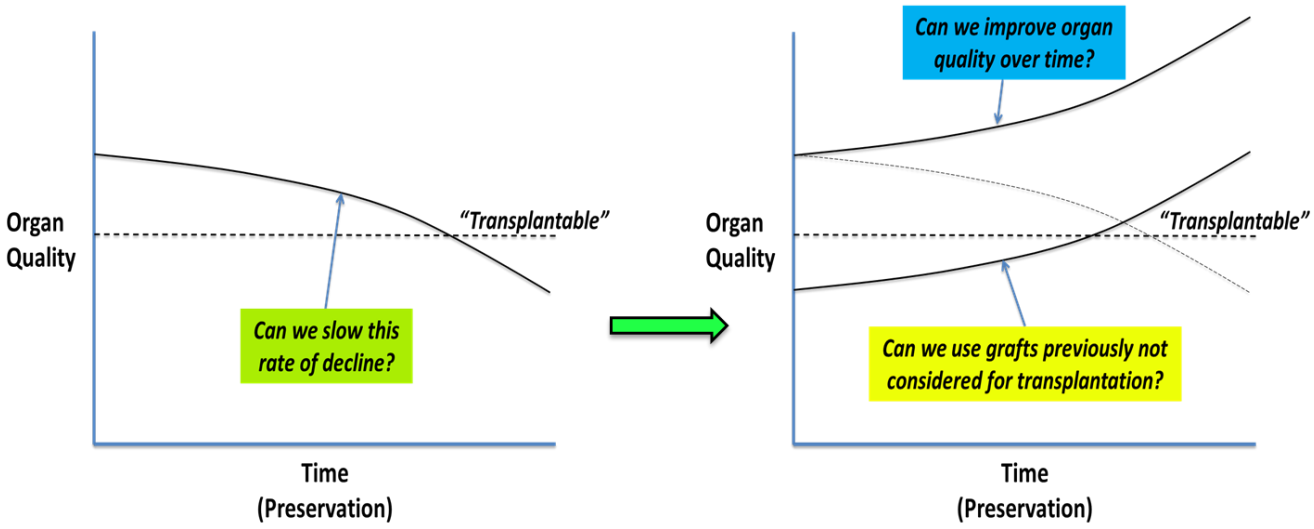


Figure 6. Organ procurement and preservation practices impact organ quality at transplant. Organ quality is a function of storage/preservation time and donor-related factors, and most organ preservation research is aimed at reducing the organ's rate of decline prior to transplantation. A more radical approach is indicated on the right, which will be reliant upon advanced therapeutics delivered using such methods as machine perfusion.

13.4 References

1. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Eng J Med.* 2009; 360: 7-19.
2. Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial S, Machine Preservation Trial Study G. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Eng J Med.* 2012; 366: 770-771.
3. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg.* 2010; 252: 756-764.
4. Watson CJ, Wells AC, Roberts RJ, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant*. 2010; 10: 1991-1999.
5. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013; 13: 1246-1252.
6. Matsuno N, Konno YN, Jyojima Y, et al. Machine perfusion preservation for kidney grafts with a high creatinine from uncontrolled donation after cardiac death. *Transplant Proc.* 2010; 42: 155-158.
7. Thuillier R, Allain G, Celhay O, et al. Benefits of active oxygenation during hypothermic machine perfusion of kidneys in a preclinical model of deceased after cardiac death donors. *J Surg Res.* 2013; 184: 1174-1181.
8. Hoyer DP, Gallinat A, Swoboda S, et al. Subnormothermic machine perfusion for preservation of porcine kidneys in a donation after circulatory death model. *Transpl Int*. 2014; 27: 1097-1106.
9. Guarrera JV, Polyak M, O'Mar Arrington B, Kapur S, Stubenbord WT, Kinkhabwala M. Pulsatile machine perfusion with Vasosol solution improves early graft function after cadaveric renal transplantation. *Transplantation*. 2004; 77: 1264-1268.
10. Bathini V, McGregor T, McAlister VC, Luke PP, Sener A. Renal perfusion pump vs cold storage for donation after cardiac death kidneys: a systematic review. *J Urol.* 2013; 189: 2214-2220.
11. Lam VW, Laurence JM, Richardson AJ, Pleass HC, Allen RD. Hypothermic machine perfusion in deceased donor kidney transplantation: a systematic review. *J Surg Res.* 2013; 180: 176-182.
12. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. Preservation solutions for static cold storage of kidney allografts: a systematic review and meta-analysis. *Am J Transplant*. 2012; 12: 896-906.
13. Jiao B, Liu S, Liu H, Cheng D, Cheng Y, Liu Y. Hypothermic machine perfusion reduces delayed graft function and improves one-year graft survival of kidneys from expanded criteria donors: a meta-analysis. *PLoS ONE*. 2013; 8: e81826-e81826.
14. Wight JP, Chilcott JB, Holmes MW, Brewer N. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. *Clin Transplant*. 2003; 17: 293-307.
15. Hameed AM, Pleass HC, Wong G, Hawthorne WJ. Maximizing kidneys for transplantation using machine perfusion: from the past to the future: A comprehensive systematic review and meta-analysis. *Medicine (Baltimore).* 2016; 95: e5083.
16. TSANZ. Annual Newsletter 2016 2016. Available at: https://www.tsanz.com.au/documents/2016Newsletter.pdf. Acces date: November 16, 2018.

17. Gill J, Dong J, Eng M, Landsberg D, Gill JS. Pulsatile perfusion reduces the risk of delayed graft function in deceased donor kidney transplants, irrespective of donor type and cold ischemic time. *Transplantation*. 2014; 97: 668-674.
18. Kaths JM, Echeverri J, Linares I, et al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant*. 2017; 17: 2580-2590.
19. Kaths JM, Cen JY, Chun YM, et al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. *Am J Transplant*. 2017. 17: 957-969.
20. Kaths JM, Spetzler VN, Goldaracena N, et al. Normothermic Ex Vivo Kidney Perfusion for the Preservation of Kidney Grafts prior to Transplantation. *J Vis Exp*. 2015; 101: e52909.
21. Echeverri J, Goldaracena N, Kaths JM, et al. Comparison of BQ123, Epoprostenol, and Verapamil as Vasodilators During Normothermic Ex Vivo Liver Machine Perfusion. *Transplantation*. 2018; 102: 601-608.
22. Mancina E, Kalenski J, Paschenda P, et al. Determination of the preferred conditions for the isolated perfusion of porcine kidneys. *Eur Surg Res*. 2015; 54: 44-54.
23. Hameed AM, Miraziz R, Lu DB, et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ J Surg*. 2018; 88: E429-E434.
24. Vercaemst L. Hemolysis in cardiac surgery patients undergoing cardiopulmonary bypass: a review in search of a treatment algorithm. *J Extra Corpor Technol*. 2008; 40: 257-267.
25. Hameed A, Dervish S, Rogers N, Pleass H, Hawthorne W. A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transpl Int*. 2018. *Epub ahead of print*. DOI: 10.1111/tri.13361.
26. O'Neill S, Gallagher K, Hughes J, Wigmore SJ, Ross JA, Harrison EM. Challenges in early clinical drug development for ischemia-reperfusion injury in kidney transplantation. *Exp Opin Drug Discov*. 2015; 10: 753-762.
27. Hesketh EE, Czopek A, Clay M, et al. Renal ischaemia reperfusion injury: a mouse model of injury and regeneration. *J Vis Exp*. 2014; 88: 51816.
28. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017; 7: e012237.
29. Kaths JM, Cen JY, Chun YM, et al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. *Am J Transplant*. 2017; 17: 957-969.
30. Kaths JM, Echeverri J, Linares I, et al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant*. 2017; 17: 2580-2590.
31. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct*. 2017; 3: e140.
32. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg*. 2008; 95: 111-118.
33. Schopp I, Reissberg E, Luer B, Efferz P, Minor T. Controlled Rewarming after Hypothermia: Adding a New Principle to Renal Preservation. *Clin Transl Sci*. 2015; 8: 475-478.

34. von Horn C, Minor T. Improved approach for normothermic machine perfusion of cold stored kidney grafts. *Am J Transl Res*. 2018; 10: 1921-1929.
35. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transpl Int*. 2015; 28: 665-676.
36. Opelz G, Terasaki PI. Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. *Transplantation*. 1982; 33: 64-68.
37. De Deken J, Kocabayoglu P, Moers C. Hypothermic machine perfusion in kidney transplantation. *Curr Opin Organ Transplant*. 2016; 21: 294-300.
38. Churchill TA. Organ Preservation for Transplantation. Functional Metabolism: John Wiley & Sons, Inc.; 2005: pp. 529-555.
39. Ray C, Sohrabi S, Talbot D. Correspondence Re: Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation. *N Eng J Med*. 2009; 360: 1460-1461.
40. Dutkowski P, Schlegel A, de Oliveira M, Mullhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. *J Hepatol*. 2014; 60: 765-772.
41. Dutkowski P, Polak WG, Muiesan P, et al. First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. *Ann Surg*. 2015; 262: 764-771.
42. Schlegel A, Kron P, Graf R, Clavien PA, Dutkowski P. Hypothermic Oxygenated Perfusion (HOPE) downregulates the immune response in a rat model of liver transplantation. *Ann Surg*. 2014; 260: 931-938.
43. Schlegel A, Muller X, Dutkowski P. Hypothermic Machine Preservation of the Liver: State of the Art. *Curr Transplant Rep*. 2018; 5: 93-102.
44. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*. 2014; 515: 431-435.
45. Boteon YL, Boteon AP, Attard J, Wallace L, Bhogal RH, Afford SC. Impact of machine perfusion of the liver on post-transplant biliary complications: A systematic review. *World J Transplant*. 2018; 8: 220-231.
46. Jochmans I, Akhtar MZ, Nasralla D, et al. Past, Present, and Future of Dynamic Kidney and Liver Preservation and Resuscitation. *Am J Transplant*. 2016; 16: 2545-2555.
47. COPE Consortium. COPE Work Programme - Trials 2018. Available at: http://cope-eu.com/work%20programme/trials.html. Accessed November 15, 2018.
48. Kaths JM, Paul A, Robinson LA, Selzner M. Ex vivo machine perfusion for renal graft preservation. *Transplant Rev*. 2018; 32: 1-9.
49. Gage F, Leeser DB, Porterfield NK, et al. Room temperature pulsatile perfusion of renal allografts with Lifor compared with hypothermic machine pump solution. *Transplant. Proc*. 2009; 41: 3571-3574.
50. Urcuyo D, Blum MF, Liu Q, et al. Development of a prolonged warm ex vivo perfusion model for kidneys donated after cardiac death. *Int J Artif Organs*. 2017; 40: 265-271.
51. Sammut IA, Burton K, Balogun E, et al. Time-dependent impairment of mitochondrial function after storage and transplantation of rabbit kidneys. *Transplantation*. 2000; 69: 1265-1275.
52. Minor T, Efferz P, Fox M, Wohlschlaeger J, Luer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am J Transplant*. 2013; 13: 1450-1460.
53. Brasile L, Stubenitsky BM, Booster MH, Arenada D, Haisch C, Kootstra G. Hypothermia--a limiting factor in using warm ischemically damaged kidneys. *Am J Transplant*. 2001; 1: 316-320.

54. Stubenitsky BM, Booster MH, Brasile L, Araneda D, Haisch CE, Kootstra G. Exsanguinous metabolic support perfusion--a new strategy to improve graft function after kidney transplantation. *Transplantation*. 2000; 70: 1254-1258.
55. Brasile L, Buelow R, Stubenitsky BM, Kootstra G. Induction of heme oxygenase-1 in kidneys during ex vivo warm perfusion. *Transplantation*. 2003; 76: 1145-1149.
56. Brasile L, Stubenitsky B, Haisch CE, Kon M, Kootstra G. Potential of repairing ischemically damaged kidneys ex vivo. *Transplant Proc.* 2005; 37: 375-376.
57. Fondevila C, Hessheimer AJ, Maathuis MH, et al. Superior preservation of DCD livers with continuous normothermic perfusion. *Ann Surg.* 2011; 254: 1000-1007.
58. He X, Guo Z, Zhao Q, et al. The first case of ischemia-free organ transplantation in humans: A proof of concept. *Am J Transplant.* 2018; 18: 737-744.
59. Min CG, Papas KK. Recent developments in persufflation for organ preservation. *Curr Opin Organ Transplant*. 2018; 23: 330-335.
60. Suszynski TM, Rizzari MD, Scott WE, Tempelman LA, Taylor MJ, Papas KK. Persufflation (or Gaseous Oxygen Perfusion) as a Method of Organ Preservation. *Cryobiology*. 2012; 64: 125-143.
61. Kalenski J, Mancina E, Paschenda P, et al. Comparison of Aerobic Preservation by Venous Systemic Oxygen Persufflation or Oxygenated Machine Perfusion of Warm-Ischemia-Damaged Porcine Kidneys. *Eur Surg Res.* 2016; 57: 10-21.
62. Treckmann J, Nagelschmidt M, Minor T, Saner F, Saad S, Paul A. Function and quality of kidneys after cold storage, machine perfusion, or retrograde oxygen persufflation: results from a porcine autotransplantation model. *Cryobiology*. 2009; 59: 19-23.
63. O'Callaghan JM, Pall KT, Pengel LHM. Supplemental oxygen during hypothermic kidney preservation: A systematic review. *Transplant Rev.* 2017; 31: 172-179.
64. Watson CJE, Kosmoliaptsis V, Randle LV, et al. Normothermic Perfusion in the Assessment and Preservation of Declined Livers Before Transplantation: Hyperoxia and Vasoplegia-Important Lessons From the First 12 Cases. *Transplantation*. 2017; 101: 1084-1098.
65. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018; 557: 50-56.
66. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. *Br J Surg.* 2015; 102: 1433-1440.
67. Hosgood SA, Thompson E, Moore T, Wilson CH, Nicholson ML. Normothermic machine perfusion for the assessment and transplantation of declined human kidneys from donation after circulatory death donors. *Br J Surg.* 2018; 105: 388-394.
68. Hosgood SA, Nicholson ML. An Assessment of Urinary Biomarkers in a Series of Declined Human Kidneys Measured During Ex Vivo Normothermic Kidney Perfusion. *Transplantation*. 2017; 101: 2120-2125.
69. Kaths JM, Hamar M, Echeverri J, et al. Normothermic ex vivo kidney perfusion for graft quality assessment prior to transplantation. *Am J Transplant*. 2018; 18: 580-589.
70. Messina M, Diena D, Dellepiane S, et al. Long-Term Outcomes and Discard Rate of Kidneys by Decade of Extended Criteria Donor Age. *Clin J Am Soc Nephrol.* 2017; 12: 323-331.
71. Weissenbacher A, Lo Faro L, Boubriak O, et al. Twenty-four-hour normothermic perfusion of discarded human kidneys with urine recirculation. *Am J Transplant*. 2018. *Epub ahead of print*. DOI: 10.1111/ajt.14932
72. Kaths JM, Spetzler VN, Goldaracena N, et al. Normothermic Ex Vivo Kidney Perfusion for the Preservation of Kidney Grafts prior to Transplantation. *J Vis Exp*. 2015; 101: e52909.

73. Kabagambe SK, Palma IP, Smolin Y, et al. Combined Ex Vivo Hypothermic and Normothermic Perfusion for Assessment of High-Risk Deceased Donor Human Kidneys for Transplantation. *Transplantation*. 2018. *Epub ahead of print*. DOI: 10.1097/TP.0000000000002299
74. Tietjen GT, Hosgood SA, DiRito J, et al. Nanoparticle targeting to the endothelium during normothermic machine perfusion of human kidneys. *Sci Transl Med*. 2017; 9.
75. Hosgood SA, Barlow AD, Yates PJ, Snoeijs MG, van Heurn EL, Nicholson ML. A pilot study assessing the feasibility of a short period of normothermic preservation in an experimental model of non heart beating donor kidneys. *J Surg Res.* 2011; 171: 283-290.
76. Chadha R, Hossain MA, Bagul A. Optimising organs for transplantation: is normothermic machine perfusion the answer? *Expert Rev Med Devices*. 2016; 13: 221-223.
77. Hosgood SA, Heurn E, Nicholson ML. Normothermic machine perfusion of the kidney: better conditioning and repair? *Transpl Int*. 2015; 28: 657-664.
78. Hosgood SA, Bagul A, Kaushik M, Rimoldi J, Gadepalli RS, Nicholson ML. Application of nitric oxide and carbon monoxide in a model of renal preservation. *Br J Surg*. 2008; 95: 1060-1067.
79. Yang B, Hosgood SA, Bagul A, Waller HL, Nicholson ML. Erythropoietin regulates apoptosis, inflammation and tissue remodelling via caspase-3 and IL-1beta in isolated hemoperfused kidneys. *Eur J Pharmacol.* 2011; 660: 420-430.
80. Maria S-PJ, Marco E, James H, et al. Mesenchymal Stromal Cells as Anti-Inflammatory and Regenerative Mediators for Donor Kidneys During Normothermic Machine Perfusion. *Stem Cells Dev*. 2017; 26: 1162-1170.
81. Figueiredo C, Carvalho-Oliveira M, Chen-Wacker C, et al. Immunoengineering of the vascular endothelium to silence MHC expression during normothermic ex vivo lung perfusion. *Hum Gene Ther*. 2018.
82. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol*. 2014; 4: 1201-1228.
83. Laing RW, Bhogal RH, Wallace L, et al. The Use of an Acellular Oxygen Carrier in a Human Liver Model of Normothermic Machine Perfusion. *Transplantation*. 2017; 101: 2746-2756.
84. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet*. 2015; 385: 2585-2591.
85. Bral M, Gala-Lopez B, Thiesen A, et al. Determination of Minimal Hemoglobin Level Necessary for Normothermic Porcine Ex Situ Liver Perfusion. *Transplantation*. 2018; 102: 1284-1292.
86. Vries Y, Leeuwen OB, Matton APM, Fujiyoshi M, Meijer VE, Porte RJ. Ex situ normothermic machine perfusion of donor livers using a haemoglobin-based oxygen carrier: a viable alternative to red blood cells. *Transpl Int*. 2018; 31: 1281-1282.
87. Hamar M, Urbanellis P, Kaths MJ, et al. Normothermic Ex Vivo Kidney Perfusion Reduces Warm Ischemic Injury of Porcine Kidney Grafts Retrieved After Circulatory Death. *Transplantation*. 2018; 102: 1262-1270.
88. Stone JP, Ball AL, Critchley WR, et al. Ex Vivo Normothermic Perfusion Induces Donor-Derived Leukocyte Mobilization and Removal Prior to Renal Transplantation. *KI Rep*. 2016; 1: 230-239.
89. Yeung JC, Zamel R, Bai X, et al. Towards Donor Lung Recovery - Gene Expression Changes During Ex Vivo Lung Perfusion. *J Heart Lung Transplant*. 2015; 34: S39-S40.

90. Hosgood SA, Moore T, Kleverlaan T, Adams T, Nicholson ML. Haemoadsorption reduces the inflammatory response and improves blood flow during ex vivo renal perfusion in an experimental model. *J Transl Med*. 2017; 15: 216.
91. Hosgood SA, Patel M, Nicholson ML. The conditioning effect of ex vivo normothermic perfusion in an experimental kidney model. *J Surg Res.* 2013; 182: 153-160.
92. Cavaillon JM. Pro- versus anti-inflammatory cytokines: myth or reality. *Cell Mol Biol*. 2001; 47: 695-702.
93. Opal SM, DePalo VA. Anti-inflammatory cytokines. *Chest*. 2000; 117: 1162-1172.
94. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta Mol Cell Res*. 2011; 1813: 878-888.
95. Nechemia-Arbely Y, Barkan D, Pizov G, et al. IL-6/IL-6R axis plays a critical role in acute kidney injury. *J Am Soc Nephrol*. 2008; 19: 1106-1115.
96. O'Neill S, Harrison EM, Ross JA, Wigmore SJ, Hughes J. Heat-Shock Proteins and Acute Ischaemic Kidney Injury. *Nephron Exp Nephrol*. 2014; 126: 167-174.
97. Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, Giffard RG. Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. *Ann N Y Acad Sci.* 2005; 1053: 74-83.
98. Latchman DS. Heat shock proteins and cardiac protection. *Cardiovasc Res.* 2001; 51: 637-646.
99. Richter K, Haslbeck M, Buchner J. The Heat Shock Response: Life on the Verge of Death. *Mol Cell*. 2010; 40: 253-266.
100. Kume M, Yamamoto Y, Saad S, et al. Ischemic preconditioning of the liver in rats: Implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. *J Lab Clin Med.* 1996; 128: 251-258.
101. Konstantinov IE, Arab S, Li J, et al. The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. *J Thorac Cardiovasc Surg*. 2005; 130: 1326-1332.
102. Gassanov N, Nia AM, Caglayan E, Er F. Remote Ischemic Preconditioning and Renoprotection: From Myth to a Novel Therapeutic Option? *J Am Soc Nephrol*. 2014; 25: 216-224.
103. Das DK, Maulik N. Cardiac genomic response following preconditioning stimulus. *Cardiovasc. Res.* 2006; 70: 254-263.
104. Ghinolfi D, Rreka E, De Tata V, et al. Pilot, open, randomized, prospective trial for normothermic machine perfusion evaluation in liver transplantation from older donors. *Liver Transplant*. 2018. *Epub ahead of print*; DOI: 10.1002/lt.25362
105. Daugaard M, Rohde M, Jäättelä M. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* 2007; 581: 3702-3710.
106. Minor T, von Horn C, Paul A. Role of temperature in reconditioning and evaluation of cold preserved kidney and liver grafts. *Curr Opin Organ Transplant*. 2017; 22: 267-273.
107. Harper S, Hosgood S, Kay M, Nicholson M. Leucocyte depletion improves renal function during reperfusion using an experimental isolated haemoperfused organ preservation system. *Br J Surg.* 2006; 93: 623-629.
108. Yang B, Hosgood SA, Harper SJF, Nicholson ML. Leucocyte Depletion Improves Renal Function in Porcine Kidney Hemoreperfusion Through Reduction of Myeloperoxidase+ Cells, Caspase-3, IL-1β, and Tubular Apoptosis1. *J Surg Res.* 2010; 164: e315-e324.
109. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017; 7.

110. Hameed AM, Wong G, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. *HPB (Oxford)*. 2017; 19: 933-943.
111. Hameed AM, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: working towards a unified approach. *Liver Transplant*. 2017; 23: 1615-1627.
112. Adam R, Delvart V, Karam V, et al. Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant*. 2015; 15: 395-406.
113. Stewart ZA, Cameron AM, Singer AL, Dagher NN, Montgomery RA, Segev DL. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation. *Am J Transplant*. 2009; 9: 217-221.
114. de Boer JD, Strelniece A, van Rosmalen M, et al. The Effect of Histidine-tryptophan-ketoglutarate Solution and University of Wisconsin Solution: An Analysis of the Eurotransplant Registry. *Transplantation*. 2018; 102: 1870-1877.
115. D'Amico F, Vitale A, Gringeri E, et al. Liver transplantation using suboptimal grafts: impact of donor harvesting technique. *Liver Transplant*. 2007; 13: 1444-1450.
116. Hameed AM, Pang T, Yoon P, et al. Aortic Versus Dual Perfusion for Retrieval of the Liver After Brain Death: A National Registry Analysis. *Liver Transplant*. 2018; 24: 1536-1544.
117. Ghinolfi D, Tincani G, Rreka E, et al. Dual aortic and portal perfusion at procurement prevents ischaemic-type biliary lesions in liver transplantation when using octogenarian donors: a retrospective cohort study. *Transpl Int*. 2018. *Epub ahead of print*; DOI: 10.1111/tri.13342.

APPENDIX

SDC Chapter 3

SDC 1: Search strategy – combined search of EMBASE, Medline and Cochrane databases.

Number	Search terms
1	(Kidney OR renal).mp
2	((Dynamic perfusion) OR (machine perfusion)).mp
3	1 AND 2

SDC 2: Baseline human study characteristics.

Author	Year	Study Period	Study Type	Kidney allocation	Centre(s)	Donor types	Study groups	Patients, *n*	Ischemic times (median)		Ages, mean (yr)	
									CIT (hr)	*WIT* (min)*	*Donor*	*Recipient*
Moers et al. [16]	2009	2005-2006	P (RCT)	Paired kidneys$^{\phi}$	Multicenter (Europe)	DBD/DCD/ECD	HMP	336	15	NR	51	53
							CS	336	15	NR	51	52
*Treckmann et al.^* [29]	2011	2005-2006				DBD/ECD	HMP	91	13	NR	66	65
							CS	91	13	NR	66	65
*Jochmans et al.^* [30]	2010	2005-2007				DCD	HMP	82	15	16	43	49
							CS	82	15.9	16	43	52
*Gallinat et al.^* [71]	2012	2005-2007				DBD	HMP	85	11	NR	**70**	66
							CS	85	10.5	NR	**70**	66
Gill et al. [72]	2014	2000-2011	R	NR	Multicenter (SRTR database, USA)	DBD/DCD/ECD	HMP	23799	12.1-18	NR	NR	NR
							CS	70910	12.1-18	NR	NR	NR
Opelz et al. [73]	2007	1990-2005	R	NR	Multicenter (CTS database, Europe, North America & Australia)	NR	HMP	2202	NR	NR	19-64	19-64
							CS	85944	NR	NR	19-64	19-64
Sung et al. [74]	2008	1999-2005	R	NR	Multicenter (SRTR/OPTN database, USA)	DBD/DCD/ECD	HMP	8886	NR	NR	NR	NR
							CS	55092	NR	NR	NR	NR
van der Vliet et al. [35]	2001	NR	P (RCT)	Paired kidneys	Multicenter (3 centers in Netherlands)	DCD	HMP	35	25**	28.4**	36.6**	NR
							CS	36	23**	28.4**	36.6**	NR

Kosieradzki et al. [75]	1999	NR	R	Paired kidneys	Single (Warsaw, Poland)	NR	HMP	38	27.5	NR	NR	37
							CS	38	33.7	NR	NR	36
Gage et al. [76]	1997	1993-1996	R	Matched pairs (unclear if same donor)	Single (Washington, USA)	DBD	HMP	25	22	NR	40	NR
							CS	25	19.9	NR	40	NR
Matsuno et al. [37]	1994	NR	P (RCT)	Paired kidneys	Single (Tokyo, Japan)	DCD	HMP	13	11.9	0***	50.1	38.5
							CS	13	6.1	0***	50.1	41
Merion et al. [77]	1990	1987	P	Paired kidneys	Multicenter (Michigan, USA)	NR	HMP	51	21	1	NR	39
							CS	51	21.8	1	NR	40
Jaffers et al. [78]	1989	1984-1986	R	N/A	Single (San Antonio, USA)	DBD	HMP	68	> 24	< 5	NR	NR
							CS	33	< 24	< 5	NR	NR
Mendez et al. [33]	1987	1985	P	NR	Single (LA, USA)	NR	HMP	26	< 36	NR	NR	NR
							CS	26	< 36	NR	NR	NR
Halloran et al. [34]	1987	1983-1984	P (RCT)	Donor random-ization (i.e. both kidneys from same donor either CS or MP)	Multicenter (Ontario, Canada)	NR	HMP	91	30.5	3.8	38	38.1
							CS	90	27.7	3.4	29.7	38.7
Heil et al. [79]	1987	NR	P (RCT)	Donor random-ization (i.e. both kidneys from same donor either CS or MP)	Single (Minnesota, USA)	NR	HMP	27	NR	NR	NR	NR
							CS	27	NR	NR	NR	NR

Alijani et al. [80]	1985	NR	P (RCT)	Paired kidneys	Multicenter (Washington, USA)	NR	HMP	29	32.5	NR	NR	NR
							CS	29	29.7	NR	NR	NR
Toledo-Pereyra [36]	1983	NR	P	Paired kidneys	Single center (Michigan, USA)	NR	HMP	10^^	19.5	NR	NR	NR
							CS	10^^	22.7	NR	NR	NR
Stratta et al. [61]	2007	2001-2006	R	NR	Single (North Carolina, USA)	DBD/ECD	HMP	114	24.5	NR	61.6	55.4
							CS	27	19	NR	60.5	60.1
Forde et al. [81]	2016	2003-2011	R	NR	Single (Dublin, Ireland)	DBD/ECD	HMP	93	15.6	NR	58.3	59.2
							CS	93	17.9	NR	55.7	59.9
Burgos Revilla et al. [82]	2015	2012-2014	P	All patients MP	Single (Madrid, Spain)	DBD/ECD	HMP (1 group)	90	18	NR	73.6	57.6
Dion et al. [83]	2015	2008-2010	R	Random allocation amongst retrospective cohort	Single (Ontario, Canada)	DBD/DCD/ECD	HMP	15	18.8	53.2	37.4	45.6
							CS	15	8.8	48.1	37.4	51.1
Guy et al. [84]	2015	2012-2013	P	NR	Single center (Birmingham, UK)	DBD/DCD/ECD	HMP	74	23.9	NR	52	51
							CS	101	13	NR	51	50
Wszola et al. [85]	2014	2005-2006	R	All ECD, unstable donor and elevated Cr donor kidneys used MP	Single (Warsaw, Poland)	DBD/ECD	HMP	26	28.5	30.2	47	46
							CS	36	24.2	33.3	50.4	44.5
Chueh et al. [86]	2014	2007-2012	R	Surgeon decision	Single (Cleveland, USA)	NR	HMP	28	23.4	NR	39.5	50.1
							CS	77	21.8	NR	32.9	48.7

Wszola et al. [87]	2013	2009-2011	P	Paired kidneys	Single (Warsaw, Poland)	?DBD/ECD	HMP1 (Waters-MOX-100)	25	28	NR	NR	46.4
							HMP2 (LifePort)	25	28	NR	NR	53.5
Sedigh et al. [88]	2013	2009-2012	R	Unpaired kidneys	Single (Uppsala, Sweden)	?DBD/ECD	HMP	52	12.8	NR	59	61
							CS	87	11.7	NR	59	58
Cannon et al. [89]	2013	2005-2011	R	NR	Multicenter (UNOS database, USA)	DBD/DCD/ECD	HMP	2290^^	23.4	NR	41.3	53.5
							CS	2290^^	17.4	NR	41.3	53.4
*Cannon et al.^^^* [90]	2015	2005-2011				DBD	HMP	1665^^	22.8	NR	36.8	51.6
							CS	1665^^	16.3	NR	36.8	51.7
Pieter Hoogland et al. [91]	2013	1997-2008	?P	NR	Single (Maastricht, Netherlands)	DCD	HMP (1 group)	335	27	26	45	51
Ciancio et al. [92]	2012	2000-2006	R	Paired kidneys (both to same device for differing time periods)	Single (Miami, USA)	DBD/DCD/ECD	HMP1 (shorter preservation)	66	27.6	NR	36.9	53.8
							HMP2 (longer preservation)	66	36	NR	36.9	49.3
Cantafio et al. [93]	2011	1993-2008	R	NR	Multicenter (UNOS/OPTN database, USA)	DCD	HMP	3781	12-24	11-20	41-50	51-60
							CS	2276	12-24	11-20	31-40	51-60
Lodhi et al. [94]	2012	2000-2010	R	NR	Multicenter (SRTR database, USA)	DBD/DCD	HMP	10861	12-24	NR	33.5	50.2
							CS	43275	12-24	NR	33.5	50.2

Patel et al. [95]	2012	2006-2009	R	N/A[α]	?Single (Buffalo, USA)	?DBD/ECD	HMP1[α]	82	10.8	NR	50.3	53
							HMP2[α]	83	10.8	NR	50.3	53
Watson et al. [15]	2010	2006-2007	P (RCT)	Paired kidneys	Multicenter (UK)	DCD	HMP	45	13.9	15	45.6	50.3
							CS	45	14.3	15	45.6	48.6
Matsuno et al. [28]	2010	NR	?R	NR	Single (Tokyo, Japan)	DCD	HMP (1 group)	17	23.3	7.9	44.5	41.6
Ciancio et al. [96]	2010	2000-2006	R	All had MP	Single (Miami, USA)	DBD/DCD/ECD	HMP (1 group)	339	33.2	NR	35	50.2
Kwiatkowski et al. [97]	2009	NR	P	Paired kidneys	Single (Warsaw, Poland)	NR	HMP	37	34.5	NR	36	37
							CS	37	27.5	NR	36	40
Reznik et al. [38]	2008	2005-2007	P	Paired kidneys	NR (Russia)	DCD	HMP	21	NR	42.7	49.3	45.2
							CS	17	NR	42.7	49.3	37.9
Moustafellos et al. [98]	2007	2004-2006	R	NR	Single (Oxford, UK)	DCD	HMP	18	15.2	NR	36.3	44.1
							CS	18	16.7	NR	54.5	50.4
Matsuno et al. [27]	2006	NR	NR	NR	Single (Tokyo, Japan)	DBD/DCD	HMP (1 group)	88	15.1	13.6	46.2	38.7
Balupuri et al. [99]	2001	1998-2001	NR	N/A	Single (Newcastle, UK)	DCD	HMP (1 group)	28	NR	NR	NR	NR
Kumar et al. [100]	1991	1983-1990	R	NR	?Single (Philadelphia, USA)	DBD	HMP	11	54	NR	NR	NR
							CS	56	57.8	NR	NR	NR
Opelz & Terasaki [11]	1982	1970-1980	R	NR	Multicenter (North America)	NR	HMP	5066	12-24	0-10	NR	NR
							CS	2326	12-24	0-10	NR	NR
Barry et al. [101]	1980	1974-1978	R	Change in policy from MP to CS	Single (Hawaai, USA)	DBD	HMP	37	23	< 10	NR	NR
							CS	40	23	< 10	NR	NR
Mozes et al. [31]	1985	1982-1984	P (RCT)	Paired kidneys	Multicenter (Chicago, USA)	DBD	HMP	93	35.1	NR	26.5	NR
							CS	94	32.8	NR	26.5	NR

Rosenthal et al. [13]	1984	1980-1983	R	NR	Single (Pittsburgh, USA)	DBD	HMP	86	24	< 1	NR	NR
							CS	113	23.8	< 1	NR	NR
Abboud et al. [32]	2011	2007-2009	P	Paired kidneys	Single (Paris, France)	DBD/ECD	HMP	22	20.3	NR	57.8	56.5
							CS	22	22.2	NR	57.8	53
Plata-Munoz et al. [102]	2008	2002-2005	R	NR	Single (Oxford, UK)	DCD	HMP	30	18.6	18	41.6	47.2
							CS	30	17.9	18.5	40.3	54.1
Matsuoka et al. [103]	2006	2000-2003	R	NR	Multicenter (UNOS database, USA)	DBD/DCD/ECD	HMP	912	18.9	NR	61.1	56
							CS	3706	20.1	NR	59.8	54.5
Buchanan et al. [104]	2008	1995-2004	R	NR	Multicenter (OPTN database, USA)	ECD	HMP	1114	19.9	38.8	NR	45-59
							CS	4726	20.9	32.8	NR	45-59
Polyak et al. [62]	2000	1993-1999	R	Protocol change in 1993 (MP unless specific exclusions)	Multicenter (New York, USA; all preserved in 1 lab)	DBD/ECD	HMP	402	26.6	NR	46.5	NR
							CS	248	NR	NR	42.5	NR
Kootstra et al. [105]	1997	1994-1997	R	NR	Single (Maastricht, Netherlands)	DCD	HMP (1 group)	100	NR	58.5	NR	43.1
Sy et al. [106]	1980	1972-1975	R	NR	Single (Michigan, USA)	NR	HMP (1 group)	50	NR	NR	NR	NR
Kwiatkowski et al. [107]	2001	1994-1999	R	NR	Single (Warsaw, Poland)	DBD	HMP (1 group)	234	34	NR	36	37.3
Nicholson & Hosgood [26]	2013	2008-2012	P	WP introduced 2010	Single (Leicester, UK)	ECD	**WP**	18	12.3[β]	NR	61	58
							CS	47	12.9[β]	NR	62	56

Veller et al. [108]	1994	1989-1992	P (RCT)	Paired kidneys	Single (Johannesburg, South Africa)	DBD	HMP	18	19	NR	NR	34
							CS	18	18	NR	NR	34
		1989-1990	R	Non-paired	Single (Johannesburg, South Africa)	NR	HMP	57^^	20	NR	NR	36
							CS	62^^	19	NR	NR	37
Elec et al. [109]	2014	2012	R	NR	Single (Cluj, Romania)	DBD	HMP (1 group)	82	NR	NR	NR	NR
Guarrera et al. [63]	2004	1998-2000	P	MP preferred policy	Multicenter (New York, USA; all preserved in 1 lab)	DBD	HMP1[γ]	119	26.1	NR	41.1	47
							HMP2[γ]	201	27.2	NR	45	46.1
							HMP3[γ]	212	25.4	NR	39.6	45.8
Schold et al. [110]	2005	1994-2003	R	Paired kidneys^^	Multicenter (SRTR database, USA)	NR	HMP	907^^	20	NR	NR	NR
							CS	907^^	21	NR	NR	NR
Barber et al. [111]	1988	1986-1987	R	?MP preferred policy from 1987 onwards	Single (Alabama, USA)	NR	HMP	138	27.7	NR	NR	NR
							CS	83	16.3	NR	NR	NR
Sellers et al. [112]	2000	1990-1995	R	MP preferred policy	Single (Alabama, USA)	NR	HMP	568	24.1	NR	34.1	NR
							CS	268	24.8	NR	27.9	NR
Guarrera_1 et al. [64]	2004	2000-2001	P	Paired kidneys	Multicenter (New York, USA; all preserved in 1 lab)	DBD	HMP1[ζ]	81	28	NR	62.8	41.9
							HMP2[ζ]	81	27.1	NR	64.2	39.4

Median	-	-	-	-	-	-	-	**CS**: 34.5 **MP**: 82	**CS**: 19.5 **MP**: 23.4	**CS**: 28.4 **MP**: 26	**CS**: 45.6 **MP**: 45	**CS**: 50.8 **MP**: 49.2
Range	1980-2016	1970-2014	-	-	-	-	-	-	**CS**: 6.1-57.8 **MP**: 10.8-54	**CS**: 0-48.1 **MP**: 0-58.5	**CS**: 26.5-70 **MP**: 26.5-73.6	**CS**: 34-66 **MP**: 34-66
Totals (%)	*63 studies (100)*	-	22 P[€] studies (34.9)	17 paired kidney analyses[€] (27.0)	-	**DBD**: 31 studies (49.2) **DCD**: 23 studies (36.5) **ECD**: 21 studies (33.3)	**CS**: 48 studies (76.2) **HMP**: 62 studies (98.4) **WP**: 1 study (1.6)	**CS**: 275723 **MP**: 67215[ϖ]	-	-	-	-

Cr – creatinine; CIT – cold ischemic time; CS – cold (static) storage; CTS – Collaborative Transplant Study; DBD – donation after brain death; DCD – donation after circulatory death; ECD – expanded criteria donor; HMP – hypothermic machine perfusion; MPS – machine perfusion solution; NR – not recorded; OPTN – Organ Procurement & Transplantation Network; P – prospective; R – retrospective; RCT – randomized control trial; SRTR – Scientific Registry of Transplant Recipients; UNOS – United Network for Organ Sharing; WIT – warm ischemic time; WP – warm (machine) perfusion

* Initial WIT
** Mean
*** *In situ* cooling commenced immediately after circulatory arrest, hence time stated as 0 means (mean given here)
^ Subgroups of Moers *et al*. [16] study +/- some recruitment of further patients (same methods) as per power calculations
^^ Only paired kidneys included here
^^^ Subgroup of Cannon *et al.* [89] study
[α] Based on increase or decrease in terminal flows after perfusion
[β] Total ischemic time
[γ] Three groups based on perfusate used for MP (Modified MPS, Belzer MPS, or Belzer II MPS)
[ζ] Two groups based on perfusate used for MP (Vasosol or Belzer MPS)
[ϕ] Denotes each study group patient received a kidney from the same donor, e.g. 1 to HMP patient, 1 to CS patient
[ϖ] Includes 18 patients with WP
[€] Excluding sub-group studies

SDC 3: Baseline animal study characteristics.

Author, Year	Species/sex* (weight)	Study groups (duration)	Animals, *n*	Model (Experimental Period)	Ischemic times	
					CIT[η] *(hr)*	*WIT (min)*[φ]
Hosgood et al., 2011_1 [113]	Large white pigs (60-70 kg)	HMP (18 hr)	NR	DCD^ (10 min) *Ex vivo* perfusion** (3 hr)	18	10
		CS (18 hr)	NR		18	10
		CS (4 hr) → HMP (14 hr)	NR		18	10
Minor et al., 2015 [60]	German landrace pigs (25-30 kg)	HMP [Custodiol-N] (20 hr)	NR	DBD^ Auto-transplant *** (7 d)	20.4	2-4
		HMP [UW[α]] (20 hr)	NR		20.5	2-4
Gallinat et al., 2014 [114]	German landrace pigs (25-30 kg)	CS (18 hr)	6	DBD *Ex vivo* perfusion (1.5 hr)	18	Min^^
		CS (18 hr) → HMP (1 hr)	6		19	Min
		CS (18 hr) → HMP (4 hr)	6		23	Min
Hoyer et al., 2014 [68]	Female landrace pigs (~30 kg)	HMP (21 hr)	5	DCD (30 min) *Ex vivo* perfusion (2 hr)	21	30
		HMP (21 hr) + 21% O_2	5		21	30
		HMP (21 hr) + 100% O_2	5		21	30
Thuillier et al., 2013 [40]	Large white pigs (30-35 kg)	HMP (22 hr)	4	DCD (60 min) Auto-transplant (3 m)	22	60
		HMP (22 hr) + 100% O_2	4		22	60
Gallinat et al., 2012_2 [39]	German landrace pigs (25-30 kg)	HMP (21 hr)	5	DBD Auto-transplant (7 d)	21	Min
		HMP (21 hr) + O_2!	5		21	Min
Codas et al., 2012 [115]	Large white pigs (~40 kg)	CS [UW] (22 hr)	7	DCD (60 min) Auto-transplant (1 m)	22	60
		HMP [UW] (22 hr)	7		22	60
		CS [IGL-1] (22 hr)	7		22	60
		HMP [IGL-1] (22 hr)	7		22	60
Gallinat et al., 2012_1 [116]	German landrace pigs (25-30 kg)	CS (21 hr)	5	DBD Auto-transplant (7 d)	21	Min
		HMP (21 hr)	5		21	Min
		CS (19 hr) → HMP (2 hr)	5		21	Min
Schreinemachers et al., 2010 [117]	Female landrace pigs (~30 kg)	CS [HTK] (20 hr)	6	DCD (30 min) Auto-transplant (7 d)	20.2	30
		CS [PS] (20 hr)	6		20.1	30
		HMP (20 hr)	6		20.2	30
Hosgood et al., 2010 [118]	Large white pigs (60-70 kg)	CS [HOC] (18 hr)	6	DCD (10 min) *Ex vivo* perfusion (3 hr)	18	10
		CS [HTK] (18 hr)	6		18	10
		CS [UW] (18 hr)	6		18	10
		HMP (18 hr)	6		18	10
Koetting et al., 2010 [67]	Female landrace pigs (25-30 kg)	CS (20 hr)	6	DBD *Ex vivo* perfusion (1.5 hr)	20	Min
		CS (18 hr) → HMP + 21% O_2 (2 hr)	6		20	Min
		CS (18 hr) → HMP + 100% O_2 (2 hr)	6		20	Min
Le Manna et al., 2009 [119]	Large white (female) pigs (weight NR)	CS (15 hr)	6[β]	DCD (10/15/30 min) Auto-transplant (4 d)	15	10/15/30
		HMP (15 hr)	6[β]		15	10/15/30
Treckmann et al., 2009 [70]	German landrace pigs (weight NR)	CS (4 hr)	7	DCD (60 min) Auto-transplant (7 d)	4	60
		HMP (4 hr)	5		4	60
		ROP (4 hr)	6		4	60

Maathuis et al., 2007 [45]	German landrace pigs (20-30 kg)	CS (20 hr)	5	DBD Auto-transplant (7 d)	20.4	2-6
		HMP at 30 mmHg (20 hr)	5		20.4	2-6
		HMP at 60 mmHg (20 hr)	5		20.4	2-6
Manekeller et al., 2005 [120]	German landrace pigs (20-25 kg)	HMP [UW] (18 hr)	NR	DCD (40 min) Allotransplant[γ] (6 d)	18	40
		HMP [HTK] (18 hr)	NR		18	40
Lindell et al., 2005 [121]	Female beagle dogs (5-10 kg)	CS (24 *or* 72 hr)	NR	DCD (60/75 min) Auto-transplant (10 d)	24/72	60/75
		HMP [UW1] (24 *or* 72 hr)	NR		24/72	60/75
		HMP [UW2] (24 *or* 72 hr)	NR		24/72	60/75
Minor et al., 2005 [43]	German landrace pigs (20-25 kg)	CS (18 hr)	5	DCD (40 min) Allotransplant (7 d)	18	40
		HMP [HTK] (18 hr)	5		18	40
		HMP [UW] (18 hr)	5		18	40
Nicholson et al., 2004 [122]	Large white (female) pigs (35-70 kg)	CS (24 hr)	10[δ]	DBD & DCD (30 min) Auto-transplant (14 d)	23.3	Min/30
		HMP (24 hr)	10[δ]		22.9	Min/30
Hansen et al., 1997 [123]	New Zealand white rabbits (weight NR)	CS (24 hr)	NR	DBD & DCD (60/90 min) *Ex vivo* perfusion (0.75 hr)	24	Min/60/90
		HMP (24 hr)	NR		24	Min/60/90
Booster et al., 1993 [124]	Female beagle dogs (10-12 kg)	CS (24 hr)	6	DCD (30 min) Auto-transplant (14 d)	24	30
		CS (2 hr) → HMP (22 hr)	6		24	30
McAnulty et al., 1989 [125]	Female mongrel dogs (~20 kg)	HMP (120 hr)	4	DBD Auto-transplant (10 d)	120	Min
		HMP + 0.5 mM Ca (120 hr)	8		120	Min
		HMP + 1.5 mM Ca (120 hr)	12		120	Min
		HMP + 0.5 mM Ca & Ch (120 hr)	8		120	Min
		HMP + 1.5 mM Ca & Ch (120 hr)	6		120	Min
Gallinat et al., 2013 [47]	German (female) landrace pigs (25-30 kg)	CS (19.5 hr)	6	(A) DBD *Ex vivo* perfusion (time NR)	19.5	Min
		CS (18 hr) → HMP, non-pulsatile (1.5 hr)	6		19.5	Min
		CS (18 hr) → HMP, pulsatile (1.5 hr)	6		19.5	Min
		CS (19.5 hr)	5	(B) DBD Auto-transplant (7 d)	19.5	Min
		CS (18 hr) → HMP, pulsatile (1.5 hr)	5		19.5	Min
Brasile et al., 2001 [56]	Foxhounds (20-30 kg)	CS (18 hr)	4	DCD (30 min) Auto-transplant (14 d)	18	30
		WP (18 hr)	4		18	30
		CS (18 hr) → WP (3 hr)	4		21	30
		CS (18 hr) → WP (18 hr)	4		36	30
		WP (18 hr) → CS (12 hr)	4		30	30
		WP (18 hr) → CS (24 hr)	4		42	30
Brasile et al., 2002 [126]	Canine (type NR)	WP (18 hr)	10[ε]	DCD (45/120 min) Auto-transplant (time NR)	18	45/120
		Re-implantation without WP[ζ]	4[ε]		WIT	45/120
Brasile et al., 2002_1 [127]	Foxhounds (20-30 kg)	WP (18 hr)	5	DCD (120 min) Auto-transplant (10 d)	18	120
		HMP (18 hr)	2		18	120
		Re-implantation without WP[ζ]	2		WIT	120

Brasile et al., 2005 [65]	Canine (type NR)	WP (24 hr)	2	DCD (120 min) Auto-transplant (time NR)	24	120
		WP + FGF (24 hr)	4		24	120
		HMP (24 hr)	2		24	120
		Re-implantation without MP[ζ]	2		WIT	120
Bagul et al., 2008 [128]	Large white pigs (60-70 kg)	CS (2 hr)	6	DCD (10 min) *Ex vivo* perfusion (3 hr)	2	10
		CS (18 hr)	6		18	10
		HMP (18 hr)	6		18	10
		CS (16 hr) → WP (2 hr)	6		18	10
Stubenitsky et al., 2000 [129]	Foxhounds (35-40 kg)	CS (24 hr)	11	DCD (30 min) Auto-transplant (14 d)	24	30
		CS (24 hr) → WP (3 hr)	11		27	30
van der Wijk et al., 1980 [130]	Mongrel dogs (~22 kg)	HMP (96 hr)	6	DBD Auto-transplant (14 d)	96	Min
		HMP (48 hr) → WP (4 hr) → HMP (44 hr)	6		96	Min
		HMP (144 hr)	6		144	Min
		HMP (72 hr) → WP (4 hr) → HMP (68 hr)	6		144	Min
Rijkmans et al., 1984 [131]	Mongrel dogs (21-24 kg)	HMP (144 hr)	8	DBD Auto-transplant (14 d)	144	Min
		HMP (72 hr) → WP (3 hr) → HMP (69 hr)	11		144	Min
Hosgood et al., 2013 [132]	Large white pigs (60-70 kg)	CS (24 hr)	6	DCD (10 min) *Ex vivo* perfusion (3 hr)	24	10
		CS (23 hr) → WP (1 hr)	6		24	10
Hosgood et al., 2011_2 [133]	Male landrace pigs (37-44 kg)	HMP (22 hr)	6	DCD (30 min) Auto-transplant (10 d)	22	31.5
		HMP (20 hr) → WP (2 hr)	6		21.6	33
Patel et al., 2014 [134]	Large white pigs (60-70 kg)	CS (24 hr)	6	DCD (10 min) *Ex vivo* perfusion (3 hr)	24	10
		CS (23 hr) → WP at 55 mmHg (1 hr)	6		24	10
		CS (23 hr) → WP at 75 mmHg (1 hr)	6		24	10
Hoyer et al., 2014 [59]	Female landrace pigs (~30 kg)	CS (7 hr)	5	DCD (30 min) *Ex vivo* perfusion (2 hr)	7	30
		HMP (7 hr)	5		7	30
		WP (7 hr)	5		7	30
Schopp et al., 2015 [58]	German landrace pigs (25-30 kg)	HMP (18 hr)	6	DBD *Ex vivo* perfusion (1.5 hr)	18	Min
		CS (18 hr) → WP (3 hr)	6		21	Min
		CS (18 hr) → HMP + O_2 (3 hr)	6		21	Min
Metcalfe et al., 2002 [135]	Large white pigs (80-100 kg)	CS (2 hr) → HMP (16 hr)	6	DBD *Ex vivo* perfusion (2 hr)	18	8
		CS (2 hr) → WP (16 hr)	6		18.1	8
Lindell et al., 2013 [136]	Adult beagle dogs (weight NR)	HMP, RM3 (24 hr)	8	DCD (45 min) Auto-transplant (7 d)	24	45
		HMP, LifePort (24 hr)	8		24	45
		HMP, LifePort/non-pulsatile (24 hr)	4		24	45
Yland et al., 1996 [44]	Adult mongrel dogs (18-23 kg)	CS (72 hr)	6	DBD Auto-transplant (15 d)	72	Min
		HMP at high flow (72 hr)	6		72	Min
		HMP at low flow (72 hr)	6		72	Min

Totals (%)	Porcine: 25 studies (65.8)	CS[θ]: 23 studies (60.5)	CS[θ]: 161 (27.6)	DBD: 15 studies (39.5)	CS[†]: 20.1 (2-72)	CS[†]: 25 (0-120)
	Canine: 12 studies (31.6)	HMP[ι]: 33 studies (86.8)	HMP[ι]: 286 (49.1)	DCD: 23 studies (60.5)	HMP[†]: 21 (4-144)	HMP[†]: 30 (0-120)
	Rabbit: 1 study (2.6)	WP[ι]: 14 studies (36.8)	WP[ι]: 122 (20.9)	Auto-transplant: 23 studies (60.5)	WP[†]: 24[η] (7-144)	WP[†]: 30 (0-120)
	Other: N/A	Other: 1 study (2.6)	Other: 14 (2.4)	Allotransplant: 2 studies (5.3)		
	Overall: 38 studies		Overall: 583 (100)	*Ex vivo* perfusion: 13 studies (34.2)		

Ca – calcium; Ch – chlorpromazine; CS – cold (static) storage; CIT – cold ischemic time; d – day(s); DBD – donation after brain death; DCD – donation after circulatory death; F/U – follow-up; FGF – fibroblast growth factor; HMP – hypothermic machine perfusion; HOC – hyperosmolar citrate; min – minimal; HTK – histidine-tryptophan-ketoglutarate; IGL – Institut Georges Lopez; NR – not recorded; O_2 – oxygen; PS – polysol; ROP – retrograde oxygen persufflation; WIT – (initial) warm ischemic time; WP – warm (machine) perfusion; UW – University of Wisconsin

* If specified
** *Ex vivo* perfusion indicates preservation post-nephrectomy (either by cold storage or machine preservation) followed by perfusion in an external circuit, using either blood or an alternative solution
*** Auto-transplant indicates preservation post-nephrectomy (either by cold storage or machine preservation) followed by re-implantation in the animal, along with contralateral nephrectomy
ϕ Initial WIT
^ A DCD model involved the artificial creation of a warm ischemic period prior to nephrectomy through the ligation or clamping or renal vessels for a defined period of time (indicated in parentheses); this did not occur in the DBD model experiments (WIT here is simply the period from sacrifice (if applicable) to nephrectomy)
^^ WIT for DBD recorded as "min" when NR (minimised simply by experimental design; also see note ^)
! Percentage NR
α See Table 1 for further details regarding preservation solutions
β Each group was split into a further 3 groups by WIT
γ Allotransplant denotes transplantation into another animal of the same species, necessitating immunosuppressive therapy
δ Each group was split into a further 2 groups ('0' and 30 min WIT)
ε Each group was split into a further 2 groups by WIT
ζ In this control group, kidneys were reimplanted post-warm ischemia without any further preservation
η In studies with WP, value given is the total ischemic time
θ Studies in which there was a comparator group using CS only
ι HMP value includes any studies where HMP was used with/without CS, whilst WP value includes any studies where WP was used with/without CS and/or HMP
† Expressed as median (range) of all studies

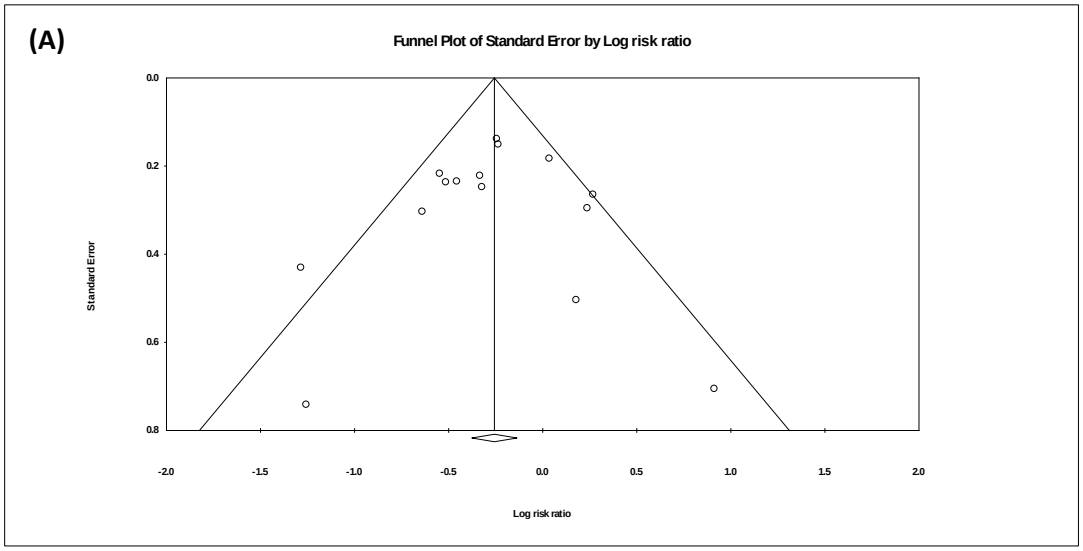
SDC 4: Summary of *human* meta-analyses. Data expressed as RR, ± 95% CI (I^2; *n* studies) for DGF and PNF, and HR for graft survival.

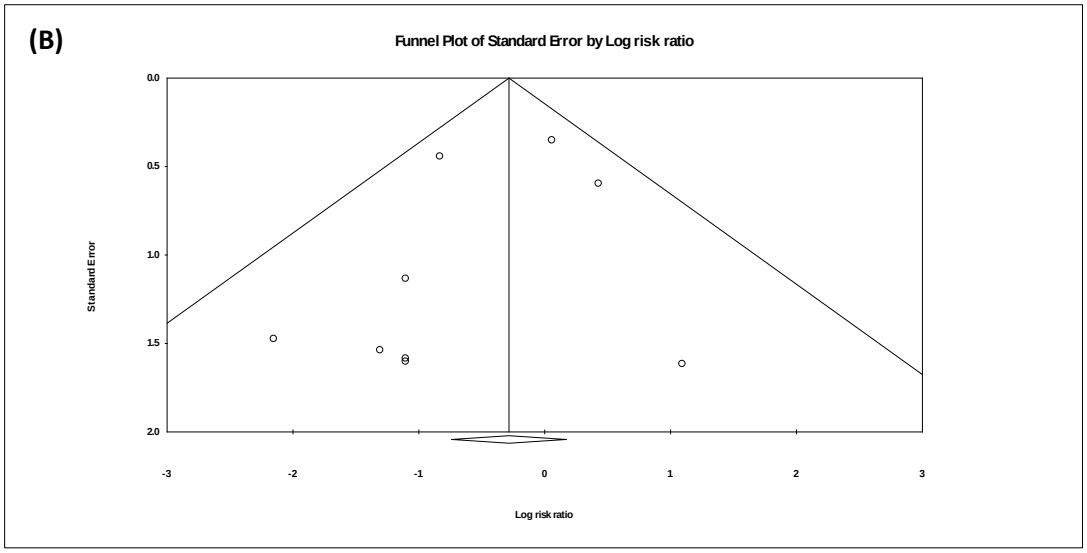
Parameter	**HMP vs. CS**	**HMP vs. CS (DCD)**	**HMP vs. CS (ECD)**
DGF	0.77, 0.69-0.87† (45.2; 15)	0.78, 0.66-0.91$^{\varepsilon}$ (0; 6)	0.67, 0.42-1.08$^{\zeta *}$ (32.0; 2)
PNF	0.75, 0.47-1.19$^{\zeta}$ (0; 9)	1.04, 0.44-2.49$^{\zeta}$ (0; 5)	0.28, 0.09-0.89$^{\Phi}$ (0; 2)
Graft failure (1-year)	1.25, 0.20-7.62$^{\zeta}$ (88.9; 2)	NA**	NA**

CI – confidence interval; CS – cold (static) storage; DBD – donation after brain death; DCD – donation after circulatory death; DGF – delayed graft function; ECD – expanded criteria donor; HMP – hypothermic machine perfusion; HR – hazard ratio; PNF – primary non-function; MP – machine perfusion; NA – not applicable; OR – odds ratio; WP – warm (normothermic) perfusion

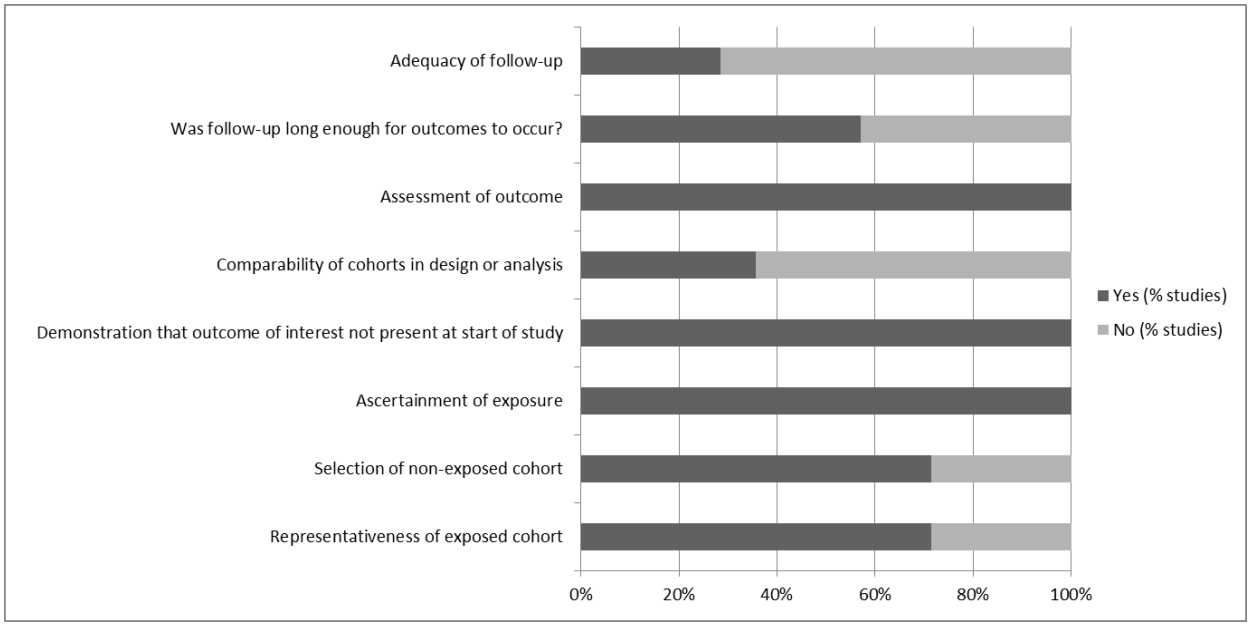
* p = 0.097; only two studies available for this comparison
** Insufficient data to perform meta-analysis
$^{\zeta}$ p not significant
$^{\Phi}$ p < 0.05
$^{\varepsilon}$ p < 0.01
† p < 0.001

SDC 5: Funnel plots for the assessment of publication bias in prospective *human* studies comparing HMP to CS in terms of (A) DGF and (B) PNF.





SDC 6: Quality/risk of bias assessment for cohort studies included in the *human* meta-analyses using the Newcastle-Ottawa Quality Assessment Scale.



SDC 7: Quality/risk of bias assessment for randomized control trial data included in *human* meta-analyses using the Cochrane Collaboration's tool for assessing risk of bias. *+* Low risk of bias, – High risk of bias, *?* Unclear/uncertain risk of bias.

Study	*Adequate sequence generation*	*Allocation concealment*	*Blinding*	*Incomplete outcome data addressed*	*Free of selective reporting*	*Free of other bias*
Alijani et al. (1985) [93]	?	–	–	?	–	?
Mozes et al. (1985) [115]	?	–	–	+	+	?
Halloran et al. (1987) [91]	?	?	?	+	+	?
Heil et al. (1987) [92]	+	+	–	?	+	?
Matsuno et al. (1994) [45]	?	–	–	+	+	?
Veller et al. (1994) [121]	?	?	?	+	+	?
van der Vliet et al. (2001) [85]	?	–	–	+	?	?
Moers et al. (2009) [21]	+	+	?	+	+	?
*Jochmans et al. (2010)** [80]	+	+	–	+	+	?
Watson et al. (2010) [20]	+	+	?	+	+	?

* This study is an extension of the Moers *et al*.[21] paper, with the recruitment of additional patients

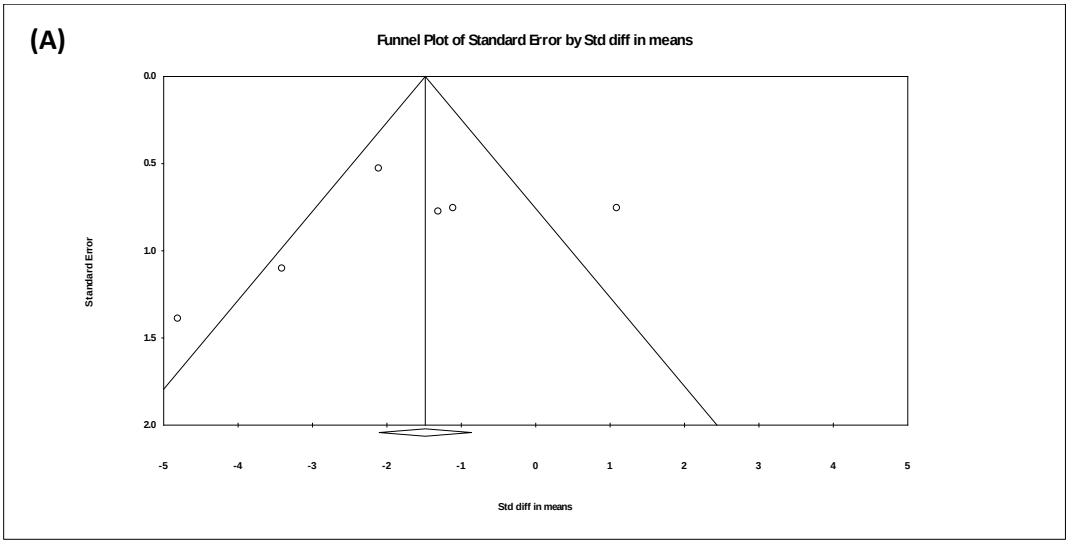
SDC 8: Summary of *animal* meta-analyses. Data expressed as SMD, ± 95% CI (I^2; *n* experimental groups).

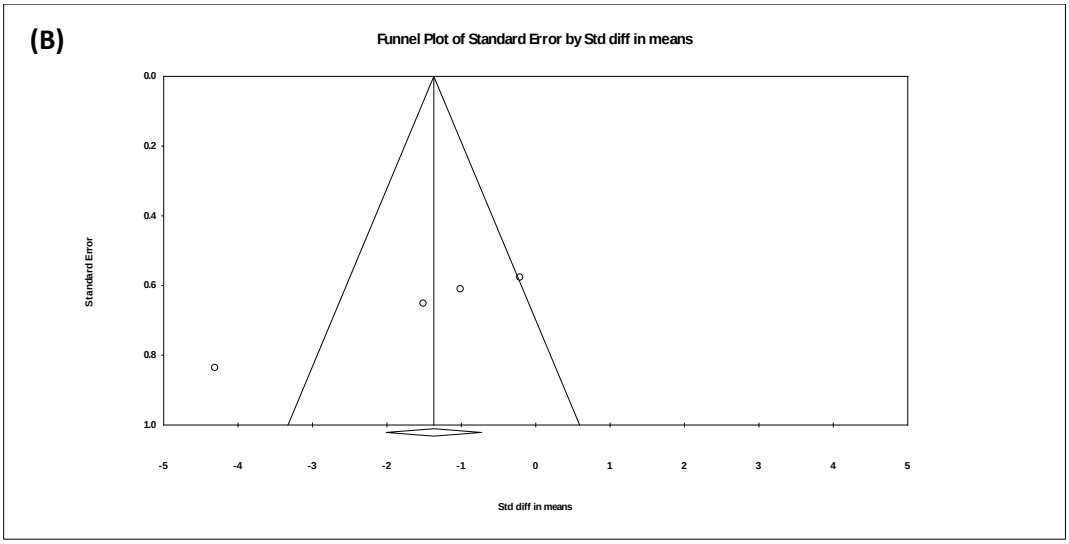
Parameter	**WP vs. CS**	**WP vs. HMP**	**HMP-Ox vs. HMP no-Ox**
Creatinine, peak	-1.72 (-3.09 to -0.34)$^{\Phi}$ (77.2; 6)	-1.66 (-3.19 to -0.14)$^{\Phi}$ (82.0; 4)	-0.39 (-1.85 to 1.08)$^{\zeta}$ (55.8; 2)
CrCl, peak	2.08 (-1.83 to 6.00)$^{\zeta}$ (89.9; 2)	0.83 (-0.50 to 2.15)$^{\zeta}$ (69.2; 3)	1.18 (-0.39 to 2.76)$^{\zeta}$ (79.4; 4)
FeNa, peak	NA*	NA*	-1.54 (-2.54 to -0.54)$^{\varepsilon}$ (0; 2)
Survival	NA*	1.29 (0.52 to 2.07)$^{\varepsilon}$ (46.9; 5)	NA*

CI – confidence interval; CS – cold (static) storage; DCD – donation after circulatory death; HMP – hypothermic machine perfusion; HMP-Ox – oxygenated HMP; HMP no-Ox – non-oxygenated HMP; NA – not applicable; SMD – standardized mean difference; WP – warm (normothermic) perfusion

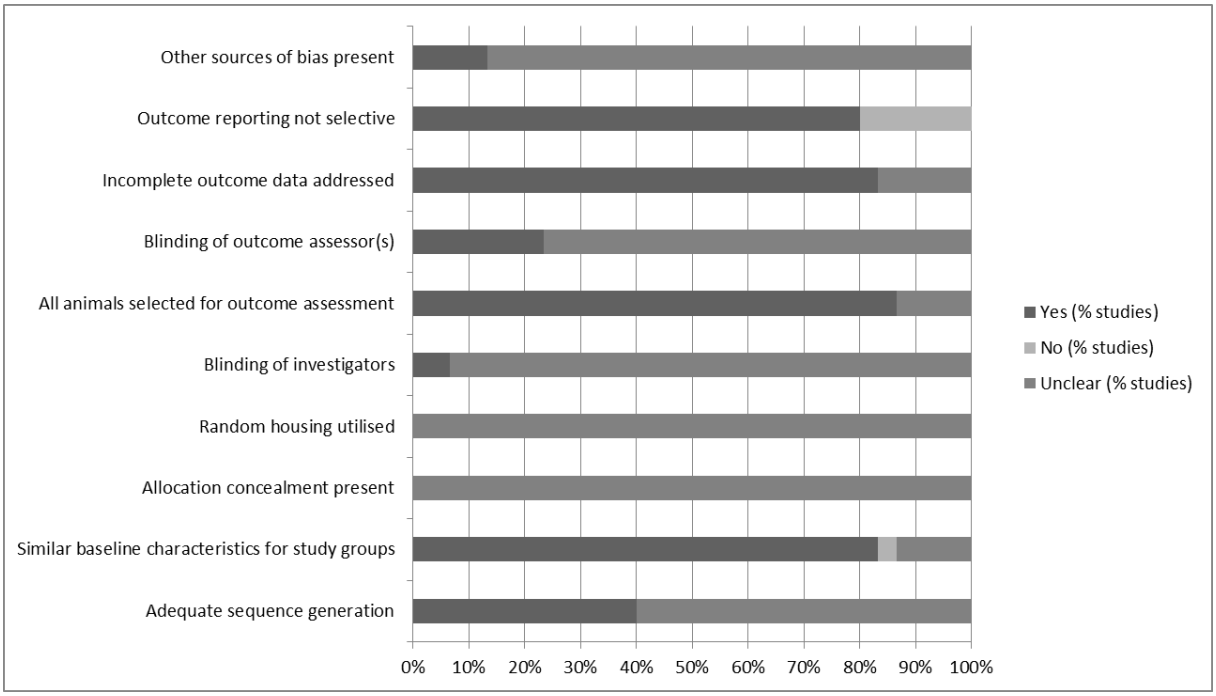
* Insufficient data for meta-analysis
$^{\zeta}$ p not significant
$^{\Phi}$ $p < 0.05$
$^{\varepsilon}$ $p < 0.01$
† $p < 0.001$

SDC 9: Funnel plots for the assessment of publication bias – *animal* studies. SMD for peak creatinine in (A) WP compared to CS studies, and (B) WP compared to HMP studies.



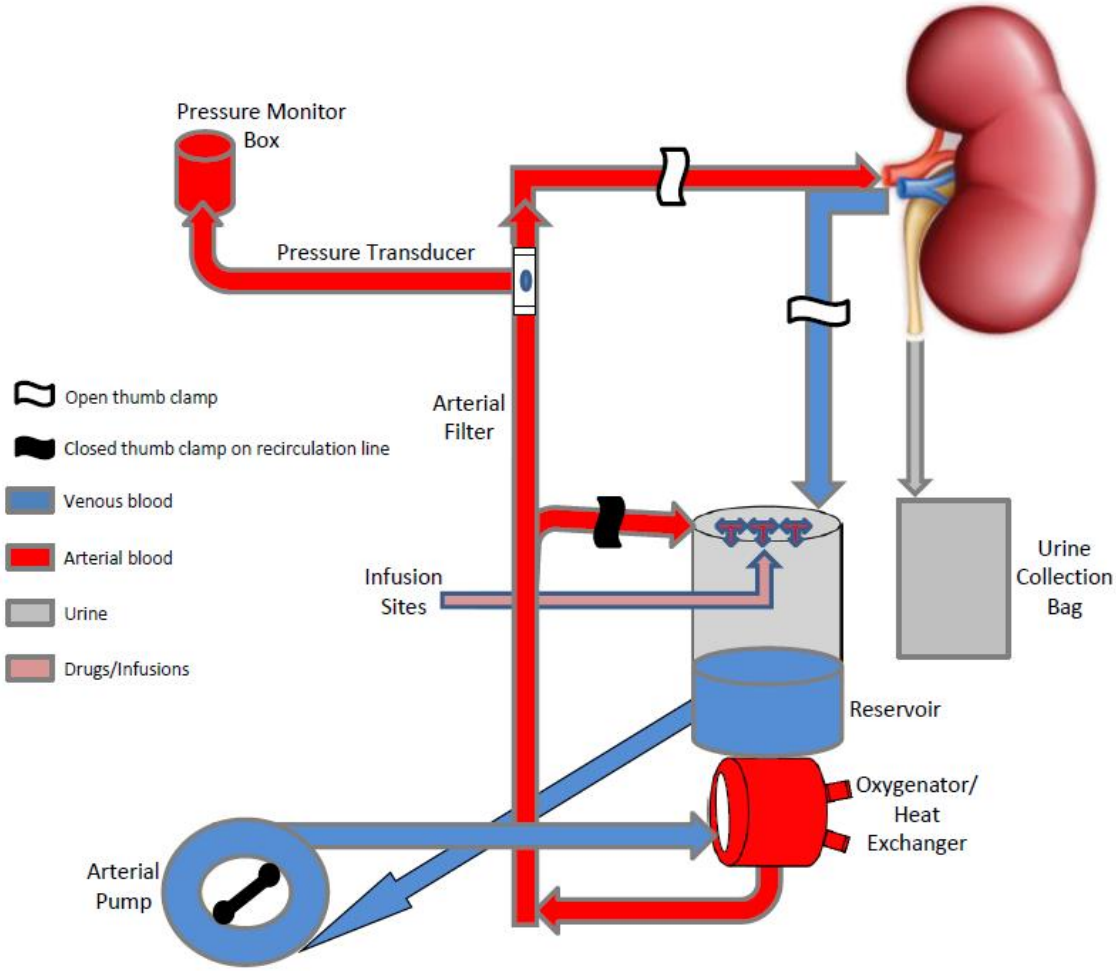


SDC 10: Quality/risk of bias assessment for *animal* experiments included in quantitative analyses using SYRCLE's risk of bias tool for animal studies.



SDC Chapter 4

SDC 1: NMP circuit components. A packed red blood cell-based perfusion fluid is added to the reservoir, and pumped into the renal artery after passing through an oxygenator and heat exchanger. The fluid recirculates through the circuit via a cannula inserted in the renal artery.



SDC 2: Device components and baseline perfusion solution.

Device Components

Renal artery and vein cannulas were attached to ¼ inch polyvinyl chloride (PVC), synthetic X-coating tubing (Lovell Surgical Supplies, Carrum Downs, Australia) using luer lock connections – the arterial and venous limbs of the "kidney line," respectively (Fig. S1). Venous effluent drained into the fluid/venous reservoir with integrated oxygenator/heat exchanger and arterial filter (Terumo Capiox FX05, Macquarie Park, Australia), with subsequent outflow via ¼ inch PVC tubing to the roller pump (Stockert SIII, LivaNova Australia, Dandenong South, Australia). After perfusate passage via the roller pump, it was circulated through the integrated oxygenator and heat exchanger, before entry into the renal arterial line. Circulation fluid temperature was controlled using a heater unit (Hemotherm, Cincinnati Sub-Zero Products Inc, Cincinnati, USA).

Baseline perfusion solution

The initial perfusion solution utilized was adapted from Nicholson and Hosgood.[1, 2] One unit of packed red blood cells (PRBCs; 200-250 ml) were employed for each kidney perfusion, and were isolated from autologous whole blood after centrifugation and washing in Hartmann's solution. The PRBCs were resuspended in 500 ml of Hartmann's solution; mannitol (25 ml, 10%) and heparin (2000 units) were added to the perfusion solution. Two separate infusion pumps were set up to infuse (1) 5% dextrose (5 ml/hr), and (2) M199 nutrient solution with ultraglutamine (Sartorius AG, Goettingen, Germany), to which was added multivitamins (Cernevit; Baxter Healthcare Pty Ltd, Old Toongabbie, Australia) and 12.5 units of insulin (Actrapid, Novo Nordisk Pharmaceuticals Ltd, Baulkham Hills, Australia) (20 ml/hr). Further Hartmann's solution was added to the reservoir to directly replace urine output (UO).

References

1. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct* 2017;**3**(3): e140.

2. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant* 2013;**13**(5): 1246-52.

SDC 3: Retrieval details for stage 1 and 2.

Porcine kidney retrieval (Stage 1)
General anaesthesia was induced with 16mg/kg intravenous thiopentone (Pentothal, Abbott Australasia Pty Ltd, Sydney, Australia) and maintained with 1–2% isoflurane (Zeneca Ltd, Macclesfield, UK) in oxygen after intubation.
A midline laparotomy was performed, after which the right and left kidney, aorta, and inferior vena cava (IVC) were dissected. The pig was exsanguinated using an infra-renal aortic catheter. Whole blood was collected for subsequent PRBC isolation into tubes containing Anticoagulant-Citrate-Dextrose solution A (ACD-A) (Aurora Bioscience, Bella Vista, Australia) in a 0.15:1 ratio (one part whole blood, 0.15 parts ACD-A).[1] Upon confirmation of cardiac arrest, the supra-coeliac aorta was cross-clamped and abdominal perfusion was commenced via the aortic catheter. A total of 3 L of University of Wisconsin (UW; Bridge to Life Ltd, Columbia, USA) solution was used, with 25,000 units of heparin added to the first (1 L) bag. Residual blood and perfusion solution was vented via the IVC. Both kidneys were then removed in the cold phase. UW solution (250 ml) was infused via the renal arteries on the back-table; the kidneys were subsequently stored in UW solution, overlying 0.9% sodium chloride ice slush, whilst the NMP circuit was prepared.

Porcine kidney retrieval, DCD model (Stage 2)
Pigs were anaesthetized as per Stage 1, and whole blood collected in the same manner. In addition, the renal artery and renal vein were dissected bilaterally and then clamped with vascular clamps to induce warm ischaemia (30 minutes). Specifically, no intravenous heparin was given prior to circulatory arrest in this animal model, as in the clinical DCD setting this is not legal within NSW. During warm ischaemia, the renal arteries and veins were divided and cannulated (heparin tips connected to a ¼ inch adaptor with luer locks; Medtronic, Minneapolis, USA and LivaNova Australia, Dandenong, Australia) intra-corporeally. The ureters were cannulated using 16 G intra-venous catheters (Terumo Surflo Catheters, Macquarie Park, Australia). Thirty minutes later, both kidneys were perfused via the renal artery using 500 ml of cold UW solution containing 10,000 units of heparin/liter. The kidneys were subsequently stored in sterile bags containing UW solution at 4 ^{o}C overnight for approximately 23 hours.

References

1. Wilson ME, Hung JC. Evaluation of heparin and anticoagulant citrate dextrose in the preparation of technetium-99m-red blood cells with UltraTag RBC kit. *J Nucl Med* 1992;**33**(2): 306-308.

SDC 4: Perfusion fluid/condition modifications.

Perfusion fluid/condition modifications

Colloid

The perfusion fluid was altered to include a colloid (Gelofusine, B. Braun Australia Pty Ltd, Bella Vista, Australia), as its exclusion from the perfusate can artificially elevate UO.[1] This perfusion fluid was based upon both the Cambridge and Toronto groups, albeit with some modifications;[2-4] we utilized 1 unit of washed PRBCs, 250 ml Gelofusine, 150 ml Hartmann's, 10 ml 8.4% Sodium Bicarbonate, 50 ml of 10% Mannitol, and 2000 units of heparin. Additional infusions were as outlined above.

Vasodilator

Verapamil infusion (0.25 mg/hr; Isoptin, Abbott GmbH & Co KG, Macquarie Park, Australia) was added to the arterial limb of the circuit to counteract the elevated renal arterial pressures seen at relatively low flow rates in kidneys 1-6.[1]

Pressure-based perfusion

Flow rates were set at 0.25 L/min for the first four kidneys regardless of arterial pressure. In the final two perfused porcine kidneys, blood flows were manually adjusted to maintain an arterial perfusion pressure range of 90-100 mmHg during perfusion.[1, 4]

Leucocyte depletion

Leucocyte-depleted blood ameliorates renal ischaemia-reperfusion injury during NMP.[5] After PRBC isolation by centrifugation, addition leucocyte depletion was undertaken by passing the PRBC suspension through a leucocyte filter (Imugard III-RC, Terumo, Tokyo, Japan).

Carbogen (5% CO_2 in 95% O_2)

Acid-base regulation of the perfusion solution may be aided by the addition of CO_2 to the oxygen supply ("carbogen") to allow adequate function of the bicarbonate buffer system.[6, 7] Carbogen (BOC Australia, North Ryde, Australia) was supplied to the circuit, instead of 100% O_2, at a flow rate of 2 L/min for the final kidney.

References

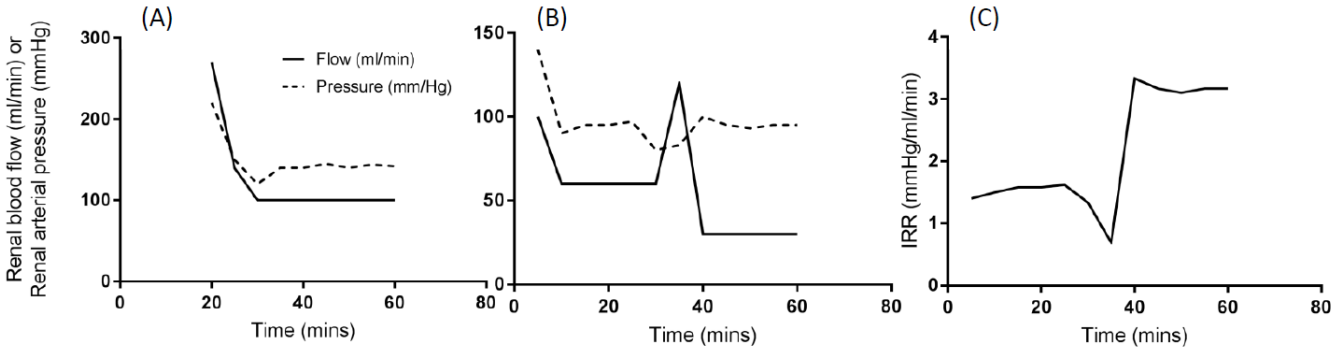
1. Kaths JM, Spetzler VN, Goldaracena N, Echeverri J, Louis KS, Foltys DB et al. Normothermic Ex Vivo Kidney Perfusion for the Preservation of Kidney Grafts prior to Transplantation. *J Vis Exp* 2015;**101**: e52909.
2. Kaths JM, Echeverri J, Linares I, Cen JY, Ganesh S, Hamar Met al. Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *Am J Transplant* 2017. Epub ahead of print, DOI 10.1111/ajt.14294
3. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct* 2017;**3**(3): e140.
4. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant* 2013;**13**(5): 1246-52.
5. Harper S, Hosgood S, Kay M, Nicholson M. Leucocyte depletion improves renal function during reperfusion using an experimental isolated haemoperfused organ preservation system. *Br J Surg* 2006;**93**(5): 623-9.
6. Daniel CR, Labens R, Argyle D, Licka TF. Extracorporeal perfusion of isolated organs of large animals - Bridging the gap between in vitro and in vivo studies. *ALTEX* 2017. Epub ahead of print, DOI 10.14573/altex.1611291
7. Mancina E, Kalenski J, Paschenda P, Beckers C, Bleilevens C, Boor Pet al. Determination of the preferred conditions for the isolated perfusion of porcine kidneys. *Eur Surg Res* 2015;**54**(1-2): 44-54.

SDC 5: Kidney retrieval and NMP characteristics.

NMP Kidney Number	***Post-Retrieval Macroscopic Perfusion Appearance***	***Post-NMP Macroscopic Perfusion Appearance***	***Urine Production (1 hr) (ml)***	***Other Comments***
3	Uniform, pale	Uniform, pink	3550	Graft oedema
4	Uniform, dark	Moderate/patchy	810	Graft oedema & capsular tearing; rose-coloured urine towards end of NMP[‡]
5	Moderate/patchy	Uniform, pink anteriorly; moderate/patchy posteriorly	230	Graft oedema; petechial haemorrhages kidney surface end-NMP[†]; rose-coloured urine[‡]
6	Moderate/patchy	Poor/mottled	500	Graft oedema; petechial haemorrhages kidney surface end-NMP[†]; rose-coloured urine[‡]
7	Moderate/patchy[§]	Poor/mottled	100	Frank, bloody urine; lower pole ?thrombosis
8	Moderate/patchy[§]	Poor/mottled	270	Rose-coloured urine[‡]

[†] Blood collected and likely clotted in perfusion chamber prior to returning to reservoir
[‡] Also occasionally noted by Nicholson & Hosgood (Personal Communication, June 30, 2017)
[§] Gross clots expelled during back-table cold perfusion; difficult to perfuse on back-table

SDC 6: Pressure-flow curves during NMP. (A) A representative example of flow-controlled perfusion [kidney 6]; (B) A representative example of pressure-controlled perfusion [kidney 8]; and (C) IRR during NMP [kidney 8]. IRR – intra-renal resistance.



SDC Chapter 6

SDC 1: Detailed methods explaining animal methods, sample processing, and analyses.

PART 1. Comparison of IRI targets – murine model

Animals

Male C57BL/6 mice (weight 25.3 ± 1.3 g; 10-12 weeks age) were obtained from the Animal Resources Centre (Canning Vale, Australia), acclimatized and allowed free access to food and water until surgery.

IRI model

General anesthesia (GA) was induced using intra-peritoneal ketamine (100 mg/kg) and xylazine (8 mg/kg). The abdomen was shaved, prepared with povidone-iodine, and a midline laparotomy performed. The small intestine was wrapped in gauze moistened with 3 ml of 0.9% sodium chloride (NaCl) (36-37° C), and placed outside the operating field. The right renal pedicle was ligated (6-0 silk tie) prior to a right nephrectomy. Each drug/combination was diluted to a total volume of 0.25 ml in 0.9% NaCl, and injected intra-venously using a 30 G needle. The left renal pedicle was then clamped for 25 minutes using an arterial microvascular clamp (Roboz Surgical Instrument Co., MD, USA). Mouse temperature was maintained at 36° C (RightTemp Temperature Monitor and Homeothermic Warming Control Module [Kent Scientific, CT, USA]). Kidney reperfusion was confirmed by the return of its original color. Warmed 0.9% NaCl (0.3 ml) was instilled into the peritoneal cavity, abdominal contents were replaced anatomically, and the defect was closed using 6-0 PDS. All mice were given buprenorphine (0.1 mg/kg) subcutaneously at defined intervals post-operatively (at least 2 doses).

Study groups and pharmacotherapeutic agents

Mice were treated with the following agent(s) [n.b. these products are still mainly investigational for the purposes described here]:

Group I – 0.9% NaCl (vehicle control) only

Group II – rTM (Asahi Kasei Pharma Co., Tokyo, Japan), 1 mg/kg body weight[28]

Group III –sCR1 (CDX-1135; Celldex Therapeutics, MA, USA), 25 μg/g body weight[29]

Group IV – αCD47Ab (MIAP 301 [sc-12731]; Santa Cruz Biotechnology, TX, USA), 0.8 μg/g body weight[30]

Group V – combination of best 2 performing drugs, determined by relative serum creatinine (Cr) decrease compared to vehicle controls – αCD47Ab (0.8 μg/g body weight) and sCR1 (25 μg/g body weight) given as a single combined dose.

Blood and renal tissue samples
Mice were culled 24 hours after reperfusion under GA. Blood samples were taken from the IVC immediately prior to exsanguination. The left kidney was removed and processed – samples were stored in 10% formalin, RNA*later* RNA stabilization solution (Ambion/Thermo Fisher Scientific, TX, USA), and also snap frozen in dry ice (with or without OCT media [Tissue-Tek, ProSciTech, Australia]). Serum samples were analyzed for urea and Cr levels using the Dimension Vista 1500 Lab System (Siemens, Munich, Germany).

Histology – Hematoxylin and Eosin (H&E)
Paraffin-embedded sections (6 μm thickness) were stained with H&E. Renal damage at the corticomedullary junction was scored by 2 blinded renal histopathologists. Six regions of interest were taken per section, and tubular damage was scored from 0-5 as described previously.[21]

Immunohistochemistry
Immunohistochemistry was performed using the Leica Bond Rx Automated Research Stainer (Leica Biosystems, Wetzlar, Germany) and the Bond Polymer Refine Detection Kit (Leica Biosystems, Newcastle upon Tyne, UK), on formalin-fixed, paraffin-embedded sections (6 μm). An optimized staining protocol was developed – 3-4% hydrogen peroxide block (20 mins), primary antibody (60 mins) and secondary antibody (30 mins) incubation, administration of poly-HRP IgG reagent for localization of rabbit (secondary) antibodies (8 mins), application of 3,3'-Diaminobenzidine tetrahydrochloride hydrate (DAB) (5 mins), and hematoxylin counterstaining (5 mins). Slides were cover-slipped using mounting media (Dako/Agilent Technologies, CA, USA). Neutrophils were detected using primary rat anti-mouse Ly-6G/Ly-6C antibody (RB6-8C5) at a 1:200 dilution (Biolegend, CA, USA), and secondary rabbit anti-rat IgG (BA-4001) at a 1:200 dilution (Vector Laboratories, CA, USA). Positively stained cells were counted from 5 high-power fields (HPF) at the corticomedullary junction in each section.
Reactive oxygen species (ROS) characterization – Cytochrome C and Amplex Red

Measurement of Superoxide ($O2^{\bullet -}$) in particulate fractions using cytochrome c
Whole kidney tissue was homogenized in ice-cold phosphate buffer (PBS) and scraped in lysis buffer (8 mM potassium, sodium phosphate buffer pH 7.0, 131 mM NaCl, 340 mM sucrose, 2 mM NaN3, 5 mM MgCl2, 1 mM EGTA, 1 mM EDTA and protease inhibitors [Roche Diagnostics GmbH, Mannheim, Germany]). Tissue was further lysed by five freeze/thaw cycles, and passage through a 30-gauge (G) needle 5 times. The lysate was centrifuged at 1000 g (5 min; 4°C) to remove unbroken cells, nuclei and debris. Extreme care was taken to maintain the lysate at a temperature close to 0 °C. The cell lysate was centrifuged at 28,000 g (15 min; 4°C). The supernatant was removed, membranes were resuspended in lysis buffer, and protein concentration was measured using the Bradford microplate method.

Superoxide production in particulate fractions (20 μg/ml) of untreated, αCD47Ab-, rTM-, or sCR1-treated mice was measured in 0.1 ml of oxidase assay buffer (65 mM sodium phosphate buffer pH 7.0, 1 mM EGTA, 10 μM FAD, 1 mM MgCl2, 2 mM NaN3 and 0.2 mM cytochrome c [Sigma-Aldrich]). Superoxide production was initiated by the addition of 180 μM NADPH and was calculated from the initial linear rate of superoxide dismutase (SOD) (150 U/ml) (Sigma-Aldrich) inhibitable cytochrome c reduction quantified at 550 nm using an extinction coefficient of 21.1 mM-1 cm-1 (Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader).

Hydrogen peroxide (H_2O_2)-generating activity

Whole kidney tissue was homogenized in ice-cold disruption buffer (PBS containing 0.1 mM EDTA, 10% glycerol, protease inhibitor cocktail, and 0.1 mM phenylmethylsulfonyl fluoride [Sigma-Aldrich]), and further lysed as for superoxide. Lysate (50 µg/ml) was added to the assay mixture (25 mM Hepes, pH 7.4, containing 0.12 M NaCl, 3 mM KCl, 1 mM MgCl2, 0.1 mM Amplex red [Invitrogen, CA, USA], and 0.32 U/ml HRP). The reaction was initiated by the addition of 36 µM NADPH. Fluorescence measurements were made using a Biotek Synergy 4 hybrid multimode microplate reader with a 530/25-excitation and a 590/35-emission filter. The reaction was monitored at 25°C (15 min); the emission increase was linear during this interval. To confirm the H_2O_2 signal, catalase (300 U/ml; Sigma-Aldrich) was added in parallel wells, and the catalase-inhibitable rate of H_2O_2 production was quantified from an H_2O_2 standard curve.

Inflammatory markers – pro-inflammatory cytokine/chemokine mRNA expression

Kidney tissue sections stored in RNA*later* were homogenized, and RNA was extracted using the ISOLATE II RNA Mini Kit as per manufacturer's instructions (Bioline/Meridian Life Science, TN, USA). One microgram of RNA was reverse transcribed using the SensiFAST cDNA synthesis kit (Bioline/Meridian Life Science). cDNA amplification was performed in triplicate in volumes of 10 µL, consisting of cDNA, SensiFAST Probe No-ROX, and the relevant gene-specific primer/Taqman probes (HPRT1 – MM00446968_m1; IL-6 – MM00446190_m1; TNF-α – MM00443258_m1; IL-1β – MM00434228_m1; CCL2 – MM00441242_m1; CXCL2 – MM00436450_m1) (Thermo Fisher Scientific, MA, USA). Real-time polymerase chain reaction (RT-PCR) was performed on a Bio-Rad CFX384 machine – 95^{o}C for 10 mins, 95^{o}C for 30 sec (40 cycles), and 60^{o}C for 45 sec (40 cycles). The ΔΔCt method was used to calculate expression fold changes normalized to HPRT1, with the 0.9% NaCl group utilized as the control.

Immunofluorescence

Complement C3 and C9 staining was ascertained using immunofluorescence. Cryosections (7 µm thickness) were fixed for 10 mins using 4% paraformaldehyde, followed by blocking at room temperature with 1% BSA, 0.1% Tween 20, and 22.5 mg/ml glycine in PBS (30 minutes). The primary antibody of interest (complement C3 [Thermo Fisher Scientific] or C9 polyclonal antibodies [Abcam, Cambridge, UK]) was added to separate cryosections at a 1:250 dilution in blocking solution, and left in a humidified chamber overnight (4^{o}C). Sections were incubated with goat anti-rabbit Alexa Fluor 647 secondary antibody (Thermo Fisher Scientific) at a 1:400 dilution at room temperature (1 hour), co-stained with DAPI (1 min), and then cover-slipped. Staining was visualized using a confocal microscope, and quantified using Image J.

TUNEL staining

Cellular death was ascertained using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining, performed with a commercially available kit (*In Situ* Cell Death Detection Kit, TMR Red; Sigma-Aldrich/Merck, MO, USA), as per the manufacturer's instructions. Staining was visualized by confocal microscopy; TUNEL-positive cells were counted from 3-5 HPF in each section.

PART 2. Direct intra-renal delivery of αCD47Ab using NMP – porcine DCD model

Animals

Female adult outbred Landrace pigs (70.7 ± 14.2 kg) obtained from a certified animal supplier were acclimatized and allowed free access to food until 12 hours before surgery. Water was available *ad libitum* until the time of surgery.

Porcine kidney retrieval – DCD model

Kidney retrieval and a DCD model was established as previously described.[1] In brief,
In brief – All operative procedures were performed under general anesthetic, including pre-medication with intramuscular 1 mg/kg Ilium Xylazil (Xylazine, Troy Laboratories Pty Ltd, Sydney, Australia), 25 mg/kg Tiletamine combined with 25 mg/kg Zolazepam (Zoletil 100, Virbac Australia Pty Ltd, Sydney, Australia), and 1mg/kg Azaperone (Stresnil, Boehringer Ingelheim Pty Ltd, Sydney, Australia). General anesthesia was subsequently induced with 16 mg/kg intravenous thiopentone (Pentothal, Abbott Australasia Pty Ltd, Sydney, Australia) and maintained with 1–2% isoflurane (Zeneca Ltd, Macclesfield, UK) in oxygen after intubation. NaCl (0.9%) was given intravenously at 60 ml/hr for the surgical duration. After a midline laparotomy, the renal pedicles and aorta were exposed/mobilized. The infra-renal aorta was cannulated using a TUR giving set (Baxter Healthcare, IL, USA), through which each pig was exsanguinated; blood was collected into tubes containing Anticoagulant-Citrate-Dextrose solution A (ACD-A) (Aurora Bioscience, Bella Vista, Australia).[2] During exsanguination, the renal pedicle was clamped for 10 mins to simulate warm ischemia in a DCD setting. The renal artery was cannulated intra-corporeally using heparin tips cannulas (Medtronic, Minneapolis, USA), and the ureter was cannulated using a 12 G intra-venous catheter (Terumo Surflo Catheters, Tokyo, Japan). After exactly 10 mins, the kidney was cold-perfused via the renal artery with 500 ml University of Wisconsin (UW) solution containing 10,000 IU/L heparin. The 2 experimental groups were – (i) control kidneys (no further additives); (ii) treatment kidneys (given the best performing anti-IRI agent from the murine study, i.e. [porcine/human-specific] αCD47Ab – BRIC-126 [sc-59079], Santa Cruz Biotechnology) via the renal artery (100 µg diluted in 10 ml UW solution), immediately after the initial UW flush. All kidneys were stored in UW solution prior to NMP (at 4° C for 6 hrs).

Normothermic machine perfusion

Kidney NMP was performed using a modified cardio-pulmonary bypass circuit, as described previously.[1] In brief, packed red blood cells (PRBCs) were isolated from autologous whole blood, and leucocyte-depleted using a leucocyte filter (Imugard III-RC, Terumo, Tokyo, Japan). PRBCs (230 ml) were added to a reservoir (with integrated oxygenator, heat exchanger, and arterial filter) (Terumo Capiox FX05, Tokyo, Japan), along with 150 ml Hartmann's solution, 250 ml Gelofusine (B. Braun Australia Pty Ltd, Bella Vista, Australia), 18 ml sodium bicarbonate 8.4%, 50 ml mannitol 10%, 2000 IU of unfractionated heparin, 5 ml calcium gluconate 0.22 mmol/ml, and 25 ml water for injection. Cr (Merck, Darmstadt, Germany) was added to achieve a concentration of 1000 µmol/L to allow for subsequent creatinine clearance (CrCl) calculation.[3]

The kidney was flushed with Hartmann's solution to remove residual UW solution, weighed, and perfused through the renal artery at a mean pressure of 75-85 mmHg and temperature of 37° C (1 hr). The 1 hour time period was chosen as it has been shown to be effective in human kidney transplantation after initial CS, and is now the subject of a multi-center RCT in the UK.[4, 5] The kidney was placed in a customized 3D-printed copolyester perfusion chamber during NMP.[6] Continuous infusions of verapamil (0.5 mg/hr), 5% dextrose (5 ml/hr), and M199 nutrient solution containing 100 IU of actrapid and multivitamins (1 vial of Soluvit N dissolved in Vitalipid N; Fresenius Kabi, Bad Homburg, Germany) (100 ml at 20 ml/hr) were also provided. Immediately prior to starting NMP in treatment kidneys, 200 µg of αCD47Ab (BRIC-126) was directly injected into the renal arterial line (i.e. ~0.8 µg/g of kidney weight).

Renal tissue, blood, and urine samples
Sequential kidney biopsies were taken just prior to the commencement of NMP (end CS) and at the end of NMP (1 hr), and processed as above. Perfusate blood samples taken from the arterial arm of the circuit (immediately after commencement, and just prior to cessation, of NMP) were analyzed for sodium (Na), creatinine (Cr), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST). Urine samples were taken at the end of NMP and analyzed for Cr and Na. All automated analyses were conducted using the Dimension Vista 1500 Lab System (Siemens). Blood gas analyses (arterial and venous) for pH, partial pressure of oxygen and carbon dioxide, base excess (BE), lactate, and bicarbonate levels were also conducted at the start and end of NMP using the i-STAT Alinity (Abbott, IL, USA).

Calculations
Renal blood flow (RBF) was adjusted to a kidney weight of 250 g and recorded at 5 min intervals. Intra-renal resistance (IRR; pressure/flow) was also calculated at each corresponding time point. Urine output (UO) was measured at the end of NMP. CrCl, fractional excretion of sodium (FeNa), and renal oxygen consumption were calculated as described elsewhere.[3]

Histology
H&E was performed as above. Sections were scored from 0-3 (from least to most severe) by a blinded renal histopathologist based on the extent of tubular dilatation, tubular debris, cytoplasmic vacuolation, and inflammatory cell infiltration.[7, 8]

Inflammatory markers – pro-inflammatory cytokine/chemokine mRNA expression
RT-PCR was performed as described above using the following porcine-specific primers: HPRT1 (Ss03388274_m1), IL-6 (Ss03384604_u1), TNF-α (Ss03391318_g1), IL-1β (Ss03393804_m1), and IL-18 (Ss03391203_m1) (Thermo Fisher Scientific).

CD47 antibody binding to renal tissue – immunofluorescence

αCD47Ab binding to porcine renal tissue was visualized on cryosections fixed with 96% ethanol (room temperature), permeabilized using 0.1% Triton X-100 in PBS (10 minutes), and blocked using 1% BSA and 22.5 mg/ml glycine in PBS (25 minutes). CD47 BRIC-126 is a mouse monoclonal antibody; goat anti-mouse secondary antibody conjugated to Alexa Fluor 647 dye (Thermo Fisher Scientific) was therefore added to the sections (1:400 dilution), and left in a humidified chamber (45 mins). Samples were co-stained with DAPI, and cover-slipped. Fluorescence signaling was visualized using confocal microscopy.

Renal oxidative stress

Porcine renal tissue oxidative stress was quantified using dihydroethidium (DHE) (Thermo Fisher Scientific), indicative of tissue levels of superoxide. DHE (10 µM in PBS) was added to unfixed cryosections at 37° C in a light-protected humidified chamber (22 min). Slides were co-stained with DAPI and mounted. Fluorescence was visualized using confocal microscopy. DHE staining density was quantified using Image J software.

TUNEL staining

TUNEL staining was performed as described above.

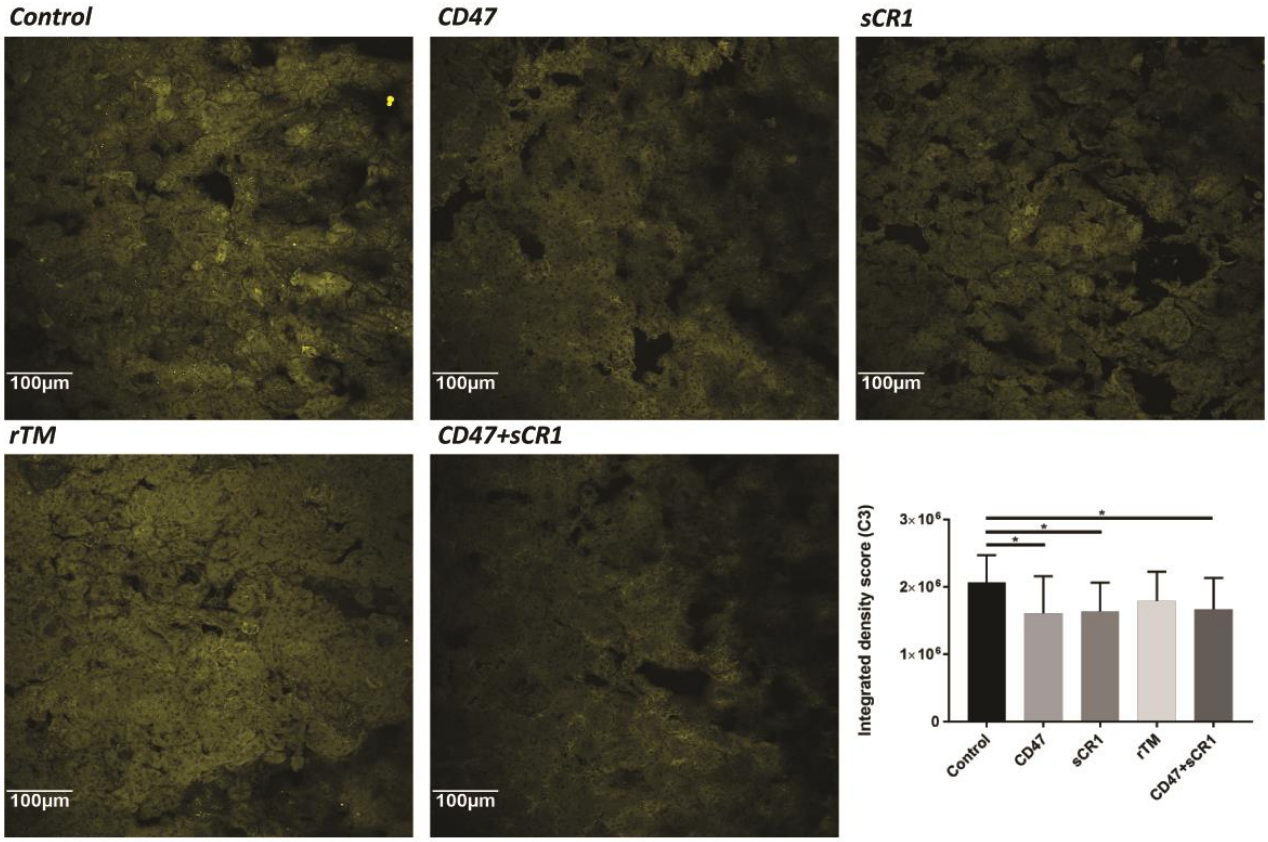
Statistical analyses

Data is presented as mean ± standard deviation (SD). Continuous parametric variables were compared using the unpaired student's t-test. In the event that more than 2 groups of parametric variables were to be compared, the ANOVA test was utilized. Area under the curve (AUC) was calculated for RBF and IRR prior to further statistical comparisons. GraphPad Prism v. 7.02 was used for all statistical analyses. A p-value of <0.05 was deemed statistically significant.

References

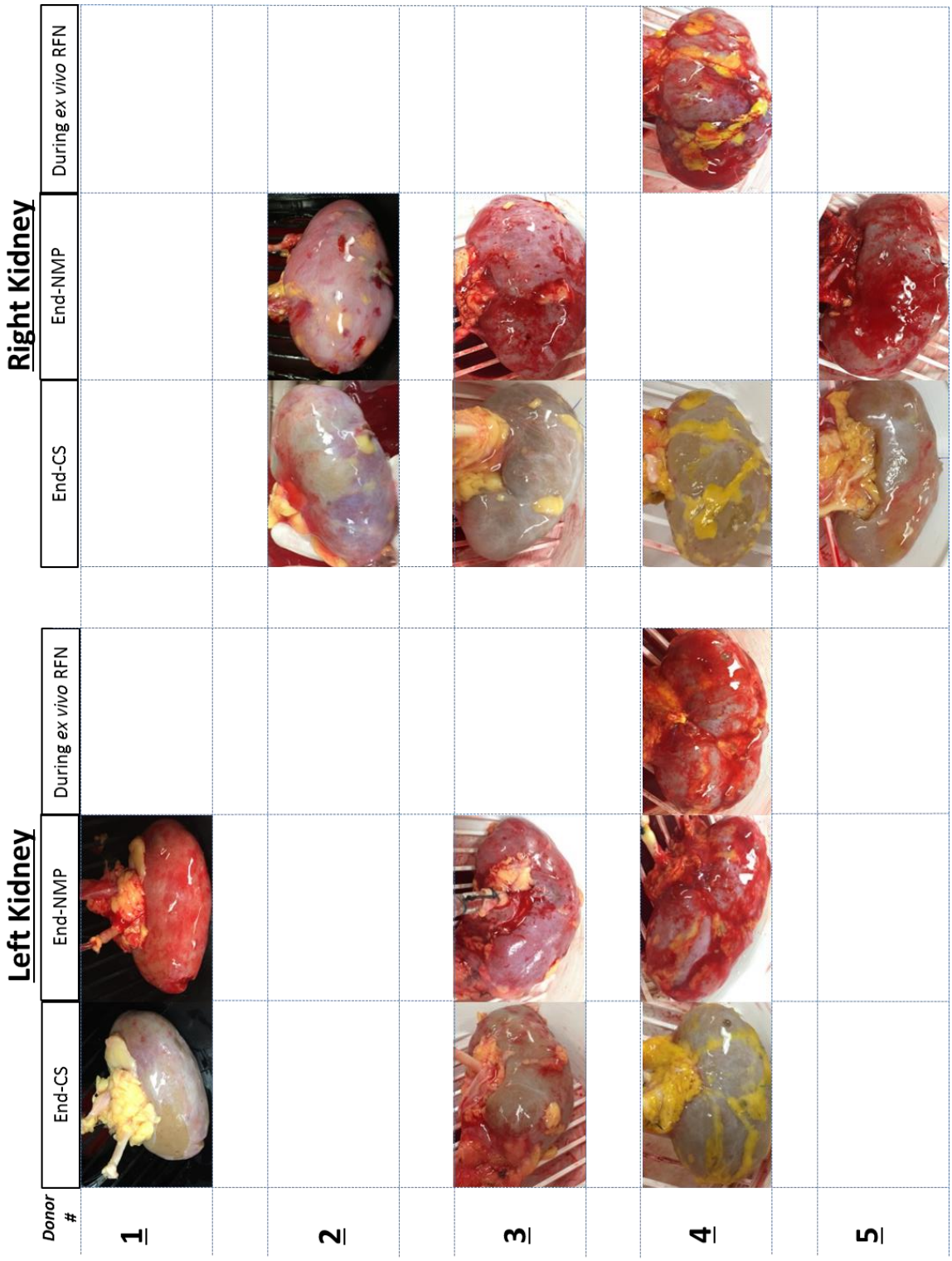
1. Hameed AM, Miraziz R, Lu DB, et al. Extra-corporeal normothermic machine perfusion of the porcine kidney: working towards future utilization in Australasia. *ANZ J Surg*. 2018; 88: E429-434.
2. Wilson ME, Hung JC. Evaluation of heparin and anticoagulant citrate dextrose in the preparation of technetium-99m-red blood cells with UltraTag RBC kit. *J Nucl Med.* 1992; 33: 306-308.
3. Adams TD, Patel M, Hosgood SA, Nicholson ML. Lowering Perfusate Temperature From 37 degrees C to 32 degrees C Diminishes Function in a Porcine Model of Ex Vivo Kidney Perfusion. *Transplant Direct*. 2017; 3: e140.
4. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013; 13: 1246-1252.
5. Hosgood SA, Saeb-Parsy K, Wilson C, Callaghan C, Collett D, Nicholson ML. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017; 7.
6. Hameed A, Dervish S, Rogers N, Pleass H, Hawthorne W. A novel, customized 3D-printed perfusion chamber for normothermic machine perfusion of the kidney. *Transpl Int*. 2018. Epub ahead of print; DOI: 10.1111/tri.13361
7. Bagul A, Hosgood SA, Kaushik M, Kay MD, Waller HL, Nicholson ML. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. *Br J Surg.* 2008; 95: 111-118.
8. Hosgood SA, Barlow AD, Yates PJ, Snoeijs MG, van Heurn EL, Nicholson ML. A pilot study assessing the feasibility of a short period of normothermic preservation in an experimental model of non heart beating donor kidneys. *J Surg Res.* 2011; 171: 283-290.

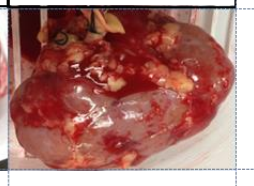
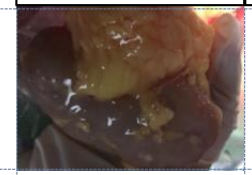
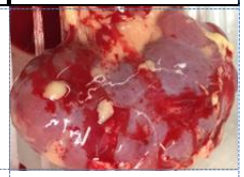
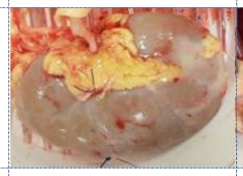
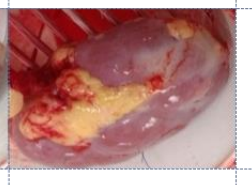
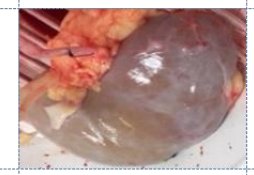
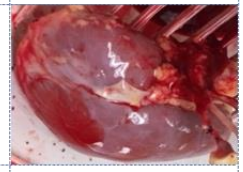
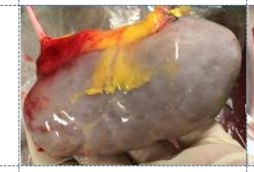
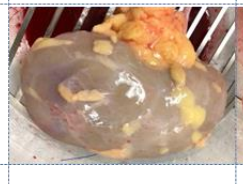
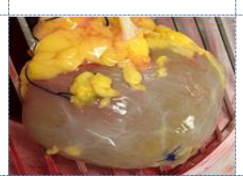
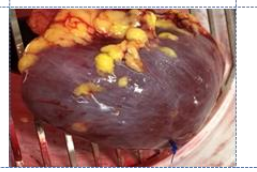
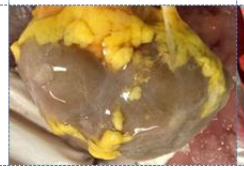
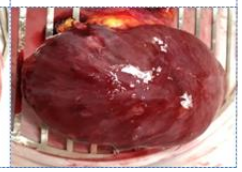
SDC 2: Complement C3 staining in all murine study groups 24 hrs of induction of ischemia-reperfusion injury, as visualized by immunofluorescence (20 x). Data shown as mean ± SD; n = 5-6/group. *p<0.05.



SDC Chapter 7

SDC 1: Pictorial representation of all donor kidneys at the end of cold (static) storage (CS), NMP, and *ex vivo* whole blood reperfusion (RFN), as appropriate.



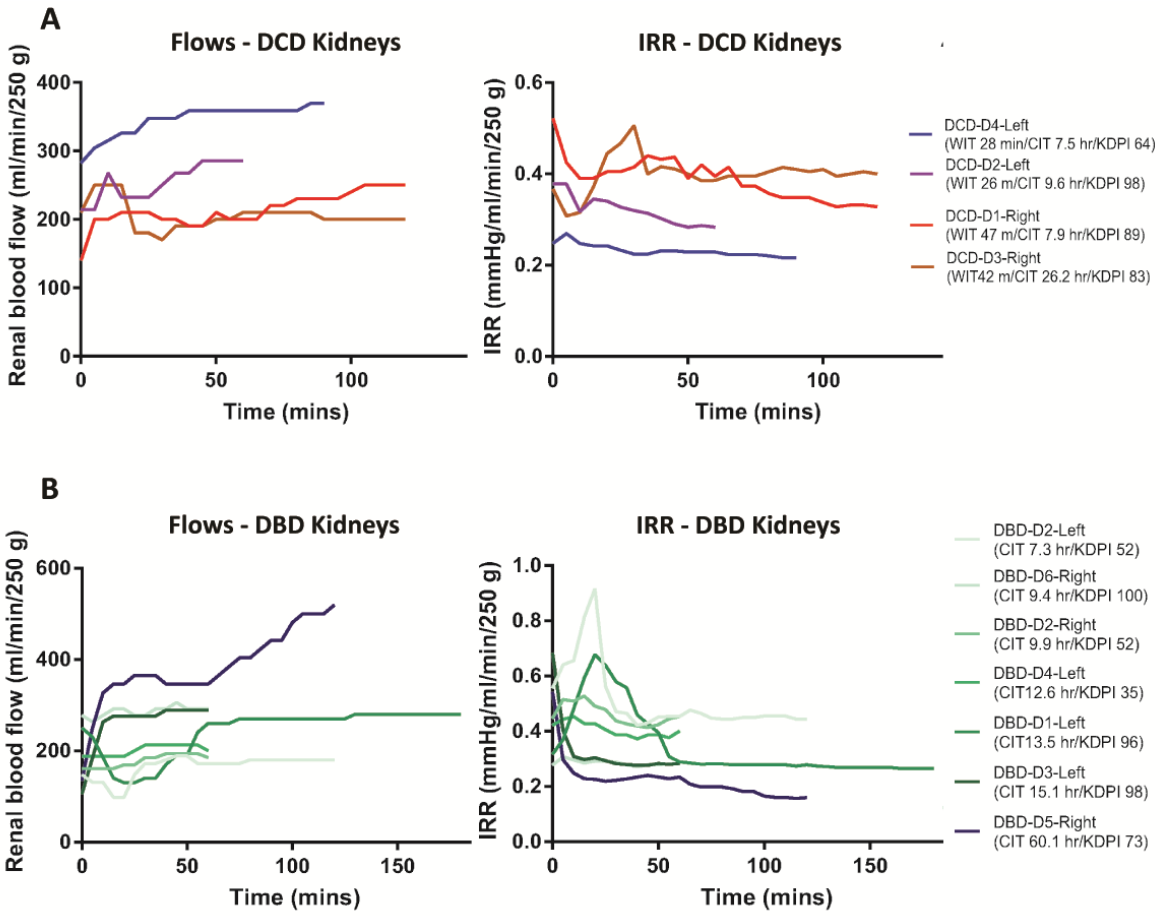
	Left Kidney			Right Kidney		
Donor #	End-CS	End-NMP	During *ex vivo* RFN	End-CS	End-NMP	During *ex vivo* RFN
6						
7						
8						
9						
10						

SDC 2: Renal tubular injury scores at selected time-points in kidneys undergoing NMP.

Kidney	***End CS***	***60 min NMP***	***120 min NMP***	***180 min NMP***
DBD-D1	1 (focal 2*)	2	1 (focal 2*)	1 (focal 2*)
DBD-D2-L	1 (focal 2**)	1 (focal 2*)	1 (focal 2*)	
DBD-D2-R	1 (focal 2*)	1 (focal 2**)		
DBD-D3-L	1	1		
DBD-D4-L	0-1	1		
DBD-D5	0	1***	1	
DBD-D6-R	1 (focal 2**)	1		
DCD-D1	1	1		
DCD-D2-L	1 (focal 2*)	1		
DCD-D3	0	0-1		
DCD-D4	1	1 (focal 2**)	1 (focal 2****)	

***** Sloughed cells
** Casts and sloughed cells
*** Few casts only
**** 90 min sample; occasional casts

SDC 3: Renal blood flow (RBF) and intra-renal resistance (IRR) in (A) DCD, and (B) DBD kidneys, arranged by cold (CIT) and/or warm (WIT) ischemia times, as applicable.

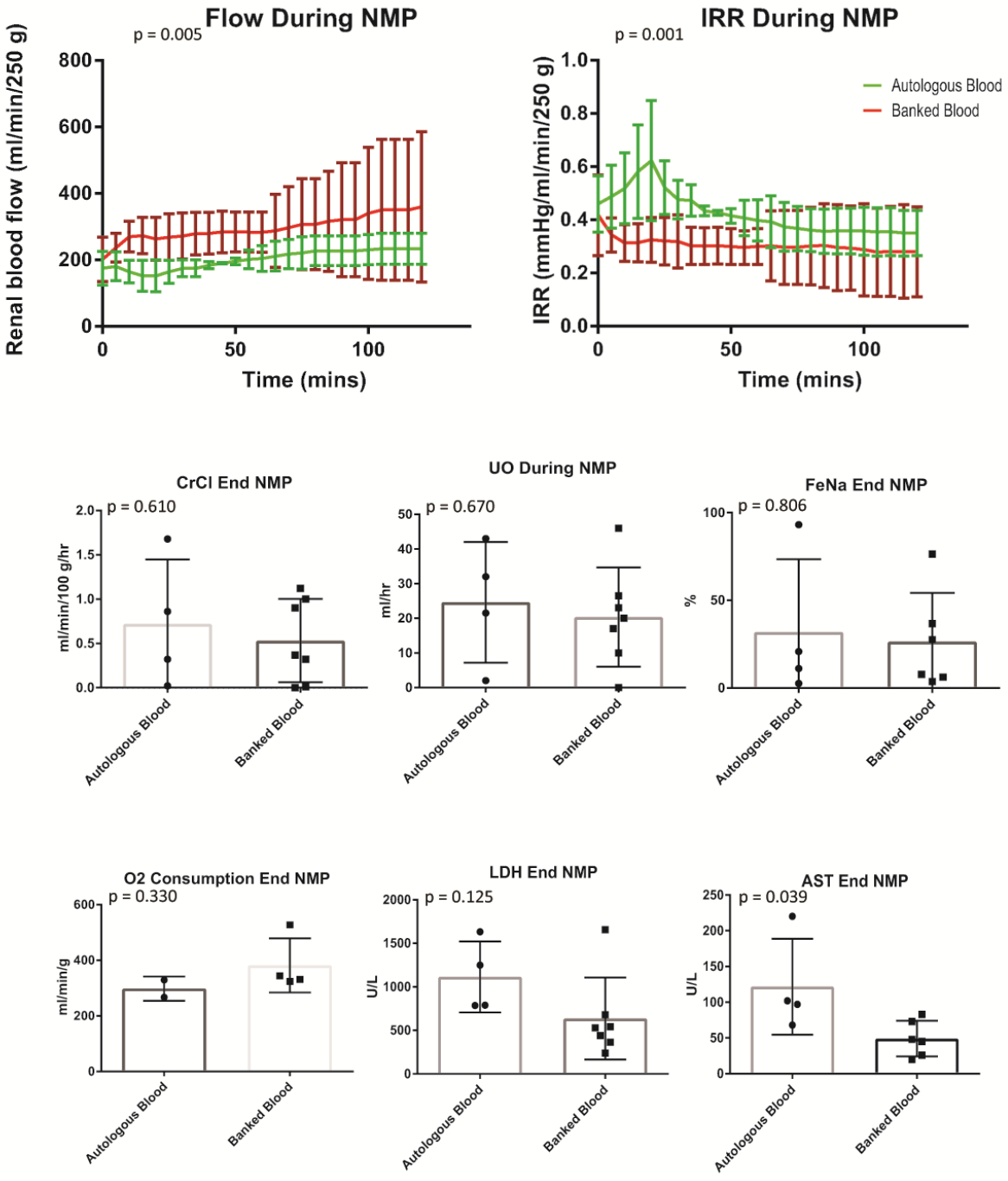


SDC 4: Comparative perfusate baseline hematologic and biochemical parameters at the start of NMP in kidneys perfused with autologous or banked (allogeneic) blood.

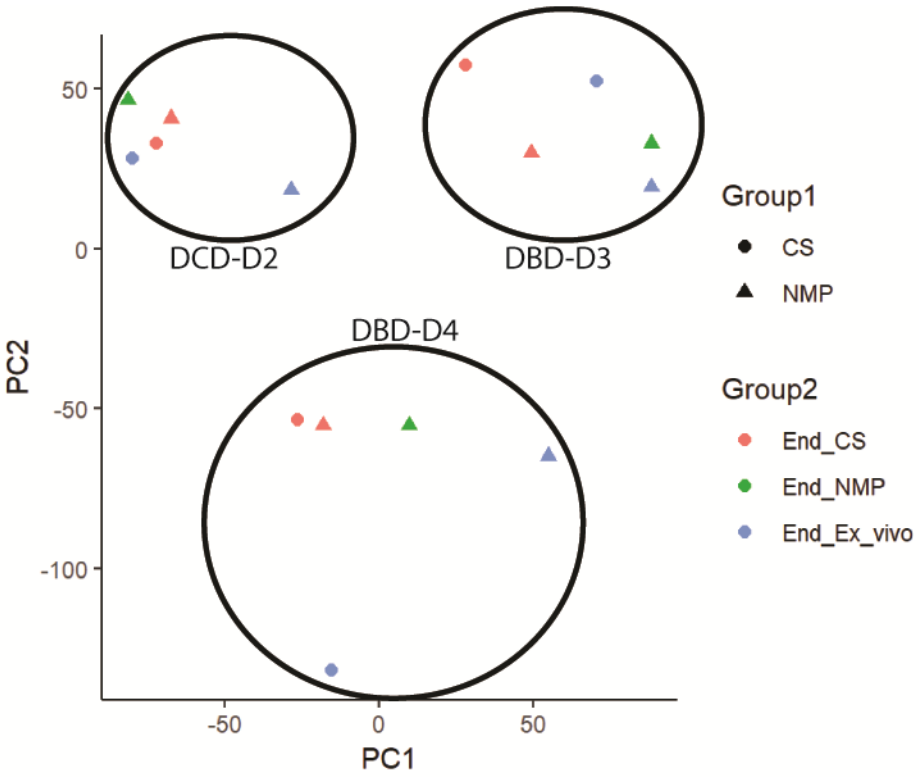
Characteristic	**Autologous Blood (Mean, SD)**	**Banked Blood (Mean, SD)**	**p-value**
Hemoglobin (g/L)	43.8 (25.2)	65.3 (13.7)	0.094
White cell count (x 10^9/L)	0.15 (0.1)	0.06 (0.05)	0.071
Platelet count (x 10^9/L)	45 (19.9)	0.9 (1.5)	< 0.001
Hematocrit (%)	14.3 (8.7)	20.7 (5.2)	0.150
Sodium (mmol/L)	142 (1.4)	143.9 (4.0)	0.404
Potassium (mmol/L)	5.4 (0.4)*	7.6 (1.5)*	0.107
Bicarbonate (mmol/L)	13 (4.2)	14.3 (1.9)	0.491

* Values for 2 autologous samples and 1 banked sample missing due to sample hemolysis (post/during collection)

SDC 5: Comparison of NMP using autologous or banked (allogeneic) packed red blood cells (PRBCs). UPPER PANELS – Flow and intra-renal resistance (IRR) during NMP using each source of blood. LOWER PANELS – Comparative renal glomerular, tubular, and functional parameters after NMP with banked versus autologous PRBCs. AST – aspartate aminotransferase; CrCl – creatinine clearance; FeNa – fractional excretion of sodium; LDH – lactate dehydrogenase; UO – urine output.



SDC 6: Principal component analysis (PCA) for all paired kidney samples that underwent whole transcriptome RNA sequencing.



SDC 7: Differentially expressed genes and pathways in paired kidneys after NMP (in comparison to the end-CS samples from the same kidneys [NMP group]).

SDC 8: Differentially expressed genes and pathways after *ex vivo* whole blood reperfusion in paired kidneys having NMP (in comparison to the end-NMP samples from the same kidneys).

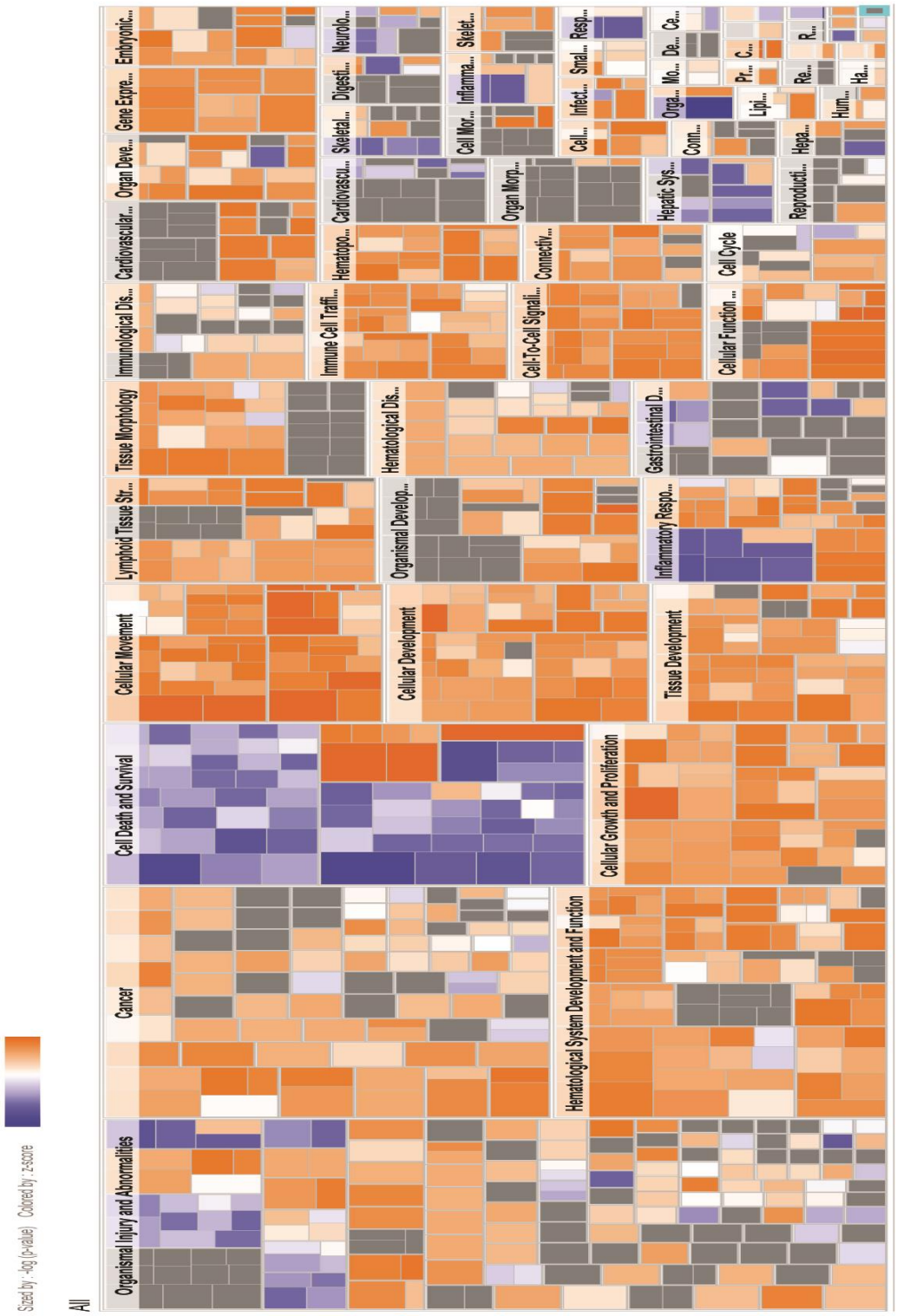
SDC 9: Differentially expressed genes and pathways after *ex vivo* whole blood reperfusion in paired kidneys having NMP compared to CS alone.

SDC 11: IPA – Tabulation of Diseases/Functions outlined in SDC 10.

Excel spreadsheets – accessible via the following link:

https://www.dropbox.com/sh/d5r4r44aexver9c/AACkytA6dRLnm38bQjiOxVb9a?dl=0

SDC 10: IPA – Diseases/functions activated and/or repressed by NMP in comparison to paired kidneys having CS alone (sampled at the end of simulated transplantation). Each large box indicates a Disease/Function category, whilst each small box represents a distinct Disease/Function process (annotation). Boxes are colored based on z-score (orange indicates an increase in the predicted pathway activation state, and blue indicates a decrease).



SDC 12: Remuzzi scores (including tubular injury scores) after simulated transplantation in paired kidneys having CS alone or NMP.

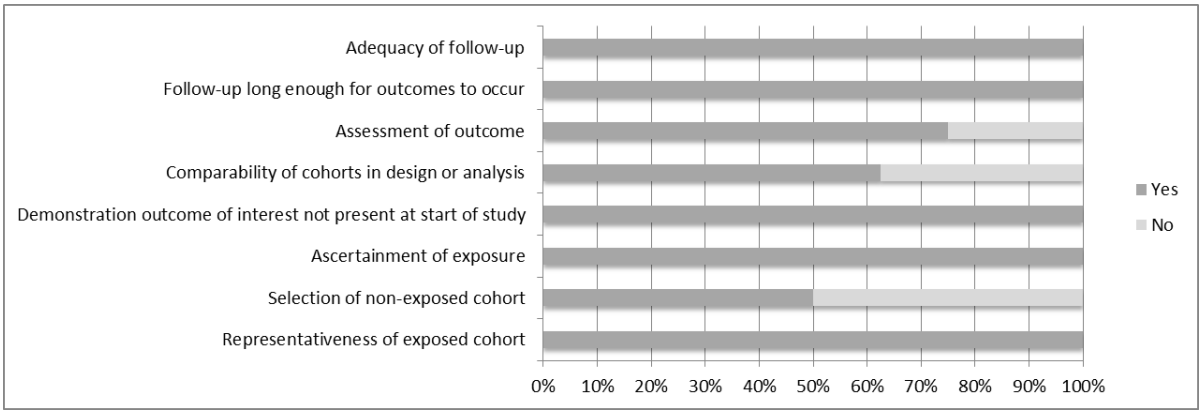
Pair No.	*Treatment Group*	*No. Glomeruli*	*% Sclerosed*	*% Chronic Damage (Tubular atrophy/Interstitial fibrosis)*	*Arteriolar Hyalinosis*	*Intimal Elastosis*	*Acute Tubular Injury*
1	NMP	22	5	5	1	NA	1
1	CS	20	5	5	1	NA	1
2	NMP	40	5	5	1	2	1 (with focal 2)
2	CS	30	10	5	1	1	1-2
3	NMP	60	0	2	0	0	1 (with focal 2)
3	CS	35	2	2	1	NA	1
4	NMP	225	4	5	1	1	1-2
4	CS	280	3	3	1	2	1 (with focal 2)

SDC Chapter 9

SDC 1: Search strategy – databases searched simultaneously using Ovid: Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Embase, Ovid Medline, and Epub Ahead of Print, In-Process & Other Non-Indexed Citations. Last search date 25 January, 2017.

Number	Search terms
1	transplant.mp. or exp transplantation/
2	pancreas surgery/ or pancreas/ or pancrea*.mp. or exp kidney pancreas transplantation/
3	(university of wisconsin or UW or HTK or histidine* or collins or hyperosmolar citrate or HOC or celsior or IGL-1 or institut-George* or custodial or belzer or MPS or KPS or marshall* or hypertonic citrate or soltran or ross).mp
4	1 AND 2 AND 3
5	REMOVE DUPLICATES FROM 4

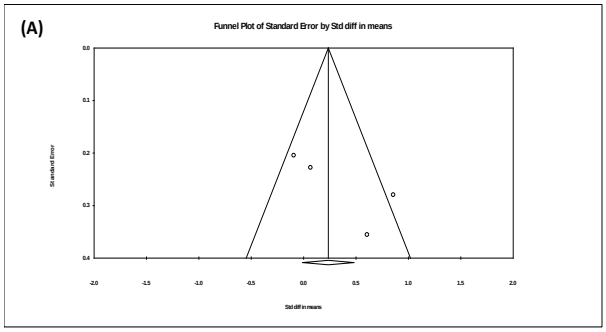
SDC 2: Newcastle-Ottawa Quality Assessment Scale – bias assessment of cohort studies included in meta-analyses for procurement and preservation of the pancreas.

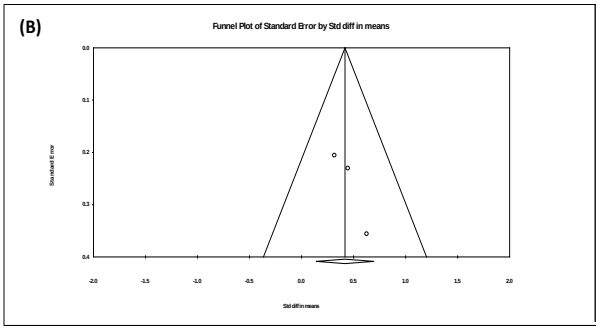


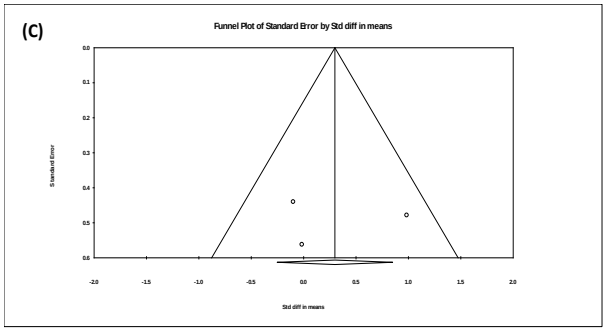
SDC 3: Cochrane Collaboration's tool for assessing risk of bias – bias assessment of randomized trials. *?* Unclear risk of bias; *+* High risk of bias; – Low risk of bias.

Study	***Random sequence generation***	***Allocation concealment***	***Blinding of participants & personnel***	***Blinding of outcome assessment***	***Incomplete outcome data***	***Selective reporting***
Boggi *et al*., 2004	*?*	*?*	*+*	*+*	–	*?*
Nicoluzzi *et al*., 2008	–	*?*	*?*	*?*	–	*?*
Schneeberger *et al*., 2009	*?*	*?*	*?*	*?*	–	*?*

SDC 4: Funnel plots for the assessment of publication bias in studies comparing UW to HTK aortic perfusion and preservation for the parameters of (A) peak amylase, (B) peak lipase, and (C) thrombotic graft loss rates.







SDC 5: Grading of Recommendations, Assessment, Development and Evaluations (GRADE) – assessment of studies comparing UW or HTK aortic-only perfusion and cold static storage.

Outcome type	**No. of studies (Type of study)**	**Risk of bias/Quality of evidence**	**Consistency**	**Directness**	**Precision**	**Publication bias**	**Overall effect size estimate (95% CI)**	**Quality of evidence**
Peak amylase	4 (4 cohort)	No serious risk of bias; observational evidence (+2)	Moderate inconsistency; I^2 = 67.0 (-1)	Direct (0)	Small sample size (-1)	No important publication bias	0.32 (-0.13-0.76) (0)	Very low
Peak lipase	3 (3 cohort)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	0.42 (0.14-0.69) (0)	Very low
Hospital LOS	2 (2 cohort)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Wide CI (-1)	Cannot confidently assess (0)	2.92 (-0.04-5.87) (0)	Very low
Thrombotic graft loss	3 (3 cohort)	No serious risk of bias; observational evidence (+2)	Some inconsistency; I^2 = 36.9	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	1.50 (0.55-4.11) (0)	Very low
One-month graft survival	3 (3 cohort)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Wide CI (-1)	Cannot confidently assess (0)	2.22 (0.83-5.94) (+1)	Low

CI – confidence interval; HTK – histidine-tryptophan-ketoglutarate; LOS – length of stay; UW – University of Wisconsin

SDC Chapter 10

SDC 1: Search strategy – databases searched simultaneously: Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Embase, Ovid Medline, and Epub Ahead of Print, In-Process & Other Non-Indexed Citations. Last search date 30 January, 2017.

Number	Search terms
1	transplant.mp. or exp transplantation/
2	exp liver preservation/ or exp liver transplantation/ or liver/ or liver.mp. or exp liver perfusion/ or hepatic.mp
3	(university of wisconsin or UW or HTK or histidine* or collins or hyperosmolar citrate or HOC or celsior or IGL-1 or institut-George* or custodial or belzer or MPS or KPS or marshall* or hypertonic citrate or soltran or ross).mp
4	1 AND 2 AND 3
5	REMOVE DUPLICATES FROM 4

Additional search:

Number	Search terms
1	transplant.mp. or exp transplantation/
2	exp liver preservation/ or exp liver transplantation/ or liver/ or liver.mp. or exp liver perfusion/ or hepatic.mp
3	(aortic perfusion or dual perfusion or aortic cooling or dual cooling or portal perfusion or portal cooling).mp
4	1 AND 2 AND 3
5	REMOVE DUPLICATES FROM 4

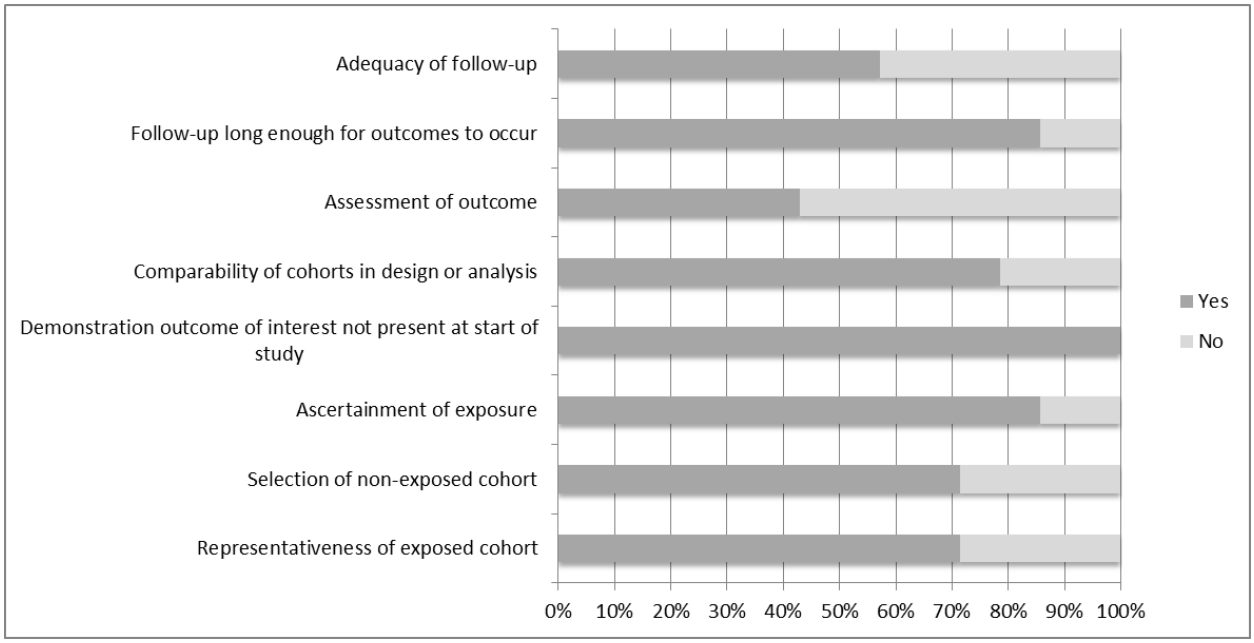
Additional search #2 (donation after circulatory death liver transplantation (April 26, 2017):

Number	Search terms
1	exp liver preservation/ or exp liver transplantation/ or liver/ or liver.mp. or exp liver perfusion/ or hepatic.mp
2	(donation after cardiac death or donation after circulatory death or DCD or nonheart beating donor or non-heart beating donor).mp
3	1 AND 2
4	(university of wisconsin or UW or HTK or histidine* or collins or hyperosmolar citrate or HOC or celsior or IGL-1 or institut-George* or custodial or belzer or MPS or KPS or marshall* or hypertonic citrate or soltran or ross).mp
4	3 AND 4
5	REMOVE DUPLICATES FROM 4

SDC 2: Cochrane Collaboration's tool for assessing risk of bias – bias assessment of randomized trials included in meta-analyses. *?* Unclear risk of bias; *+* High risk of bias; – Low risk of bias.

Study	***Random sequence generation***	***Allocation concealment***	***Blinding of participants & personnel***	***Blinding of outcome assessment***	***Incomplete outcome data***	***Selective reporting***
Cavallari *et al*., 2003	–	*?*	*+*	*?*	–	–
Chui *et al*., 1998	–	–	*+*	*?*	–	*?*
Erhard *et al*., 1994	*?*	*+*	*?*	–	–	*?*
Garcia-Gil *et al*., 2011	–	–	*?*	*?*	–	–
Meine *et al*., 2006	–	*?*	*+*	*?*	–	–
Nardo *et al*., 2001	*?*	*?*	*+*	*?*	–	*?*

SDC 3: Newcastle-Ottawa Quality Assessment Scale – bias assessment of cohort studies included in meta-analyses.



SDC 4: Additional article donor, recipient and preservation characteristics. No statistically significant differences between groups unless otherwise indicated.

LIVER Studies	Primary Study Purpose	Comparator Groups	Liver Allocation (% Local)	Preservation Solution Volume (CS) (L)	Donor Cause of Death (%)	Donor Liver Steatosis (Mean)	Use of Split Livers	Recipient Sex (% Male)	Recipient Transplant Due to HCV/HBV (%)
Aortic vs Dual Perfusion (UW)									
Anthuber et al., 1993	Comparison of aortic vs dual perfusion	UW perfusion/CS (Ao)	100	NR	Trauma 48.6 CVA/ICH 31.1	NR	NR	67.2	NR
		UW perfusion/CS (Du)	100	NR	Trauma 42.3 CVA/ICH 48.1	NR	NR	57.7	NR
Boillot et al., 1993	Comparison of aortic vs dual perfusion	UW perfusion/CS (Ao)	NR	NR	NR	NR	7 of 61 (11.5%)	NR	NR
		UW perfusion/CS (Du)	NR	NR	NR	NR		NR	NR
Chui et al., 1998	Comparison of aortic vs dual perfusion	Marshall (Ross) pre-flush + UW perfusion/CS (Ao)	NR	NR	NR	NR*	NR	75	NR
		Marshall (Ross) pre-flush + UW perfusion/CS (Du)	NR	NR	NR	NR*	NR	70	NR
De Goyet et al., 1994	Comparison of aortic vs dual perfusion	UW perfusion/CS (Ao)	NR	1-2	Trauma 59.2 CVA/ICH 31.6	NR	NR	NR	NR
		UW perfusion/CS (Du)	NR	1-2	Trauma 65.6 CVA/ICH 21.9	NR	NR	NR	NR
Gabel et al., 2001	Comparison of aortic vs dual perfusion	UW perfusion/CS (Ao)	NR	NR	NR	NR	NR	50.0	NR
		UW perfusion/CS (Du)	NR	NR	NR	NR	NR	50.0	NR
UW vs HTK Perfusion (Ao)									
Avolio et al., 2006	Comparison of UW vs HTK perfusion/CS	UW perfusion/CS	NR	NR	NR	NR*	NR	81.0	NR
		HTK perfusion/CS	NR	NR	NR	NR*	NR	78.6	NR
Mangus et al., 2006; Mangus et al., 2008	Comparison of UW vs HTK perfusion/CS	UW perfusion/CS	NR	NR	NR	< 30%	NR	69.0	NR
		HTK perfusion/CS	NR	NR	NR	< 30%	NR	63.0	NR
UW vs HTK Perfusion (Du)									
Erhard et al., 1994	Comparison of UW vs HTK perfusion/CS	UW perfusion/CS	56.7	NR	NR	NR	NR	NR	NR
		HTK perfusion/CS	36.7	NR	NR	NR	NR	NR	NR
Hatano et al., 1997	Comparison of UW vs HTK perfusion/CS	UW perfusion/CS	NR	NR	Trauma 44.4 CVA/ICH 55.6	NR[¥]	2 of 18 (11.1%)	NR	16.7
		HTK perfusion/CS	NR	NR	Trauma 46.7 CVA/ICH 43.3	NR[¥]	1 of 30 (3.3%)	NR	
Meine et al., 2006	Comparison of UW vs HTK perfusion/CS	UW perfusion/CS	NR	1	NR	< 30%[Ø]	NR	64.6	61.6
		HTK perfusion/CS	NR	1	NR	< 30%[Ø]	NR	62.2	83.8

Moench et al., 2006	ITBL in HTK compared to UW perfusion/CS	UW perfusion/CS	NR	NR	NR	< 30%	NR	NR	18.0
		HTK perfusion/CS	NR	NR	NR	< 30%	NR	NR	
UW vs Celsior Perfusion (Du)									
Cavallari et al., 2003	Comparison of UW vs Celsior perfusion/CS	UW perfusion/CS	100	NR	Trauma 39.0 CVA/ICH 54.4	18%	Nil	71.1	65.5
		Celsior perfusion/CS	100	NR	Trauma 44.0 CVA/ICH 45.3	17%	Nil	71.1	63.0
Garcia-Gil et al. 2006; Garcia-Gil et al., 2011	Comparison of UW vs Celsior perfusion/CS	UW perfusion/CS	Local	NR	Trauma 33.3 CVA/ICH 66.7	< 30%**	NR	76.5	19.6 (HCV only)
		Celsior perfusion/CS	Local	NR	Trauma 25.5 CVA/ICH 74.5	< 30%**	NR	72.5	33.3 (HCV only)
Lopez-Andujar et al., 2009	Comparison of UW vs Celsior perfusion/CS	UW perfusion/CS	NR	NR	Trauma 20.2 CVA/ICH 67.3	< 30%	Nil	73.1	58.7
		Celsior perfusion/CS	NR	NR	Trauma 16.3 CVA/ICH 73.9	< 30%	Nil	81.5	65.2
Nardo et al., 2001	Comparison of UW vs Celsior perfusion/CS	UW perfusion/CS	NR	NR	Trauma 35.0 CVA/ICH 56.7	NR[γ]	NR	61.7	55.0
		Celsior perfusion/CS	NR	NR	Trauma 39.6 CVA/ICH 58.5	NR[γ]	NR	52.8	50.9
HTK vs IGL-1 Perfusion (Du)									
Meine et al., 2015	Comparison of HTK vs IGL-1 perfusion/CS	HTK perfusion/CS	NR	NR	Trauma 24.6 CVA/ICH 73.8	NR[€]	NR	63.1	56.9
		IGL-1 perfusion/CS	NR	NR	Trauma 30.1 CVA/ICH 66.4	NR[€]	NR	67.3	54.9
Wiederkehr et al., 2014	Comparison of HTK vs IGL-1 perfusion/CS	HTK perfusion/CS	NR	NR	NR	NR	NR	83.2	NR[κ]
		IGL-1 perfusion/CS	NR	NR	NR	NR	NR	71.7	NR[κ]
Other Comparisons									
Dondero et al., 2010	Comparison of UW vs IGL-1 perfusion/CS (Du)	UW perfusion/CS	NR	1	NR	< 30%[ξ]	Nil	73.9	13.0
		IGL-1 perfusion/CS	NR	1	NR	< 30%[ξ]	Nil	70.8	18.0
Nardo et al., 2005	Comparison of Celsior vs HTK perfusion/CS (Du)	HTK perfusion/CS	NR	NR	Trauma 20.0 CVA/ICH 80.0	NR[δ]	NR	35.0	55.0
		Celsior perfusion/CS	NR	NR	Trauma 20.0 CVA/ICH 80.0	NR[δ]	NR	55.0	50.0

Ao – Aortic-only perfusion; ALT – alanine aminotransferase; AST – aspartate aminotransferase; CS – cold storage; CVA – cerebrovascular accident; Du – dual perfusion; HBV – hepatitis B virus; HCV – hepatitis C virus; HTK – histidine-tryptophan-ketoglutarate; ICH – intra-cranial hemorrhage (spontaneous); IGL-1 – Institut Georges Lopez; L – litres; NR – not recorded; Tx – transplant; UW – University of Wisconsin

* Grossly fatty livers excluded

** Nil in 88% of cases (includes patients across both study groups)

[¥] Two grafts in UW and HTK groups each with steatosis ("moderate" in HTK group, and "mild" and "moderate" in UW group)

[Φ] In UW group, 20.3% of livers had steatosis (> 60% in 1.5%), whilst 18.9% of HTK grafts had steatosis (> 60% in 5.4%)

[δ] In HTK group, 30% and 10% had mild and moderate steatosis, respectively, and no graft had severe steatosis; in Celsior group, respective values were 35%, 5% and 0

[γ] In the UW group, 23.3% of livers had mild or moderate steatosis compared to 24.5% in the HTK group; there were no cases with severe steatosis

[€] Grafts with steatosis > 50% excluded; steatosis of some degree (not defined) present in 13.8% and 14.2% of HTK and IGL-1 grafts, respectively

[ξ] In the UW group, 55.5% of grafts had no steatosis compared to 56.3% of IGL-1 grafts

[κ] Indications for transplant in both groups were similar, as per the text

SDC 5: Grading of Recommendations, Assessment, Development and Evaluations (GRADE) – assessment of studies included in meta-analyses.

Outcome type	No. of studies (Type of study)	Risk of bias/Quality of evidence	Consistency	Directness	Precision	Publication bias	Overall effect size estimate (95% CI)	Quality of evidence
Aortic vs. Dual (UW)								
Peak ALT	4 (3 cohort, 1 RCT)	No serious risk of bias; observational evidence (+2)	Minor inconsistency; I^2 = 18.3 (0)	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	0.24 (0.01-0.47) (0)	Very low
PNF	5 (4 cohort, 1 RCT)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	1.81 (0.84-3.88) (0)	Very low
UW vs. HTK (Dual)								
Peak ALT	3 (2 cohort, 1 RCT)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	-0.00 (-0.27-0.26) (0)	Very low
Peak AST	3 (2 cohort, 1 RCT)	No serious risk of bias; observational evidence (+2)	No inconsistency; I^2 = 0	Direct (0)	Small sample size (-1)	Cannot confidently assess (0)	-0.05 (-0.31-0.22) (0)	Very low
UW vs. Celsior (Dual)								
Thrombotic graft loss	3 (3 RCT)	No serious risk of bias; RCT evidence (+4)	No inconsistency; I^2 = 0	Direct (0)	Wide CI (-1)	Cannot confidently assess (0)	0.85 (0.23-3.14) (0)	Moderate
PNF	3 (3 RCT)	No serious risk of bias; RCT evidence (+4)	No inconsistency; I^2 = 0	Direct (0)	Wide CI (-1)	Cannot confidently assess (0)	0.61 (0.14-2.62) (0)	Moderate
1-year graft survival	4 (4 RCT)	No serious risk of bias; RCT evidence (+4)	No inconsistency; I^2 = 0	Direct (0)	Wide CI (-1)	Cannot confidently assess (0)	1.02 (0.71-1.47) (0)	Moderate

ALT – alanine aminotransferase; AST – aspartate aminotransferase; CI – confidence interval; HTK – histidine-tryptophan-ketoglutarate; PNF – primary non-function; UW – University of Wisconsin

SDC Chapter 12

SDC 1: Baseline transplantation characteristics stratified by graft shipping.

	Shipped	**Unshipped**	*p-value*
Transplants, *n* (%)	256 (18.5)	1125 (81.4)	NA
Donor Age (SD)	40.2 (19.8)	43.1 (17.6)	***0.030***
Donor COD (%) • Trauma • CVA/ICH • Anoxia • Other	 • 60 (23.4) • 121 (47.3) • 59 (23.0) • 16 (6.3)	 • 272 (24.2) • 548 (48.7) • 220 (19.6) • 85 (7.6)	0.611
CIT (hr) (SD)	7.1 (2.5)	6.8 (2.5)	0.097
SWIT (min) (SD)	43.4 (14.2)	42.7 (15.8)	0.535
Perfusion Technique • Aortic • Dual	 • 188 (73.4) • 68 (26.6)	 • 768 (68.3) • 357 (31.7)	0.115
Recipient Age (SD)	52.4 (10.7)	51.6 (11.3)	0.315
Recipient Primary Diagnosis (%) • Fulminant/Subacute Hepatic Failure • Cholestatic Cirrhosis • HBV/HCV-related Cirrhosis • Alcoholic Cirrhosis • HCC • NAFLD/NASH-related Cirrhosis • Other	 • 23 (9.0) • 30 (11.7) • 77 (30.1) • 47 (18.4) • 33 (12.9) • 21 (8.2) • 25 (9.8)	 • 104 (9.2) • 156 (13.9) • 306 (27.2) • 160 (14.2) • 182 (16.2) • 76 (6.8) • 141 (12.5)	0.309
Recipient MELD (IQR)	16 (11-25)	17 (12-25)	0.292

BMI – body mass index; CIT – cold ischemic time; COD – cause of death; CVA – cerebrovascular accident; HBV – hepatitis B virus; HCV – hepatitis C virus; HCC – hepatocellular carcinoma; ICH – intracerebral hemorrhage; IQR – inter-quartile range; MELD – model for end-stage liver disease score; NA – not applicable; NAFLD – non-alcoholic fatty liver disease; NASH – non-alcoholic steato-hepatitis; SD – standard deviation; SWIT – secondary warm ischemic time

The Evolution of Kidney Transplantation Surgery Into the Robotic Era and Its Prospects for Obese Recipients

Ahmer M. Hameed, MS,[1,2,3] Jinna Yao, MS,[1] Richard D.M Allen,[1,2,3] Wayne J. Hawthorne, PhD,[1,2,3] Henry C. Pleass, MD,[1,2,3] and Howard Lau[1,4]

Abstract: Robotic-assisted kidney transplantation (RAKT) represents the most recent innovation in the evolution of kidney transplantation surgery. Vascular techniques enabling kidney transplantation have existed since the early 20th century and contributed to the first successful open kidney transplant procedure in 1954. Technical advances have since facilitated minimally invasive laparoscopic and robotic techniques in live-donor surgery, and subsequently for the recipient procedure. This review follows the development of surgical techniques for kidney transplantation, with a special focus on the advent of robotic-assisted transplantation because of its potential to facilitate transplantation of those deemed previously too obese to transplant by standard means. The different techniques, indications, advantages, disadvantages, and future directions of this approach will be explored in detail. Robot-assisted kidney transplantation may become the preferred means of transplanting morbidly obese recipients, although its availability to such recipients remains extremely limited and strategies targeting weight loss pretransplantation should never be abandoned in favor of a "RAKT-first" approach.

(*Transplantation* 2018;102: 1650–1665)

Kidney transplantation is the most commonly performed solid organ transplant procedure. Establishment of its safety and success has built on an effective method for vascular and ureteric anastomoses that provided the basis for subsequent open, laparoscopic, and robotic transplantation. Each technique has different advantages and disadvantages and is suitable for different patient subsets. In particular, obese recipients have traditionally suffered reduced access to transplantation using the open approach, which at least is in part attributable to impaired access to iliac vessels, greater surgical morbidity and inferior graft outcomes. In this overview, we outline the evolution of kidney transplantation surgery, focusing on the indications and techniques used for robot assisted surgery, and its possible implications for obese patients requiring kidney transplantation.

Received 16 January 2018. Revision received 1 June 2018.

Accepted 8 June 2018.

[1] *Department of Surgery, Westmead Hospital, Sydney, Australia.*

[2] *Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney, Australia.*

[3] *Discipline of Surgery, Sydney Medical School, University of Sydney, Sydney, Australia.*

[4] *Discipline of Surgery, School of Medicine, Western Sydney University, Sydney, Australia.*

The authors declare no funding or conflicts of interest.

A.M.H. participated in article content design, writing, and revision. J.Y. produced the supplementary digital content and participated in article writing, and revision. R.A. participated in article content writing, and revision. W.H. participated in article content writing, and revision. H.P. participated in article content design/outline, writing, and revision. H.L. participated in article content design/outline, writing, and revision.

Correspondence: Howard Lau, FRACS, Department of Surgery, Westmead Hospital, Cnr Darcy Road and Hawkesbury Road, Westmead, NSW 2145, Australia. (drhlau@gmail.com).

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

ISSN: 0041-1337/18/10210-1650

DOI: 10.1097/TP.0000000000002328

VASCULAR ANASTOMOSES AND THE ADVENT OF KIDNEY TRANSPLANTATION

Vascular surgical techniques existed before the work of French Surgeon, Alexis Carrel. However, he is credited for providing the impetus for their implementation in organ transplantation after conducting extensive work in animal organ transplantation, including the auto and allotransplantation of kidneys in collaboration with Charles Guthrie.,[1,2] Carrel's vascular suturing technique, initially published in 1902 and later refined through this collaboration, consisted of a triangulation method for end-to-end anastomoses, using 3 circumferential stay sutures and traction on 2 of 3 sutures while undertaking anastomoses in any particular segment.[2,3] Particular emphasis was placed on the use of fine suturing material and precise instruments.[4] The "Carrel Patch," practical only with the use of deceased donor renal arteries, was also developed during this period, with the aim of minimizing the thrombotic risk associated with small vessel anastomoses.[3]

CLINICAL KIDNEY TRANSPLANTATION AND OPEN SURGERY

After the development of these vascular suturing techniques, Ukrainian surgeon Yuri Voronoy performed the first human kidney transplants, with a succession of 6 human kidney allografts transplanted into the thigh of uremic patients between 1933 and 1949.[5] However, he was unable to achieve successful graft function because of issues related to prolonged warm ischemia and/or donor-recipient mismatch.

Successful human kidney transplantation with subsequent recipient graft function was not performed until Michon in 1953 undertook the first living-donor kidney transplant. It rejected after 3 weeks. Murray subsequently performed the first successful living-donor kidney transplant in the following year[1,6] The kidney donor, an identical twin of the recipient, underwent an open nephrectomy in an adjacent operating theater. Murray used an oblique right lower quadrant incision in the recipient, with the renal artery anastomosed to the internal iliac artery in an end-to-end fashion, and the renal vein to the common iliac vein using an end-to-side anastomosis.[6,7]

Open kidney transplantation is most commonly undertaken using an oblique (Rutherford Morison) muscle-cutting incision, or the pararectal (Alexandre/'hockey stick') incision. An incision of 15 to 20 cm is required for appropriate exposure, and is often longer in the obese. It is either parallel to the inguinal ligament with extension toward the anterior superior iliac spine (Rutherford Morison), or down the lateral margin of the rectus abdominis muscle (pararectal). In comparison to nonobese patients, wound dehiscence, incisional hernia, and wound infection rates, and hospital length-of-stay, are higher in obese open kidney transplant recipients.[8]

OBESITY AND ACCESS DISPARITIES IN OPEN KIDNEY TRANSPLANTATION

Obesity is a major public health concern, and carries specific risks for patients with end-stage renal disease (ESRD). Furthermore, obesity is a significant problem in patients with ESRD, associated with both its incidence and progression.[9,10] Global prevalence data continue to show an increase in the proportion of the obese among the general population and its subsequent adverse effect on healthcare-related costs.[11-13]

Although obesity is not an absolute contraindication to transplantation in most national guidelines, it is quite clear that comorbid obesity restricts such patients' access to donor kidneys.[14-21] The US data show that obesity independently decreases the chances of transplantation (hazard ratio [HR], 0.93), with this trend compounded in proportion to the degree of obesity (HR, 0.56 in the morbidly obese), despite these patients being listed for transplantation.[17] Furthermore, obese recipients are more likely to be bypassed during kidney allocation.[17] European data are concordant with these findings, also showing that each annual 1 kg/m^2 decrease in body mass index (BMI) enhanced the likelihood of transplantation by approximately 10%.[18] Transplantation access may be disparate between obese men and women, with Gill et al[22] showing a reduced likelihood for transplantation among women with a BMI $\geq$ 25 kg/m^2. In comparison, this trend was only seen in men with a BMI $\geq$ 35 kg/m^2. In Australia and New Zealand, obese ESRD patients are less likely to be placed on an active kidney transplant waitlist.[23] Bariatric surgery is likely to allow a greater proportion of morbidly obese dialysis patients to achieve and maintain sufficient weight, become waitlisted and eventually receive a kidney transplant. However, this surgery carries specific risks in dialysis patients and is not always readily available.[19,24-28] Advances in surgical technologies that improve the ease of transplantation in confined spaces, such as robotic transplantation surgery, may help more obese patients escape the rigors of dialysis and improve their quality of life.[29,30]

KIDNEY TRANSPLANTATION OUTCOMES IN OBESE RECIPIENTS

Approaching potential open kidney transplantation in obese recipients warrants special consideration of the added technical challenges of the implantation procedure itself, as well as surgical/wound-related complications and their impact on short- and longer-term graft function.

Obesity significantly compounds the technical difficulty of kidney transplantation surgery using traditional open approaches.[21,31] Unsurprisingly, postoperative complications are higher in this recipient cohort, including wound dehiscence, infection, and lymphocele formation rates.[8,32-37] A positive relationship between obesity, wound infection, and dehiscence, in addition to incisional hernias has been confirmed in a meta-analysis.[8] However, interpretation is made difficult by the limited number of patients in the obese cohort, and the subsequent tendency to use a relatively low BMI of 30 kg/m^2 or greater to define obesity. There is a likely incremental effect the greater the patient's BMI is above this "cutoff."[34]

Adverse impacts of obesity on longer-term clinical outcomes, particularly for well-screened recipients, are less clear. As with any patient population or subtype, the risks and benefits of transplantation in this cohort need to be balanced against remaining on dialysis, with further consideration given to the individual patient's comorbidities and functional status. Transplantation still offers obese dialysis patients a significant survival benefit, albeit less so in patients with a BMI of 40 kg/m^2 or greater.[38,39] Furthermore, patient quality of life after transplantation is not negatively impacted by pretransplantation obesity.[40]

There is also conflicting evidence for the correlation between obesity and poorer patient and graft survival.[41] Obesity as a risk factor for delayed graft function (DGF) was shown in a meta-analysis of 21 studies.[42] A recent analysis of more than 191000 patients from the Scientific Registry of Transplant Recipients database also showed a correlation between obesity and adverse graft outcomes independent of comorbidities such as diabetes mellitus.[41] The authors found that the OR increased from BMI class I to III for DGF (1.47 to 2.43), acute rejection (1.14 to 1.26), and the HR increased for graft failure (1.02 to 1.25). In contrast, an analysis of Australia and New Zealand Dialysis and Transplant registry data showed no association between obesity and graft or patient survival, after accounting for appropriate confounders.[43] Other authors suggest that obesity confers similar risks of graft failure to such factors as recipient diabetes mellitus.[44,45] The confounding nature of BMI as a measure of obesity in contrast to waist circumference also needs to be considered, in addition

to the relative importance of weight gain after transplantation rather than pretransplant weight per se.[45]

Overall, although surgical and/or wound-related complications are increased with obesity, its impacts on graft and patient survival are not absolute, and obesity alone should in no way preclude transplantation. Kidney transplantation in this population still has significant potential with respect to patient survival benefit, as discussed, and remains a superior option compared with the significantly reduced survivals associated with staying on dialysis. Evolving surgical techniques which address the technical challenges in this patient cohort may help reduce immediate surgical morbidity, and thus begin to address disparities in access to transplantation.

Minimally invasive techniques have the potential to benefit transplant recipients of any weight, but in particular, may be 1 approach that helps mitigate donor kidney access disparities for the obese. We will next discuss the evolution of kidney transplant surgery to incorporate such practices, especially focusing on robotic surgery. A historical timeline depicting the evolution of donor and recipient renal transplant surgical techniques is presented in Figure 1.

LAPAROSCOPIC KIDNEY DONOR SURGERY

The introduction of laparoscopic surgery was a major advance in living donor kidney transplantation. The donor patients, preferring smaller incisions that minimize the cutting of muscles, have largely driven this. The first laparoscopic donor nephrectomy (LDN) was reported by Ratner et al[46] in 1995, with the donor discharged home 1 day after surgery. Laparoscopic donor nephrectomy allows for reduced hospital length-of-stay and postoperative analgesic requirements in comparison to open surgery, without adverse impacts on recipient graft outcomes.[47,48] Furthermore, recipient blood loss is not increased, and it is possible to achieve similar operating times to open donor surgery with operator experience.[47,49,50]

Significant variations in LDN technique exist, and include a purely laparoscopic approach, hand-assisted LDN, retroperitoneal LDN, and robot-assisted LDN. The purely intraperitoneal laparoscopic technique is technically more challenging than hand-assisted LDN. Surgeons without other laparoscopic surgery practice are likely to have a preference for the latter, allowing them to safely meet donor patient and referring clinician demand for minimally invasive surgery. By using their nondominant hand as a retractor and a finger to assist dissection, they are afforded a greater margin of safety in the event of a vascular catastrophe. To support this approach, it also can be argued that regardless, the donor kidney still needs to be removed through a hole in the abdominal wall. Retroperitoneal mobilization and removal of the donor kidney is a routine surgical approach for urologists. Although smaller than that for open surgery, abdominal wall muscles need to be divided or retracted to remove the kidney with this approach. Prospective randomized trials comparing different approaches, including the impact of hand assistance, retroperitoneal LDN, and the safety and utility of right versus left LDN, have been conducted and summarized in other reviews.[51-56]

Robot-assisted LDN was first reported in living donors by the University of Illinois (Chicago group) in 2002.[57,58] There is a suggestion that this approach, which facilitates dissection of the renal artery behind the inferior vena cava, may allow the retrieval of right-sided arterial graft with less divided early branches in comparison to LDN.[59] However, without a significant reconstructive component to donor surgery and the increased costs associated with the robot-assisted approach, its utilization above LDN alone is hard to justify on a routine basis.[60] Furthermore, right kidney donation and/or the donation of kidneys with multiple renal arteries and/or from selected obese donors is not precluded by a purely laparoscopic approach.[61-68] Indeed, a more important consideration is a short right renal vein, probably a greater technical challenge for the recipient surgeon and more likely to preclude use of a right donor kidney than multiple right renal artery branches.[69]

The live-donor nephrectomy is a unique procedure in that the operation is performed on a very healthy patient for wholly altruistic reasons. As such, it is paramount that this procedure is undertaken by highly trained providers, and any procedural risks are as close to absolutely negated. Although operative complications are not significantly higher in laparoscopic donors, the rare but potentially catastrophic complication of a loss of vascular control can be difficult to manage especially if this occurs postoperatively.[48,70] Laparoscopic donor surgery has therefore evolved to incorporate safer methods of securing the renal artery stump using transfixion methods such as a vascular stapler and/or locking

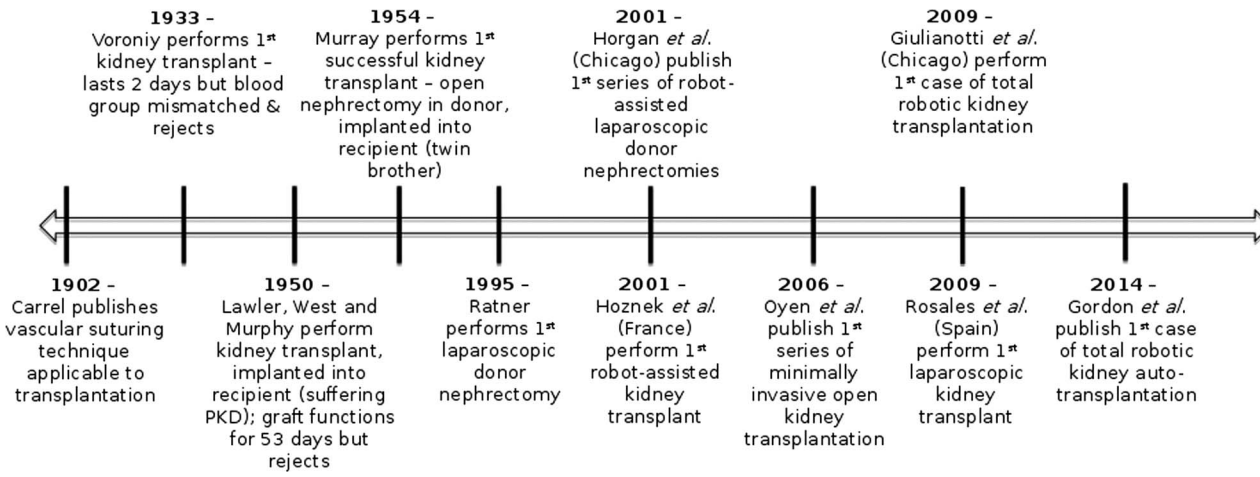


FIGURE 1. Major milestones in the evolution of kidney transplantation donor and recipient surgical techniques. PKD, polycystic kidney disease.

vascular clips such as the Hem-o-Lok (Teleflex, Morrisville, NC).[71,72] However, no single technique is fail-safe, and there have still been reported cases of severe arterial hemorrhage despite the use of these transfixion methods, including vascular staplers.[72-74] The use of locking clips alone is especially problematic in the event of a short renal artery stump, with a risk of delayed clip failure. Furthermore, the manufacturer withdrew support of the use of Hem-o-Lok as the primary method of arterial control during live-donor nephrectomies in 2006.[75] Some surgeons in our unit prefer the combined utilization of a noncutting stapler such as the Endo-TA, in addition to the deployment of the Hem-o-Lok clip on the most proximal staple line.[76] This approach allows for maximal vessel length in addition to a further failsafe against the loss of vascular control (Figure 2).

THE QUEST FOR MINIMALLY INVASIVE TRANSPLANTATION (RECIPIENT) SURGERY

Upon establishment of minimally invasive live-donor techniques, the logical next step was the attempt to use minimally invasive procedures in the kidney transplant recipient to minimize the size and morbidity of the incision associated with open surgery (minimally invasive open kidney transplantation [MIOKT]). Laparoscopic and robot-assisted kidney transplantation (RAKT) was the natural progression for surgeons with appropriate skills and access to equipment. The challenge was to perform such surgery without compromising graft and patient outcomes.

Minimally invasive open kidney transplantation modifies the existing open approach by using a smaller incision size that is 5 to 10 cm, depending on the size of the donor kidney. Øyen et al[77] in 2006 were the first to describe the technique. They used a 7- to 9-cm transverse incision superior to the inguinal ligament to obtain adequate exposure of the iliac vessels. It often necessitated division of the conjoint tendon. Median BMI in a cohort of 21 MIOKT patients was 25.7 kg/m^2 in comparison to 24.4 kg/m^2 for the control group of 21 conventional open-kidney transplants recipients (COKT). Overall, operative time and postoperative stay was impressively less in the MIOKT group (118 minutes vs 187 minutes, and 8.2 days vs 12.4 days, respectively), without any significant differences in surgical complications and DGF.[77] Minimally invasive open kidney transplantation has been described in various publications since this initial report, with some evidence of less requirement of analgesia and return to normal activity in the MIOKT compared with COKT groups. Importantly, short- to medium-term graft outcomes do not seem to be negatively impacted.[78-82]

Laparoscopic kidney transplantation (LKT) endeavors to further reduce incision size while enhancing the surgeon's field of view. The most difficult part of such a procedure is the successful achievement of intracorporeal anastomoses using instruments with limited rotational degrees of freedom. Laparoscopic kidney transplantation requires an access point for the kidney, either via an abdominal incision (up to 7 cm) or the transvaginal route, in addition to the insertion of up to 4 laparoscopic instrument ports. Rosales et al[83] performed the first LKT in 2009. The recipient had a BMI of 22 kg/m^2, and intra-abdominal access was achieved using a 7-cm Pfannenstiel incision, and 3 right-sided access ports. The secondary warm ischemic (anastomotic) time (SWIT) was prolonged at 53 minutes, although an attempt was made to cool the kidney by topical means using ice slush and surface irrigation with cold saline.[83]

Modi et al's group from India has the greatest published experience of LKT.[84-87] A series of 72 patients with an average BMI of 20.5 kg/m^2 underwent LKT after laparoscopic live-donor nephrectomy was compared to a cohort of 145 COKT patients.[86] LKT was conducted using a Pfannenstiel incision, and 4 left-sided abdominal ports. The iliac vessels were accessed transperitoneally and topical cooling was not applied during the anastomoses. Before wound closure, the kidney was fixed in position using a peritoneal flap. Mean wound length in the LKT group was 5.5 cm, in comparison to 17.8 cm in the COKT group. However, the anastomotic and rewarming times were significantly longer in the LKT group (50.3 vs 27.1 minutes, and 60.3 vs 30.3 minutes, respectively). Analgesic requirement was significantly reduced in LKT patients. Although the estimated glomerular filtration rate at 7 and 30 days postoperatively was also significantly lower in the LKT group, estimated glomerular filtration rate values in both groups converged between 3 and 18 months.[86] Modi et al[87] later demonstrated that the transvaginal route could be used in women who had previously undergone vaginal delivery, precluding the need for the Pfannenstiel incision. Mean kidney rewarming time was still prolonged at

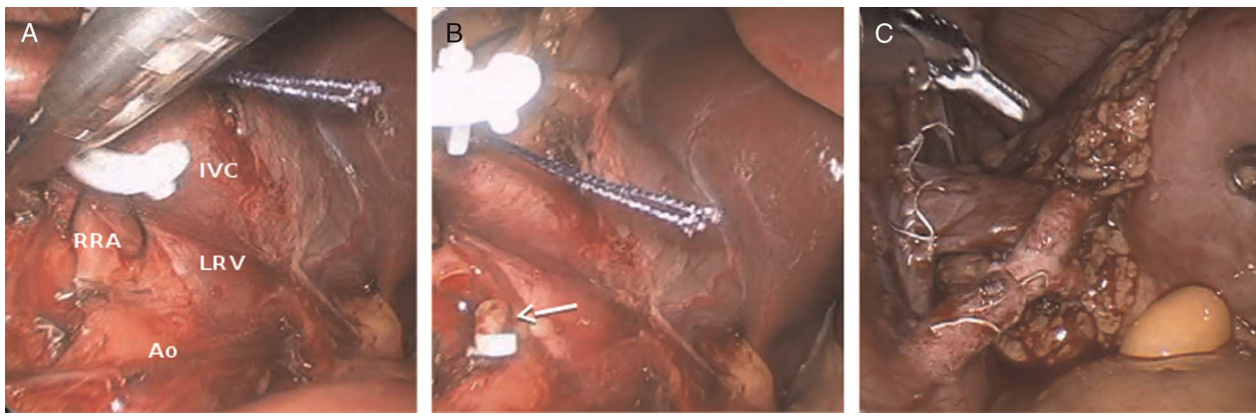


FIGURE 2. Our center's technique for securing the renal artery stump during laparoscopic donor nephrectomy. A, Endo-TA stapler deployed across renal artery stump, B, Secure renal artery stump after transection, with Hem-o-lok clip additionally used across the stapler line close the origin of the artery (arrow), and C, Renal artery and vein visualized after autotransplantation and reperfusion. Ao, aorta; IVC, inferior vena cava; LRV, left renal vein; RRA, right renal artery.

62.9 minutes, therefore extending the ischemic time, whereas analgesic requirements were only marginally less than required when an abdominal incision was used.[87]

There are several limitations attributable to LKT based on the current published experience. The prolonged rewarming time with its potential adverse impact on graft function is probably the most important consideration. Furthermore, LKT is currently only applicable to a very small subset of kidney transplant recipients, with procedures performed by a few select surgeons. Published studies have largely included low risk and thin patients, and explicitly excluded obese recipients and those with significant atherosclerotic disease, and previous lower abdominal surgery. Establishment of pneumoperitoneum for the purposes of LKT may also be problematic, with contribution to respiratory acidosis, and impaired renal perfusion.[86,88] Modi et al, however, suggest that these effects can be ameliorated by maintaining insufflation pressures less than 10 to 12 mm Hg.[86] Regardless, it is probable that LKT is not likely to be feasible in the near future for the majority of kidney transplant recipients, especially those that are overweight or obese.

THE ERA OF ROBOTIC KIDNEY TRANSPLANTATION

Robotic-assisted Kidney Transplantation

The promise and potential of robotic surgery stems from its ability to facilitate complex operations using smaller incisions while simultaneously providing an enhanced field of view and a higher degree of instrument maneuverability. These characteristics are especially beneficial in obese patients who are not suitable for LKT and traditionally require long incisions prone to multiple complications, as already discussed. The da Vinci Surgical System (Intuitive Surgical, CA) has been used in all published reports to date (Table 1), and consists of a "master/slave" set-up. A patient side-cart containing multiple robotic arms must be docked onto the patient, and procedural assistance is provided by assistant surgeon(s) at the patient's side. Robotic-assisted kidney transplantation using systems, such as the da Vinci robot, allows 7 degrees of freedom at the instrument tip, in addition to a 3-dimensional view and significant ergonometric advantages compared to laparoscopy. It also eliminates tremor.[108]

RAKT—Uptake and Variations in Reported Technique Over Time

In 2001, Hoznek et al[89] performed the first reported partial RAKT. Although a full-length open incision was used in the left lower quadrant, this case report nevertheless demonstrated that anastomoses required for transplantation could be performed using a robotic system. Due to robot-specific issues identified by this group, such as a lack of fine haptic feedback, a prolonged SWIT of 57 minutes, and high costs, the adoption of RAKT has been slow. In 2009, Geffner and colleagues performed transperitoneal robotic vascular anastomoses and ureteric implantation after having placed the kidney extraperitoneally through a reduced length iliac fossa incision.[109] A group from Chicago later undertook the first total RAKT, with the primary aim of allowing safe and successful transplantation in a morbidly obese patient.[29] A 7-cm periumbilical incision was used to insert the graft, making use of a hand access device (Lap Disc, Ethicon, Cincinnati, OH). Robot redocking was required for the ureteroneocystostomy component of the procedure, and the final graft position was intraperitoneal.[29]

Boggi et al[90] then described the first purely RAKT procedure in Europe. The primary difference in their approach was that the ureteric implantation was performed in an open fashion after conducting the vascular anastomoses robotically. The graft was also placed in a retroperitoneal pocket before closure.[90] Further evolutionary steps in RAKT were introduced by Menon and colleagues[92] from Detroit in the United States and Medanta in India. The major differences in the approach used by these groups from that of the Chicago group, included the introduction of regional hypothermia to counteract the effects of prolongation of SWIT, extraperitoneal repositioning of the graft after implantation to avoid the risk of torsion, and the avoidance of robotic redocking for performance of the ureteroneocystostomy.[95]

All of the aforementioned approaches required a 4- to 7-cm incision for graft insertion. An alternative used by some centers in female recipients is transvaginal graft insertion.[99,103,110-112] Suitability for the transvaginal route requires preoperative assessment. For example, Modi et al in the situation of LKT, ensure both an absence of local infection and/or malignancy, in addition to sufficient vaginal laxity.[87] It is likely that only women who have previously undergone normal vaginal delivery will be suitable for this approach, and gynecologic input is essential. Bacteriologic contamination can potentially be reduced by kidney insertion using a sterile bag or wound retractor. Analgesic requirements are likely to be reduced, although data are very limited.[87,103]

RAKT—A Summary of Techniques

Techniques used in all published RAKT articles to date are summarized in Table 1. The RAKT technique does not require significant variation for obese recipients.

Entering the Abdomen

Robotic-assisted kidney transplantation requires port placement for visualization of the surgical field and controlled intra-abdominal access of robotic instruments. There is no set port placement for the performance of RAKT but is not dissimilar to common positioning used for other pelvic procedures, such as prostatectomies and cystectomies. Access points used by the groups in Chicago and Detroit/Medanta are summarized in Figure 3.[29,93,95] Port types common to all centers include those for robotic arms, bedside assistant port(s), and a camera port. The size of each individual port tends to range between 8 and 12 mm, depending on the system used.

Recipient Vascular and Bladder Dissection

The external iliac vessels are prepared for anastomoses in the vast majority of published reports, although Boggi et al used the common iliacs (Table 1).[90] Vascular access is most commonly achieved by the transperitoneal approach. Peritoneal flaps may be created if the graft is to be retroperitonealized after implantation.[90,93] The catheterized bladder may be prepared at this stage,[93,95] or after vascular anastomoses are completed.[29]

Graft Preparation and Insertion

Meticulous back-table preparation of the graft is essential to minimize recipient blood loss in RAKT because the

TABLE 1.

Published articles describing the utilization of RAKT

Author, year study type	Study center(s)	Indication(s) for robotic transplant/BMI Donor type(s)	Robotic technique—Ports[a]/ incisions/anastomoses (graft placement)	Patients (n) – Robotic vs standard groups	SWIT/rewarming time (min)—Robotic vs standard groups	Cooling technique employed during SWIT	Postoperative outcomes and/or complications
Hoznek et al, 2002[89]	Creteil, France	Nil specific (BMI, NR) DBD Donor	Left lower quadrant *open* incision; 2 × robotic arms +1 × central camera arm Da Vinci Robot EIV/EIA/bladder (extraperitoneal)	1 (RAKT)	57 (SWIT)	Ice-cooled pads placed on kidney during anastomoses	Unclear if DGF by dialysis criteria; Cr drop from 8.5 to 1.7 mg/dL on day 8
Case report							
Giulianotti et al, 2010[29]	Chicago, IL	Morbid obesity (BMI, 41)	Periumbilical incision (7 cm); 4 × additional ports Da Vinci Robot Anastomoses to EIV/EIA/bladder (intraperitoneal)	1 (RAKT)	50 (SWIT)	Nil	D/C on POD 5; Cr 1.3 mg/dL on day 5
Case report		DBD donor					
Boggi et al, 2011[90]	Pisa, Italy	Nil specific (BMI, 21.9)	Pfannenstiel incision (7 cm); 3 × additional ports Da Vinci Robot CIV/CIA (retroperitonealized); bladder anastomoses w/o robot	1 (RAKT)	51 (SWIT)	Nil	D/C on POD 10; Cr 1.4 mg/dL on day 10 and 3 months postoperatively
Case report		Living donor					
Oberholzer et al, 2013[30]	Chicago, IL	Obesity (mean BMI in RAKT group 42.6 vs 38.1 in retrospective control COKT cohort)	Periumbilical incision (7 cm); 4 additional × ports Da Vinci Robot Anastomoses to EIV/EIA/bladder (intraperitoneal)	28 (RAKT)[b] 28 (COKT)	47.7 (SWIT; RAKT) 49.2 (SWIT; COKT)	Nil	DGF in 1/28 RAKT, 0/28 COKT; Wound complications 1/28 RAKT, 8/28 COKT ($P < 0.05$); No significant differences between study groups with respect to acute rejection, graft/patient survival at 6 months; total hospital costs significantly greater in RAKT group; initial hospital LOS 8.2 RAKT, 8.1 COKT; Conversion to open surgery in 2/39 patients[b]
Cohort study (prospective RAKT, retrospective + matched COKT)		2/28 in each group Deceased donors; 26/28 in each group Living donors					

Continued next page

TABLE 1. (Continued)

Author, year study type	Study center(s)	Indication(s) for robotic transplant/BMI Donor type(s)	Robotic technique—Ports[a]/ incisions/anastomoses (graft placement)	Patients (n) – Robotic vs standard groups	SWIT/rewarming time (min)—Robotic vs standard groups	Cooling technique employed during SWIT	Postoperative outcomes and/or complications
Abaza et al, 2014[91]	Columbus/Detroit, MI; Medanta, India	Nil specific (BMI NR)	Periumbilical incision, vertical (4-5 cm); 4 × additional ports Da Vinci Robot EIV/EIA/Bladder (retroperitonealized)	29 (RAKT)	NR	Kidney introduced in gauze jacket containing ice slush; 120-180 ml ice slush instilled onto surgical bed + then onto graft	100% graft survival at 3 months; other results not clarified
Prospective case series		Living donors					
Menon et al, 2014a[92]	Medanta, India; Columbus/Detroit, MI	Exclusion criteri—2nd Tx; iliac blockage >30%; previous major abdominal surgery; immunologically high risk Tx; dual/ multiorgan Tx (phase 1 study) (mean BMI, 24.2; max, 29.8)	As per Abaza et al, 2014 (Retroperitonealized—peritoneal flap used)	7 (RAKT)	51.4 (Rewarming Time)[c]	Ice slush instilled onto surgical bed; mean kidney temperature at reperfusion 22.5 °C	DGF in 0/7 patients; Wound complications NR; Mean Cr at POD 7 was 1.2 mg/dL
Prospective case series		Living donors					
Menon et al, 2014b,[93] Sood et al, 2014,[94] Sood et al, 2015,[95] Sood et al, 2016[96]	Medanta, India; Columbus/Detroit, MI	Exclusion criteria—as per Menon et al, 2014a (phase 2a study) (mean BMI, 24.1; max, 42.3)	Periumbilical incision, vertical (4-5 cm); 4 × additional ports Da Vinci Robot EIV/EIA/bladder (retroperitonealized)	54 (RAKT)[d]	42.9 (Rewarming time)[c]	Kidney introduced in gauze jacket containing ice slush; 180-240 ml ice slush instilled onto surgical bed + then onto graft; mean kidney temperature at reperfusion 19.2 °C	DGF in 0/54; Wound complications in 0/25[e]; Acute rejection in 7/54; Mean Cr at 6 months was 1.2 mg/dL; Graft survival 52/52 (death-censored) at 6 months; Graft thrombosis in 0/54; Conversion to open surgery in 0 patients
Prospective case series		Living donors					
Tsai et al, 2014[97]	Taipei, Taiwan	Nil specific recorded (mean BMI, 22.8; max, 28.2)	Modified oblique (Gibson) incision (7-9 cm); 2 × additional ports; no gas insufflation Da Vinci Robot EIV/EIA (extraperitoneal at all times); bladder anastomoses w/o robot	10 (RAKT) 1 (COKT)[f]	67.4 (SWIT)	Unclear	DGF in 1/10; Cr at D/C was 1.3 mg/dL; Wound complications in 0/10; Acute rejection in 2/10; Graft survival 100% at mean F/U of 6.9 months
Case series		3/10 DBD donors; 7/10 Living donors					

Ayloo et al, 2015[98]	Chicago, IL	Morbid obesity (BMI, 42)	Upper midline incision (?length); 4 × ports [*combined sleeve gastrectomy + RAKT* – redocking required] Da Vinci Robot Anastomoses to EIV/EIA/bladder (intraperitoneal)	1 (RAKT) + Sleeve Gastrectomy	40 (SWIT)	Nil	Nil DGF; D/C on POD 4; Nil nutritional deficits; Nil wound complications
Case report		Living donor					
Doumerc et al, 2015[99]	Toulouse, France	Nil specific recorded (BMI, NR)	Transvaginal graft insertion; 4× additional ports Da Vinci Robot EIV/EIA/Bladder (retroperitonealized)	1 (RAKT)	55 (SWIT)	Unclear	Not specifically recorded
Case report		Living donor					
Frongia et al, 2015[100]	Cagliari, Italy	Nil-specific recorded (BMI, 23.8)	Upper midline incision (7 cm); 7× additional ports Da Vinci Robot EIV/EIA/bladder—right side followed by left side (Intraperitoneal)	1 (RAKT) – *Dual* Kidney Tx	45 (SWIT, Right) 49 (SWIT, Left)	Kidney introduced in gauze jacket containing ice slush	D/C on POD 7; Cr 1.1 mg/dL on day 7; Cr 1.3 mg/dL at 24 months; Nil wound infection
Case report		DBD donor (marginal)					
Breda et al, 2016,[101] Breda et al, 2017[102]	Barcelona, Spain	Nil specific recorded (mean BMI 26, max 33)	Periumbilical incision, vertical (6 cm); 4× additional ports Da Vinci Robot EIV/EIA/bladder (retroperitonealized)	17 (RAKT)	51.5 (Rewarming Time)[c]	Kidney introduced in gauze jacket containing ice slush + topical application of ice slush onto graft	DGF in 1/17; Mean LOS 6 days; Cr 1.8 mg/dL at 7 days and 1.4 mg/dL at 1 month; Nil wound complications; Vascular Thrombosis in 1/17 (with graft loss); Conversion to open surgery in 0 patients
Case series		Living donors					
Doumerc et al, 2016[103]	Toulouse, France	\Nil-specific recorded (BMI NR)	As per Doumerc et al, 2015; transvaginal removal of kidney from donor, and transvaginal insertion into recipient	1 (RAKT)	45 (SWIT)	Unclear	D/C on POD 4; Cr ~1.1 mg/dL on day of D/C;? No need for postoperative analgesia; Nil significant complications reported
Case report		Living donor					
Tugcu et al, 2016[104]	Istanbul, Turkey	Nil specific recorded (mean BMI, 22.6)	Periumbilical incision (4-5 cm); 4 additional ports Da Vinci Robot EIV/EIA/Bladder (retroperitonealized)	15 (RAKT)	73.3 (rewarming time)[c]	Kidney introduced in drape jacket containing ice slush; ~220 mL ice slush instilled onto graft during SWIT	Mean Cr at D/C 1.5 mg/dL; Ileus in 2 patients (requiring laparotomy) attributed to excess ice slush use; Conversion to open surgery in 0 patients
Case series		Living donors					

Continued next page

TABLE 1. (Continued)

Author, year study type	Study center(s)	Indication(s) for robotic transplant/BMI Donor type(s)	Robotic technique—Ports[a]/ incisions/anastomoses (graft placement)	Patients (n) – Robotic vs standard groups	SWIT/rewarming time (min)—Robotic vs standard groups	Cooling technique employed during SWIT	Postoperative outcomes and/or complications
Doumerc et al, 2017[105]	Toulouse, France	Morbid obesity (BMI, 37-40)	Supraumbilical incision (4 cm); 4× additional ports Da Vinci Robot EIV/EIA/bladder (retroperitonealized)	2 (RAKT)	44.5 (SWIT, mean)	Unclear	Patient 1: D/C on POD 10; Cr 2.5 mg/dL on day of D/C; Nil surgical complications Patient 2: D/C on POD 9; Cr 2.3 mg/dL on day of D/C; Nil surgical complications
Case series		1/2 Deceased donor; 1/2 living donor					
Garcia-Roca et al, 2017[106]	Chicago, IL; UNOS Registry	Obesity (only patients with a BMI ≥ 40 were included in analyses)	As per Oberholzer et al, 2013	67 (RAKT)[g] 545 (COKT)	NR	Unclear	DGF 2/67 RAKT patients, 31/545 COKT patients ($P > 0.05$); Acute rejection in 2/67 RAKT, 10/545 COKT ($P > 0.05$); Thrombotic graft loss 0/67 RAKT, 7/545 COKT ($P > 0.05$); No differences in 1 and 3-year patient and graft survival between both groups; no significant differences in serum Cr up to 3 y postoperatively; no significant differences in hospital stay or readmissions between both groups
Retrospective cohort/ registry analysis		Living donors					
Breda et al, 2018[107]	Europe (8 centers—Spain, Turkey, France, Germany, Belgium, Italy)	Nil specific recorded (patients with BMI ≤ 40 were explicitly excluded) (median BMI, 25.2)	As per Breda et al, 2016 and 2017; transvaginal graft insertion in 4/120 patients	120 (RAKT)	50.0 (Rewarming time)[c]	Kidney introduced in gauze jacket containing ice slush + topical application of ice slush onto graft	DGF 5/120 patients; Median LOS 7 days; Cr 1.5 mg/dL at 7 and 30 days; Thrombotic graft loss (arterial) 3/120 patients; Bleeding requiring laparotomy in 5/120; Conversion to open surgery in 2/120
Prospective case series		2/120 Deceased donors; 118/120 living donors					

[a] In addition to any ports placed via hand access device/main incision.
[b] Only 28/39 patients included analyses due to at least 6-month follow-up.
[c] Time with ice slush present (after kidney removal from cold static storage, until reperfusion).
[d] Results discussed for only 54/67 patients due to at least 6-month follow-up; some patients overlap with Menon et al, 2014a.
[e] Not reported in most recent paper.
[f] Planned robotic case, but did not proceed with robot docking due to significant adhesions around femoral vessels.
[g] Overlap with patients/data from Oberholzer et al, 2013.
CIA, common iliac artery; CIV, common iliac vein; Cr, creatinine; DBD, donation after brain death; D/C, discharge; EIA, external iliac artery; EIV, external iliac vein; F/U, follow-up; LOS, length-of-stay; NR, not recorded; IIA, internal iliac artery; POD, postoperative day; Tx, transplant; UNOS, United Network of Organ Sharing; w/o, without.

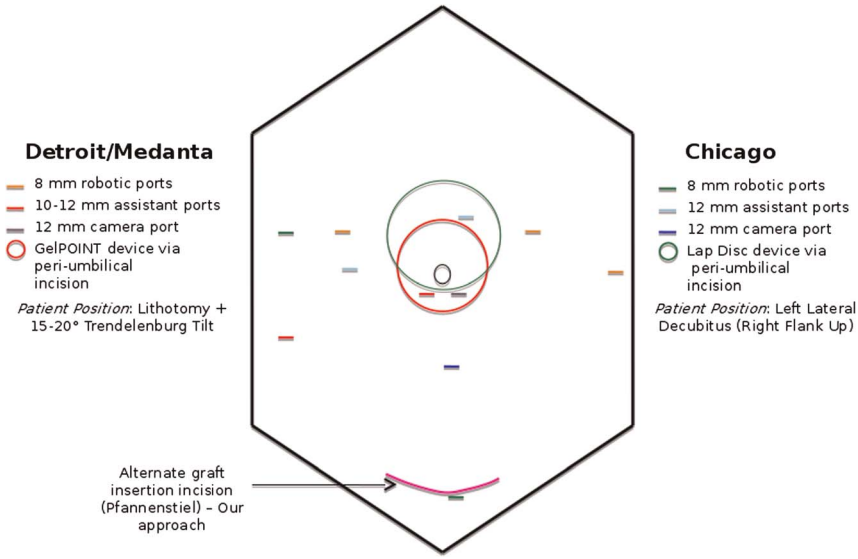


FIGURE 3. Robot-assisted kidney transplantation port placement/access points—2 common techniques.

surgeon does not have the ability to readily apply pressure over bleeding site(s), and the extensive use of suction results in a loss of pneumoperitoneum. A useful technique described by the Chicago group in the context of robotic simultaneous pancreas/kidney transplantation involves a back-table arterial flush using University of Wisconsin solution containing methylene blue dye to identify any obvious vascular leaks.[113] Estimated blood loss does not appear to be higher after RAKT in comparison to conventional techniques, as reported by the current major proponents of RAKT.[30,93]

Graft insertion requires an incision that can accommodate kidney size. Transabdominal incisions have been described in a periumbilical position, directed vertically,[29,93,102] horizontally in the suprapubic region (Pfannenstiel),[90] or upper midline.[100,105] Gel-based hand-assisted devices are generally required to seal these incisions, and include the Lap Disc (Ethicon, Cincinnati, OH) and GelPOINT devices (Applied Medical Inc., Rancho Santa Margarita, CA); further ports can also be placed through these.[29,90,93] Alternatively, the aforementioned transvaginal access avoids the need for an abdominal incision. After vaginal access is created, an Alexis wound retractor (Applied Medical Inc., Rancho Santa Margarita, CA) can allow subsequent graft insertion.[99]

Incorrect kidney orientation during implantation can be avoided by marking the lower pole of the kidney. In our practice, all kidneys are placed in a "stockinette" to allow ease of handling and manipulation of the graft during anastomoses. Easier identification of the lower pole of the kidney after it is covered by the "stockinette" is facilitated by placing a large knot inferiorly.

Patient Positioning

The Chicago technique involves left lateral decubitus patient positioning during RAKT.[29] In contrast, other RAKT units introduce a degree of Trendelenburg ("head-down") tilt (15-20 degrees) to facilitate better intraoperative exposure.[95] Anecdotally, the degree of Trendelenburg tilt may be greater in obese recipients with increased intra-abdominal fat. Important anesthetic considerations with this patient position include potential impacts on lung expansion/compliance, impaired cardiac output (due to effects in combination with pneumoperitoneum), raised intracerebral/ocular pressures, and the potential for lower limb ischemia.[95,114,115] This may necessitate the avoidance of RAKT in patients particularly at risk from these factors. Importantly, however, authors have demonstrated that a head-down tilt of up to 40 degrees does not necessarily compromise cardiorespiratory function, and cerebral perfusion and oxygenation.[116] Our view is that safety can be further enhanced by utilization of the "modified Z Trendelenburg position," which is designed to maintain the head and shoulders in the horizontal position, and significantly ameliorates any impacts of raised intraocular pressure.[117] Furthermore, patient suitability and positioning should always be planned preoperatively and discussed with the involved anesthetic team.

Kidney Transplantation

A major advantage of total RAKT is provision of significantly improved vision over open procedures while performing the vascular anastomoses. They can be achieved with standard techniques in an end-to-side or end-to-end fashion, using atraumatic vascular instruments.[29,90,93] The different types of available robotic instruments has been summarized by Boggi et al.[90] Polytetrafluoroethylene is the commonly used suture material because it displays minimal suture memory.[30,95] After the kidney transplant is revascularized and hemostasis achieved, the robot may need to be redocked for the ureteroneocystostomy, depending on the patient's original position.[29,95] The ureteric anastomosis is generally created over a ureteric stent inserted into the ureter/bladder.[29,93] Previously fashioned peritoneal flaps and/or the cecum can then be used to reposition the graft in the extraperitoneal plane.[90,93]

Graft Cooling and the Amelioration of a Prolonged SWIT

Graft insertion, positioning, and creation of vascular anastomoses may prolong the anastomotic time (SWIT) during RAKT in comparison to COKT; a summary of SWITs/rewarming times for published reports is outlined in Table 1,

and range from 40 to 73 minutes. A prolonged SWIT in turn is associated with inferior short- and longer-term graft outcomes.[118] Although there is some evidence that cumulative RAKT experience helps shorten the time taken to perform vascular anastomoses,[94,119,120] active attempts at graft cooling after its removal from cold storage may be protective even when SWIT is prolonged.[120] Menon et al introduced a technique of RAKT incorporating regional hypothermia, achieved by the instillation of ice slush onto the vascular bed and graft, after first surrounding the kidney with a gauze jacket containing ice slush during its introduction into the abdomen.[92,93] This technique has since been replicated by other groups, with kidney surface temperature before reperfusion remaining below 20°C.[95,102,104,121,122] In a porcine study, Meier et al used an alternative graft cooling technique comprising a kidney jacket through which ethanol and methylene blue was continuously recirculated at 4°C.[123] Using this system, mean kidney temperature was 6.5°C at reperfusion, with evidence that ischemia-reperfusion injury in cooled kidneys was less compared to noncooled controls.[123] At present, there is no published clinical comparison of RAKT outcomes with and without the utilization of graft cooling techniques.

In addition to active graft cooling, the impact of overall graft ischemia times can be reduced by the simultaneous commencement of recipient and live donor surgery in 2 separate operating rooms.[95,101]

RAKT and the Obese Recipient

Robotic-assisted kidney transplantation has the potential to enhance the numbers of obese patients receiving a kidney transplant. Table 1 summarizes patient BMI in each published RAKT report. It can be seen that although some centers have explicitly excluded patients with a BMI of 40 kg/m^2 or greater,[107] others have either focused purely on obese patients, or at least allowed for RAKT in the morbidly obese.[30,95,105,106,121]

Obesity has been the primary indication for RAKT by the Chicago team.[29,30,106] The rationale for this approach is to facilitate an increased transplantation rate in this recipient group while minimizing wound complications, such as surgical site infections, and thereby also indirectly targeting graft loss.[124] Obese patient selection followed standard guidelines for kidney transplant recipients. In comparison to obese COKT recipients, initial work by this group demonstrated that RAKT reduced wound complications (3.6% vs 28.6%, $P = 0.02$) with no significant adverse impact on graft outcomes, including DGF ($P = 0.99$), acute rejection ($P = 0.99$), 6-month graft survival (100% in both groups), and serum creatinine at 6 months (1.5 mg/dL vs 1.6 mg/dL, $P = 0.47$).[30]

A later analysis by the same group compared outcomes between their RAKT recipients with all COKT obese recipients registered in the United Network of Organ Sharing database.[106] Overall, there were 545 COKT recipients and 67 RAKT recipients; although both groups had a mean BMI > 40 kg/m^2, patients in the RAKT group still had significantly higher BMIs. Patient and graft survival up to 3 years after transplantation was not different (96.8% and 89.7%, respectively, for RAKT patients, compared to 94.6% and 90%, respectively, for the COKT group). None of the RAKT grafts were lost secondary to thrombosis, infection, or urologic complications. Unfortunately, wound infection rates were not available for comparison, and interestingly hospital length-of-stay was not reduced by RAKT.[106] In France, Doumerc et al[105] outlined the potential utility of RAKT to reduce the access disparity for transplanted kidneys in the obese by transplanting 2 recipients with BMIs of 37 and 40 kg/m^2, respectively, who had been previously been refused transplantation. Neither patient suffered postoperative complications, with satisfactory graft function at 3 months posttransplantation.[105]

Although bariatric surgery is not always readily available, it is certainly more widespread than RAKT and remains a feasible option that can lead to more possible transplant recipients than RAKT alone.[8,24,26-28,125] The feasibility of simultaneous RAKT and sleeve gastrectomy for weight loss has also been described by the Chicago group, with a randomized trial currently in progress.[98,126]

Overall, although these articles have demonstrated the significant potential of RAKT in obese recipients, it is unlikely that RAKT on its own will improve overall access to transplantation for obese recipients. This is in part related to the limited availability of the RAKT because of cost. Furthermore, more large-scale evidence is required for the routine implementation of such an approach. In particular, outcomes after RAKT, and especially in the morbidly obese, need to be compared with obese patients remaining on dialysis with respect to survival and quality of life. In addition, the most productive approach to reducing transplantation access disparities in obese recipients should always encompass simultaneous aggressive weight-loss strategies to mitigate the impacts of the patient's obesity on their overall health.

Utilization and Uptake of RAKT

Donor Source

Most of the world's experience in RAKT is in living donor kidney transplantation (Table 1), with very few published articles describing utilization of the robotic technique for deceased donor kidneys in a small number of patients.[29,30,97,100,105] The likely explanation for this is that living donor transplantation involves the use of ideal donor kidneys at a planned time and under controlled circumstances, allowing the preparation of the necessary equipment, and fully robotically trained theater and surgical staff.

Robotic Experience

The introduction of RAKT at a transplant center necessitates previous robotic surgical and kidney transplantation experience. Sood et al[94] demonstrated that the learning curve for RAKT is minimal in the presence of extensive robotic experience (defined by the completion of >300-2000 cases). Prolific robotic surgeons performed better than surgeons with little robotic experience (< 10 cases) but extensive COKT experience (> 2000 cases) with respect to procedure learning curves and task completion. Surgeons with both robotic (> 300 cases) and COKT (> 2000 cases) experience performed the best with regard to a lack of learning phase for completion of anastomoses.[94] Importantly, however, a lack of robotic experience did not negatively impact graft functional outcomes, with the authors' conclusion that this was related to the utilization of regional hypothermia.[94]

Breda et al,[107] in their recent publication of the European experience, outlined that all surgeons had performed significant numbers of both robotic cases and COKT (> 100 of each) and had undergone further supervised RAKT training

using animal models. Additional supervision was provided during each surgeon's initial 4 RAKT cases in human recipients. Overall, previous robotic experience in the pelvis seems to be essential to the successful and safe performance of RAKT, more so than sole COKT experience. Centers aiming to introduce RAKT need to take this into account, and potentially need to hone surgeons' skills by practicing necessary techniques on animal models or cadavers.[92,107,127] Similarly, oncology surgeons have demonstrated the advantages of activities to reduce robotic learning curves with prescribed curricula; similar models could lead to the wider implementation of RAKT.[128-133]

Costs

Any decision to implement RAKT at a transplant center must take into account the higher costs of such a system to cover the purchase of the robot itself and purpose-made disposable consumables. Large-scale cost-benefit analyses comparing RAKT and COKT have not yet been conducted, largely due to limited worldwide experience using RAKT. Oberholzer et al[30] did however outline individual transplantation costs for RAKT and COKT in their cohort of patients. When comparing the transplant procedure alone, without consideration of the cost of the robotic system itself, RAKT was still significantly more expensive per transplant (approximately 75 000 US dollars in comparison to 60 000 US dollars for COKT). Six-month hospital costs were also significantly higher in the RAKT group by approximately 20 000 US dollars per patient.[30]

It is expected that the cost of robotic surgery will decrease over time, especially as surgeons' experience and outcomes improve. In our own experience, the operating theatre costs of RAKT are very similar to robot-assisted prostatectomies (~10 000 USD). Reassuringly, in the sphere of prostate surgery, increased operating costs of the robotic procedure are offset by reductions in hospital length of stay, and cost-neutrality can be achieved after the performance of 140 cases per year.[134]

Relative Contraindications and Patient Selection

Absolute contraindications for RAKT are likely to be the same as COKT. However, factors that might limit the application RAKT now are likely to change as operator and center experience increases, and robotic technology and feedback improves. For example, initial reports from the Detroit/Medanta groups excluded recipients with significant iliac artery atherosclerosis, a previous kidney transplant, likely intra-abdominal adhesions secondary to major abdominal surgery, a high risk of rejection, and dual/multiorgan recipients.[92,93] In contrast, the Chicago team did not preclude previous transplant recipients or immunologically at-risk transplants.[30] Indeed, the robotic technique has been taken up in an expanded fashion and has been demonstrated to be possible for the performance of simultaneous pancreas/kidney transplants and dual-kidney transplantation.[100,113,135]

Recipient atherosclerotic disease is probably the most important consideration with respect to appropriate patient selection due to the potential for disaster if clamps do not achieve adequate vascular control, compounded by the loss of haptic feedback with the robot that precludes accurate intraoperative assessment. In the interests of patient safety, and especially during the early implementation of RAKT, it is probably prudent to continue to be wary of recipients with significant risk factors for atherosclerosis, and preoperative computed tomography should be used to identify any subclinical iliac vessel disease.[107]

Autotransplantation and RAKT Beyond Obesity

Robotic assistance can be used to achieve complete intracorporeal autotransplantation for such indications as hematuria loin-pain syndrome and obstructing ureteric strictures. Gordon et al[136] published the first report of a completely robotic kidney auto-transplant, using intracorporeal cooling with a continuous, cold Hartmann's flush that was delivered via the renal artery before anastomoses. This therefore meant that the kidney did not need to be extracted via a separate abdominal incision for ex vivo cooling before reimplantation. Case reports of total robotic autotransplantation have subsequently been reported by other groups.[137,138]

Our center has commenced the implementation of RAKT specifically for kidney autotransplantation. We propose an expanded utilization of robot-assisted renal autotransplantation itself in procedures requiring the repair of complex renal artery aneurysms. In our first case demonstrating this approach (Figure 4; SDC 1, http://links.lww.com/TP/B592), a 22-year-old patient with a proximal left renal artery stenosis, and poststenotic aneurysm located just proximal to the renal artery bifurcation, underwent a LDN, ex vivo vascular bench repair, and subsequent heterotopic RAKT. This patient had poorly controlled hypertension despite the use of multiple agents. In this case, the renal artery anastomosis to the external iliac artery was performed in and end-to-side manner before the renal vein. One week after surgery, the patient was normotensive without need for antihypertensive medication.

Overall Outcomes and Complications After RAKT

Table 1 summarizes outcomes after RAKT in the published reports to date. Early experience with totally robotic kidney transplantation indicated a slower creatinine decline after RAKT patients compared with COKT. This difference was no longer significant at 6 months.[30] It is likely that the creation of pneumoperitoneum during RAKT contributes to this initial reduction in graft function.[30,139] For this reason, the Detroit/Medanta system reduces gas insufflation pressure from 15 to 8 mm Hg after kidney revascularization, although any beneficial effects of this are still unclear.[93]

Currently, there is minimal published evidence comparing the efficacy of RAKT to other kidney transplantation approaches. Published articles and conference proceedings are dominated by case reports or case series. No randomized control trials have been published. Nevertheless, comparative graft function and survival does not seem to differ in selected patients when compared to COKT.[106,121] Sood et al[121] prospectively compared RAKT (n = 59) and COKT (n = 168) patients in phase 2B of their study based in Medanta and Detroit, showing no difference in graft and patient survival. In concordance with results from the Chicago group, wound complications were reduced when RAKT was used, in addition to postoperative pain and subsequent analgesic requirements.[30,121]

Conversion to open surgery is uncommon, and was reported in only 5 of 256 patients in published articles where this outcome was commented on.[30,95,97,102,104,107] Lymphocele formation is potentially reduced after RAKT and is likely explained by the intraperitoneal rather than conventional

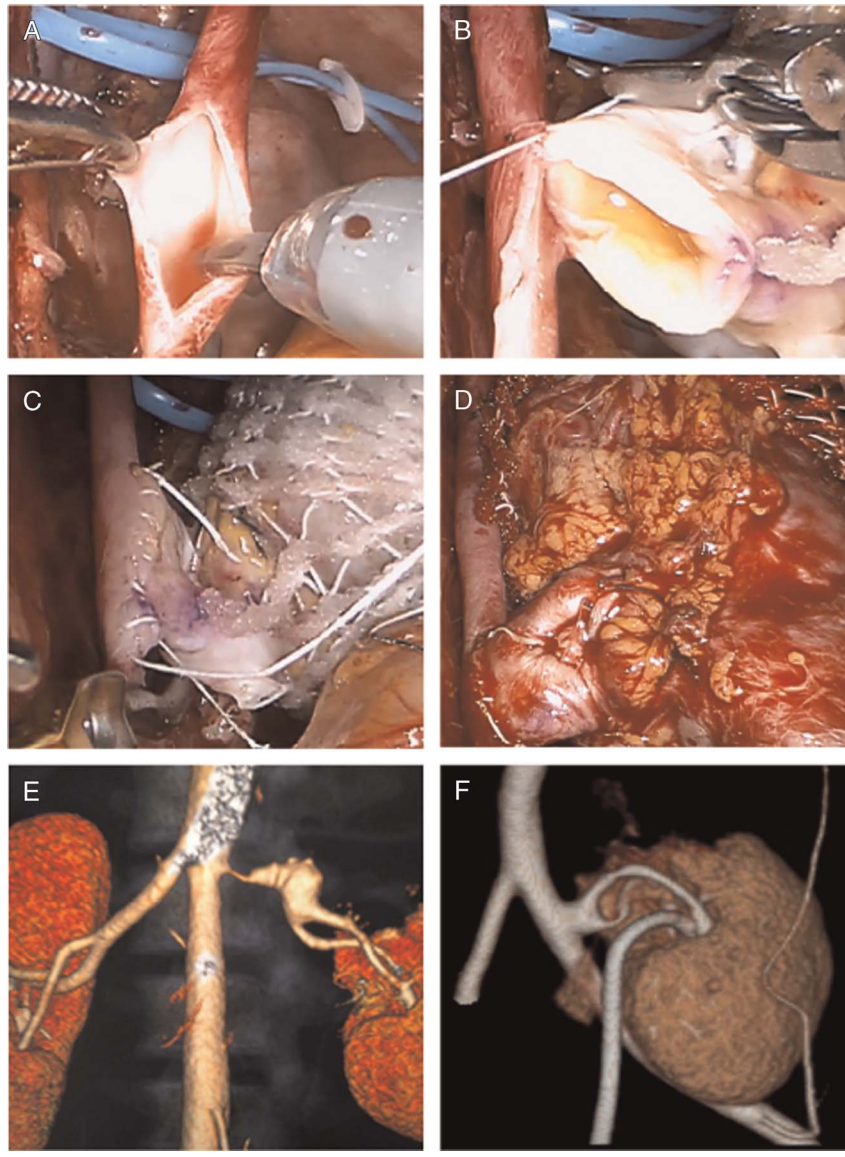


FIGURE 4. Robot-assisted autotransplantation of the kidney in the case of a complex left renal artery aneurysm. After laparoscopic donor nephrectomy and ex vivo bench repair, the kidney was heterotopically autotransplanted using a total robotic approach. A, Arteriotomy, B, Commencement of arterial anastomosis (n.b. severely atherosclerotic renal artery), C, Completion of arterial anastomosis, and D, Reperfusion after completion of vascular anastomoses. E-F, Preprocedural and postprocedural CT angiograms. n.b. A previous attempt at endoluminal repair caused thrombosis of an upper pole renal artery, thereby rendering the superior pole nonperfused.

extraperitoneal surgery.[121,140] In the consideration of graft placement, torsion is a theoretical risk of intraperitoneal positioning. However, this complication has not yet been described by the Chicago group, which routinely uses such a technique. Furthermore, intraperitoneal positioning necessitates a laparoscopic procedure under general anesthetic when a renal biopsy is required. This is unnecessary when the kidney is extraperitonealized.[30,108] Regional hypothermia induced through the instillation of ice slush has been blamed for ileus in 2 cases in 1 series, although a reduction in the amount of slush used may prevent this from occurring.[104] Graft thrombosis after RAKT necessitating transplant nephrectomy has been reported in the literature, although this is reassuringly uncommon.[107,122]

When considering minimally invasive kidney transplantation techniques in general, there is 1 recently published systematic review comparing such an approach to COKT.[141] Not surprisingly, overall quality of evidence for the included studies was low. However, this review did cautiously conclude that surgical/wound complications and patient recovery may be enhanced by minimally invasive transplantation without negatively impacting graft outcomes.[141]

CONCLUSIONS AND FUTURE DIRECTIONS

Robotic-assisted kidney transplantation presents the latest innovation in the evolution of kidney transplantation surgery. It is a highly specialized procedure performed only by a limited number of kidney transplant centers. A variety of technical variations exist, with differences in patient positioning, incision(s), port placement, graft placement, and the utilization of kidney cooling techniques. All techniques still require an incision ranging from 4 to 7 cm for graft insertion, depending on kidney size. Ischemic times may be prolonged

with the robotic approach, although active cooling of the graft before reperfusion should at least partially ameliorate this problem. The majority of RAKT has been undertaken using live-donor kidneys and in carefully selected recipients. Randomized controlled trials, and indeed even prospective or retrospective cohort studies, are still required to compare and confirm the long-term safety and efficacy of RAKT in preference to COKT.

The future expansion in the implementation of RAKT requires the appropriate facilities and technical expertise, and must be balanced with the increased costs of such an approach. New RAKT programs must involve surgeons with both extensive open transplantation and robotic surgical expertise, potentially supplemented by formal robotic training curricula and training using animal and/or cadaveric models, such that patient safety is maintained, especially for obese recipients. Furthermore, the increased costs of RAKT must be balanced against any potential benefits conferred to recipients.

Although bariatric surgery is an important and feasible consideration in morbidly obese ESRD patients, and is more commonly available, it is not without complications and does not guarantee absolute success. Especially in the patients who either fail bariatric surgery, or in whom this option is not available, RAKT presents a unique opportunity for safe and effective transplantation. In comparison to obese recipients undergoing open transplantation, RAKT improves wound/surgery-related outcomes, without compromising graft function, and has the potential to allow transplantation in recipients previously excluded exclusively due to morbid obesity. Initial costs will certainly be higher; however, these should decrease as center volume and outcomes improve further still. Especially when considering that transplantation in the obese offers a survival advantage in comparison to remaining on dialysis, the authors believe that in the ensuing years, RAKT for morbidly obese recipients who are unable to lose weight will become the preferred option for this otherwise largely dialysis-dependent group, although availability of this approach remains limited and overall access disparities cannot be addressed by RAKT alone. Furthermore, we hope that RAKT will never abrogate the need to encourage obese recipients to continue to pursue weight-loss strategies for the ongoing optimization of their general health.

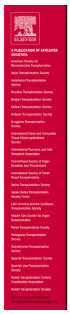
REFERENCES

1. Groth CG, Brent LB, Calne RY, et al. Historic landmarks in clinical transplantation: conclusions from the consensus conference at the University of California, Los Angeles. *World J Surg*. 2000;24:834–843.
2. Hamilton D. 1—Alexis Carrel and the early days of tissue transplantation. *Transplant Rev*. 1988;2:1–15.
3. Sade RM. Transplantation at 100 years: Alexis Carrel, pioneer surgeon. *Ann Thorac Surg*. 2005;80:2415–2418.
4. Barker CF, Markmann JF. Historical overview of transplantation. *Cold Spring Harb Perspect Med*. 2013;3:a014977.
5. Matevossian E, Kern H, Huser N, et al. Surgeon Yurii Voronoy (1895-1961) - a pioneer in the history of clinical transplantation: in memoriam at the 75th anniversary of the first human kidney transplantation. *Transpl Int*. 2009;22:1132–1139.
6. Harrison JH, Merrill JP, Murray JE. Renal homotransplantation in identical twins. *Surg Forum*. 1956;6:432–436.
7. Murray JE, Merrill JP, Harrison JH. Renal homotransplantation in identical twins. 1955. *J Am Soc Nephrol*. 2001;12:201–204.
8. Lafranca JA, IJermans JN, Betjes MG, et al. Body mass index and outcome in renal transplant recipients: a systematic review and meta-analysis. *BMC Med*. 2015;13:111.
9. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Nephrol Dial Transplant*. 2017;32:203–210.
10. Vivante A, Golan E, Tzur D, et al. Body mass index in 1.2 million adolescents and risk for end-stage renal disease. *Arch Intern Med*. 2012;172:1644–1650.
11. The GBDOC, Ng M, Fleming T, et al. Global, regional and national prevalence of overweight and obesity in children and adults 1980-2013: a systematic analysis. *Lancet*. 2014;384:766–781.
12. Tremmel M, Gerdtham UG, Nilsson PM, et al. Economic burden of obesity: a systematic literature review. *Int J Environ Res Public Health*. 2017;14:e435.
13. Flegal KM, Kruszon-Moran D, Carroll MD, et al. Trends in obesity among adults in the united states, 2005 to 2014. *JAMA*. 2016;315:2284–2291.
14. Dudley C, Bright R, Harden P. *Assessment of the Potential Kidney Transplant Recipient*. 5th ed. The Renal Association: UK; 2011.
15. Campbell S, Pilmore H, Gracey D, et al. KHA-CARI guideline: recipient assessment for transplantation. *Nephrology (Carlton)*. 2013;18:455–462.
16. Knoll G, Cockfield S, Blydt-Hansen T, et al. Canadian Society of Transplantation consensus guidelines on eligibility for kidney transplantation. *CMAJ*. 2005;173:S1–S25.
17. Segev DL, Simpkins CE, Thompson RE, et al. Obesity impacts access to kidney transplantation. *J Am Soc Nephrol*. 2008;19:349–355.
18. Lassalle M, Fezeu LK, Couchoud C, et al. Obesity and access to kidney transplantation in patients starting dialysis: a prospective cohort study. *PLoS ONE*. 2017;12:e0176616.
19. Huang E, Shye M, Elashoff D, et al. Incidence of conversion to active waitlist status among temporarily inactive obese renal transplant candidates. *Transplantation*. 2014;98:177–186.
20. Lesage J, Gill JS. Management of the obese kidney transplant candidate. *Transplant Rev*. 2017;31:35–41.
21. Hossain M, Woywodt A, Augustine T, et al. Obesity and listing for renal transplantation: weighing the evidence for a growing problem. *Clin Kidney J*. 2017;10:703–708.
22. Gill JS, Hendren E, Dong J, et al. Differential association of body mass index with access to kidney transplantation in men and women. *Clin J Am Soc Nephrol*. 2014;9:951–959.
23. Ladhani M. Obesity in the end stage kidney disease population. In: *Australian Society of Nephrology Annual Scientific Meeting*. 2016.
24. Modanlou KA, Muthyala U, Xiao H, et al. Bariatric surgery among kidney transplant candidates and recipients: analysis of the United States Renal Data System and literature review. *Transplantation*. 2009;87:1167–1173.
25. Johansen KL. Obesity and body composition for transplant wait-list candidacy—challenging or maintaining the BMI limits? *J Ren Nutr*. 2013;23:207–209.
26. Alexander JW, Goodman HR, Gersin K, et al. Gastric bypass in morbidly obese patients with chronic renal failure and kidney transplant. *Transplantation*. 2004;78:469–474.
27. Freeman CM, Woodle ES, Shi J, et al. Addressing morbid obesity as a barrier to renal transplantation with laparoscopic sleeve gastrectomy. *Am J Transplant*. 2015;15:1360–1368.
28. MacLaughlin HL, Hall WL, Patel AG, et al. Laparoscopic sleeve gastrectomy is a novel and effective treatment for obesity in patients with chronic kidney disease. *Obes Surg*. 2012;22:119–123.
29. Giulianotti P, Gorodner V, Sbrana F, et al. Robotic transabdominal kidney transplantation in a morbidly obese patient. *Am J Transplant*. 2010;10:1478–1482.
30. Oberholzer J, Giulianotti P, Danielson KK, et al. Minimally invasive robotic kidney transplantation for obese patients previously denied access to transplantation. *Am J Transplant*. 2013;13:721–728.
31. Dudley C, Harden P. Renal Association Clinical Practice Guideline on the assessment of the potential kidney transplant recipient. *Nephron Clin Pract*. 2011;118(Suppl 1):c209–c224.
32. Furriel F, Parada B, Campos L, et al. Pretransplantation overweight and obesity: does it really affect kidney transplantation outcomes? *Transplant Proc*. 2011;43:95–99.
33. Johnson DW, Isbel NM, Brown AM, et al. The effect of obesity on renal transplant outcomes. *Transplantation*. 2002;74:675–681.
34. Tran M-H, Foster CE, Kalantar-Zadeh K, et al. Kidney transplantation in obese patients. *World J Transplant*. 2016;6:135–143.
35. Kuo JH, Wong MS, Perez RV, et al. Renal transplant wound complications in the modern era of obesity. *J Surg Res*. 2012;173:216–223.

36. Espejo B, Torres A, Valentin M, et al. Obesity favors surgical and infectious complications after renal transplantation. *Transplant Proc*. 2003; 35:1762–1763.
37. Chung H, Lam VW, Yuen LP, et al. Renal transplantation: better fat than thin. *J Surg Res*. 2015;194:644–652.
38. Gill JS, Lan J, Dong J, et al. The survival benefit of kidney transplantation in obese patients. *Am J Transplant*. 2013;13:2083–2090.
39. Glanton CW, Kao TC, Cruess D, et al. Impact of renal transplantation on survival in end-stage renal disease patients with elevated body mass index. *Kidney Int*. 2003;63:647–653.
40. Zaydfudim V, Feurer ID, Moore DR, et al. Pre-transplant overweight and obesity do not affect physical quality of life after kidney transplantation. *J Am Coll Surg*. 2010;210:336–344.
41. Meier-Kriesche HU, Arndorfer JA, Kaplan B. The impact of body mass index on renal transplant outcomes: a significant independent risk factor for graft failure and patient death. *Transplantation*. 2002; 73:70–74.
42. Nicoletto BB, Fonseca NK, Manfro RC, et al. Effects of obesity on kidney transplantation outcomes: a systematic review and meta-analysis. *Transplantation*. 2014;98:167–176.
43. Chang SH, Coates PT, McDonald SP. Effects of body mass index at transplant on outcomes of kidney transplantation. *Transplantation*. 2007;84:981–987.
44. Gore JL, Pham PT, Danovitch GM, et al. Obesity and outcome following renal transplantation. *Am J Transplant*. 2006;6:357–363.
45. Khwaja A, El-Nahas M. Transplantation in the obese: separating myth from reality. *Nephrol Dial Transplant*. 2012;27:3732–3735.
46. Ratner LE, Ciseck LJ, Moore RG, et al. Laparoscopic live donor nephrectomy. *Transplantation*. 1995;60:1047–1049.
47. Nanidis TG, Antcliffe D, Kokkinos C, et al. Laparoscopic versus open live donor nephrectomy in renal transplantation: a meta-analysis. *Ann Surg*. 2008;247:58–70.
48. Nicholson ML, Kaushik M, Lewis GR, et al. Randomized clinical trial of laparoscopic versus open donor nephrectomy. *Br J Surg*. 2010;97: 21–28.
49. Stifelman MD, Hull D, Sosa RE, et al. Hand assisted laparoscopic donor nephrectomy: a comparison with the open approach. *J Urol*. 2001;166: 444–448.
50. Rawlins MC, Hefty TL, Brown SL, et al. Learning laparoscopic donor nephrectomy safely: a report on 100 cases. *Arch Surg*. 2002;137:531–535.
51. Bargman V, Sundaram CP, Bernie J, et al. Randomized trial of laparoscopic donor nephrectomy with and without hand assistance. *J Endourol*. 2006; 20:717–722.
52. Minnee RC, Bemelman WA, Maartense S, et al. Left or right kidney in hand-assisted donor nephrectomy? A randomized controlled trial. *Transplantation*. 2008;85:203–208.
53. Kok NF, Lind MY, Hansson BM, et al. Comparison of laparoscopic and mini incision open donor nephrectomy: single blind, randomised controlled clinical trial. *BMJ*. 2006;333:221.
54. Dols LF, Kok NF, d'Ancona FC, et al. Randomized controlled trial comparing hand-assisted retroperitoneoscopic versus standard laparoscopic donor nephrectomy. *Transplantation*. 2014;97:161–167.
55. Dols LF, Kok NF, Ijzermans JN. Live donor nephrectomy: a review of evidence for surgical techniques. *Transpl Int*. 2010;23:121–130.
56. Özdemir-van Brunschot DM, Koning GG, van Laarhoven KC, et al. A comparison of technique modifications in laparoscopic donor nephrectomy: a systematic review and meta-analysis. *PLoS One*. 2015;10: e0121131.
57. Horgan S, Vanuno D. Robots in laparoscopic surgery. *J Laparoendosc Adv Surg Tech A*. 2001;11:415–419.
58. Horgan S, Vanuno D, Sileri P, et al. Robotic-assisted laparoscopic donor nephrectomy for kidney transplantation. *Transplantation*. 2002;73:1474–1479.
59. Bhattu AS, Ganpule A, Sabnis RB, et al. Robot-assisted laparoscopic donor nephrectomy vs standard laparoscopic donor nephrectomy: a prospective randomized comparative study. *J Endourol*. 2015;29:1334–1340.
60. Monn MF, Gramm AR, Bahler CD, et al. Economic and utilization analysis of robot-assisted versus laparoscopic live donor nephrectomy. *J Endourol*. 2014;28:780–783.
61. Crane C, Lam VW, Alsakran A, et al. Are there anatomical barriers to laparoscopic donor nephrectomy? *ANZ J Surg*. 2010;80:781–785.
62. Bachir BG, Hussein M, Nasr R, et al. Evaluation of right versus left laparoscopic donor nephrectomy. *Exp Clin Transplant*. 2011;9:310–314.
63. Kapoor A, Lambe S, Kling AL, et al. Outcomes of laparoscopic donor nephrectomy in the presence of multiple renal arteries. *Urology Annals*. 2011;3:62–65.
64. Troppmann C, Wiesmann K, McVicar JP, et al. Increased transplantation of kidneys with multiple renal arteries in the laparoscopic live donor nephrectomy era: surgical technique and surgical and nonsurgical donor and recipient outcomes. *Arch Surg*. 2001;136:897–907.
65. Kay MD, Brook N, Kaushik M, et al. Comparison of right and left laparoscopic live donor nephrectomy. *BJU Int*. 2006;98:843–844.
66. Buell JF, Edye M, Johnson M, et al. Are concerns over right laparoscopic donor nephrectomy unwarranted? *Ann Surg*. 2001;233:645–651.
67. Kuo PC, Plotkin JS, Stevens S, et al. Outcomes of laparoscopic donor nephrectomy in obese patients. *Transplantation*. 2000;69:180–182.
68. Heimbach JK, Taler SJ, Prieto M, et al. Obesity in living kidney donors: clinical characteristics and outcomes in the era of laparoscopic donor nephrectomy. *Am J Transplant*. 2005;5:1057–1064.
69. Allen RDM, Pleass HC. Donor and recipient kidney transplantation surgery. In: Turner N, Lameire N, Goldsmith DJ, et al., editors. *Oxford Textbook of Clinical Nephrology*. 4th ed. Oxford: Oxford University Press; 2016:2378–2389.
70. Oyen O, Andersen M, Mathisen L, et al. Laparoscopic versus open living-donor nephrectomy: experiences from a prospective, randomized, single-center study focusing on donor safety. *Transplantation*. 2005; 79:1236–1240.
71. Ponsky L, Cherullo E, Moinzadeh A, et al. The hem-o-lok clip is safe for laparoscopic nephrectomy: a multi-institutional review. *Urology*. 2008; 71:593–596.
72. Friedman AL, Peters TG, Jones KW, et al. Fatal and nonfatal hemorrhagic complications of living kidney donation. *Ann Surg*. 2006;243: 126–130.
73. Hsi RS, Ojogho ON, Baldwin DD. Analysis of techniques to secure the renal hilum during laparoscopic donor nephrectomy: review of the FDA database. *Urology*. 2009;74:142–147.
74. Meng MV. Reported failures of the polymer self-locking (Hem-o-lok) clip: review of data from the Food and Drug Administration. *J Endourol*. 2006; 20:1054–1057.
75. Friedman AL, Peters TG, Ratner LE. Regulatory failure contributing to deaths of live kidney donors. *Am J Transplant*. 2012;12:829–834.
76. Bernie JE, Sundaram CP, Guise AI. Laparoscopic vascular control techniques in donor nephrectomy: effects on vessel length. *JSLS*. 2006;10: 141–144.
77. Øyen O, Scholz T, Hartmann A, et al. Minimally invasive kidney transplantation: the first experience. *Transplant Proc*. 2006;38:2798–2802.
78. Kim S-D, Kim J-I, Moon I-S, et al. Comparison of minimal skin incision technique in living kidney transplantation and conventional kidney transplantation. *Chin Med J (Engl)*. 2016;129:917–921.
79. Kacar S, Eroglu A, Tilif S, et al. Minimally invasive kidney transplantation. *Transplant Proc*. 2013;45:926–928.
80. Mun SP, Chang JH, Kim KJ, et al. Minimally invasive video-assisted kidney transplantation (MIVAKT). *J Surg Res*. 2007;141:204–210.
81. Park SC, Kim SD, Kim JI, et al. Minimal skin incision in living kidney transplantation. *Transplant Proc*. 2008;40:2347–2348.
82. Malinka T, Banz VM, Wagner J, et al. Incision length for kidney transplantation does not influence short- or long-term outcome: a prospective randomized controlled trial. *Clin Transplant*. 2013;27:E538–E545.
83. Rosales A, Salvador JT, Urdaneta G, et al. *Eur Urol*. 2010;57:164–167.
84. Modi P, Rizvi J, Pal B, et al. Laparoscopic kidney transplantation: an initial experience. *Am J Transplant*. 2011;11:1320–1324.
85. Modi P, Thyagaraj K, Rizvi S, et al. Laparoscopic en bloc kidney transplantation. *Indian J Urol*. 2012;28:362–365.
86. Modi P, Pal B, Modi J, et al. Retroperitoneoscopic living-donor nephrectomy and laparoscopic kidney transplantation: experience of initial 72 cases. *Transplantation*. 2013;95:100–105.
87. Modi P, Pal B, Kumar S, et al. Laparoscopic transplantation following transvaginal insertion of the kidney: description of technique and outcome. *Am J Transplant*. 2015;15:1915–1922.
88. Lindberg F, Bergqvist D, Björck M, et al. Renal hemodynamics during carbon dioxide pneumoperitoneum: an experimental study in pigs. *Surg Endosc*. 2003;17:480–484.
89. Hoznek A, Zaki SK, Samadi DB, et al. Robotic assisted kidney transplantation: an initial experience. *J Urol*. 2002;167:1604–1606.
90. Boggi U, Vistoli F, Signori S, et al. Robotic renal transplantation: first European case. *Transpl Int*. 2011;24:213–218.
91. Abaza R, Ghani KR, Sood A, et al. Robotic kidney transplantation with intraoperative regional hypothermia. *BJU Int*. 2014;113:679–681.
92. Menon M, Abaza R, Sood A, et al. Robotic kidney transplantation with regional hypothermia: evolution of a novel procedure utilizing the IDEAL guidelines (IDEAL phase 0 and 1). *Eur Urol*. 2014;65:1001.

93. Menon M, Sood A, Bhandari M, et al. Robotic kidney transplantation with regional hypothermia: a step-by-step description of the Vattikuti Urology Institute-Medanta technique (IDEAL phase 2a). *Eur Urol*. 2014;65:991.
94. Sood A, Ghani KR, Ahlawat R, et al. Application of the statistical process control method for prospective patient safety monitoring during the learning phase: robotic kidney transplantation with regional hypothermia (IDEAL Phase 2a–b). *Eur Urol*. 2014;66:371–378.
95. Sood A, Ghosh P, Jeong W, et al. Minimally invasive kidney transplantation: perioperative considerations and key 6-month outcomes. *Transplantation*. 2015;99:316–323.
96. Sood A, McCulloch P, Dahm P, et al. Ontogeny of a surgical technique: robotic kidney transplantation with regional hypothermia. *Int J Surg*. 2016;25:158–161.
97. Tsai MK, Lee CY, Yang CY, et al. Robot-assisted renal transplantation in the retroperitoneum. *Transpl Int*. 2014;27:452–457.
98. Ayloo SM, D'Amico G, West-Thielke P, et al. Combined robot-assisted kidney transplantation and sleeve gastrectomy in a morbidly obese recipient. *Transplantation*. 2015;99:1495–1498.
99. Doumerc N, Roumiguie M, Rischmann P, et al. Totally robotic approach with transvaginal insertion for kidney transplantation. *Eur Urol*. 2015;68:1103–1104.
100. Frongia M, Cadoni R, Solinas A. First robotic-assisted dual kidney transplant: surgical technique and report of a case with 24-month follow-up. *Transplant Direct*. 2015;1:e34.
101. Breda A, Gausa L, Territo A, et al. Robotic-assisted kidney transplantation: our first case. *World J Urol*. 2016;34:443–447.
102. Breda A, Territo A, Gausa L, et al. Robotic kidney transplantation: one year after the beginning. *World J Urol*. 2017;35:1507–1515.
103. Doumerc N, Beauval JB, Rostaing L, et al. A new surgical area opened in renal transplantation: a pure robot-assisted approach for both living donor nephrectomy and kidney transplantation using transvaginal route. *Transpl Int*. 2016;29:122–123.
104. Tuğcu V, Şener NC, Şahin S, et al. Robotic kidney transplantation: the Bakırköy experience. *Turk J Urol*. 2016;42:295–298.
105. Doumerc N, Roumiguie M, Beauval JB, et al. Robotic kidney transplantation for morbidly obese patients excluded from traditional transplantation. *Obes Surg*. 2017;27:1056–1057.
106. Garcia-Roca R, Garcia-Aroz S, Tzvetanov I, et al. Single center experience with robotic kidney transplantation for recipients with bmi of 40 kg/m2 or greater: a comparison with the UNOS registry. *Transplantation*. 2017;101:191–196.
107. Breda A, Territo A, Gausa L, et al. Robot-assisted kidney transplantation: the European experience. *Eur Urol*. 73:273–281.
108. Modi P, Pal B, Modi J, et al. Robotic assisted kidney transplantation. *Indian J Urol*. 2014;30:287–292.
109. Merion RM, Sung RS. The cutting edge of transplant surgery: committed to leading the way in the 21st century. *Am J Transplant*. 2010;10:36–78.
110. Raveendran V, Adiyat KT, Koduveli RM, et al. Robotic renal transplant recipient surgery with vaginally inserted allograft. *J Robotic Surg*. 2017;11:473–477.
111. Modi P, Pal B, Kumar S, et al. Transvaginal insertion of kidney and robotic kidney transplantation: first 19 cases. *Indian J Transplant*. 2016;10:96–97.
112. Modi P, Pal B, Kumar S, et al. Kidney Insertion Through Vagina and Robotic Assisted Laparoscopic Kidney Transplantation: A Pilot Study. In: *Paper presented at: American Transplant Congress; 2017*.
113. Yeh C, Spaggiari M, Oberholzer J. Robotic simultaneous pancreas kidney transplantation in obese recipient. Paper presented at: 26th International Congress of the Transplantation. Society; 2016; Hong Kong.
114. Lee JR. Anesthetic considerations for robotic surgery. *Korean J Anesthesiol*. 2014;66:3–11.
115. Awad H, Santilli S, Ohr M, et al. The effects of steep trendelenburg positioning on intraocular pressure during robotic radical prostatectomy. *Anesth Analg*. 2009;109:473–478.
116. Kalmar AF, Foubert L, Hendrickx JF, et al. Influence of steep Trendelenburg position and CO(2) pneumoperitoneum on cardiovascular, cerebrovascular, and respiratory homeostasis during robotic prostatectomy. *Br J Anaesth*. 2010;104:433–439.
117. Raz O, Boesel TW, Arianayagam M, et al. The effect of the modified Z trendelenburg position on intraocular pressure during robotic assisted laparoscopic radical prostatectomy: a randomized, controlled study. *J Urol*. 2015;193:1213–1219.
118. Heylen L, Naesens M, Jochmans I, et al. The effect of anastomosis time on outcome in recipients of kidneys donated after brain death: a cohort study. *Am J Transplant*. 2015;15:2900–2907.
119. Lucereau B, Thaveau F, Lejay A, et al. Learning curve of robotic-assisted anastomosis: shorter than the laparoscopic technique? An educational study. *Ann Vasc Surg*. 2016;33:39–44.
120. Arora S, Tugcu V, Sood A, et al. Learning curve of a new sugical procedure: experience from a new center adopting robotic kidney transplant. In: *Paper presented at: 112th Annual Meeting of the American Urological Association*.
121. Sood A, Ghosh P, Jeong W, et al. Robotic kidney transplantation with regional hypothermia: results from a prospective two-arm non-randomized controlled trial (IDEAL Phase 2B). Paper presented at: Annual Meeting of the American Urological Association; 2016; San Diego, USA.
122. Breda A, Territo A, Guasa L, et al. Robotic kidney transplantation: european one-year data. In: *Paper presented at: 112th Annual Meeting of the American Urological Association*. 2017.
123. Meier RPH, Piller V, Hagen ME, et al. Intra-abdominal cooling system limits ischemia-reperfusion injury during robot-assisted renal transplantation. *Am J Transplant*. 2018;18:53–62.
124. Lynch RJ, Ranney DN, Shijie C, et al. Obesity, surgical site infection, and outcome following renal transplantation. *Ann Surg*. 2009;250:1014–1020.
125. Takata MC, Campos GM, Ciovica R, et al. Laparoscopic bariatric surgery improves candidacy in morbidly obese patients awaiting transplantation. *Surg Obes Relat Dis*. 2008;4:159–165.
126. Tzvetanov I, D'Amico G, Georgiev G, et al. Combined robotic kidney transplantation and sleeve gastrectomy in obese recipients: initial results from prospective randomized study [abstract]. In: *American Transplant Congress*. 2015.
127. Hagen ME, Pugin F, Bucher P, et al. Robotic kidney implantation for kidney transplantation: initial experience. *J Robot Surg*. 2010;4:271–276.
128. Ahmed K, Khan R, Mottrie A, et al. Development of a standardised training curriculum for robotic surgery: a consensus statement from an international multidisciplinary group of experts. *BJU Int*. 2015;116:93–101.
129. Knab LM, Zureikat AH, Zeh HJ 3rd, et al. Towards standardized robotic surgery in gastrointestinal oncology. *Langenbecks Arch Surg*. 2017;402:1003–1014.
130. Hogg ME, Besselink MG, Clavien PA, et al. Training in minimally invasive pancreatic resections: a paradigm shift away from "see one, do one, teach one". *HPB*. 2017;19:234–245.
131. Hogg ME, Tam V, Zenati M, et al. Mastery-based virtual reality robotic simulation curriculum: the first step toward operative robotic proficiency. *J Surg Educ*. 2017;74:477–485.
132. King JC, Zeh HJ 3rd, Zureikat AH, et al. Safety in numbers: progressive implementation of a robotics program in an academic surgical oncology practice. *Surg Innov*. 2016;23:407–414.
133. Shakir M, Boone BA, Polanco PM, et al. The learning curve for robotic distal pancreatectomy: an analysis of outcomes of the first 100 consecutive cases at a high-volume pancreatic centre. *HPB*. 2015;17:580–586.
134. Basto M, Sathianathen N, Te Marvelde L, et al. Patterns-of-care and health economic analysis of robot-assisted radical prostatectomy in the Australian public health system. *BJU Int*. 2016;117:930–939.
135. Boggi U, Signori S, Vistoli F, et al. Laparoscopic robot-assisted pancreas transplantation: first world experience. *Transplantation*. 2012;93:201–206.
136. Gordon ZN, Angell J, Abaza R. Completely intracorporeal robotic renal autotransplantation. *J Urol*. 2014;192:1516–1522.
137. Lee JY, Alzahrani T, Ordon M. Intra-corporeal robotic renal autotransplantation. *Can Urol Assoc J*. 2015;9:E748–E749.
138. Gill B, Ramirez D, Krishnamurthi V, et al. Completely intracorporeal robotic renal autotransplantation. In: *Paper presented at: Annual Meeting of the American Urological Association*. San Diego, USA; 2016.
139. London ET, Ho HS, Neuhaus AM, et al. Effect of intravascular volume expansion on renal function during prolonged CO2 pneumoperitoneum. *Ann Surg*. 2000;231:195–201.
140. Modi P, Pal B, Kumar S, et al. Technique and outcome of robotic kidney transplantation in 161 adult recipients. The 26th International Congress of the Transplantation. Society; 2016; Hong Kong.
141. Wagenaar S, Nederhoed JH, Hoksbergen AWJ, et al. Minimally invasive, laparoscopic, and robotic-assisted techniques versus open techniques for kidney transplant recipients: a systematic review. *Eur Urol*. 2017;72:205–217.

Accepted Manuscript



TRANSPLANTATION PROCEEDINGS

Techniques to ameliorate the impact of second warm ischemic time on kidney transplantation outcomes

Ahmer M. Hameed, (MBBS, MS), Lawrence Yuen, (FRACS), Tony Pang, (FRACS), Natasha Rogers, Wayne J. Hawthorne, (MD, PhD), Henry C. Pleass, (MD, FRACS

PII: S0041-1345(18)30750-4

DOI: 10.1016/j.transproceed.2018.09.003

Reference: TPS 28778

To appear in: *Transplantation Proceedings*

Received Date: 4 June 2018

Revised Date: 25 August 2018

Accepted Date: 5 September 2018

Please cite this article as: Hameed AM, Yuen L, Pang T, Rogers N, Hawthorne WJ, Pleass HC, Techniques to ameliorate the impact of second warm ischemic time on kidney transplantation outcomes, *Transplantation Proceedings* (2018), doi: 10.1016/j.transproceed.2018.09.003.

TITLE PAGE

Manuscript Title Here: Techniques to ameliorate the impact of second warm ischemic time on kidney transplantation outcomes

My manuscript is submitted as an original work: ✓

Authors: Ahmer M. Hameed (MBBS, MS)[a,b,c], Lawrence Yuen (FRACS)[a,c], Tony Pang (FRACS) [a,c], Natasha Rogers [b,c,d], Wayne J. Hawthorne (MD, PhD)[a,b,c] & Henry C. Pleass (MD, FRACS)[a,c]

Affiliations:

a. Department of Surgery, Westmead Hospital, Sydney, Australia
b. Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney, Australia
c. Sydney Medical School, University of Sydney, Sydney, Australia
d. Department of Renal Medicine, Westmead Hospital, Sydney, Australia

Email addresses of authors: ahmer.hameed@sydney.edu.au; tony.pang@sydney.edu.au; lawrence.yuen@sydney.edu.au; natasha.rogers@sydney.edu.au; wayne.hawthorne@sydney.edu.au

Corresponding author: Prof Henry Pleass. Department of Surgery, Westmead Hospital, Cnr Darcy Road and Hawkesbury Road, Westmead, NSW 2145, Australia. Tel: +61 2 8890 7738. Email: henry.pleass@sydney.edu.au

Grant information: N/A

Key words: Kidney; cooling; SWIT

Abbreviations: CIT – cold ischemic time; CS – cold static storage; DGF – delayed graft function; IRI – ischemia-reperfusion injury; KDPI – kidney donor profile index; MP – machine perfusion; SWIT – second warm ischemia time; TIT – total ischemic time; UW – University of Wisconsin

Tables: 2

Figures: 1 (color – Yes / **No**)

Abstract

Anastomosing the renal artery and vein in transplant recipients without a cooling mechanism exposes the kidney to temperatures exceeding the metabolic threshold (15-18 °C), at which the protective effects of renal hypothermia are lost. This anastomotic time, or second warm ischemic time, can be deleterious to graft outcomes, especially if it is prolonged. Techniques to ameliorate organ warming prior to reperfusion have been designed, and range from simpler surface cooling techniques, to organ immersion in bags of ice slush, and the application of 'jackets' that incorporate their own internal cooling mechanism. The efficacy of these methods with respect to the minimization of kidney temperature prior to reperfusion and subsequent effects on graft outcomes are discussed using clinical and experimental data, in the setting of open, laparoscopic, and robotic kidney transplantation.

Introduction

Kidney transplantation outcomes are a complex function of donor, procurement, preservation, and recipient characteristics. Significant prominence is traditionally given by transplantation teams to maintenance of an appropriately cold environment during organ procurement and transportation. In recent years much research has concentrated on the impact of cold ischemic time (CIT) and technologies such as machine perfusion (MP) to reduce its impacts on kidney allograft outcomes.[1, 2] However, often less importance is given to temperature dynamics during the second warm ischemia time (SWIT), denoting the time from the commencement of vascular anastomoses until reperfusion.

Ischemia-reperfusion injury (IRI) is a key component of early and potentially longer-term graft outcomes, and is significantly impacted by the duration of ischemia and preservation temperature. An important aim during procurement and preservation, therefore, is both the minimization of the total ischemic time (TIT) and also induction of organ hypothermia, such that the kidney's metabolism is significantly reduced during the time it has no blood and/or oxygen supply. Core organ cooling is achieved during retrieval by *in situ* perfusion of the kidney with cold preservation fluid; core cooling is the primary mechanism by which renal hypothermia is induced in preparation for transportation to the transplant center. Ideally, the temperature should be maintained at less than 15-18 °C; above this threshold, the degree of renal glomerular and tubular metabolic activity results in warm ischemic damage.[3-5]

Maintenance of appropriate renal hypothermia is therefore not only important during preservation/transportation, but also when the kidney is removed from its cooled transport media in preparation for anastomoses. Delayed graft function (DGF) is a function of donor and transplantation factors, with likely contributing mechanisms including kidney re-warming during the SWIT, in addition to other considerations such as surgical inexperience with a prolonged anastomotic time. A prolonged duration of anastomoses (greater than 35-45 mins) significantly increases the risk of delayed graft function (DGF) and histologic injury, including interstitial fibrosis, and may independently reduce graft

survival.[6, 7] DGF itself is also associated with poorer longer-term graft survival.[8, 9] Moreover, an increased SWIT potentially explains the poorer graft survival of right compared to left deceased donor kidneys.[10]

SWIT may in fact have a pivotal influence on transplantation outcomes for higher kidney donor profile index (KDPI) and donation after circulatory death kidneys, and is an area of potential research growth, particularly with the advent of MP technology. Strategies that reduce the SWIT and/or its impacts should therefore be employed. An important area of investigation here is the reduction of core kidney temperature during the SWIT as a means to maintain low levels of renal metabolism right up until reperfusion, to thereby minimize the IRI hit. Using current technologies, this cannot be achieved using core cooling methods (i.e. direct intra-vascular perfusion) during anastomoses, but rather relies upon external methods and/or devices. In this review, we aim to investigate this concept further in the setting of open, laparoscopic and/or robotic renal transplantation.

Methods

The Cochrane, Embase and Medline databases (January 1975- September 2017) were searched using the following keywords:

(Kidney or renal) AND (transplant* or kidney transplantation or transplantation) AND (second warm ischemic time or SWIT or anastomosis or anastomotic time) AND (cool or temp*).

All relevant experimental and clinical articles exploring active kidney cooling techniques during the SWIT in open, laparoscopic and/or robotic renal transplantation were extracted, and effects on kidney temperature and clinicopathologic outcomes were recorded.

Study evidence levels and grades of recommendation were assessed based on the guidelines provided by the Oxford Centre for Evidence-Based Medicine.[11] This system classifies studies from level 1 to level 5, from a high to low level of evidence. A final, cumulative recommendation grade from A to D (highest to lowest) is given based on these evidence levels, which estimates the confidence that can be placed in the suggested efficacy of the approach utilized based on the consistency and quality of studies.

Results

Temperature changes during renal transplantation

Kidney temperature alterations during living donor transplantation were measured in 152 patients by Kuipers *et al*, and during deceased donor transplantation in 65 patients by Feuillu *et al*.[4, 5] Grafts from both studies were flushed and stored in University of Wisconsin (UW) solution. Kuipers *et al*. covered grafts with a wet, cold sponge during implantation, with surface temperature measurements taken using an infra-red thermometer; in contrast, Feuillu *et al*. utilized cooled paper towels on the back-table, then flushed the kidney with cold serum, followed by immersion in cold serum until anastomoses. Temperature here was measured at a depth of 15 mm. Comparative temperature changes in these studies during the SWIT are summarized in Figure 1. Mean kidney temperature prior to reperfusion (i.e. end-SWIT) in both studies was 25.5 and 26.7 °C, respectively, with the so-called 15-18 °C metabolic threshold exceeded between an anastomotic time of 10-20 minutes.[3-5]

Porcine, *ex vivo* studies have explored kidney temperature changes depending on kidney surface and size. The posterior kidney surface in contact with the iliac fossa itself warms at a faster rate in comparison to the anterior surface exposed to the ambient environment, and organ warming is inversely proportional to the kidney's size/weight.[12, 13]

Cooling techniques

A variety of cooling methods applicable to the SWIT have been tested in the clinical and experimental setting, and are summarized in Table 1. Broadly, these can be classified as topical/surface cooling techniques overlying the isolated kidney, kidney immersion in bags/stockinettes containing ice slush with or without additional cold preservation solution, and/or the application of a shell/jacket containing a mechanical cooling system around the kidney.

Only the former two have been utilized in clinical transplantation, with the use of cooling shells/jackets at this stage largely restricted to the experimental setting. Kidney placement in a bag/stockinette necessitates small holes being made in the receptacle at the region of the hilum such that the renal vessels can be brought out for anastomoses. The bag is prevented from obscuring the operating field by obliquely affixing it to the wound edge/drapes, although intuitively this is more likely to occur in comparison to when less bulky receptacles are used for the kidney.[14, 15]

Thermodynamic effects

Each technique causes differential cooling of the kidney cortex and medulla. Kidney temperatures at different time points for each study are outlined in Table 1. Assuming a SWIT of 30 minutes, median cortical and medullary temperatures, respectively, at reperfusion were as follows – 22.1 and 22.5 °C for surface/topical cooling methods,[5, 13, 16, 17] 6.5 and 6.3 °C for shell/jacket-based approaches,[18-21] and 4 and 5 °C when ice bag/stockinettes were utilized.[15, 22, 23]

Impacts on graft outcomes

Table 1 outlines the clinic-pathologic and/or other effects of cooling systems in comparison to no cooling. Topical/surface cooling systems are utilized at a minimum by most units, and as such no comparative data was available for this subset. In contrast, clinical transplantation outcomes were documented by two study groups utilizing the ice bag/stockinette method; one study group suggested there were no significant outcome differences when this technique was employed in comparison to standard methods, whilst the other group found better 14-day estimated glomerular filtration rates and a lower cumulative delayed graft function and acute rejection rate in the ice bag group.[15, 22, 24] Application of a shell/jacket, which also incorporated a mechanical cooling system, appeared to be beneficial in all animal studies applying such systems.[18, 19, 21]

Robotic or Laparoscopic Applications

SWIT can potentially be further prolonged during kidney transplantation in the setting of robotic or laparoscopic transplantation. Laven *et al*. investigated kidney cooling in the context of laparoscopic surgery, whilst Meier *et al*. and Menon *et al*. outlined cooling techniques for use in robotic transplantation.[17, 21, 25] Meier *et al*. employed a jacket-based cooling system, and demonstrated continuous, adequate cooling despite a mean anastomotic time of 70.4 minutes in the robotic transplantation group.[21] In contrast, Menon *et al*. used primarily topical cooling methods to achieve a higher mean temperature (20.3 ℃) prior to reperfusion.[17] A continuous kidney surface infusion of microparticulate ice slurry was delivered in the laparoscopic ischemia-reperfusion study, and was able to cool the renal cortex to 15 ℃.[25]

Overall Comparison of Techniques

Relative advantages and disadvantages of the different cooling techniques, accounting for the evidence presented above, are compared in Table 2.

Levels of Evidence

Study level of evidence is indicated in Table 1. For human studies investigating topical cooling methods, the overall Grade of Recommendation is C, owing to a preponderance of case series or case control studies with small patient numbers. All papers investigating cooling shells/jackets were conducted using animal models, and thus overall evidence Grade is D. In contrast, published articles outlining ice bag immersion techniques had an overall evidence grade of C, as these consisted of prospective studies with inconsistent results or case series.

Discussion & Conclusions

The second warm ischemic time is an important period in kidney transplantation, as the kidney is transferred from a cold setting to the warm environment of the abdominal cavity. Unless methods are employed to actively cool the kidney, the kidney's temperature will naturally equilibrate to its surroundings. The more time the organ spends without reperfusion and restoration of oxygenation, the closer its temperature will get to its ambient environment. Kidney cooling techniques should therefore be especially important when the SWIT is expected to be prolonged, such as in laparoscopic or robotic transplantation, or in obese recipients. A prolonged SWIT in association with ineffective organ cooling may translate to poorer short and longer term graft outcomes.

The ischemic insult sustained during the second warm ischemic time may be modulated by techniques that cool the kidney during this time period. A variety of options are available, and can fit within the broad categories of surface (or topical) cooling using cold fluid, kidney immersion in a receptacle incorporating an ice slush, and/or sandwiching the kidney within a temperature-modulating jacket. The latter two methods both appear to achieve renal temperatures of less than 10 °C at the time of reperfusion, although temperature monitoring was more consistent in studies employing cooling jackets.

The clinical and/or pathologic implications of renal cooling systems have been less uniformly explored, and comparisons between different systems are largely lacking. Within these studies, a link between higher temperature and the occurrence of DGF was made by Szostek *et al*. and Kaminska *et al*., although the latter study compared cumulative DGF and acute rejection between cooled and non-cooled kidneys.[16, 24] In contrast, one larger cohort study found no difference in outcomes, including DGF, between kidneys cooled using an ice bag compared to controls.[14] Kidney temperatures were not recorded in this study however, in comparison to the 4 °C maintained by Kaminska *et al*. All animal studies investigating the utility of cooling jackets demonstrated a superior reduction of ischemia-

reperfusion injury compared to controls, although only Meier *et al*. tested their device in a transplantation setting, and therefore further applicability to the clinical setting is uncertain.[18, 19, 21]

In considering the evidence and studies presented, it can be concluded that effective cooling techniques can be instituted such that the renal parenchyma can be cooled to below the metabolic threshold during vascular anastomoses. This is most likely to be achieved by kidney immersion in cool solution or application of a cooling jacket/shell, with the latter appearing to be more effective as a consistent, low temperature is easier to achieve. Downstream effects with respect to graft function and patient outcomes are largely unknown, however. Furthermore, the overall quality of published studies was poor; all study summations lead to a Grading of Recommendation of only C or D, thereby indicating the need for higher quality evidence.

Additional considerations must be made in conjunction with kidney anastomotic cooling techniques. MP preservation is being increasingly utilized either continuously or for a brief period prior to organ implantation. The use of cooling techniques during SWIT has not been investigated in the context of MP, and whether these would have synergistic or competing effects. Furthermore, effective cooling during the SWIT entails an abrupt temperature shift from hypo- to normothermia upon reperfusion, which does not occur if the graft is allowed to naturally warm during anastomoses. There is at least some evidence that suggests abrupt temperature shifts may in fact be detrimental, compromising mitochondrial integrity.[2, 26]

Overall, a large, multi-center randomized control trial comparing different cooling modalities with conventional techniques is still required to prove the advantageous nature of such approaches. Such a trial would be especially useful in transplantation procedures with longer SWITs. Simple, cost-effective solutions should be trialed as a priority, before the widespread implementation of more complex and/or expensive techniques can be justified. These techniques also need to be investigated in the context of the increasing utilization of modalities such as machine perfusion preservation. Furthermore, the need for

time-efficient vascular anastomoses without unnecessary prolongation of the SWIT should always be recognized; external cooling technologies are ideally deployed with this concept always in mind.

Figure Legends

Fig. 1 Comparative kidney temperatures during the SWIT in two studies. Data expressed as means, with upper and lower temperature limits. CS – cold storage

Acknowledgments/Funding

This work was supported by the Royal Australasian College of Surgeons – Sir Roy McCaughey Surgical Research Fellowship (for AH)

References

[1] I. Jochmans, M.L. Nicholson, S.A. Hosgood, Kidney perfusion: some like it hot others prefer to keep it cool. Curr Opin Organ Transplant 2017;22:260-66.
[2] T. Minor, C. von Horn, A. Paul, Role of temperature in reconditioning and evaluation of cold preserved kidney and liver grafts. Curr Opin Organ Transplant 2017;22:267-73.
[3] J.P. Ward, Determination of the Optimum temperature for regional renal hypothermia during temporary renal ischaemia. Br J Urol 1975;47:17-24.
[4] T.G.J. Kuipers, J. Hellegering, M. El Moumni, C. Krikke, J.W. Haveman, S.P. Berger, H.G. Leuvenink, R.A. Pol, Kidney temperature course during living organ procurement and transplantation. Transpl Int 2017;30:162-69.
[5] B. Feuillu, L. Cormier, L. Frimat, M. Kessler, M. Amrani, P. Mangin, J. Hubert, Kidney warming during transplantation. Transpl Int 2003;16:307-12.
[6] L. Heylen, M. Naesens, I. Jochmans, D. Monbaliu, E. Lerut, K. Claes, S. Heye, P. Verhamme, W. Coosemans, B. Bammens, P. Evenepoel, B. Meijers, D. Kuypers, B. Sprangers, J. Pirenne, The effect of anastomosis time on outcome in recipients of kidneys donated after brain death: a cohort study. Am J Transplant 2015;15:2900-7.
[7] K.K. Tennankore, S.J. Kim, I.P. Alwayn, B.A. Kiberd, Prolonged warm ischemia time is associated with graft failure and mortality after kidney transplantation. Kidney Int. 2016;89:648-58.
[8] W.H. Lim, S.P. McDonald, G.R. Russ, J.R. Chapman, M.K. Ma, H. Pleass, B. Jaques, G. Wong, Association between delayed graft function and graft loss in donation after cardiac death kidney transplants - a paired kidney registry analysis. Transplantation 2017:101:1139-43.
[9] W.H. Lim, S.P. McDonald, S.E. Kennedy, N. Larkins, G. Wong, Association Between Slow and Delayed Graft Function With Graft Outcomes in Pediatric and Adolescent Deceased Donor Kidney Transplant Recipients. Transplantation 2017;101:1906-12.
[10] H. Vacher-Coponat, S. McDonald, P. Clayton, A. Loundou, R.D. Allen, S.J. Chadban, Inferior early posttransplant outcomes for recipients of right versus left deceased donor kidneys: an ANZDATA registry analysis. Am J Transplant 2013;13:399-405.
[11] Oxford Centre for Evidence-based Medicine – Levels of Evidence, University of Oxford. https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/; 2017 [accessed 12 December 2017].
[12] A.C. Wylds, M.T. Richard, A.M. Karow, A model for thermal gradients during renal vascular anastomoses. J Surg Res 1987;43:532-8.
[13] B. Doorschodt, D. Naafs, J.V. Vlekkert, P. Zum Vorde Sive Vording, J. Van Dijk, T. Van Gulik, Rewarming gradients in porcine kidney grafts during simulated second warm ischemic time. Transplant Proc 1997;29:3420-21.
[14] F. Karipineni, S. Campos, A. Parsikia, J.B. Durinka, P.N. Chang, K. Khanmoradi, R. Zaki, J. Ortiz, Elimination of warm ischemia using the Ice Bag Technique does not decrease delayed graft function. Int J Surg 2014;12:551-6.
[15] I.S. Gill, L.C. Munch, B.A. Lucas, Use of a stockinette to minimize warm ischemia during renal transplant vascular anastomoses. J Urol 1994;152:2053-4.
[16] M. Szostek, M. Pacholczyk, B. Lagiewska, R. Danielewicz, J. Walaszwski, W. Rowinski, Effective surface cooling of the kidney during vascular anastomosis decreases the risk of delayed kidney function after transplantation. Transpl Int 1996;9 Suppl 1:S84-5.
[17] M. Menon, A. Sood, M. Bhandari, V. Kher, P. Ghosh, R. Abaza, W. Jeong, K.R. Ghani, R.K. Kumar, P. Modi, R. Ahlawat, Robotic kidney transplantation with regional hypothermia: a step-by-step description of the Vattikuti Urology Institute-Medanta technique (IDEAL phase 2a). Eur Urol 2014;65:991-1000.

[18] T.A. Creagh, F. McLoughlin, P.J. Broe, P.A. McLean, D.M. Murphy, D.J. Bouchier-Hayes, A novel method of induced renal hypothermia. J Urol 1992;147:249-52.
[19] F. Desgrandchamps, M. Eugene, Y. Tuchschmid, F. Muller, P. Teillac, J.M. Idatte, A. Le Duc, [Cooling shell in renal transplantation. Thermometric evaluation of a prototype]. Prog Urol 1996;6:25-9.
[20] J.L. Forsythe, P.M. Dunnigan, G. Proud, T.W. Lennard, R.M. Taylor, Reducing renal injury during transplantation. Br J Surg 1989;76:999-1001.
[21] R.P.H. Meier, V. Piller, M.E. Hagen, C. Joliat, J.B. Buchs, A. Nastasi, R. Ruttimann, N.C. Buchs, S. Moll, J.P. Vallee, F. Lazeyras, P. Morel, L. Buhler, Intra-Abdominal Cooling System Limits Ischemia-Reperfusion Injury During Robot-Assisted Renal Transplantation. Am J Transplant 2018;18:53-62.
[22] D. Kaminska, K. Koscielska-Kasprzak, P. Chudoba, M. Klinger, Kidney Injury Due to Warm Ischemia During Transplantation Can Be Reduced. Am J Transplant 2016;16:1639.
[23] A. Pupka, P. Chudoba, D. Patrzalek, D. Janczak, P. Szyber, The modification of renal transplantation with the usage of own polyethylene receptacle. Polim Med 2003;33:33-7.
[24] D. Kaminska, K. Koscielska-Kasprzak, P. Chudoba, A. Halon, O. Mazanowska, A. Gomolkiewicz, P. Dziegiel, D. Drulis-Fajdasz, M. Myszka, A. Lepiesza, W. Polak, M. Boratynska, M. Klinger, The influence of warm ischemia elimination on kidney injury during transplantation - clinical and molecular study. Sci Rep 2016;6:36118.
[25] B.A. Laven, K.E. Kasza, D.E. Rapp, M.A. Orvieto, M.B. Lyon, J.J. Oras, D.G. Beiser, T.L. Vanden Hoek, H. Son, A.L. Shalhav, A pilot study of ice-slurry application for inducing laparoscopic renal hypothermia. BJU Int 2007;99:166-70.
[26] I. Schopp, E. Reissberg, B. Luer, P. Efferz, T. Minor, Controlled Rewarming after Hypothermia: Adding a New Principle to Renal Preservation. Clin Transl Sci 2015;8:475-8.
[27] C.B. Anderson, R.J. Graff, W.T. Newton, A method of facilitating renal transplantation with the use of stockinet. Am J Surg 1973;126:124-5.
[28] A. Lepiesza, P. Chudoba, D. Kaminska, A. Pupka, P. Zaleska, [Methods of Reduction of Warm Ischemic Time in Kidney Transplantation and Their Role of Early and Late Outcomes]. Polim Med 2016;46:71-77.
[29] J. Ortiz, M. Siddeswarappa, S. Sea, A. Parsikia, S. Campos, K. Khanmoradi, R. Zaki, The Elimination of Warm Ischemic Time in Kidney Transplantation Using the Ice Bag Technique: A Feasibility Study. J Exp Clin Med 2011;3:187-90.
[30] E. Schenkman, M. Goldinger, W.F. Tarry, D.L. Lamm, Preventing warm ischemia with a polyurethane bag during renal transplantation. Urology 1997;50:436-7.

Table 1. Cooling techniques applied during the SWIT with associated thermodynamic, histologic and/or clinical implications.

Author, Yr; Centre	Level of Evidence	Cooling Technique during Anastomoses	Thermodynamic Effects (Cooled Kidneys)	Clinicopathologic/Other Implications
Surface/Topical Cooling				
Anderson et al., 1973[27] St Louis, USA	4 (Case Series)	Kidney placed in stockinette & intermittently washed with iced saline. *Transplantation setting* [Human]	NR	NR
Doorschodt et al., 1997[13] Amsterdam, The Netherlands	5 (Animal Study)	Kidney wrapped in gauze, with surface irrigation using cold 0.9% saline (4 °C) at 5 minute intervals *Isolated kidney model, without transplantation* [Porcine]	Kidney cortex: 14 °C at 10 mins & 27 °C at 30 mins; 30 °C at 60 mins Kidney medulla: 10-11 °C at 10 mins & 21 °C at 30 mins; 28 °C at 60 mins[a]	NR
Feuillu et al., 2003[5] Nancy, France	3b (Case-Control Study)	Ice-cooled paper tissues placed on kidney during back-table preparation; flushed with and then immersed in cold serum until ready for anastomoses, at which point it was intermittently surface cooled with cold serum. *Transplantation setting* [Human]	See Fig. 1 Kidney temperature as a function of time (t) was expressed by the following equation: Temperature = 7.2ln(t) – 0.6	In comparing kidneys with and without DGF, there were no significant differences in kidney temperature at the commencement, during, & end of anastomoses.**
Laven et al., 2007[25] Chicago, USA	5 (Animal Study)	Microparticulate ice slurry delivered laparoscopically to kidneys (surface) through a modified suction/irrigation cannula; flow of slurry maintained by peristaltic pump. *In vivo ischemia-reperfusion model without transplantation* [Porcine] [***Laparoscopic***]	Kidney cortex: 15 ^{o}C achieved by 16.5 mins Kidney medulla: NR	NR.
Menon et al., 2014[17] Detroit, USA & Gurgaon, India	4 (Case Series)	Kidney wrapped in gauze 'jacket', also containing ice slush; pelvic bed is lined with ice slush & then the kidney/gauze jacket is introduced via hand-access port; further ice slush is introduced onto the kidney/gauze once in pelvis. *Transplantation setting* [Human] [***Robotic***]	Kidney cortex: 20.3 ^{o}C achieved at end-SWIT (mean 46.6 mins) Kidney medulla: NR	DGF in 0 out of 25 patients (living donor kidney transplants)[b]
Szostek et al.,	3b (Case Control	Kidney placed in holding net, onto which a 0.9%	Kidney cortex: 19 ^{o}C achieved at end-SWIT	In comparing kidneys with and without DGF,

1996[16] Warsaw, Poland	Study)	saline infusion (4 °C) was continuously applied (i.e. to kidney surface) during anastomoses. *Transplantation setting* [Human]	(mean 33.6 mins) Kidney medulla: NR Surface kidney temperature increased by 0.3 ^{o}C/min	temperature at end-SWIT was significantly higher in the former group (19.7 compared to 14.9 ^{o}C).
Cooling Jackets				
Creagh et al., 1992[18] Dublin, Ireland	5 (Animal Study)	Frozen polymer gel cylinders enclosed in metallized polyester laminate jacket; kidney is sandwiched between two layers of this. *In vivo ischemia-reperfusion model without transplantation* [Canine]	Kidney cortex (surface): 10 °C achieved by 5 mins & 2 °C at 12 minutes; 7 °C at 120 mins Kidney medulla (core): 10 °C achieved by 10 mins & 4 °C at 17 mins; 12 °C at 120 mins	Significantly lower serum Ur, Cr, & histologic damage in dogs with cooled compared to non-cooled kidneys.
Desgrandchamps et al., 1996[19] Paris, France	5 (Animal Study)	Cooling shell (two magnetic half-shells; 10 mm thickness each) applied around kidney – shells contain Multitherm sponge (freezes when water evaporated), impregnated with water and overlaid by a metallic mesh. *In vivo ischemia-reperfusion model without transplantation* [Porcine]	Kidney cortex: 10 °C achieved by 5 mins & 7-8 °C at 20 minutes; 7-8 °C at 60 mins Kidney medulla: 10 °C achieved by 15 mins; 7-8 °C at 60 mins	No surface damage of kidneys; greater UO in cooled compared to non-cooled kidneys ($p = 0.06$); better tubular function in cooled compared to non-cooled kidneys.
Forsythe et al., 1989[20] Newcastle upon Tyne, UK	5 (Animal Study)	Double-layered, biocompatible plastic jacket (8-10 mm thick); between the layers is a weave of plastic enclosing trapped air (which has low thermal conductivity). *Isolated kidney model, without transplantation* [Porcine]	Kidney cortex: NR Kidney medulla: 1.8 °C at 10 mins; 8 °C at 45 mins Core kidney temperature increased by 0.9 °C/5 min, between 5-45 mins	NR
Meier et al., 2017[21] Geneva, Switzerland	5 (Animal Study)	Kidney sandwiched between a double sheath (external thickness 5 mm) enclosing a silicone tubing system, through which ethanol and methylene blue (4 °C) are continuously circulated during vascular anastomoses. *Transplantation setting* [Porcine] [***Robotic***]	Kidney cortex: 6.5 ^{o}C achieved at end-SWIT (mean 70.4 mins) Kidney medulla: Max. difference between cortex and medulla was 1.4 ^{o}C Surface temperature at reperfusion was 28.7 ^{o}C if cooling system not utilized	No significant differences in UO between cooled & non-cooled kidneys over 7 hrs; less parenchymal heterogeneity/perfusion defects seen on MRI in cooled kidneys; greater histologic damage (at 7 hrs) in non-cooled kidneys.
Ice Slush/Ice Bag Techniques				
Gill et al., 1994[15]	4 (Case Series)	Single-ply stockinette containing fine-consistency ice slush used to enclose the kidney during vascular anastomoses; ice slush	Kidney cortex: NR Kidney medulla: Temperature maintained between 4- 6 ^{o}C during anastomoses	No complications related to the use of the stockinette. Other data NR.

Lexington, USA		intermittently replaced to maintain target temperature. *Transplantation setting* [Human]		
Kaminska et al.; Lepiesza et al.; Pupka et al., 2016[22-24, 28] Wroclaw, Poland	2b (Randomized Control Study)	High-density polyethylene bag (0.04 mm thickness) consisting of 3 compartments – kidney in central compartment, surrounded on either side by cold saline/ice slush. *Transplantation setting* [Human]	Kidney temperature maintained at approximately 4 °C [c]	Paired kidney analysis – eGFR at 14 days post-transplantation was 40% higher in non-cooled kidneys ($p < 0.05$), but no significant differences beyond this time-point up to 5 yrs; *cumulative* DGF and one-year acute rejection rates significantly lower in cooled compared to non-cooled kidney transplant recipients; no significant difference in histology approximately 30 mins post-reperfusion
Karipineni et al.; Ortiz et al., 2014[14, 29] Philadelphia, USA	2b (Cohort Study)	Plastic sterile bag (transport bag) containing ice slush and preservation fluid; kidney is introduced into this bag. *Transplantation setting* [Human]	NR	No difference in outcomes between cooled and non-cooled kidneys (DGF, one-year acute rejection, one-year graft survival).
Schenkman et al., 1997[30] West Virginia, USA	4 (Case Series)	Polyurethane bag containing ice slush, into which the kidney is placed in preparation for vascular anastomoses. *Transplantation setting* [Human]	NR	No complications related to the use of the polyurethane bag. Other data NR.

Cr – creatinine; DGF – delayed graft function; eGFR – estimated glomerular filtration rate; MRI – magnetic resonance imaging; NR – not recorded; UO – urine output; Ur – urea

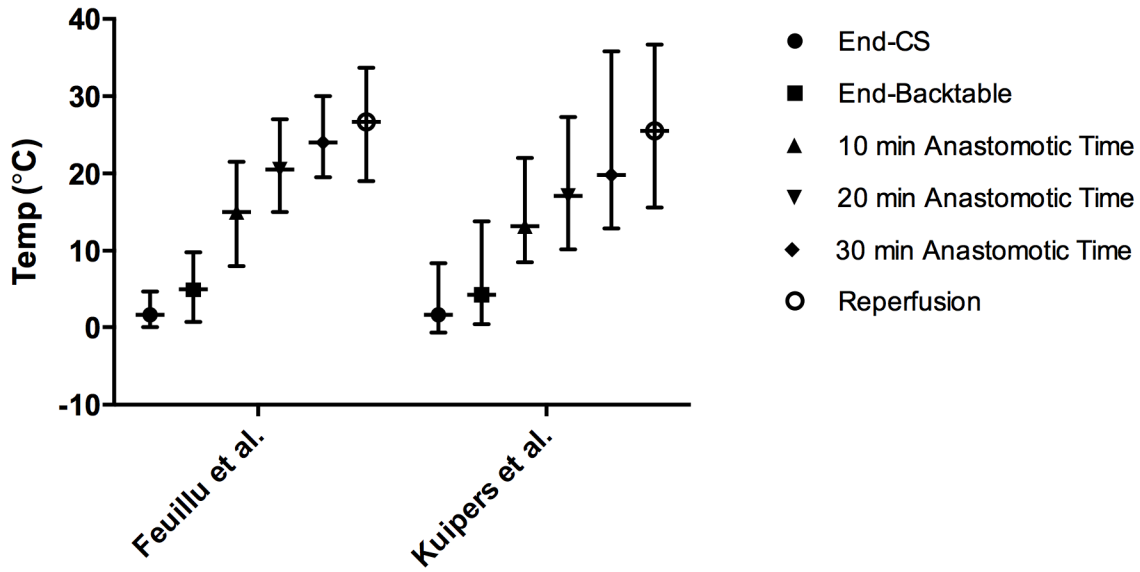
[a] Temperatures provided recorded without surface cooling; however, text states surface cooling did not significantly reduce cortical and medullary temperatures in comparison to those recorded here

[b] No comparison conducted with non-cooled kidneys

[c] Unclear if surface or core temperature; temperature trends NR

Table 2. Relative advantages and disadvantages of different anastomotic kidney cooling techniques.

Cooling Technique	Advantages	Disadvantages
Topical/surface cooling	• Simple • Cheap	• Ineffective temperature drop and/or control
Ice slush/bag immersion	• Relatively simple • Cheap	• Bulky – may obscure surgeon view • Fluid may drip into operative field • Ice slush may need to be intermittently replenished to maintain cooling efficacy
Cooling jackets	• Temperature control superior to other approaches	• More costly • No evidence from human studies



Highlights

- A prolonged anastomotic time beyond 35-45 minutes negatively affects kidney function post-transplantation.
- The effects of this can potentially be countered by cooling the kidney during anastomoses such that its temperature is maintained below the metabolic threshold.
- Cooling mechanisms include ice bags for organ immersion, surface cooling methods, and/or specifically designed kidney cooling jackets.
- There is some clinical evidence for the use of the ice bag technique, however kidney cooling jackets have currently only been used in the experimental setting.

Chapter 9

Pancreas Retrieval for Whole Organ and Islet Cell Transplantation

Wayne J. Hawthorne, Ahmer Hameed and Henry Pleass

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75151

Abstract

For more than five decades, we have been refining advances in pancreas whole organ and islet cell transplantation as clinical therapies to treat the ever-increasing number of patients suffering from type-1-diabetes. Research and clinical practice have contributed to making both whole organ and cellular transplantation viable therapeutic options for a broader range of patients. Furthermore, both forms of clinical transplantation results have progressively improved, due to the ongoing refinement of organ donation and its various technical processes, combined with the evolution of immunosuppression and patient care now offering excellent long-term treatment for both type-1-diabetes and concomitant renal failure. This chapter provides an overview on how this has been undertaken and achieved over decades to ultimately provide outstanding outcomes on par with other organ transplantation results. Briefly, we cover the history of pancreas retrieval procedures, the importance of donor selection, the intricate processes of the organ donor operation, preservation of the pancreas, and the ideal ways to best improve outcomes for transplantation. Improving and providing the optimal donor and preservation conditions underpinning the success of subsequent whole pancreas or islet transplantation as a safe, effective, and feasible therapeutic option for an increasing number of patients suffering from type-1-diabetes.

Keywords: diabetes, insulin, islet, islet cell, islet cell allotransplantation, islet cell transplantation, islet cell isolation, organ perfusion, organ retrieval, renal failure, type 1 diabetes, whole organ transplantation

1. Introduction

Since Kelly and colleagues performed the first whole pancreas transplant in 1966, significant advancements in pancreas transplantation have been made. [1] There was a gap following the first series of whole pancreas transplants due to poor graft outcomes with significant impact from poor organ preservation of the pancreas playing a major role. It took almost 20 years for the development of newer surgical techniques including use of newly developed perfusion solutions, segmental grafts, advances in ductal drainage including bladder drainage, and effective immunosuppression regimens such that whole organ transplantation burgeoned, with great advances made by Sutherland and colleagues at the University of Minnesota [2]. However, it was not until much later following many years of experimental research that pancreata for islet cell isolation and transplantation became a reality. Over the past two decades in particular a great deal of effort has underpinned making islet cell transplantation a viable therapy for a broader range of patients with type 1 diabetes (T1D). Clinical results have progressively improved, now demonstrating outcomes on par with other organ transplants, specifically in terms of insulin independence, and graft and patient survival [3]. We are now at the point where islet cell transplantation, in the form of allotransplantation, like its forebear whole organ transplantation, has become widely accepted as a clinical therapy for patients affected by T1D.

Now more than five decades on and with many organ donor operations having been performed since the advent of organ donor procedures as we know them, we have refined and perfected the organ donor process since the first organ retrieval of a brain dead donor in 1963 [4] and the subsequent adoption of the "Acceptance of Brain Death for Organ Donation" issued by the Ad Hoc Committee of the Harvard Medical School [5]. We have seen an increasing emergence of specialized organ retrieval teams with focus on the overwhelming need to improve organ donor rates for the ever increasing recipient patient population [6]. Always a dedicated surgical pursuit, research into organ donation and the surgical retrieval process for the pancreas and most other organs has often been overlooked in favor of recipient-related research into the prevention of rejection, and improving immunosuppression and tissue matching. This is particularly problematic when it comes to whole pancreas and islet transplantation as the pancreas is a less retrieval tolerant organ than other solid organs, and requires extra attention both during and after retrieval to ensure that the organ's valuable islets, which are especially susceptible to hypoxia and the ischemic insult, are effectively preserved [7, 8].

In this chapter we provide a general overview of Pancreas Retrieval for both Whole Organ and Islet Cell Transplantation, but it should be noted that there are clear overlaps in this process for both whole organ and cellular transplantation. As such the way the processes are performed can be utilized for retrieval for either type of subsequent transplant. Overall, we have seen significant improvements to pancreas transplantation results, in particular in the islet cell arena, due to the significant research undertaken to improve graft outcomes by improving donor selection and organ procurement and preservation [9]. On the recipient side we have also further improved outcomes with changes to the transplant and to the

pharmacological treatment of recipients such as newer focused monoclonal immunosuppressive strategies that better control graft rejection [9].

This chapter focuses on the optimal process for deceased donor pancreas retrieval and its role in maximizing graft function and survival. However, with a great number of processes to outline, only the major ones will be covered in this chapter. In particular, we will emphasize major improvements in donor selection, surgical retrieval techniques, pancreas retrieval in the context of multi-organ donors, back-table preparation of the pancreas, perfusion fluid types, and future perspectives including the utilization of technologies such as machine perfusion and persufflation. These factors will be discussed in the context of improved outcomes to the engraftment, function and survival of the transplants. It is also acknowledged that there remains the ongoing need for further improvements to both whole organ and islet cell transplantation, however both techniques clearly offer safe and achievable therapeutic options for the ever-expanding number of patients suffering from T1D [10].

2. Historical timeline

The original retrieval processes of the modern era were initially developed for and used in kidney only retrieval surgery. As per **Figure 1** the procedure first introduced in 1963 utilized cold lactated Ringer's or low-molecular-weight dextran solutions infused directly into the renal artery of the retrieved kidneys, performed only after their removal from the donor [11]. These were the beginnings of modern donor retrieval but they were less than ideal techniques due to the time taken to perfuse the organs, and therefore a number of more active and by far more effective methods of perfusion and cooling of organs were subsequently developed in order to minimize ischemic insult and subsequent damage to organs. These techniques were based upon the concepts from cardiothoracic surgery, involving active patient cooling during procedures to prevent ischemic damage [12, 13]. The transplant fraternity quickly adopted these intravascular perfusion-related cooling techniques, which were standardly utilized as a first step in the preservation of all whole-organ grafts. The currently accepted modern cadaveric donor procedure is performed using some basal form of the *ex situ* techniques developed and performed in the mid to late 60's by Starzl and colleagues [14] for not only kidneys but also incorporating the pancreas and liver. Further refinements saw the perfusion and addition of heparin to the perfusate solutions and also the donor. Ensuring removal of blood by *ex situ* perfusion as described by Belzer et al. [15] resulted in improved but only satisfactory kidney preservation of several days. However, this technique was eventually abandoned in most kidney transplant centers when it was learned that the quality of 2-day preservation was no better than with the simpler *"iced slush"* methods [16].

The underpinning method of *iced slush* for shipping was based around experimental work on kidneys [17]. This research and practice focused on perfusion fluids of differing intracellular and extracellular fluids consisting of electrolytes with varying osmotic and oncotic effects that were infused into the allograft before placing it in a cold storage container. Collins

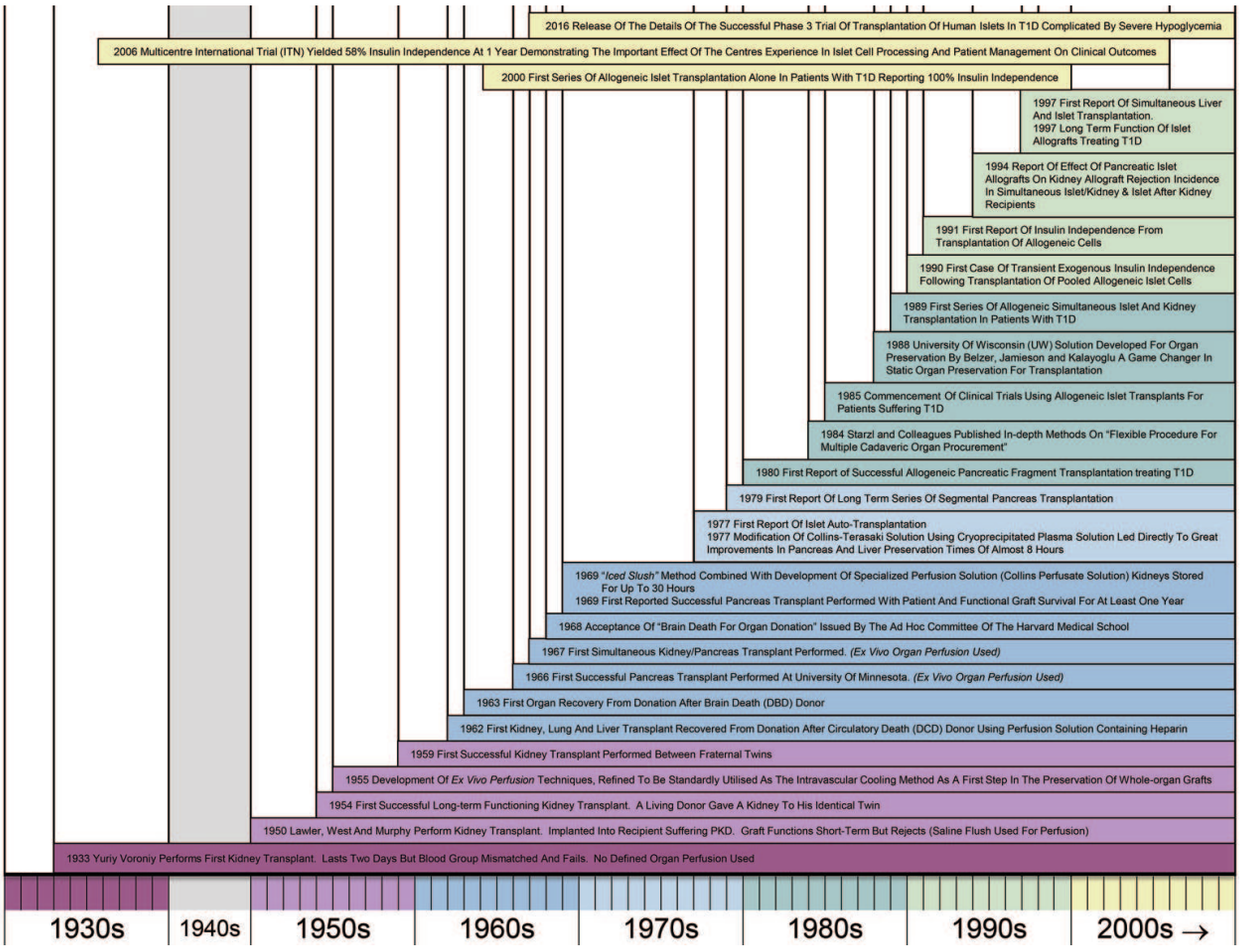


Figure 1. A time line in the significant development of transplantation over the years with focus on the techniques used for whole pancreas and islet cell transplantation.

and colleagues developed a relatively simple technique (infusion of mannitol, phenoxybenzamine, and their Collins perfusate) providing good preservation in kidneys stored for up to 30 hours [17]. Other perfusates, such as Ringer's lactate and 10% invert-sugar solutions, gave inferior results. The new perfusate solution and technique extended the time of simple ice storage from 12 hours to 30 hours. Continuous hypothermic perfusion saw further additions by Ackerman and Snell [18] and Merkel, Jonasson, and Bergan [19] who following many organ donor studies developed the widely accepted and much more simplified core cooling. These utilized cold perfusion solutions with the infusion of the fluids being performed directly to the vascular bed of all the organs via the distal aorta and demonstrated significant improvement for the pancreas but they were still less than ideal for this most sensitive organ. However, the development of these techniques used throughout the 70's meant that organs could generally be removed without causing issues when retrieving multiple organs, which included the liver and sometimes pancreas. Kidney preservation became more feasible along with the other abdominal organs seeing times of 1 to 2 days, long enough to allow tissue matching and sharing of organs between hospital units even interstate or in Europe between countries. However, these were purely focused still on the kidneys rather than the other abdominal organs and as such a number of groups undertook experiments focusing on other organs including the pancreas and liver; landmark papers included those by Benichou

et al. [20], using the Collins-Terasaki solution, and de Gruyl et al. [21] using cryoprecipitated plasma perfusion preservation of duct-ligated pancreatic allografts, along with Wall et al. [22] using similar plasma-like solution. This led directly to great improvements in pancreas and liver preservation and allowed organ sharing amongst transplant centers, although the preservation period was still limited to less than 8 hours.

The development of University of Wisconsin (UW) solution for organ preservation by Belzer, Jamieson, and Kalayoglu in the 1980s was a game changer in static organ preservation for transplantation [23]. This new flushout solution for preservation of the pancreas was tested in the dog model of segmental pancreas autotransplantation. The solution has an osmolality of 320 mOsm/L (K+ = 120 mM, Na + = 30 mM), and contains lactobionate, and raffinose as impermeants. The role of hydroxyethyl starch (HES), the colloid component of UW solution, was shown to be particularly important for pancreas preservation, in comparison to the liver and kidney [24]. UW perfusate solution preservation almost tripled the time of safe preservation of the various organs, including the pancreas, making national sharing of most organs a viable and practical process [25].

However, despite significant success the preservation or extended preservation of the pancreas still required further refinement, and significant research using animal models of static perfusion were pursued, in particular for use in islet cell transplantation. Along with perfusion fluids a number of standardly used retrieval techniques became more readily adopted [19]. However, until 1981 transplantation of the extra-renal organs was an unusual event such that the focus of perfusion only really focused on kidneys. By the mid-1980s, it became apparent that multiple organs would start to become transplanted in earnest, with liver, pancreas and thoracic organ transplant procedures becoming more widely accepted. A safe and effective method for multi-organ procurement and preservation was required by which the abdominal organs, kidneys, liver, and pancreas, could all be suitably retrieved using the same solution. At this stage Starzl and colleagues published an in-depth method on their "flexible procedure for multiple cadaveric organ procurement" [26], which was adopted by many centers worldwide.

However, even at this point the pancreas was often over-looked with the focus on the kidneys and liver as the principal organs to be retrieved. Starzl's publication stated "*If the whole pancreas is transplanted as we recommend, the combination of liver and pancreas removal is incompatible*" and it was often the case when surgical teams were procuring the liver and pancreas together that there were issues relating to the suitable separation of their vasculature [27]. At this time, our own surgical team also retrieved the pancreas with the liver, but always removed liver to the back-table before the pancreas and kidneys. The major perceived reason for this was the need for the life-saving liver to take priority. Furthermore, as the portal vein and the branches of the celiac trunk, drain or supply both organs, preference was given to sacrificing the pancreas' vessels instead of the liver. It was a number of years before this routine surgical practice would change.

Whole organ research utilized canine models as the dog pancreas is more anatomically similar to humans in comparison to the tri-lobed porcine pancreas. These models allowed replication of the clinical situation and further refinement of the retrieval and transplant procedures [28, 29]. From these came the widespread implementation of newer perfusion fluids such as UW solution, and the utilization of vascular extension grafts to the pancreatic vasculature helped resolve the situation of shortened pancreatic inflow and outflow conduits due to preference to

the liver in combined retrievals [30]. The other major change to the procedure was the adoption of an *en bloc* liver pancreas retrieval technique, where both organs were rapidly removed in a bloodless field post perfusion, then separated on the back-table. Furthermore, the sharing of organs from a common donor by recipient teams from different units became routine by the early-1990s, in particular due to the use of UW solution, which had clearly been shown to be a real advantage in pancreas retrieval both experimentally and clinically [31].

In the 1990's the focus on research and advances relating to the retrieval process started to shift, with attention once again shifting to the perfusate solutions, which were thought to be especially impactful for islet cell transplantation. A number of groups also investigated additives to the perfusate solutions such as the use of antibodies to reduce inflammation and further improve graft outcomes, although this was met with limited success [32]. In the 2000's it became generally accepted this was achieved via cannulation of the aorta alone, with or without additional access to the portal venous system with variations that have been seen specifically in relation to multiorgan retrieval where some groups chose to perform 'dual' perfusion technique which are all discussed in greater detail later in this chapter [33, 34].

3. Use of the pancreas for whole organ or cellular transplantation—donor selection

Underpinning the entire transplantation process, regardless of whether the donor is for whole pancreas or islet cell transplantation, is appropriate donor selection such that the donor organ is of a suitable size and quality to allow for use in either type of therapy. In order to be utilized in clinical transplantation, it is imperative that the donor be appropriately screened to ensure the organ to be retrieved is free from any disease that may subsequently manifest in the donor, including cancer, and infections with viruses, bacteria, fungi, or prions [9]. It is paramount that we avoid the more commonly occurring diseases when screening the donor before accepting the pancreas for organ donor retrieval and subsequent clinical transplantation. Infectious risk factors depend on the history of patient, any underlying disease of the organ donor, and the immunosuppressive treatment administered to the recipient [35]. Transmission of most pathogens is possible, but their frequency varies according to the endemic population from the transplanted organ, the selected immunosuppressive therapy and prophylaxis utilized in the recipient, and also at the donor procedure [36]. Obviously, there are many more variables with regards to organ donor selection criteria, and these will be discussed in more detail in the following sections.

4. Pancreas retrieval

4.1. Surgical techniques

Pancreas retrieval for both whole organ and cellular transplantation necessitates meticulous surgical technique. In comparison to the liver and kidneys, the pancreas is more commonly damaged at retrieval, which subsequently results in non-utilization of a significant proportion

of procured pancreata [37]. The organ must be procured without any parenchymal and/or capsular breach, and its arterial inflow and venous outflow vessels must be clearly identified (tagged) and maintained for subsequent back-table reconstruction when used for whole pancreas [38]. The extent of organ and vascular dissection depends upon whether the retrieval is from a brain-dead (DBD) or circulatory death (DCD) donor; a large proportion of pancreas dissection can be undertaken in the warm phase for DBD donors, whilst pursuit of the DCD pathway necessitates wholly cold-phase dissection, which is potentially more difficult as appropriate anatomy is harder to identify.

4.1.1. Anatomical considerations

The pancreas is situated in the retroperitoneum, nestled within the curvature of the duodenum. Important relations are both kidneys posteriorly, the spleen laterally and attached to the pancreas via its pedicle contained within the lienorenal ligament, the superior mesenteric vessels, bile duct, and portal vein in the region of the pancreatic head/neck, the inferior vena cava (IVC) deep to the head and portal vein, and the aorta, left suprarenal gland and left renal vein deep to the body. Pancreatic blood supply is primarily derived from the celiac artery in origin via the splenic and superior mesenteric arteries (via the inferior pancreaticoduodenal artery), and also the gastroduodenal artery (via the superior pancreaticoduodenal artery). The celiac trunk gives off the splenic artery, which emerges at the upper pancreatic border and runs along this border in a tortuous fashion until turning towards the splenic hilum within the lienorenal ligament. The superior mesenteric artery (SMA) emerges from the aorta inferior to the celiac trunk, and is directed inferiorly on the posterior aspect of the pancreatic neck, to then lie on the uncinate process and then the 3rd part of the duodenum prior to entering the root of the mesentery. Venous drainage occurs via the splenic vein for a large part of the pancreas, whilst the superior and inferior pancreaticoduodenal veins drain the head into the superior mesenteric vein (SMV) and portal vein confluence. It is the shared vasculature of the pancreas with the liver that often causes retrieval issues as the origin of the splenic artery is from the celiac, and the outflow of the splenic vein is through the portal vein, necessitating delicate surgical dissection and care in separation to ensure shared and usable vasculature for both organs [39].

4.1.2. DBD retrievals—pancreas-specific considerations

Pancreas retrieval in the DBD donor is a controlled process that allows significant preliminary organ and vascular pedicle dissection. The Cattell-Braasch maneuver is utilized to expose the aorta and IVC distally, with the proximal extent of dissection limited by the SMA overlying the left renal vein; this maneuver will incorporate mobilization of the small bowel mesentery and pancreatic head/duodenum [40]. Our approach to exposure and dissection of the remaining pancreas [41] is to access the lesser sac by mobilization of the greater curvature of the stomach; the greater omentum is detached at its origin using ultrasonic shears (Harmonic Scalpel) as per **Figure 2**. The short gastric vessels are also detached using this method at the upper portion of the greater curvature. The splenic flexure of the large bowel can thence be mobilized onto the lower pole of the spleen. Once the spleen is free of its surrounding attachments, it can be lifted and used as a handle to mobilize the tail and body of the pancreas without directly

handling the pancreas itself. The Harmonic Scalpel is also very useful in the dissection of the superior and inferior pancreatic borders, particularly the relatively vascular splenic flexure of the colon. The posterior surface of the pancreas can be mobilized with standard electrocautery in a relatively bloodless plane. The SMA/SMV pedicle inferior to the pancreas needs to be skeletonized such that it can be divided using a vascular stapler prior to pancreas removal in the cold phase. Superiorly, the bile duct is ligated and transected proximal to the point of ligation; residual bile is flushed out its open proximal end using saline instilled into the gallbladder. We will also free attachments around the gastroduodenal junction and duodenojejunal flexure, which are then identified with circumferential vessel loops for stapled division later in the cold phase. The inferior mesenteric vein is ligated *in situ* post perfusion as subsequent retraction of the divided vessel may make it difficult to identify on the back-table. Diluted povidone-iodine solution is instilled into the duodenum via a nasogastric tube as a decontamination step, and is subsequently removed through the same route. Some authors report concerns with subsequent duodenal mucosal toxicity related to instillation of povidone-iodine, and

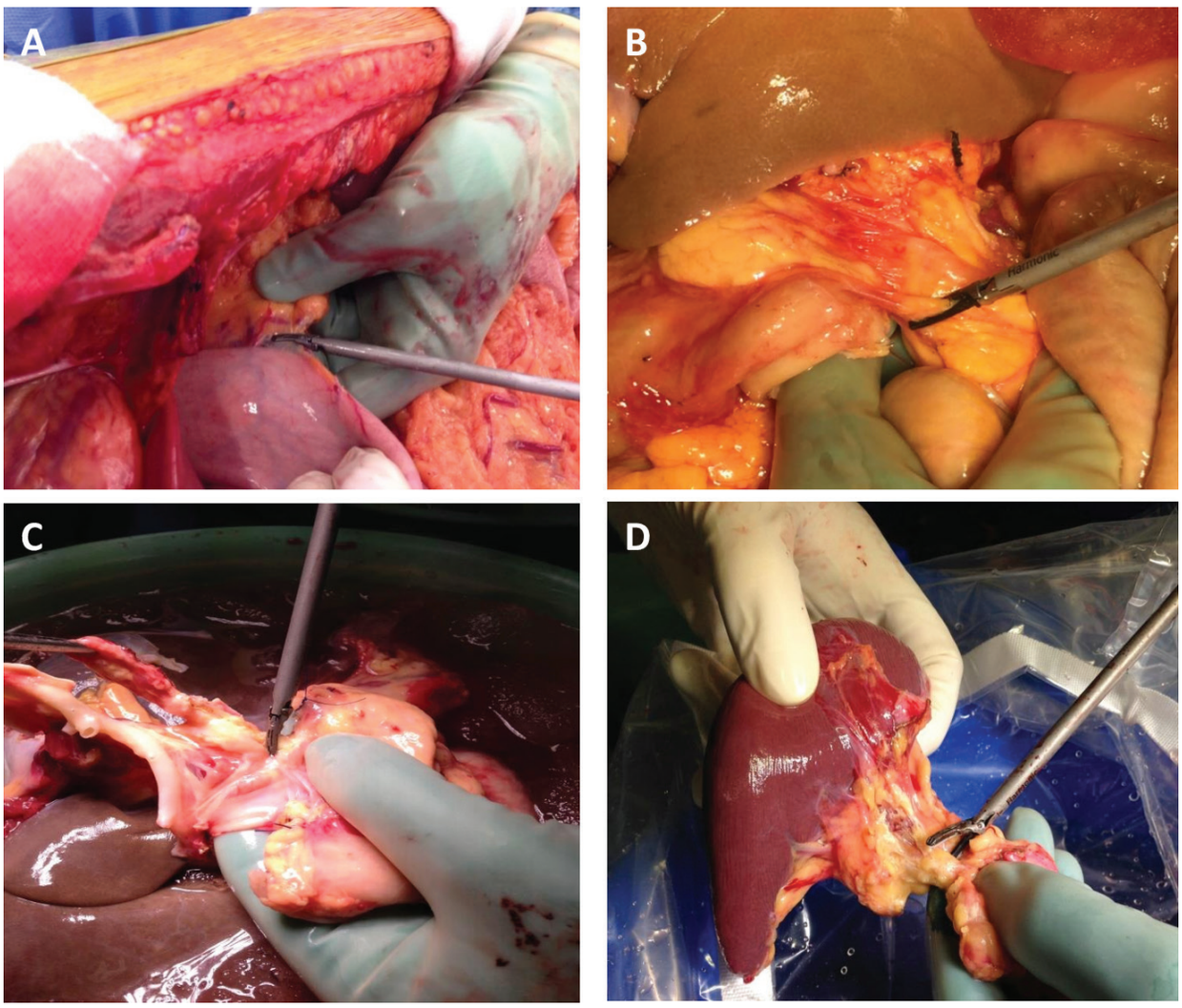


Figure 2. The harmonic scalpels utilization during pancreas procurement. (A) Mobilization of the greater curvature of the stomach, (B) creation of the superior mesenteric pedicle (cold phase), and back-table separation of (C) the liver-pancreas block, and (D) the pancreas and spleen.

suggest additional back-table flushing of the duodenum with an alternate solution [42, 43]. Alternatively, duodenal decontamination can be completed using an antibiotic solution, such as amphotericin [40]. However, the most important factor is to utilize a decontamination procedure to reduce the potential risk of cross infection to the recipient. Our own unit has utilized povidone-iodine solution instilled into the duodenum via a nasogastric tube as a decontamination step in more than 500 SPK transplants at our own center with no duodenal mucosal toxicity identified [44]. In the cold phase the duodenum is then divided above and below the pancreatic head with a linear cutting stapler, after carefully withdrawing the nasogastric tube from the duodenum into the body of the stomach. Any remaining superior mesenteric pedicle dissection is also completed, and a vascular (cutting) stapler is utilized to divide this pedicle. It is of paramount importance that the pancreas is not injured during this step as this will cause serious issues in both whole organ and islet cell transplantation. Furthermore, if the mesenteric pedicle is divided too close to the pancreas, or includes part of the uncinate process, there is a risk that blood supply to the pancreatic head via the inferior pancreaticoduodenal branch of the SMA will be compromised, creating a significant problem for whole organ transplantation [40, 45, 46]. Additionally, for the whole organ transplant an arterial and venous conduit should be retrieved for back-table pancreatic vascular reconstruction. This usually consists of a segment of the external iliac vein for use as a portal vein extension graft if required, and the common iliac artery bifurcation, including a length of the internal and external iliac arteries, to fashion a Y-graft connecting the native SMA and splenic artery. It is essential that the common iliac bifurcation is not damaged during this process [45]. Like a number of other major units our center preferentially retrieves the pancreas *en bloc* with the liver, with separation of both organs performed on the back-table (see below) [47].

4.1.3. DCD retrievals

DCD pancreas retrieval is technically feasible, and can achieve excellent outcomes in selected donors certainly in the whole organ arena (see Outcomes, below). In contrast to DBD procurement, the first step in all DCD retrievals after a rapid laparotomy is cannulation and cold perfusion via the aorta [48, 49]. Venous venting is conducted via the IVC. Alternatively, if local laws allow, an *in situ* flush can be achieved using femoral cannulae inserted prior to the withdrawal of life support [49, 50]. Ante-mortem interventions including heparinization have been shown to also provide significant improvements to pancreas retrieval outcomes in the DCD setting [51]. Standard pancreas retrieval can then be undertaken as described for DBD donors, although donor hemostasis is no longer a concern and therefore sharp dissection is commonly utilized. The use of energy devices such as the Harmonic Scalpel at this stage may help minimize recipient bleeding however, as described in the DBD setting.

4.1.4. Pancreas retrieval and multi-organ donors

Pancreas retrieval is almost never undertaken in isolation, but rather it is usually procured in the context of a multi-organ retrieval, often in the presence of multiple retrieval teams. Meticulous retrieval technique therefore needs to be maintained and balanced in the presence of these competing factors, especially in the presence of concomitant liver procurement, which is still given preference owing to the critical requirement of liver transplant recipients.

Pancreas-alone donors are uncommon in this day and age due to developments in procurement and preservation techniques. Some authors raised concerns that combined liver-pancreas retrieval, in contrast to pancreas retrieval alone, resulted in significant "flush" injury to the pancreas owing to a higher volume of perfusion solution and the utilization of dual aorto-portal cannulation in the combined donors [52]. However, other studies clearly demonstrated that multi-organ retrieval, including combined liver-pancreas retrieval, was not detrimental to pancreas transplantation outcomes [53–58]. Another factor that previously precluded combined liver-pancreas procurement was aberrant hepatic arterial anatomy, in particular the presence of an aberrant or accessory right hepatic artery originating from the superior mesenteric artery [58]. Abandoning retrieval of the pancreas due to this situation is now rare, as a preserved length of the right hepatic artery originating from the SMA stump can effectively be anastomosed to the GDA as part of a back-table reconstructive procedure [45, 46]. It is only when the right hepatic artery is within the substance of the pancreas that whole pancreas retrieval should be precluded in favor of the liver [59] but the pancreas can still be retrieved for islet cell isolation as the pancreas can still be readily perfused, and on the back table the vessels readily separated, including if necessary taking them from the body of the pancreas [9]. However, if this is undertaken then care should be taken to not damage the parenchyma of the pancreas as this makes the distension of the pancreas with collagenase for digestion more difficult [9]. Over the last 25 years and more than 1000 retrievals the authors have never found any anatomical vascular anomaly to prevent an *en bloc* liver-pancreas retrieval, although this is cited as a common reason to decline pancreas retrieval worldwide.

4.1.5. Back-table separation of the liver-pancreas block and further back-table preparation of the pancreas

The combined liver-pancreas block is taken to the back-table for separation. The aortic segment is divided such that the proximal portion of the SMA remains with the pancreas, whilst the celiac axis remains in continuity with the liver. Superior to the pancreatic head, the portal vein is divided approximately 1 cm from the pancreas, whilst the splenic artery is divided closer to its emergence from the celiac axis [45, 46]. The GDA is ligated and divided prior to entering the pancreas; a longer length remains with the liver. The splenic artery and portal vein associated with the pancreas should be marked with a prolene suture to facilitate identification at the transplant center. The spleen is also routinely removed at the donor hospital, in addition to skeletonization of the pancreas prior to transportation. The Harmonic Scalpel is once again a useful tool that facilitates all pancreas-related back-table work if the graft is to be used for whole pancreas transplantation [41]. Limited back-table perfusion of the pancreas with UW solution is employed to ensure no blood is left within the organ or its vessels, whilst minimizing the risk of graft pancreatitis or edema.

In pancreas retrievals for islet cell isolation, the author's use a similar en bloc technique, with careful mobilization of the pancreas prior to aortic cannulation as per **Figure 3**. However, there is no need for meticulous hemostasis post perfusion and it is not necessary to remove the bulk of the tissues as this can be performed at the islet isolation facility. At the isolation center, the duodenum, spleen, and connective, extracapsular and vascular tissues are removed from the pancreas prior to it being cannulated to allow infusion of the digestive collagenase enzyme for

http://dx.doi.org/10.5772/intechopen.75151

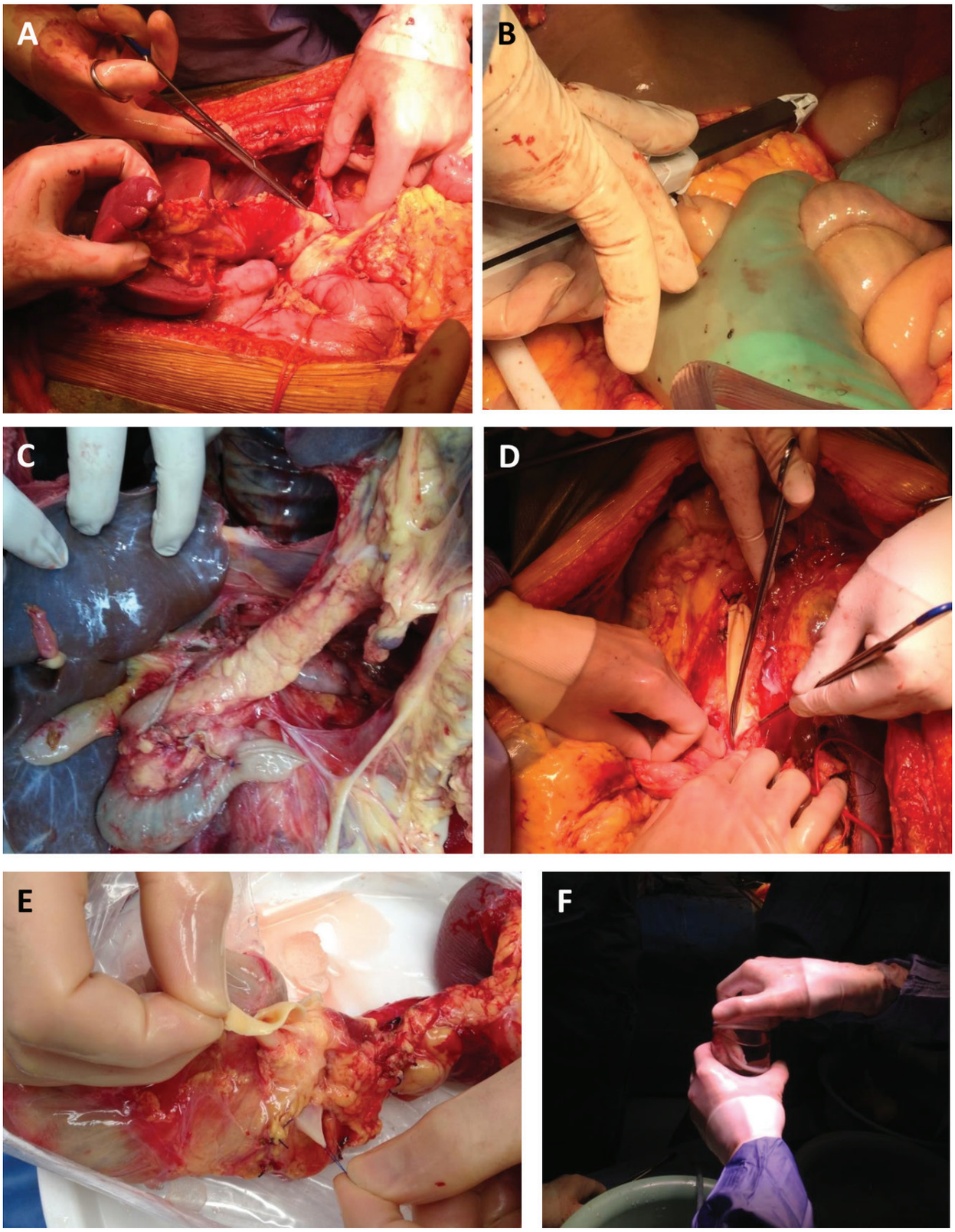


Figure 3. Procurement of the DBD pancreas. (A) Skeletonization of the pancreas, using the spleen as a handle, (B) stapled division of the superior mesenteric pedicle, (C) liver and pancreas ready for en bloc removal, (D) division of the aorta, (E) pancreas appearance after back-table preparation (n.b. Portal vein and superior mesenteric artery), and (F) back-table packing of iliac conduits in preservation solution.

islet cell isolation [9]. As such there is also no need for preservation of pancreas vasculature, which can be given wholly to the liver when separated on the back-table.

4.1.6. Pancreatic inspection and graft assessment

The pancreas must be closely inspected during the retrieval process, and any concerns regarding organ quality and/or integrity should be fully conveyed to the implanting surgeon. Graft assessment should include qualities inherent to the donor pancreas, in addition to any retrieval-related damage, and should be made both *in situ* and on the back-table as per **Figure 3**. The pancreas should be assessed for parenchymal damage, capsular breach, and/or hematoma(s). Furthermore, other important factors that may preclude further transplantation individually or in combination should be identified, including fibrosis, mass(es), high intra-parenchymal fat content, calcification, edema, and/or significantly diseased arteries [40, 46, 59]. It is important to note that much of this assessment is highly subjective, and an "acceptable" pancreatic appearance and/or texture will vary from center-to-center. Obviously some of the co-factors such as high intra-parenchymal fat content, calcification, edema, and/or significantly diseased arteries do not preclude the pancreas from being used for cellular transplantation. As an example, high intra-parenchymal fat content has been shown to be an advantage when performing islet isolation. Additionally, calcification, edema, and/or significantly diseased arteries do not affect the pancreas when used for islet cell isolation as all blood vessels and extraneous tissues are stripped from the pancreas prior to its use. The pancreas should not be discarded without direct consultation with the recipient team and exploration of its use for cellular transplantation if precluded from whole organ transplantation [9].

4.1.7. Packaging the organ for transport

Following perfusion, back-table dissection, and final inspection, the pancreas can then be packed into a suitable transport container along with perfusate solution to ensure ongoing exposure to cold preservation solution. Our unit uses the sterile triple plastic bag technique whereby the organ and a suitable volume of organ perfusion fluid is instilled into the first sterile plastic bag, without dilution from iced slush. All air is removed from the bag, prior to sealing it with a zip-tie or heavy tie. This bag is then placed inside a second sterile plastic bag filled with iced slush, ensuring close and adequate cooling of the perfusate-filled inner bag. These two bags are then placed inside a third sterile plastic bag that is securely sealed, double tied, and appropriately labeled to identify the organ and contents of the bags. Additional vessels retrieved for back-table reconstruction of the whole pancreas may also be packed into the triple sterile plastic bag set with the pancreas, or alternatively are placed inside a sterile vessel jar filled with preservation solution as per **Figure 3**, which is then double-bagged in sterile plastic bags, the first of which contains iced slush. The sealed pancreas and vessels are thence transported in a suitable, insulated iced shipping container. The container is labeled with its contents along with the contact details for both the donor and recipient coordinators.

4.2. In situ perfusion and cold static preservation

The function of *in situ* perfusion of the pancreas, as with other organs, is to achieve rapid removal of residual blood, whilst simultaneously cooling the organ and exposing it to preservation fluid media for subsequent cold static storage (CS).

http://dx.doi.org/10.5772/intechopen.75151

4.2.1. Perfusion route

In order to achieve adequate *in situ* pancreatic perfusion during abdominal organ perfusion as a whole, the aorta must be securely cannulated and flushed using pressure such that perfusion media can flow into the pancreas via the superior mesenteric, gastroduodenal, and splenic arteries. Once perfusion fluid has traversed the pancreas, it must be allowed to exit the donor's vasculature via the systemic and/or portal routes to prevent graft edema. Aortic-only perfusion is routinely performed by our center, and subsequent venous venting is usually undertaken via the IVC in the thorax. In the event that dual aorto-portal perfusion is employed for combined liver-pancreas retrievals, portal venous access via an inferior mesenteric cannula can impede pancreatic outflow, and reduce the physiologic arterial-portal pressure difference that is required for pancreatic perfusion/flow [42, 60]. As such, in these cases, the portal vein may instead be accessed after dividing it superior to the pancreas, thereby also allowing unobstructed pancreatic venous drainage via the proximal aspect of the transected portal vein [60, 61]. A further back-table flush of the pancreas at the donor center is sometimes conducted via the splenic artery and SMA, although this step may be omitted [45, 62–65]. Evidence for or against either approach is currently lacking in both the case of whole pancreas and cellular transplantation. But preference in the cellular setting appears to favor not having any over-perfusion or edema as this can impede and dilute the infusion of the collagenase used for digestion of the pancreas in the isolation process [66].

4.2.2. Perfusion fluid types

In general, the same final fluid employed for the final *in situ* flush of the pancreas is then utilized for preservation of the organ in a bag of cold preservation fluid (CS). The preservation fluid utilized must maintain the organ at a hypothermic temperature (0–4°C), whilst simultaneously ameliorating the consequences of cold ischemia and prolonged organ immersion in fluid. As such, cold organ preservation fluids should ideally have the following properties that aim to minimize and/or reverse cellular and subcellular processes occurring within the pancreas during CS:

- Disrupted ionic pumps and ion accumulation and/or depletion, with subsequent downstream effects;
- Mitochondrial dysfunction, including reversal of the electron transport chain, and succinate accumulation;
- Altered redox potentials (RP);
- Cellular edema;
- Acidosis;
- Accumulation of reactive oxygen species (ROS);
- Adenosine triphosphate (ATP) depletion; and,
- Disruption in glycolytic pathways [67–69].

There are multiple preservation fluids currently in existence. These can be broadly classified as those that are intracellular and extracellular/intermediate in nature, based largely upon the solution's potassium content, or low viscosity compared to high viscosity solutions [70]. Common components include colloid and/or impermeants to counteract cellular edema, anti-oxidants for protection against ROS generation, ATP precursors to allow replenishment upon reperfusion, and buffers to retard the acidosis attendant with organ ischemia [70].

University of Wisconsin (UW) solution remains the most popular pancreatic preservation fluid, and was initially developed specifically for this purpose [71]. It is an intracellular solution with a high potassium content and high viscosity as it contains hydroxyethyl starch, a particularly important component for pancreas preservation [24]. UW contains other components that fulfill many ideal criteria that should be exhibited by preservation fluids, including the addition of impermeants such as raffinose, the ATP precursor adenosine, and anti-oxidants such as allopurinol. [68] Histidine-tryptophan-ketoglutarate (HTK) is another commonly utilized preservation fluid for the pancreas. In contrast to UW, HTK it is an "intermediate" solution with a significantly lower potassium and sodium concentration, thereby in effect preventing ongoing organ metabolism. HTK also has low viscosity, theoretically allowing higher flow rates, and the histidine component of HTK provides it with significant buffering capacity [68, 70]. The next most commonly studied and clinically utilized pancreas perfusion and preservation fluid is Celsior, which has similar potassium content to HTK in addition to containing histidine as a buffer. It differs from HTK in that it has much higher sodium content; furthermore, it incorporates some of the advantageous constituents of UW, including similar impermeants and one shared anti-oxidant [68, 70]. Most recently, the use of Institut Georges Lopez (IGL-1) solution has been reported in pancreatic transplantation [72]. This solution has similar constituents to UW, except the sodium and potassium concentrations are reversed such that it more closely resembles the extra-cellular environment [68]. A number of other more recently developed perfusion fluids have shown good effect in the preservation of pancreata for islet cell transplantation in particular the ET-Kyoto perfusion fluid. This fluid has a high sodium:low potassium ratio, and contains trehalose to protect the cell membrane against hypothermia and the nitric oxide donor nitroglycerin that facilitates vasodilatation [73].

National guidelines and/or protocols differ with respect to recommended perfusion and preservation fluids for the pancreas [45, 60, 74, 75]. UW and HTK solutions are the two most frequently recommended solutions for pancreas retrieval by such guidelines, although their utilization and volumes vary significantly. UK guidelines stipulate that *in situ* UW perfusion must be undertaken for pancreas retrieval, whilst Eurotransplant, German, and Australia/New Zealand guidelines allow for either UW or HTK. Furthermore, none of these guidelines preclude dual perfusion when the pancreas is being retrieved, although German standards stipulate portal venous perfusion via a catheter inserted directly into the portal vein above the pancreas/duodenum [45, 60, 74, 75]. The use of Celsior or IGL-1 solution has not yet been incorporated into major National or Regional guidelines, although both have been employed in the clinical context [64, 65, 72, 76].

A "pre-flush" is defined as a crystalloid fluid, such as Hartmann's solution, that is perfused *in situ* prior to the final flush/preservation fluid, such as UW. The pre-flush can be employed safely in pancreatic procurement, although it is not commonly utilized. The function of this pre-flush in the context of pancreas procurement is to potentially (1) reduce the amount of UW required, thereby reducing retrieval costs, and (2) to clear all blood from the vasculature such that any residual blood does not aggregate with the hydroxyethyl starch in UW [77, 78].

UW is traditionally perfused in much lower volumes in comparison to HTK, and this is also reflected in the various pancreas retrieval guidelines in existence. This is largely related to the higher viscosity of UW, in addition to the larger volume and time for HTK perfusion to achieve equilibration of electrolyte content with the extracellular milieu [79, 80]. Australian guidelines recommend a 2–4 L crystalloid/low viscosity solution *in situ* pre-flush, followed by a UW flush of at least 1–2 L; a volume range for HTK is not specified [45]. In contrast, UK guidelines state a UW flush of 50–70 ml/kg should be employed via the aorta, whilst Eurotransplant allows for 50–100 ml/kg UW or 150–300 ml/kg HTK [74, 75]. Published reports may deviate from this; perfusion volumes utilized in aortic-only perfusion range from 0.8–5.6 L, 4.9–9.7 L, and 0.8–7.9 L for UW, HTK, and Celsior respectively [81].

4.2.3. Additive(s) to perfusate

Heparin is a standard additive to the *in situ* perfusion fluid during DCD organ retrievals, including for the pancreas. Additionally, thrombolytics such as streptokinase or tissue plasminogen activator (tPA) can be added to the *in situ* perfusion fluid, or alternatively our approach is to directly inject tPA into the aorta before commencement of the cold *in situ* flush; the aim of this is to achieve a higher quality vascular flush through the clearance of microthrombi [82–84]. However no comparative evidence exists for or against the use of thrombolytics in DCD pancreas retrieval, although there is certainly enthusiasm for this approach [83, 85].

4.2.4. Two-layer method

Great focus has remained on improving the quality of pancreas transport to the islet transplant centers, including novel ways to provide oxygen rich media to the graft whilst in cold storage during shipping. In late 1988 Kuroda et al. was the first to report the use of the Two-Layer Method (TLM) for shipping of the pancreas prior to islet cell isolation [86]. The TLM uses a perfluorochemical (PFC) and the organ perfusion fluid; initially Euro-Collins' solution was used but was replaced by UW solution. The benefits of the use of the PFC are due to it being a biologically inert liquid that acts as an oxygen-supplying media. A pancreas preserved using the TLM is theoretically oxygenated through the PFC and substrates are supplied by the UW solution. This allows the pancreas preserved using the TLM to generate adenosine triphosphate during storage, prolonging the preservation time [87]. Strong debate still remains over its benefits, if any, when compared to the use of UW solution during CS [88, 89] and a recent publication of guidelines recommended against the use of the TLM for preservation of the pancreas preceding islet isolation [85].

5. Outcomes

5.1. Whole organ pancreas transplant outcomes

Vascularized pancreas transplantation outcomes have improved considerably over time. Although changes to immunosuppression and post-transplantation care can partly account for this, advances in retrieval surgery and organ preservation, in addition to better donor selection, are significant contributors [90, 91]. When exploring pancreas transplantation outcomes, it is paramount to account for the type of transplant performed, as these are associated with differential graft success and survival rates. More specifically, outcomes must be considered based on whether a simultaneous pancreas-kidney (SPK), pancreas after kidney (PAK) transplant, or pancreas transplant alone (PTA) was performed. An exploration of general pancreas transplantation outcomes is beyond the scope of this chapter, as the focus is on the specific impact of retrieval and preservation practices. Overviews investigating trends and recipient outcomes following pancreas transplantation have been published by others, including Dean et al., and Gruessner et al. [90–94]. In brief, the current expected 5-year graft and patient survival rates for pancreas (SPK) transplantation range from 73 to 82% and 89 to 93%, respectively, in the US, UK, Eurotransplant region, and Australia/New Zealand [91, 94–96]. Outcome differences are seen between SPKs, which have historically provided better results, and PTAs and PAKs, due to important variations in the type(s) of recipients for each transplant type, technical differences in the transplantation procedure, and a differential ability to diagnose and treat rejection episodes [91]. SPK transplantation is by far the most commonly performed type of pancreas transplant but islet cell transplantation has also seen a great increase in acceptance and success.

5.2. Islet cell transplant outcomes

Like its forebear, islet cell transplantation outcomes have improved considerably over time. The most impactful change was seen with advances in immunosuppression, clearly shown by the success of the Edmonton trial [97], one that revolutionized the progress of the cellular transplant. Other factors have also continued to impact the field, including post-transplantation care, advances in retrieval surgery and organ preservation, in addition to better donor selection. In brief, the most recent presentation from the Collaborative Islet transplant registry (CITR), presented the combined world islet cell transplant data where they reported that over 1055 allogeneic islet transplants have now been reported by 50 islet transplantation centers in Australia, Europe, North America, and Asia. Of these cases, islet transplant alone (ITA) was the most frequent procedure (n = 858) followed by islet after kidney (IAK) and simultaneous islet and kidney transplantation (SIK) (n = 197) [98]. More recently, according to outcomes of the Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia, the primary end point of HbA1c < 7.0% was achieved by 87.5% of subjects at 1 year and by 71% at 2 years. The median HbA1c level was 5.6% at both 1 and 2 years. Hypoglycemia awareness was restored, with highly significant improvements in Clarke and HYPO scores ($P > 0.0001$). No study-related deaths or disabilities occurred [99]. This trial clearly demonstrated the significant improvements achieved in the outcomes of islet cell transplantation and its impact on those patients suffering from hypoglycemic unawareness.

5.3. The impact of procurement practices and techniques

Pancreas procurement techniques can significantly impact subsequent transplantation outcomes, and can also prove the difference between organ utilization and discard. In particular, there is ample evidence that factors such as *en bloc* retrieval, retrieval technique and graft handling, type(s) of instruments utilized, and perfusion routes are all important determinants of graft function and transplant-related morbidity. Ensuring that pancreas retrieval is performed by an experienced pancreatic transplant surgeon can significantly minimize such retrieval-related complications and risks [100].

Pancreatic damage during retrieval is not uncommon, and may deem the organ unusable certainly for whole organ transplant. Although the rates are different between centers and of course depends upon the level of training of the surgeons performing the retrievals, a large UK registry analysis showed a greater than 50% rate of surgical damage in retrieved pancreata; furthermore, approximately 10% of grafts were subsequently discarded due to damage sustained at retrieval in this analysis [37]. This was further seen as a significant loss as the grafts were also not utilized for islet cell transplantation due to extended cold ischemic times as a result of ongoing referrals. Within the same series, parenchymal and/or vascular (arterial) damage at procurement contributed to significantly higher rates of subsequent graft loss if the pancreas proceeded to transplantation [37]. In order to minimize surgical retrieval damage it is best to ensure that the staff performing the surgery are at a more senior level, and therefore our unit always sends a senior experienced surgeon to all pancreas retrieval surgeries to ensure adequate training of junior staff and optimize graft quality.

Graft thrombosis is the most important technical cause of whole organ pancreatic allograft loss. Pancreas retrieval and surgical technique is a significant etiologic factor in the incidence of graft thrombosis [101–104]. Graft pancreatitis, which in itself is a significant risk factor for graft thrombosis, is another potentially catastrophic complication associated with morbidity and graft loss that is partly attributable to retrieval technique [100]. Excessive graft handling and poor retrieval surgical technique, including damage to the inferior pancreaticoduodenal artery, are commonly accepted causes of graft pancreatitis in the recipient. [100] The same contributing factors also have an impact on the organs when they are used for islet cell isolation [9].

En bloc procurement of the liver and pancreas is associated with better recipient outcomes owing to faster organ retrieval and therefore shorter warm ischemia times [58, 100]. Interestingly, in the aforementioned UK registry analysis between 2008 and 2012, although the vast majority of liver and pancreas retrievals were not performed *en bloc*, there was a trend favoring the *en bloc* approach with respect to reduced pancreatic retrieval injury [37].

In situ perfusion routes, in particular the utilization of dual aorto-portal perfusion in preference to aortic-only perfusion, can impact both whole organ and cellular allograft outcomes. Dual perfusion is potentially associated with increased retrieval-related pancreatic injury through a combination of flush injury (increased perfusion volumes), and/or an obstruction of pancreatic portal venous outflow secondary to catheter placement within the inferior or superior mesenteric veins [52, 58]. This ultimately impacts on the pancreas that is retrieved for whole pancreas or cellular transplantation as it can cause a significant increase in edema, and

may be associated with a higher rate of graft pancreatitis in whole organ, and poorer isolation results due to collagenase dilution in the islet isolation process. Importantly, an aortic-only perfusion technique does not seem to compromise hepatic allograft outcomes, especially in the standard criteria DBD donors from which pancreata are usually retrieved, and therefore should be considered by retrieval surgeons in these circumstances especially in centers that retrieve grafts for both whole and cellular transplantation [34, 58].

Furthermore, the specific instrument-type employed for pancreatic dissection is an important determinant of the amount of pancreatic bleeding upon reperfusion in the recipient [46]. We have shown that ultrasonic shear (e.g. Harmonic Scalpel) utilization during pancreas retrieval allows the sealing of peri-pancreatic vessels that are otherwise easily missed, thereby contributing to less bleeding and a reduced blood transfusion requirement after transplantation within the recipient [41].

5.4. The impact of perfusion and preservation fluids

Pancreas preservation by cold storage using University of Wisconsin solution has been the mainstay method used for pancreas transplantation over the past two decades. Other solutions, such as HTK, Celsior, and SCOT 15, struggled to demonstrate any advantage for short-term preservation periods. But the advent of clinical islet transplantation and the larger use of controlled DBD donors have prompted the transplantation community to develop methods for increasing pancreas graft quality while preventing ischemic reperfusion damage especially in the cellular arena. It has been thought that oxygenation by 1- or 2-layer methods during pancreas preservation, as well as the use of perfluorocarbons, may increase islet yield. Based on the former methods, there is a renewed interest in machine perfusion and oxygenation in pancreas preservation for pancreas transplantation and islet cell preparation [105].

A recent systematic review and meta-analysis by our group compared the outcomes of whole organ pancreas transplantation based on the *in situ* perfusion and subsequent preservation fluid utilized (UW, HTK, or Celsior) [81]. Ischemia-reperfusion injury of the pancreas, as reflected by post-operative peak lipase levels, was significantly lower when UW was employed as a perfusion/preservation fluid in comparison to HTK, but there was no significant difference in peak amylase. This pancreatic ischemia-reperfusion injury may translate to lower clinical graft pancreatitis rates when UW is used in comparison to HTK, although this is not a universal finding [106]. No significant disparity was observed in biochemical injury markers or graft pancreatitis rates between UW and Celsior [81].

As discussed above, post-transplantation graft thrombosis is a significant cause of graft loss. Thrombotic graft loss rates do not differ based on whether UW, HTK, or Celsior is used for *in situ* perfusion and preservation of the pancreas [81]. Furthermore, cumulative graft survival after first post-transplantation month does not favor UW over HTK, although a distinct trend favoring UW emerges at the 1-year mark [81, 106, 107]. A US registry analysis provided further evidence for this, showing a significant association between HTK perfusion/preservation and graft loss, in comparison to UW [108]. In comparison, the use of Celsior is associated with similar 1-year graft survival rates to UW [64, 76].

The comparative utility of each preservation fluid must also be considered in the context of additional donor and transplant-related factors. One important consideration when considering any possible superior preservation effects of UW is the expected pancreatic graft cold ischemic time (CIT). UW may especially be beneficial when CIT is greater than or equal to 12 hours [106, 108]. Furthermore, as already mentioned previously, pancreas retrieval is usually undertaken in the multi-organ donor setting. The perfusion/preservation fluid utilized must therefore not compromise any abdominal organ additionally procured, especially the liver. There is conflicting evidence regarding the relative efficacy of UW, HTK, Celsior, and IGL-1 for liver preservation. Some authors suggest that HTK results in inferior graft survival in comparison to UW, whilst others have reported similar survival but a reduction in post-liver transplantation biliary strictures when HTK is utilized [109, 110]. Overall, current cumulative evidence does not suggest a significant difference between these four fluids, and further research in this area is required [34].

5.5. Donation after circulatory death (DCD) vs. donation after brain death (DBD) transplantation and the importance of donor selection

With careful selection of donors, excellent whole organ pancreatic transplantation outcomes can be obtained even after DCD transplantation. The Pancreas Donor Risk Index (PDRI) is a tool that incorporates donor and preservation-related risk factors, including DCD donors, prolonged preservation time, and high body mass index (BMI), in a risk model for subsequent graft failure [111]. This model has been utilized in both the North American and European settings [111, 112]. It is important to note however that such models must not be used in isolation, and donor pancreata with one or more risk factors, including DCD donors, can still be used to achieve good outcomes. Indeed, our center's first DCD pancreas transplant was in 2007, and has been followed by a further six DCD pancreas transplants, all displaying good long-term graft function [84, 113]. Meta-analyses have shown equivalent graft and patient survival when comparing DBD and DCD pancreatic transplantation, although graft thrombosis rates are higher when DCD grafts are used [51, 114]. Importantly, this higher graft thrombosis rate can be abrogated when donor therapies such as systemic ante-mortem heparin administration are applied [51]. The use of younger donors, with a lower BMI, and low warm ischemic times, has contributed to the success of DCD whole organ pancreas transplantation [84, 115].

There has, however, been more reserved interest in DCD in pancreas for cellular transplantation as the perceived ischemic insult appears to have a much greater effect on the isolated islets for cellular transplantation than when the whole pancreas is transplanted. This is largely because the entire reserve of islets remains intact in the whole organ graft rather than being removed, and a smaller proportion is transplanted in the cellular graft [66, 99]. However, a number of encouraging studies have shown varying success. Albeit from a more advantageous DCD setting allowing earlier intervention including cannulation of the donor and antemortem heparin administration, which has, been shown to be a distinct advantage in this setting [51]. One such report from the Japanese Islet Registry reported their findings from 65 DCD islet isolations performed for 34 transplantations in 18 patients with T1DM. Despite

the fact that all recipients remained free of severe hypoglycemia, only three patients achieved insulin independence for 14, 79, and 215 days. HbA1c levels and requirement of exogenous insulin were significantly improved in all patients [116]. In the more traditional DCD setting the Edmonton group have recently reported their findings comparing islet isolations from 15 DCD and 418 DBD donors performed between September 2008 and September 2014. Compared to DBD, pancreata from DCD were procured locally and donors required less vasopressive support ($P < 0.001$ and $P = 0.023$, respectively), but the other variables were similar between groups. The metabolic function was similar between DBD and DCD, as well as the mean decrease in insulin requirement at 1-month post-transplantation (DBD: 64.82%; DCD: 60.17% reduction, $P = 0.517$). These results support the broader use of DCD pancreata for islet isolation. However, a much larger DCD islet experience will be required to truly determine non-inferiority of both short and long-term outcomes [117].

6. Future perspectives

There has been considerable interest regarding the utility and advantages of dynamic preservation methods in comparison to CS alone for organs such as the liver, kidneys, heart, and lungs. The pancreas has not remained immune to attempts adapting such techniques during the post-procurement phase, although their current clinical success remains limited. Non-static methods of preservation can potentially:

- Reduce graft discard by allowing more accurate graft assessment after retrieval in comparison to current methods, which are largely subjective; and
- Improve organ quality by reducing ischemia-reperfusion-related damage, including by the targeted delivery of pharmacotherapies aimed against ischemia-reperfusion injury, and also gene therapies and stem cells, into the pancreas.

6.1. Machine perfusion

Machine (*ex vivo*) perfusion (MP) entails cannulation and mechanical perfusion of the pancreas via its inflow vessels; perfusion fluid is re-circulated through the circuit for the duration of perfusion. Broadly, MP can be hypothermic, subnormothermic or normothermic, pulsatile or non-pulsatile, and continuous or for a limited proportion of the preservation/transport phase (e.g. pre-implantation). Current pancreatic MP work is lacking in the sphere of clinical transplantation, and is limited to pre-clinical animal and discarded human pancreas studies; only the latter will be the focus of this section, with experimental animal work summarized in detail elsewhere [118–120].

There are certain pancreas-specific issues that need to be considered with respect to MP that do not apply to other organs such as the kidney. Most importantly, the pancreas is a low-flow organ, and even relatively low pressures in a MP setup can result in significant graft edema and weight gain [121]. Furthermore, higher perfusion pressures can contribute to vascular thrombosis secondary to endothelial damage [120]. However, especially if MP is undertaken

at normal body temperature (normothermic), such risks must then be balanced against the need for adequate perfusion to sustain normal aerobic metabolism. An additional challenge during pancreatic MP is the need to adequately and safely account for the organ's exocrine output, which is stimulated during normothermic perfusion [122].

As a result of these issues, most pancreatic MP studies have been conducted in the field of islet cell transplantation rather than the whole pancreas [120, 123]. Graft edema, is disadvantageous for both whole organ and cellular transplantation. However some groups have studied its use as it theoretically facilitates the enzymatic digestion of pancreatic acinar tissue [124]. Hypothermic MP can potentially be employed to increase human islet yield, viability, and insulin secretion despite an extended CIT (> 12 hours), possibly increasing the number of pancreata that can be used for successful islet isolation [125]. Cases of human islet transplantation following MP are yet to be published, however. Whole organ pancreas MP has been investigated in the context of extended criteria organs that were not utilized for human transplantation. Some authors have shown 6 hours of oxygenated hypothermic MP using UW machine perfusion solution increases the ATP content of DCD pancreata to reach a level that is similar to DBD pancreata at baseline [126]. Graft edema can be kept to a minimum if low pressure hypothermic MP is utilized, even for as long as 24 hours [127]. Subsequent *ex vivo* normothermic perfusion can be used to simulate reperfusion at transplantation after initial hypothermic MP, and has been shown to demonstrate adequate insulin secretion by such pancreata [128].

Normothermic MP is an attractive alternative for whole pancreas preservation, and likely provides better graft viability assessment than hypothermic perfusion. Both endocrine and exocrine graft function can be assessed during perfusion by measuring C-peptide and/or insulin secretion and stimulation in response to glucose, and amylase and lipase release, respectively [122, 129]. Blood flow and resistance parameters can also be assessed using this technique, although this is also possible with hypothermic MP. However it is important to note that no defined cut-offs or validated protocols for human transplantation have been developed, and will require significantly more pre-clinical work.

6.2. Persufflation

Persufflation is a technique in which the pancreas is directly perfused with a humidified gas such as oxygen via the SMA and/or splenic arteries. Non-utilized human DBD pancreata have been perfused by this method, and subsequent graft assessment showed an increase in pancreatic ATP levels [130]. Porcine data from the same group showed significantly improved pancreatic histology after 24 hours of persufflation in comparison to utilization of the TLM [131]. Islet isolation after 24 hours of persufflation, including in human pancreata, is likely increased, compared to other methods such as the TLM [132]. This was confirmed in a later study, whereby islets of sufficient quantity and quality for transplantation were isolated from all five human pancreata that underwent persufflation using 40% humidified oxygen perfused at 10–25 mmHg [133]. Similar to MP however, pancreas persufflation has not yet been followed by clinical islet and/or whole organ pancreas transplantation although some research is now underway by a limited number of groups.

6.3. Normothermic regional perfusion

Normothermic Regional Perfusion (NRP) of the abdomen was initially utilized in Spain in the uncontrolled DCD setting, and has since been utilized in the controlled DCD setting in other European countries and Asia [134–138]. The donor's systemic arterial and venous systems are rapidly cannulated, and an *ex vivo* pump/oxygenator system is used to maintain an effective artificial circulation of the abdominal viscera. Cerebral and thoracic perfusion is avoided by clamping the supra-celiac aorta. This system reduces the organ's warm ischemic insult, and proposed benefits include facilitation of a more effective subsequent *in situ* cold flush, ATP replenishment, and reduced oxidative stress [139]. Current experience for NRP exists mainly in the sphere of kidney and liver transplantation. However, utilization of this technique for DCD pancreas preservation and transplantation is appealing, especially because DCD pancreata can have sustained, long-term graft function (as discussed above). Within the UK, five pancreata have been procured after initial NRP, resulting in two SPK transplants and one islet cell transplantation [136]. In Spain, one NRP pancreas has been transplanted in the context of a controlled DCD donor [140]. Future studies are required to more effectively classify evidence for this strategy, and define its comparative role or efficacy with respect to MP. In the DCD setting, NRP may prove to be a more feasible strategy than MP owing to the aforementioned difficulties of maintaining a pancreas on an *ex vivo* machine circuit, although no direct comparisons exist between the two methods.

7. Conclusions

This chapter outlines the numerous advances that have occurred over the past few decades in pancreas retrieval techniques for both whole organ and cellular transplantation. It clearly demonstrates the improved outcomes in both whole pancreas and islet cell transplantation from significant improvements to organ donor selection and management, and organ perfusion and retrieval surgery. We have seen insulin independence rates for more than 10 years post-transplant in both settings with minimal complications. Whole organ transplantation is obviously now a well-accepted clinical therapy for many patients worldwide. However, islet transplantation still has limited application to the broader population of patients with T1D due to its reliance on the availability of cadaveric donors and selection, isolation results and transplant engraftment, the side effects of immunosuppression and issues associated with the requirement for lifelong immunosuppression. The future holds many interesting potential new therapies that may or may not yield appropriate and safe methods for treatment of type 1 diabetes. From what has been outlined in this chapter we can see that outcomes for patients have improved significantly. If, unfortunately, patients cannot be treated prior to the advent of their type 1 diabetes then they can still be treated by transplantation. Moving forward, researchers and clinicians have numerous fronts and multiple strategies arising at different stages of development in which to be able to offer patients treatments tailored to them and their disease. In the foreseeable future, transplantation and in particular the focus on organ retrieval and organ preservation will play a significant role in further improving outcomes, particularly with newer technologies such as machine perfusion and normothermic regional perfusion. Such technologies are hoped to increase both the

number of suitable whole pancreata, as well as their quality, which will simultaneously lead to improved islet cell numbers and function in the cell therapy sphere of Diabetes care.

Acknowledgements

The authors wish to thank Callista Rainey for assistance with the figures in this chapter. The authors also wish to acknowledge support from the Royal Australasian College of Surgeons (Sir Roy McCaughey Surgical Research Fellowship).

Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

BMI	body mass index
CS	Celsior
CIT	cold ischemic time
DBD	donation after brain death
DCD	donation after circulatory death
PFC	perfluorochemical
T1D	type 1 diabetes
UW solution	University of Wisconsin
SPK	simultaneous pancreas and kidney
TLM	two-layer method
T1D	type 1 diabetes

Author details

Wayne J. Hawthorne[1,2,3]*, Ahmer Hameed[1,2,3] and Henry Pleass[1,3]

*Address all correspondence to: wayne.hawthorne@sydney.edu.au

1 Department of Surgery, Westmead Hospital, Sydney, Australia

2 Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney, Australia

3 Sydney Medical School, University of Sydney, Sydney, Australia

References

[1] Squifflet JP, Gruessner RW, Sutherland DE. The history of pancreas transplantation: Past, present and future. Acta Chirurgica Belgica. 2008;**108**:367-378

[2] Sutherland DE, Najarian JS. Transplantation of the pancreas. Transplantation Proceedings. 1979;**11**:1158-1162

[3] Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care. 2012;**35**:1436-1445. DOI: 10.2337/dc12-0063

[4] Squifflet JP. The history of transplantation at the Catholic University of Louvain-Belgium 1963-2003. Acta Chirurgica Belgica. 2003;**103**(Suppl 1):10-20. DOI: 10.1080/00015458.2003.11679382

[5] A definition of irreversible coma. Report of the ad hoc committee of the Harvard Medical School to examine the definition of brain death. Journal of the American Medical Association. 1968;**205**:337-340

[6] Bell MD. Non-heart beating organ donation: Old procurement strategy--new ethical problems. Journal of Medical Ethics. 2003;**29**:176-181

[7] Hawthorne WJ. Necessities for a clinical islet program. Advances in Experimental Medicine and Biology. 2016;**938**:67-88. DOI: 10.1007/978-3-319-39824-2_6

[8] O'Gorman D, Kin T, Murdoch T, Richer B, McGhee-Wilson D, Ryan E, et al. The standardization of pancreatic donors for islet isolation. Transplantation Proceedings. 2005;**37**:1309-1310. DOI: 10.1016/j.transproceed.2004.12.087

[9] Farney ACSD, Opara EC. Evolution of islet transplantation for the last 30 years. Pancreas. 2016;**45**:8-20. DOI: 10.1097/MPA.0000000000000391

[10] Bertuzzi FAB, Tosca MC, Galuzzi M, Bonomo M, Marazzi M, Colussi G. Islet transplantation in pediatric patients: Current indications and future perspectives. Endocrine Development. 2016;**30**:14-22. DOI: 10.1159/000439322

[11] Starzl TE. Experience in Renal Transplantation. Philadelphia: WB Saunders Co.; 1964

[12] Gwathmey O, Pierpont H. Stage occlusion and resection of the human aortic arch with hypothermia. The American Surgeon. 1955;**21**:827-834

[13] Owens JC, Prevedel AE, Swan H. Prolonged experimental occlusion of thoracic aorta during hypothermia. A.M.A. Archives of Surgery. 1955;**70**:95-97

[14] Marchioro TL, Huntley RT, Waddell WR, Starzl TE. Extracorporeal perfusion for obtaining postmortem homografts. Surgery. 1963;**54**:900-911

[15] Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. Lancet. 1967;**2**:536-538

[16] Starzl TE. History of clinical transplantation. World Journal of Surgery. 2000;**24**:759-782

[17] Collins GM, Bravo-Shugarman M, Terasaki PI. Kidney preservation for transportation. Initial perfusion and 30 hours' ice storage. Lancet. 1969;**2**:1219-1222

[18] Ackermann JR, Snell ME. Cadaveric renal transplantation: A technique for donor kidney removal. British Journal of Urology. 1968;**40**:515-521

[19] Merkel FK, Jonasson O, Bergan JJ. Procurement of cadaver donor organs: Evisceration technique. Transplantation Proceedings. 1972;**4**:585-589

[20] Benichou J, Halgrimson CG, Weil R 3rd, Koep LJ, Starzl TE. Canine and human liver preservation for 6 to 18 hr by cold infusion. Transplantation. 1977;**24**:407-411

[21] de Gruyl J, Westbroek DL, Macdicken I, Ridderhof E, Verschoor L, van Strik R. Cryoprecipitated plasma perfusion preservation and cold storage preservation of duct-ligated pancreatic allografts. The British Journal of Surgery. 1977;**64**:490-493

[22] Wall WJ, Calne RY, Herbertson BM, Baker PG, Smith DP, Underwood J, et al. Simple hypothermic preservation for transporting human livers long distances for transplantation. Report of 12 cases. Transplantation. 1977;**23**:210-216

[23] Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD, et al. Extended preservation of the liver for clinical transplantation. Lancet. 1988;**1**:617-619

[24] Ploeg RJ, Boudjema K, Marsh D, Bruijn JA, Gooszen HG, Southard JH, et al. The importance of a colloid in canine pancreas preservation. Transplantation. 1992;**53**:735-741

[25] Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation. 1988;**45**:673-676

[26] Starzl TE, Hakala TR, Shaw BW Jr, Hardesty RL, Rosenthal TJ, Griffith BP, et al. A flexible procedure for multiple cadaveric organ procurement. Surgery, Gynecology & Obstetrics. 1984;**158**:223-230

[27] Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. Surgery, Gynecology & Obstetrics. 1987;**165**:343-348

[28] Deane SA, Ekberg H, Stewart GJ, Grierson JM, Williamson P, Hawthorne W, et al. Whole pancreas grafting with exocrine drainage into the bladder: Method of choice for clinical transplantation? Transplantation Proceedings. 1988;**20**:84-86

[29] Ekberg H, Deane SA, Allen RDM, Hawthorne WJ, Williamson P, Grierson JM, et al. Monitoring of canine pancreas allograft function with measurements of urinary amylase. ANZ Journal of Surgery. 1988;**58**:583-586. DOI: 10.1111/j.1445-2197.1988.tb06198.x

[30] Deane SA, Ekberg H, Stewart GJ, Grierson JM, Williamson P, Hawthorne WJ, et al. Canine whole pancreatic transplantation with exocrine drainage into the bladder. ANZ Journal of Surgery. 1989;**59**:659-664

[31] Griffin AD, Hawthorne WJ, Allen RD, Grierson JM, Jablonski P, Howden BO, et al. Twenty-four-hour preservation of canine pancreas allografts using low-cost, low-viscosity, modified University of Wisconsin cold storage solution. Transplantation Proceedings. 1993;**25**:1595-1596

[32] Wilson TG, Hawthorne WJ, Lau H, Williamson P, Chapman JR, Grierson JM, et al. Pretreatment of canine whole pancreas allografts with monoclonal antibodies does not prolong graft survival. Transplantation Proceedings. 1990;**22**:2163-2164

[33] Lam VW, Pleass HC, Hawthorne W, Allen RD. Evolution of pancreas transplant surgery. ANZ Journal of Surgery. 2010;**80**:411-418. DOI: 10.1111/j.1445-2197.2010.05309.x

[34] Hameed AM, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation of the hepatic allograft: Working toward a unified approach. Liver Transplantation. 2017;**23**:1615-1627. DOI: 10.1002/lt.24829

[35] Greenwald MA, Kuehnert MJ, Fishman JA. Infectious disease transmission during organ and tissue transplantation. Emerging Infectious Diseases. 2012;**18**. DOI: 10.3201/eid1808.120277

[36] Martinez-Pourcher V. Infections in the transplant patient. La Revue du Praticien. 2015;**65**:1075-1078

[37] Ausania F, Drage M, Manas D, Callaghan CJ. A registry analysis of damage to the deceased donor pancreas during procurement. American Journal of Transplantation. 2015;**15**:2955-2962. DOI: 10.1111/ajt.13419

[38] Allen RD, Nankivell BJ, Hawthorne WJ, O'Connell PJ, Chapman JR. Pancreas and islet transplantation: An unfinished journey. Transplantation Proceedings. 2001;**33**:3485-3488

[39] Allen RD, Chapman JR, Hawthorne WJ, Pearl TA, Wilson TG, Lau H, et al. Advantages and disadvantages of pancreas transplantation. Transplantation Proceedings. 1992;**24**:171-172

[40] Fridell JA, Powelson JA, Kubal CA, Burke GW, Sageshima J, Rogers J, et al. Retrieval of the pancreas allograft for whole-organ transplantation. Clinical Transplantation. 2014;**28**:1313-1330. DOI: 10.1111/ctr.12459

[41] Hameed A, Yu T, Yuen L, Lam V, Ryan B, Allen R, et al. Use of the harmonic scalpel in cold phase recovery of the pancreas for transplantation: The westmead technique. Transplant International. 2016;**29**:636-638. DOI: 10.1111/tri.12777

[42] Forsythe JL. In: Garden OJ, Paterson-Brown S, editors. A Companion to Surgical Practice: Transplantation. 5th ed. Saunders Elsevier: Edinburgh; 2014

[43] Olson DW, Kadota S, Cornish A, Madsen KL, Zeng J, Jewell LD, et al. Intestinal decontamination using povidone-iodine compromises small bowel storage quality. Transplantation. 2003;**75**:1460-1462. DOI: 10.1097/01.tp.0000060871.02234.1b

[44] Thwaites SE, Lam VWT, Yao J, Kable K, Jenkins L, Chen C, et al. Surgical morbidity of simultaneous kidney and pancreas transplantation: A single-Centre experience in the Tacrolimus era. ISRN Transplantation. 2013;**2013**(6). DOI: 10.5402/2013/685850

[45] TSANZ. Guidance Document - Surgical Technique for Deceased Donor Abdominal Organ Procurement (ATCA-TSANZ Guidelines G003/2015). Sydney, Australia: TSANZ; 2015

[46] Oniscu GC, Forsythe JLR, Fung JJ. Abdominal Organ Retrieval and Transplantation Bench Surgery. Chichester, West Sussex: John Wiley & Sons; 2013

[47] Thwaites SE, Gurung B, Yao J, Kable K, Robertson P, Ryan BJ, et al. Excellent outcomes of simultaneous pancreas kidney transplantation in patients from rural and urban Australia: A national service experience. Transplantation. 2012;**94**:1230-1235. DOI: 10.1097/TP.0b013e3182708e04

[48] Jeon H, Ortiz JA, Manzarbeitia CY, Alvarez SC, Sutherland DE, Reich DJ. Combined liver and pancreas procurement from a controlled non-heart-beating donor with aberrant hepatic arterial anatomy. Transplantation. 2002;**74**:1636-1639. DOI: 10.1097/01.tp.0000038707.82035.75

[49] Reich DJ, Mulligan DC, Abt PL, Pruett TL, Abecassis MM, D'Alessandro A, et al. ASTS recommended practice guidelines for controlled donation after cardiac death organ procurement and transplantation. American Journal of Transplantation. 2009;**9**:2004-2011. DOI: 10.1111/j.1600-6143.2009.02739.x

[50] D'Alessandro AM, Hoffmann RM, Knechtle SJ, Eckhoff DE, Love RB, Kalayoglu M, et al. Successful extrarenal transplantation from non-heart-beating donors. Transplantation. 1995;**59**:977-982

[51] Shahrestani S, Webster AC, Lam VW, Yuen L, Ryan B, Pleass HC, et al. Outcomes from pancreatic transplantation in donation after cardiac death: A systematic review and meta-analysis. Transplantation. 2017;**101**:122-130. DOI: 10.1097/tp.0000000000001084

[52] Nghiem DD, Cottington EM. Pancreatic flush injury in combined pancreas-liver recovery. Transplant International. 1992;**5**:19-22

[53] Dunn DL, Morel P, Schlumpf R, Mayoral JL, Gillingham KJ, Moudry-Munns KC, et al. Evidence that combined procurement of pancreas and liver grafts does not affect transplant outcome. Transplantation. 1991;**51**:150-157

[54] D'Alessandro AM, Reed A, Hoffmann RM, Sollinger HW, Kalayoglu M, Knechtle SJ, et al. Results of combined hepatic, pancreaticoduodenal, and renal procurements. Transplantation Proceedings. 1991;**23**:2309-2311

[55] Conway MB, Saunders R, Munn SR, Perkins JD. Combined liver/pancreaticoduodenal procurement effect on allograft function. Transplantation Proceedings. 1990;**22**:429-430

[56] Spees EK, Orlowski JP, Temple DR, Kam I, Karrer IF. Efficacy of simultaneous cadaveric pancreas and liver recovery. Transplantation Proceedings. 1990;**22**:427-428

[57] Abu-Elmagd K, Fung J, Bueno J, Martin D, Madariaga JR, Mazariegos G, et al. Logistics and technique for procurement of intestinal, pancreatic, and hepatic grafts from the same donor. Annals of Surgery. 2000;**232**:680-687

[58] Brockmann JG, Vaidya A, Reddy S, Friend PJ. Retrieval of abdominal organs for transplantation. The British Journal of Surgery. 2006;**93**:133-146. DOI: 10.1002/bjs.5228

[59] Maglione M, Ploeg RJ, Friend PJ. Donor risk factors, retrieval technique, preservation and ischemia/reperfusion injury in pancreas transplantation. Current Opinion in Organ Transplantation. 2013;**18**:83-88. DOI: 10.1097/MOT.0b013e32835c29ef

[60] Wunderlich H, Brockmann JG, Voigt R, Rauchfuss F, Pascher A, Brose S, et al. DTG procurement guidelines in heart beating donors. Transplant International. 2011;**24**:733-757. DOI: 10.1111/j.1432-2277.2011.01266.x

[61] Sollinger HW, Vernon WB, D'Alessandro AM, Kalayoglu M, Stratta RJ, Belzer FO. Combined liver and pancreas procurement with Belzer-UW solution. Surgery. 1989;**106**:685-690 discussion 690-681

[62] Potdar S, Malek S, Eghtesad B, Shapiro R, Basu A, Patel K, et al. Initial experience using histidine-tryptophan-ketoglutarate solution in clinical pancreas transplantation. Clinical Transplantation. 2004;**18**:661-665. DOI: 10.1111/j.1399-0012.2004.00262.x

[63] Fridell JA, Mangus RS, Powelson JA. Histidine-tryptophan-ketoglutarate for pancreas allograft preservation: The Indiana University experience. American Journal of Transplantation. 2010;**10**:1284-1289. DOI: 10.1111/j.1600-6143.2010.03095.x

[64] Boggi U, Vistoli F, Del Chiaro M, Signori S, Croce C, Pietrabissa A, et al. Pancreas preservation with University of Wisconsin and Celsior solutions: A single-center, prospective, randomized pilot study. Transplantation. 2004;**77**:1186-1190

[65] Nicoluzzi J, Macri M, Fukushima J, Pereira A. Celsior versus Wisconsin solution in pancreas transplantation. Transplantation Proceedings. 2008;**40**:3305-3307. DOI: 10.1016/j.transproceed.2008.05.080

[66] Hawthorne WJ. Beta cell therapies for type 1 diabetes. In: Hardikar AA, editor. Pancreatic Islet Biology. Switzerland: Springer Press; 2016

[67] Churchill TA. Organ Preservation for Transplantation. Functional Metabolism. John Wiley & Sons, Inc.; 2005. pp. 529-555. DOI: 10.1002/047167558X.ch19

[68] Guibert EE, Petrenko AY, Balaban CL, Somov AY, Rodriguez JV, Fuller BJ. Organ preservation: Current concepts and new strategies for the next decade. Transfusion Medicine and Hemotherapy. 2011;**38**:125-142. DOI: 10.1159/000327033

[69] Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature. 2014;**515**:431-435. DOI: 10.1038/nature13909

[70] Bon D, Chatauret N, Giraud S, Thuillier R, Favreau F, Hauet T. New strategies to optimize kidney recovery and preservation in transplantation. Nature Reviews. Nephrology. 2012;**8**:339-347. DOI: 10.1038/nrneph.2012.83

[71] Wahlberg JA, Love R, Landegaard L, Southard JH, Belzer FO. 72-hour preservation of the canine pancreas. Transplantation. 1987;**43**:5-8

[72] Chedid MF, Grezzana-Filho TJ, Montenegro RM, Leipnitz I, Hadi RA, Chedid AD, et al. First report of human pancreas transplantation using IGL-1 preservation solution: A case series. Transplantation. 2016;**100**:e46-e47. DOI: 10.1097/tp.0000000000001242

[73] Chen F, Nakamura T, Wada H. Development of new organ preservation solutions in Kyoto University. Yonsei Medical Journal. 2004;**45**:1107-1114. DOI: 10.3349/ymj.2004.45.6.1107

[74] Eurotransplant Foundation. Eurotransplant Manual. Leiden: Netherlands: Eurotransplant; 2016

[75] Zalewska K, Ploeg R. National Standards for Organ Retrieval from Deceased Donors (NORS Retrieval Standards). UK: Bristol; 2014

[76] Manrique A, Jimenez C, Herrero ML, Meneu JC, Abradelo M, Moreno A, et al. Pancreas preservation with the University of Wisconsin versus Celsior solutions. Transplantation Proceedings. 2006;**38**:2582-2584. DOI: 10.1016/j.transproceed.2006.08.058

[77] van der Plaats A, t Hart NA, Morariu AM, Verkerke GJ, Leuvenink HG, Ploeg RJ, et al. Effect of University of Wisconsin organ-preservation solution on haemorheology. Transplant International. 2004;**17**:227-233. DOI: 10.1007/s00147-004-0705-8

[78] Gonzalez AM, Filho GJ, Pestana JO, Linhares MM, Silva MH, Moura RM, et al. Effects of Eurocollins solution as aortic flush for the procurement of human pancreas. Transplantation. 2005;**80**:1269-1274

[79] Blech M, Hummel G, Kallerhoff M, Ringert RH. Electrolyte equilibration of human kidneys during perfusion with HTK-solution according to Bretschneider. Urological Research. 1997;**25**:331-335

[80] Troisi R, Meester D, Regaert B, Jacobs B, Van den Broucke C, Cuvelier C, et al. Physiologic and metabolic results of pancreatic cold storage with Histidine-tryptophan-Ketoglutarate-HTK solution (Custodiol) in the porcine autotransplantation model. Transplant International. 2000;**13**:98-105

[81] Hameed AM, Wong G, Laurence JM, Lam VWT, Pleass HC, Hawthorne WJ. A systematic review and meta-analysis of cold in situ perfusion and preservation for pancreas transplantation. HPB: The Official Journal of the International Hepato Pancreato Biliary Association. 2017;**19**:933-943. DOI: 10.1016/j.hpb.2017.07.012

[82] Fridell JA, Mangus RS, Thomas CM, Kubal CA, Powelson JA. Donation after circulatory arrest in pancreas transplantation: A report of 10 cases. Transplantation Proceedings. 2017;**49**:2310-2314. DOI: https://doi.org/10.1016/j.transproceed.2017.10.009

[83] Rathnasamy Muthusamy AS, Friend PJ, Dor FJM, Crane JS, Papalois V, Herbert P, et al. DCD pancreas transplantation meta-analysis: Ethical and technical considerations. Transplantation. 2017;**101**:e57. DOI: 10.1097/tp.0000000000001560

[84] Shahrestani S, Robertson P, Pleass HCC, Hawthorne WJ. The authors' reply. Transplantation. 2017;**101**:e58. DOI: 10.1097/tp.0000000000001561

[85] Berney T, Boffa C, Augustine T, Badet L, de Koning E, Pratschke J, et al. Utilization of organs from donors after circulatory death for vascularized pancreas and islet of Langerhans transplantation: Recommendations from an expert group. Transplant International. 2016;**29**:798-806. DOI: 10.1111/tri.12681

[86] Kuroda YKT, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y. A new, simple method for cold storage of the pancreas using perfluorochemical. Transplantation. 1988;**46**:457-460

[87] Fujino Y. Two-layer cold storage method for pancreas and islet cell transplantation. World Journal of Gastroenterology. 2010;**16**:3235-3238. DOI: 10.3748/wjg.v16.i26.3235

[88] Hosgood SA, Nicholson ML. The role of perfluorocarbon in organ preservation. Transplantation. 2010;**89**:1169-1175. DOI: 10.1097/TP.0b013e3181da6064

[89] Iwanaga Y, Sutherland DE, Harmon JV, Papas KK. Pancreas preservation for pancreas and islet transplantation. Current Opinion in Organ Transplantation. 2008;**13**:135-141. DOI: 10.1097/MOT.0b013e3282f63942

[90] Gruessner AC, Sutherland DE, Gruessner RW. Long-term outcome after pancreas transplantation. Current Opinion in Organ Transplantation. 2012;**17**:100-105. DOI: 10.1097/MOT.0b013e32834ee700

[91] Dean PG, Kukla A, Stegall MD, Kudva YC. Pancreas transplantation. BMJ. 2017;**357**. DOI: 10.1136/bmj.j1321

[92] Dean PG, Kudva YC, Stegall MD. Long-term benefits of pancreas transplantation. Current Opinion in Organ Transplantation. 2008;**13**:85-90. DOI: 10.1097/MOT.0b013e3282f2fd7f

[93] Gruessner RWG, Gruessner AC. The current state of pancreas transplantation. Nature Reviews. Endocrinology. 2013;**9**:555-562. DOI: 10.1038/nrendo.2013.138

[94] Gruessner AC, Gruessner RW. Long-term outcome after pancreas transplantation: A registry analysis. Current Opinion in Organ Transplantation. 2016;**21**:377-385. DOI: 10.1097/mot.0000000000000331

[95] NHS Blood and Transplant. Annual Report on Pancreas and Islet Transplantation. UK: NHS; 2017

[96] ANZIPTR. ANZIPTR Report 2017 - Australia and New Zealand Islet and Pancreas Transplant Registry Data 1984-2016. Westmead: ANZIPTR; 2017

[97] Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. The New England Journal of Medicine. 2006;**355**:1318-1330. DOI: 10.1056/NEJMoa061267

[98] Barton FB. CITR Update. Melbourne: IPITA/IXA/CTS Joint Congress; 2015

[99] Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, Bellin MD, et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe Hypoglycemia. Diabetes Care. 2016;**39**:1230-1240. DOI: 10.2337/dc15-1988

[100] Nadalin S, Girotti P, Konigsrainer A. Risk factors for and management of graft pancreatitis. Current Opinion in Organ Transplantation. 2013;**18**:89-96. DOI: 10.1097/MOT.0b013e32835c6f0f

[101] Troppmann C, Gruessner AC, Benedetti E, Papalois BE, Dunn DL, Najarian JS, et al. Vascular graft thrombosis after pancreatic transplantation: Univariate and multivariate operative and nonoperative risk factor analysis. Journal of the American College of Surgeons. 1996;**182**:285-316

[102] Farney AC, Rogers J, Stratta RJ. Pancreas graft thrombosis: Causes, prevention, diagnosis, and intervention. Current Opinion in Organ Transplantation. 2012;**17**:87-92. DOI: 10.1097/MOT.0b013e32834ee717

[103] Patel SR, Hakim N. Prevention and management of graft thrombosis in pancreatic transplant. Experimental and Clinical Transplantation. 2012;**10**:282-289

[104] Troppmann C. Complications after pancreas transplantation. Current Opinion in Organ Transplantation. 2010;**15**:112-118. DOI: 10.1097/MOT.0b013e3283355349

[105] Squifflet JP, LeDinh H, de Roover A, Meurisse M. Pancreas preservation for pancreas and islet transplantation: A minireview. Transplantation Proceedings. 2011;**43**:3398-3401. DOI: 10.1016/j.transproceed.2011.09.052

[106] Alonso D, Dunn TB, Rigley T, Skorupa JY, Schriner ME, Wrenshall LE, et al. Increased pancreatitis in allografts flushed with histidine-tryptophan-ketoglutarate solution: A cautionary tale. American Journal of Transplantation. 2008;**8**:1942-1945. DOI: 10.1111/j.1600-6143.2008.02312.x

[107] Englesbe MJ, Moyer A, Kim DY, Granger DK, Pietroski R, Yoshida A, et al. Early pancreas transplant outcomes with histidine-tryptophan-ketoglutarate preservation: A multicenter study. Transplantation. 2006;**82**:136-139. DOI: 10.1097/01.tp.0000225764.21343.e3

[108] Stewart ZA, Cameron AM, Singer AL, Dagher NN, Montgomery RA, Segev DL. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation. American Journal of Transplantation. 2009;**9**:217-221. DOI: 10.1111/j.1600-6143.2008.02449.x

[109] Stewart ZA, Cameron AM, Singer AL, Montgomery RA, Segev DL. Histidine-tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. American Journal of Transplantation. 2009;**9**:286-293. DOI: 10.1111/j.1600-6143.2008.02478.x

[110] Mangus RS, Fridell JA, Vianna RM, Milgrom MA, Chestovich P, Chihara RK, et al. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution in extended criteria liver donors. Liver Transplantation. 2008;**14**:365-373. DOI: 10.1002/lt.21372

[111] Axelrod DA, Sung RS, Meyer KH, Wolfe RA, Kaufman DB. Systematic evaluation of pancreas allograft quality, outcomes and geographic variation in utilization. American Journal of Transplantation. 2010;**10**:837-845. DOI: 10.1111/j.1600-6143.2009.02996.x

[112] Blok JJ, Kopp WH, Verhagen MJ, Schaapherder AF, de Fijter JW, Putter H, et al. The value of PDRI and P-PASS as predictors of outcome after pancreas transplantation in a large European pancreas transplantation center. Pancreas. 2016;**45**:331-336. DOI: 10.1097/mpa.0000000000000485

[113] Suh N, Ryan B, Allen R, O'Connell P, Pleass H. Simultaneous pancreas and kidney transplantation from organ donation after cardiac death. ANZ Journal of Surgery. 2009;**79**:245-246. DOI: 10.1111/j.1445-2197.2009.04853.x

[114] van Loo ES, Krikke C, Hofker HS, Berger SP, Leuvenink HGD, Pol RA. Outcome of pancreas transplantation from donation after circulatory death compared to donation after brain death. Pancreatology. 2017;**17**:13-18. DOI: https://doi.org/10.1016/j.pan.2016.11.002

[115] Muthusamy AS, Mumford L, Hudson A, Fuggle SV, Friend PJ. Pancreas transplantation from donors after circulatory death from the United Kingdom. American Journal of Transplantation. 2012;**12**:2150-2156. DOI: 10.1111/j.1600-6143.2012.04075.x

[116] Saito T, Gotoh M, Satomi S, Uemoto S, Kenmochi T, Itoh T, et al. Islet transplantation using donors after cardiac death: Report of the Japan islet transplantation registry. Transplantation. 2010;**90**:740-747. DOI: 10.1097/TP.0b013e3181ecb044

[117] Andres A, Kin T, O'Gorman D, Livingstone S, Bigam D, Kneteman N, et al. Clinical islet isolation and transplantation outcomes with deceased cardiac death donors are similar to neurological determination of death donors. Transplant International. 2016;**29**:34-40. DOI: 10.1111/tri.12650

[118] Kuan KG, Wee MN, Chung WY, Kumar R, Mees ST, Dennison A, et al. Extracorporeal machine perfusion of the pancreas: Technical aspects and its clinical implications – A systematic review of experimental models. Transplantation Reviews. 2016;**30**:31-47. DOI: https://doi.org/10.1016/j.trre.2015.06.002

[119] Taylor MJ, Baicu SC. Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. Cryobiology. 2010;**60**:S20-S35. DOI: 10.1016/j.cryobiol.2009.10.006

[120] Balfoussia D, Yerrakalva D, Hamaoui K, Papalois V. Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. Experimental and Clinical Transplantation. 2012;**10**:87-100

[121] Hamaoui K, Gowers S, Sandhu B, Vallant N, Cook T, Boutelle M, et al. Development of pancreatic machine perfusion: Translational steps from porcine to human models. In: J Surg Res. 2018. DOI: 10.1016/j.jss.2017.11.052

[122] Barlow AD, Hamed MO, Mallon DH, Brais RJ, Gribble FM, Scott MA, et al. Use of ex vivo Normothermic perfusion for quality assessment of discarded human donor pancreases. American Journal of Transplantation. 2015;**15**:2475-2482. DOI: 10.1111/ajt.13303

[123] Barlow AD, Hosgood SA, Nicholson ML. Current state of pancreas preservation and implications for DCD pancreas transplantation. Transplantation. 2013;**95**:1419-1424. DOI: 10.1097/TP.0b013e318285558f

[124] Taylor MJ, Baicu S, Leman B, Greene E, Vazquez A, Brassil J. Twenty-four hour hypothermic machine perfusion preservation of porcine pancreas facilitates processing for islet isolation. Transplantation Proceedings. 2008;**40**:480-482. DOI: 10.1016/j.transproceed.2008.01.004

[125] Leeser DB, Bingaman AW, Poliakova L, Shi Q, Gage F, Bartlett ST, et al. Pulsatile pump perfusion of pancreata before human islet cell isolation. Transplantation Proceedings. 2004;**36**:1050-1051. DOI: 10.1016/j.transproceed.2004.04.041

[126] Leekmuil M, Engelse M, Ploeg RJ, de Koning E, Krikke C, Leuvenink HG, editors. Hypothermic Machine Perfusion Improves the Quality of Marginal Donor Pancreata [Abstract]. American Transplant Congress; 2015 Am J Transplant

[127] Cantarovich D, Renaudin K, Branchereau J, editors. Preservationof Human Pancreas with Hypothermic Machine Perfusion [Abstract]. Congress of the Transplantation Society. Hong Kong: Transplantation; 2016

[128] Hamaoui K, Gowers S, Sandhu B, Vallant N, Cook T, Boutelle M, et al. Development of pancreatic machine perfusion: Translational steps from porcine to human models. The Journal of Surgical Research. 2018. DOI: 10.1016/j.jss.2017.11.052

[129] Nassar A, Liu Q, Urcuyo D, Medearis S, El-Gazzaz G, Eghtesad B, et al. Establishing an ex-vivo pancreas perfusion model on discarded human grafts [abstract]. World transplant congress; 2014. Transplantation. 2014;**98**:376

[130] Scott WE, Weegman BP, Ferrer-Fabrega J, Stein SA, Anazawa T, Kirchner VA, et al. Pancreas oxygen Persufflation increases ATP levels as shown by nuclear magnetic resonance. Transplantation Proceedings. 2010;**42**:2011-2015. DOI: 10.1016/j.transproceed.2010.05.091

[131] Scott WE, O'Brien TD, Ferrer-Fabrega J, Avgoustiniatos ES, Weegman BP, Anazawa T, et al. Persufflation improves pancreas preservation when compared with the two-layer method. Transplantation Proceedings. 2010;**42**:2016-2019. DOI: 10.1016/j.transproceed.2010.05.092

[132] Scott WE, Rizzari MD, Stein ES, Avgoustiniatos ES, Suszynski TM, Weegman BP, et al., editors. Oxygen Persufflation of the Human Pancreas Increases ATP Levels and Improves Viable Islet Yields Compared with the two-Layer Method [Abstract]. Prague: IPITA; 2011

[133] Papas KK, Karatzas T, Purvis WG, Kitzmann JP, Gruessner A, O'Gorman D, et al., editors. Pancreas Oxygen Gas Perfusion (Persufflation) during Preservation Improves Clinical Islet Isolation Yields and Success Rates [Abstract]. Melbourne: IPITA; 2015

[134] Demiselle J, Augusto JF, Videcoq M, Legeard E, Dube L, Templier F, et al. Transplantation of kidneys from uncontrolled donation after circulatory determination of death: Comparison with brain death donors with or without extended criteria and impact of normothermic regional perfusion. Transplant International. 2016;**29**:432-442. DOI: 10.1111/tri.12722

[135] Butler AJ, Randle LV, Watson CJ. Normothermic regional perfusion for donation after circulatory death without prior heparinization. Transplantation. 2014;**97**:1272-1278. DOI: 10.1097/tp.0000000000000082

[136] Oniscu GC, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MT, et al. In situ normothermic regional perfusion for controlled donation after circulatory death--the United Kingdom experience. American Journal of Transplantation. 2014;**14**:2846-2854. DOI: 10.1111/ajt.12927

[137] Valero R, Cabrer C, Oppenheimer F, Trias E, Sanchez-Ibanez J, De Cabo FM, et al. Normothermic recirculation reduces primary graft dysfunction of kidneys obtained from non-heart-beating donors. Transplant International. 2000;**13**:303-310

[138] Lee JH, Hong SY, Oh C-K, Hong YS, Yim H. Kidney transplantation from a donor following cardiac death supported with extracorporeal membrane oxygenation. Journal of Korean Medical Science. 2012;**27**:115-119. DOI: 10.3346/jkms.2012.27.2.115

[139] Hessheimer AJ, Billault C, Barrou B, Fondevila C. Hypothermic or normothermic abdominal regional perfusion in high-risk donors with extended warm ischemia times: Impact on outcomes? Transplant International. 2015;**28**:700-707. DOI: 10.1111/tri.12344

[140] Minambres E, Suberviola B, Dominguez-Gil B, Rodrigo E, Ruiz-San Millan JC, Rodriguez-San Juan JC, et al. Improving the outcomes of organs obtained from controlled donation after circulatory death donors using abdominal Normothermic regional perfusion. American Journal of Transplantation. 2017;**17**:2165-2172. DOI: 10.1111/ajt.14214

Intentional gap - contents from prior letter removed

Letter

Optimal Culture Methods and Microbial Contamination During Kidney Ex Vivo Normothermic Perfusion

Sara Shahrestani, MD,[1,2,3] Ahmer Hameed, MBBS,[1,2,3] Kerry Hitos, PhD,[2,4] Henry Pleass, MD,[1,2,3] and Wayne J. Hawthorne, MD, PhD[1,2,3,5]

Received 2 April 2018. Revision received 28 April 2018.

Accepted 1 May 2018.

[1] Westmead Clinical School, Sydney Medical School, University of Sydney, NSW, Australia.

[2] Sydney Medical School, University of Sydney, NSW, Australia.

[3] The Department of Surgery, Westmead Hospital, Westmead, NSW, Australia.

[4] Westmead Research Centre for Evaluation of Surgical Outcomes, Department of Surgery, Westmead Hospital, Westmead, NSW, Australia.

[5] The Centre for Transplant & Renal Research, Westmead Millennium Institute, Westmead, NSW, Australia.

The authors declare no conflicts of interest.

S.S. participated in research design, data collection, data analysis and writing. A.H. participated in data analysis and writing. K.H. participated in research design, data analysis and writing. H.C.P. participated in research design, data analysis and writing. W.J.H. participated in research design, data analysis, and writing.

Correspondence: Wayne J. Hawthorne, MD, PhD, Department of Surgery, The University of Sydney at Westmead Hospital, Westmead, NSW, 2145, Australia. (wayneh@med.usyd.edu.au).

ISSN: 0041-1337/18/10209-e398

DOI: 10.1097/TP.0000000000002302

We read with particular interest the article entitled "Microbial contamination during kidney ex vivo normothermic perfusion."[1] In light of organ donation shortages, the utilization of ex vivo normothermic perfusion (EVNP) of the kidney has significant potential to reduce graft discard rates while simultaneously enhancing recipient outcomes.[2-3]

Ex vivo normothermic perfusion presents challenges, including the potential to introduce contaminants into the donor organ perfusion fluid. The authors examined the microbial growth rates and in both cold and warm perfusion solutions in a small sample of organs that were subsequently transplanted. The authors' comment "Two cold organ transport fluid cultures had positive growth, but with no consistency of the organisms grown from the EVNP perfusate." At our center, we have likewise found low rates of positive culture at a rate of 16.0% (N = 19/119) with direct plate culture from cold transport perfusate fluid of kidneys. However, by using BACTEC (tryptic soy broth enriched liquid media and radiometric assessment of bacterial growth, Becton Dickinson [BD], Sparks, MD) culture methods, the positive culture rate was in fact much higher at around 54.1% (N = 33/61).

Standard antimicrobial prophylaxis in kidney transplants consisted of IV cephazolin for 48 hours and oral trimethoprim/

sulfamethoxazole daily. Pancreas-kidney recipients received 100 mg of fluconazole daily for 5 days, 500 mg thrice daily of metronidazole and 1 g thrice daily of amoxicillin for 4 days. All positive cultures were communicated to the nephrologist on duty, who then assessed whether the standard antimicrobial prophylaxis was sufficient, or based on sensitivities, whether an additional antimicrobial was necessary.

Overall, 96 patients from 286 kidney and pancreas-kidney recipients (33.6%) had a wound infection or collection that cultured 1 or more distinct organisms. This consisted of 77 (80%) superficial wound infections (wound/skin infections) and 19 (19.7%) deep infections (intra-abdominal perigraft collections). Wound infections were typically treated with a combination of antimicrobial therapy and debridement, whereas deeper collections required aspiration for targeted antimicrobial therapy. An increased rate of enteric flora contamination in simultaneous pancreas and kidney transplants (26%) relative to kidney alone (9%, $P = 0.03$) may drive the increased risk of wound infection in this group, (odds ratio [OR], 44.37; 95% confidence interval [CI], 5.02-391.93; $P = 0.001$). Importantly, we found that when BACTEC was utilized in both kidney and kidney-pancreas recipients to identify the type of microbiological growth, the risk of recipient wound infection at the surgical site was significantly reduced (adjusted OR, 0.24; 95% CI, 0.07-0.86; $P = 0.029$). When standard culture was used, the odds of infection were higher (adjusted OR, 4.02; 95% CI, 1.09-14.84; $P = 0.037$), and authors speculate that this association is likely due to the poorer identification of potential infection. A systematic review and meta-analysis of culture-positive perfusion fluid found that recipient infection was far less likely when appropriate prophylaxis was given.[4] A study of liver transplants likewise found that infection occurred far less frequently (3.8% vs 43%, $P < 0.005$) in recipients with appropriate prophylaxis against the organisms in the perfusion fluid.[5]

We would recommend BACTEC for microbial culture of organ perfusion media and read with interest future works examining culture results from warm and cold perfusion media. Using BACTEC culture results to guide antimicrobial prophylaxis and treatment can have a significant effect on reducing recipient wound complications. As the use of EVNP increases, we hope future work will clarify these practices such that there is increased provision of optimal organs while minimizing procedural morbidity in transplant recipients.

REFERENCES

1. Phillips BL, Chandak P, Uwechue R, et al. Microbial contamination during kidney ex vivo normothermic perfusion. *Transplantation*. 2018; 102:e186–e188.
2. Hameed AM, Hawthorne WJ, Pleass HC. Advances in organ preservation for transplantation. *ANZ J Surg*. 2017;87:976–980.
3. Hameed AM, Pleass HC, Wong G, et al. Maximizing kidneys for transplantation using machine perfusion: from the past to the future: A comprehensive systematic review and meta-analysis. *Medicine (Baltimore)*. 2016;95:e5083.
4. Oriol I, Sabé N, Tebé C, et al. Clinical impact of culture-positive preservation fluid on solid organ transplantation: a systematic review and meta-analysis. *Transplant Rev (Orlando)*. 2018;32:85–91.
5. Janny S, Bert F, Dondero F, et al. Microbiological findings of culture-positive preservation fluid in liver transplantation. *Transpl Infect Dis*. 2011;13:9–14.

Letter

The Authors' Reply

Benedict Lyle Phillips, MSc, MRCS,[1] Pankaj Chandak, MRCS,[1] Raphael Uwechue, MRCS,[1] Claire van Nispen tot Pannerden, MSc, MD,[2] Caroline Hemsley, PhD, FRCP, FRCPath,[2] and Chris Callaghan, FRCS, PhD[1]

Received 17 May 2018.

Accepted 21 May 2018.

[1] Department of Nephrology and Transplantation, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom.

[2] Department of Clinical Infection, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom.

Funding sources from Kidney Research UK, Medical Research Council Centre for Transplantation, Guy's and St Thomas' NHS Foundation Trust, and Guy's and St Thomas' Charity.

The authors declare no conflicts of interest.

B.L.P. performed ex vivo normothermic perfusion, data collection, data analysis, and article preparation. P.C. performed ex vivo normothermic perfusion, and article preparation. R.U. performed ex vivo normothermic perfusion and article preparation. C.v.N.t.P. performed the culture preparation analysis and article preparation. C.H. performed the culture preparation analysis and article preparation. C.J.C. performed ex vivo normothermic perfusion, data analysis, and article preparation.

Correspondence: Benedict Lyle Phillips, BSc(hons) MB ChB MSc MRCS, Guy's and Saint Thomas' NHS Foundation Trust, London, United Kingdom. (Benedict.Phillips@gstt.nhs.uk).

ISSN: 0041-1337/18/10209-e399

DOI: 10.1097/TP.0000000000002303

We thank Shahrestani and colleagues[1] for their interest in our work, and for providing data on the rates of positive cultures of cold transport fluids using different culture methods. In our study, we demonstrated a high rate of apparent skin commensal contamination of the warm perfusate during ex vivo normothermic perfusion of kidneys before transplantation, but with no observed clinical implications in posttransplant infectious outcomes. Microbial growth within the perfusion fluid of ex vivo normothermic perfusion (EVNP) was initially thought to have been introduced either during EVNP or the culture-taking process. Given that the transport fluid had not grown the same organisms, we felt that these organisms were unlikely to have originated from the organ retrieval process. However, Shahrestani et al provided a valid alternative explanation for these findings by demonstrating increased sensitivity of enriched media, relative to direct plate culture. This may explain why, in our study, the kidney transport fluid grew fewer organisms after direct plate culture, compared with the EVNP perfusate, which underwent enhanced culture in 3% soybean-casein digest broth. It is therefore

Accepted: 15 May 2017

DOI: 10.1111/ctr.13016

ORIGINAL ARTICLE

WILEY Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research

Dual kidney transplant techniques: A systematic review

Annelise Cocco[1] | Sara Shahrestani[2] | Nicholas Cocco[3] | Ahmer Hameed[1] | Lawrence Yuen[1] | Brendan Ryan[1] | Wayne Hawthorne[2] | Vincent Lam[1,2] | Henry Pleass[1,2,3]

[1]Westmead Hospital, Westmead, NSW, Australia

[2]University of Sydney, Sydney, NSW, Australia

[3]Department of Surgery, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Correspondence
Henry Pleass, Department of Surgery, Westmead Hospital, Westmead, NSW, Australia.
Email: henry.pleass@sydney.edu.au

Abstract

Background: Dual kidney transplantation (DKT) was developed to improve outcomes from transplantation of extended criteria donors (ECD). This study examined which surgical techniques have been reported for DKT and whether any technique had superior patient and graft survival.

Method: Electronic databases were searched for published studies mapping to MESH terms: "kidney or renal" AND "transplan*" AND "dual or double." Single case reports, studies of patients less than 18 years old, studies which did not describe the surgical technique, and studies that did not report patient or graft survival were excluded.

Results: Fifteen reports of 434 DKT recipients were identified. Three techniques were described: bilateral placement; unilateral placement with separate anastomoses; and unilateral placement with patch anastomoses. Patient survival across all three techniques was over 95% at 1 year, and graft survival was also similar at over 90%. Rates of delayed graft function were between 20% and 30% across all techniques.

Conclusion: The three techniques have equivalent delayed graft function as well as patient and graft survival rates. This is an encouraging result as it means that the surgeon can choose to use the technique which is most appropriate for their own skills and for the patient.

KEYWORDS
dual kidney transplant, kidny transplantation, renal transplantation

1 | INTRODUCTION

Chronic kidney disease is an increasingly prevalent condition, affecting up to 16% of the adult population in several continents.[1] However, the number of organ donors across the world has remained relatively stagnant, further increasing the discrepancy between supply and demand.[2] To address the challenge of organ donation shortages, extended criteria donors (ECD) are increasingly utilized, particularly in kidney transplantation.[3] Extended criteria donors are defined by the United Network for Organ Sharing (UNOS) as donors aged over 60 or donors older than 50 with any two of the following: hypertension; cerebrovascular cause of brain death; or elevated terminal creatinine.[3] There is a known and well-demonstrated negative effect of age-related low nephron mass, particularly in extended criteria donors, which in turn leads to higher rates of delayed graft function and lower graft survival.[4]

To address the issue of low nephron mass, the transplantation of two kidneys from a marginal donor to one recipient, known as dual kidney transplantation (DKT), has been proposed as an alternative to single kidney transplant from extended criteria donors.[3] DKT has been shown to be effective when appropriate donors and recipients are chosen. Although DKT may involve greater anesthetic and surgical risk, it is associated with significantly higher rates of immediate graft function, especially when kidneys are acquired from extended criteria donors.[5]

What is not yet clear is the ideal surgical technique to perform a DKT. As dual kidney transplant becomes increasingly common with the utilization of more organs from extended criteria donors, it is important

for surgeons to be aware of the different operative approaches. The aim of this systematic review was to examine which techniques of dual kidney transplant are being performed, and whether any one of these is superior in terms of patient and graft survival. We also examined the rates of delayed graft function and surgical complications. Our hypotheses were as follows: That there would be three different surgical approached to the performance of dual renal transplant, and that none of the approaches would be demonstrably superior to the others.

2 | METHODS

This systematic review was performed by the investigators and reported in adherence to the Moose Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies.[6]

2.1 | Search strategy

The search strategy followed guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions.[7]

Electronic databases were searched, including MEDLINE via PubMed and EMBASE from January 1995 to July 2016, for published studies using the following search script, mapping to MESH terms: "kidney or renal" AND "transplan*" AND "dual or double." Two independent reviewers (AC and SS) reviewed full-text articles and any discrepancies were resolved via discussion. Once a study was selected for inclusion, its reference list was hand searched to identify further studies that could be of relevance. The search strategy is outlined in Figure 1.

2.2 | Inclusion and exclusion criteria

All studies examining outcomes from dual kidney transplants were examined. No randomized controlled trials have been performed. All donor types were included. All studies reported the method of surgical technique employed in performing DKT. If it was not clear which technique was used, an effort was made to contact the study author/s and clarify this. All studies reported transplant outcomes either alone or in comparison with single kidney transplant outcomes. Only studies that were reported in English were included.

Studies that did not describe the surgical technique that was used, studies performed in patients less than 18 years old, studies that did not describe patient and/or graft survival, and single case reports were excluded.

2.3 | Bias appraisal

We assessed the potential for bias in included studies using the Newcastle-Ottowa Quality Assessment Scale for Cohort Studies.[8]

2.4 | Data abstraction and outcomes

Each study was reviewed, and data were extracted into a predetermined template. The following data were extracted from each article: author name and year, country, study period, study type, single vs multicenter, number of patients, mean and median follow-up period, donor subtype, donor age, donor comorbidities, graft appearance, biopsy and biopsy score, cold ischemic time, warm ischemic time, surgical technique, operative time, recipient age, recipient comorbidities, recipient time on dialysis, peri-operative mortality, delayed graft function, length of hospitalization, creatinine level at follow-up, graft survival at 1 and 2 years, and patient survival at 1 and 2 years.

2.5 | Data analysis

Formal meta-analyses could not be performed due to significant study heterogeneity. Semi-quantitative summary statistics were formulated for primary and secondary outcomes of interest after their tabulation.

3 | RESULTS

3.1 | Study characteristics

We identified 15 reports for inclusion, of 434 DKT recipients (Figure 1). The number of DKT recipients per study ranged from 9 to 100; characteristics of these studies are provided in Tables 1-3.

3.2 | Surgical technique

Three different surgical techniques used to perform DKT were identified: bilateral placement, unilateral placement with separate anastomoses, and unilateral placement with patch anastomoses. We will briefly outline these three methods before comparing the outcomes from each. In the bilateral placement of DKT, separate incisions are used in each iliac fossa to place kidneys separately on both sides. Anastomoses to the iliac vessels are performed separately on either side. In the unilateral placement with separate anastomoses, only one incision in the iliac fossa is made, and kidneys are anastomosed to the iliac vessels on one side in turn. In unilateral placement with single patch anastomosis, the renal artery and vein from each kidney are anastomosed during back table preparation allowing for a single incision in one iliac fossa and single anastomoses with iliac vessels as in single kidney transplant. Further details of these techniques, as well as the outcomes of the individual papers, are discussed below.

3.3 | Bilateral placement

Ten studies examined outcomes of patients who had bilateral placement of their kidney transplants: four cohort studies, five case-control studies, and one case series. There were a total of 266 patients with a mean donor age of 67.7 and mean recipient age of 60.5. An illustration of the technique is provided in Figure 2. Andres, Lee, Remuzzi, Johnson, Moore, and D'Arcy performed end-to-side anastomoses to the iliac vessels, but did not specify to exactly which vessel although

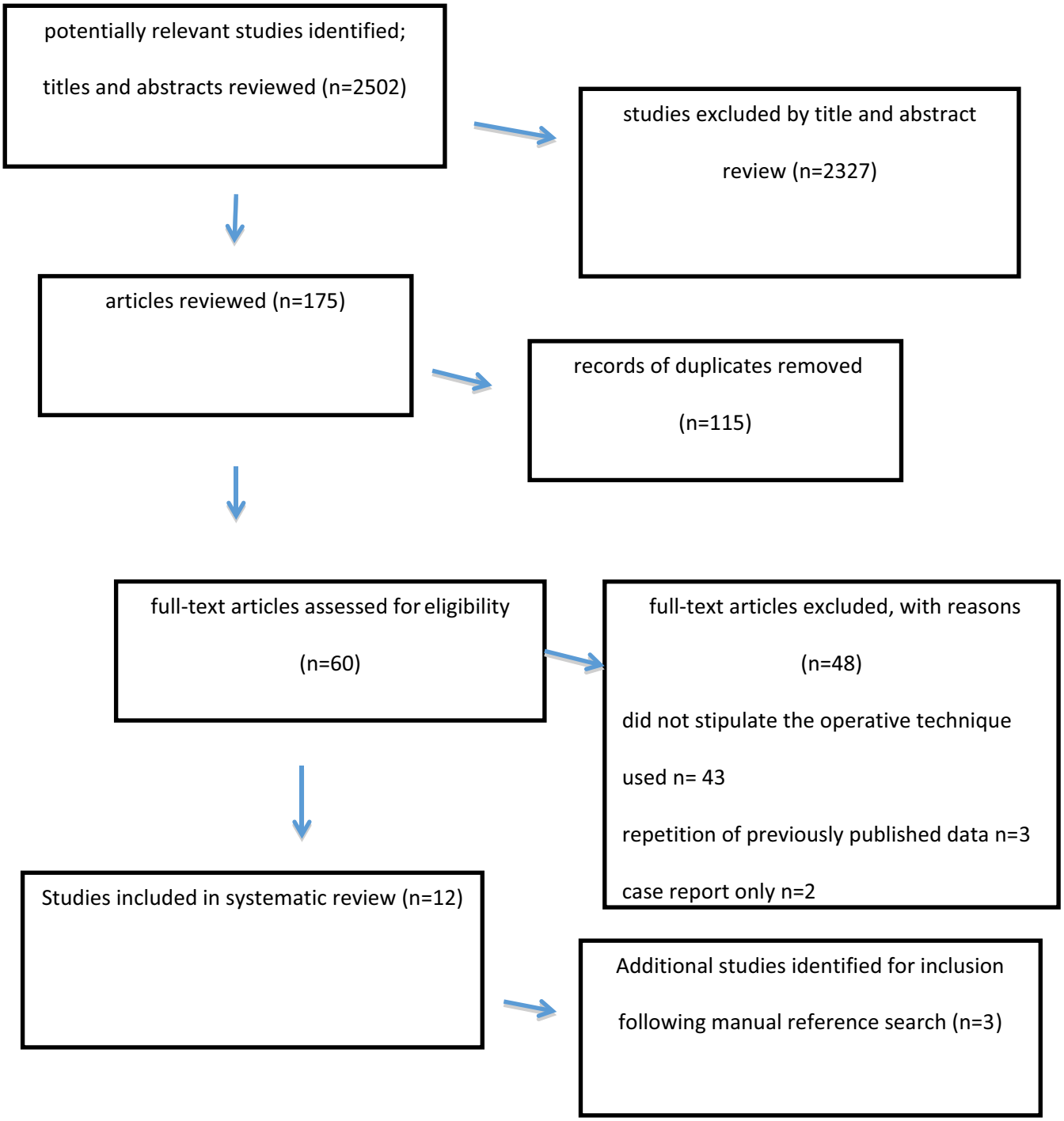


FIGURE 1 Flow chart of search strategy

it seemed to be a mixture of common, external, and occasionally internal iliac artery and vein.[5,9-13] Andres, D'Arcy, Moore, Johnson, and Lee performed bilateral ureterocystostomies.[5,9-11,13] Remuzzi did not specify how the urinary reconstruction was performed.[12] De Serres, Timsit, and Lee used the external iliac vessels, and De Serres and Lee performed extravesical ureteroneocystostomies, whereas Timsit performed pyeloureterostomy.[3,14,15] Rigotti did not specify to which vessels the renal artery and vein were anastomosed, nor were details regarding the urinary reconstruction provided.[16]

3.4 | Unilateral placement with separate anastomoses

Four studies looked at the results of unilateral placement using two separate anastomoses. One was a cohort study, one was a case control, and the other two were case series. There were a total of 146 patients with a mean donor age of 71.5 and mean recipient age of 63.2. An illustration of the technique is provided in Figure 3. In the Timset, Veroux, and Ekser studies, one kidney had anastomoses to

TABLE 1 Bilateral placement

Study	Years	Number of recipients	Donor type	Average donor age (y)	Average recipient age	CIT mean (h)	SWIT (per graft)
Andres "Double versus Single"[9]	1996-1998	21	ECD	75±7	60±5 y	22±4	Not stated
De Serres "Dual Kidney"[3]	1999-2007	63	ECD	69±8	60±9	23.1±4.5 (2nd)	Not stated
D'Arcy "Dual Kidney"[13]	2001-2008	24	ECD	60.6	60.6	17.9 (1st)	45.6 min
Lee CM "Dual Kidney"[10]	1995-1998	41	ECD	59±12	58±11	19±8	36±9
Remuzzi "Early Experience"[12]	1996-1998	24	ECD	68.7±6.8	59.4±9.9	20.3±0.3	22
Timsit "Single Graft Loss"[14]	2004-2007	41	ECD	76.5	69.4	Not stated	Not stated
Moore "Dual Kidney"[5]	2001-2006	16	ECD	65±8	49±7	22.3 (1st) 23.8 (2nd)	Not stated
Lee RS "Intermediate Outcomes"[15]	1996-1998	10	8 ECD 2 not	58.6±13.1	54.5±9.1	23.1±6.2	42.3±5.1
Johnson "Double adult renal"[11]	1994-1996	9	ECD	62.7±3.4	Not stated	23.2±2.8	Not stated
Rigotti "Short Term Outcome" [16]	1999-2001	16	ECD	72±5	63±3	16 (1st) 18 (2nd)	Not stated
Summary		266		67.67	60.51[b]		

[a]Three of these patients died. The grafts were still functioning at the time of death.
[b]Contributing data incomplete.

TABLE 2 Unilateral placement with separate anastomoses

Study	Years	Number of recipients	Donor type	Average donor age (y)	Average recipient age (y)	CIT mean (h)
Timsit "Single Graft Loss"[14]	2004-2007	14	ECD	76.5	69.4	Not stated
Veroux M "Monolateral Dual Kidney"[17]	2002-2006	23	ECD	66	66	19.6
Ekser "Technical Aspects"[4]	2003-2009	100	ECD	72.1±5.7	61.7±5.6	15.9±2.9 (2nd graft)
Gaber "Ipsilateral Placement"[18]	(published 2007)	9	ECD	Not stated	Not stated	Not stated
Summary		146		71.5[a]	63.2[a]	

[a]Contributing data incomplete.

TABLE 3 Unilateral placement with patch anastomoses

Study	Years	Number of recipients	Donor type	Average donor age (y)	Average recipient age (y)	CIT mean (h)
Ngheim "Simultaneous Double"[19]	1999-2005	12	ECD	72.2	Not stated	26.5
Veroux P[20]	2002-2007	10	ECD	73.2	55.6	22.4
Summary		22		72.7		

[a]As Ngheim stated that the operation could be done in "less than 180 min," the figure of 180 min was used to calculate the overall average surgical time for this technique.

the common iliac artery and inferior vena cava, and the second kidney was anastomosed to the external iliac artery and vein.[4,14,17] The preference for Gaber's group was to place the superior kidney above the true pelvis and anastomose the artery to the internal iliac artery, but the distal common and external were also used.[18] The first kidney's venous anastomosis was to the common iliac vein, and the second kidney was anastomosed to the external iliac vessels.[18] Ekser and Veroux both placed the right kidney superiorly as its vein can be lengthened by a segment of IVC.[4,17] Timsit performed a ureteroneocystostomy with the ureter of the lower graft, whereas both Veroux and Gaber performed a conjoined technique and Ekser performed separate ureteroneocystostomies.[4,14,17,18]

Operative time (min)	Graft thrombosis rate	Delayed graft function rate	Pt survival 1 y	Pt survival 2 y	Graft survival 1 y	Graft survival 2 y
Not stated	5 of 42 grafts (11.9%)	19%	100%	Not stated	95%	Not stated
275±80	8 of 126 grafts (6%)	27%	100%	Not stated	94%	Not stated
371 (range 165-720)	3 of 48 grafts (6.3%)	33%	Not stated	88%	Not stated	84%
Not stated	Not stated	24%	98%	86%	89%	77%
Not stated	0 of 48 grafts (0%)	20.8%	100% (at 3/12)	Not stated	100% (at 3/12)	Not stated
Not stated	4 of 82 grafts (4.9%)	0%	Not stated	Not stated	Not stated	Not stated
345±51	2 of 32 grafts (6.3%)	13%	Not stated	100%	Not stated	81%
Not stated	0 of 20 grafts (0%)	10%	70%	Not stated	100%[a]	Not stated
Not stated	Not stated	11%	100% (at 6/12)	Not stated	100% (at 6/12)	Not stated
360	Not stated	44%	100%	Not stated	93%	Not stated
315[b]	22 of 398 (5.5%)[b]	20.7%	97.3%[b]		93%[b]	

SWIT	Operative Time (min)	Graft thrombosis rate	Delayed graft function rate	Pt survival 1 y	Pt survival 2 y	Graft survival 1 y	Graft survival 2 y
Not stated	Not stated	5 of 28 grafts (18%)	0%	Not stated	Not stated	82%	Not stated
Not stated	192 min (160-260)	Not stated	13.3%	100%	100%	100%	96%
Not stated	260±35	1 of 200 grafts (0.5%)	31%	Not stated	96% (at 3 y)	Not stated	91% (at 3 y)
Not stated	219±26 min	1 of 18 grafts (5.6%)	Not stated	Not done	Not stated	94%	Not stated
	245[a]	7 of 246 grafts (2.8%)[a]	24.8%[a]		96.7%[a]	91.3%[a]	

SWIT (min)	Operative time (min)	Graft thrombosis rate	Delayed graft function rate	Pt survival 1 y	Pt survival 2 y	Graft survival 1 y	Graft survival 2 y
Not stated	"less than <180 min"[a]	1 of 24 (4%)	27%	Not stated	Not stated	96%	Not stated
Not stated	160±45 min	0 of 20 (0%)	20%	100%	90%	Not stated	90%
	171	2.27%	23.8%				

3.5 | Unilateral placement with patch anastomoses

Only two studies, both case series, looked at unilateral placement using a single patch-Ngheim "simultaneous" and Veroux "two as one."[19,20] The total number of patients was 22 with a mean donor age of 72.7. The mean recipient age in Veroux's study was 55.6, and this information is not provided in Ngheim's study. The surgical technique involved extensive back table preparation of the kidneys and is illustrated in Figure 4. Ngheim shortened the left renal vein by 3 cm and then reimplanted it on the IVC attached to the right renal vein (unless the IVC was not available, in which case they reimplanted the right end-to-side on the left and used the left as a common outflow).

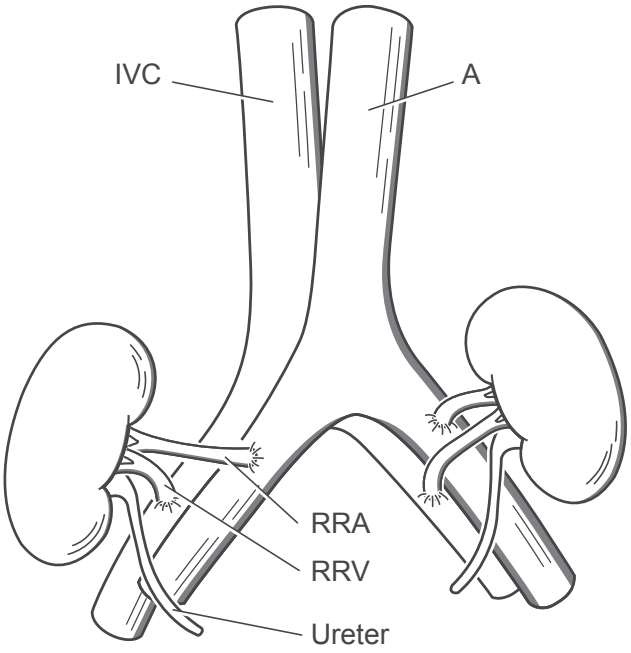


FIGURE 2 Bilateral placement

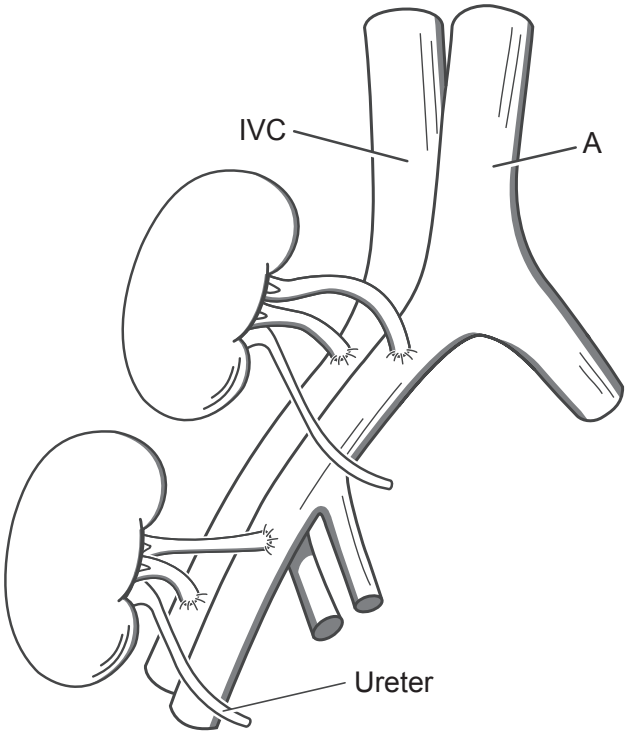


FIGURE 3 Unilateral placement with separate anastomoses

Veroux incorporated IVC into the right renal vein so that both veins were the same length and then sutured them together to create a single lumen. Both groups created the arterial patch by suturing the renal artery aortic patches together (Ngheim placed one superior to the other, Veroux had them on the same plane). Ngheim anastomosed the renal vein patch to the external iliac vein and the aortic patch to the external iliac artery. Veroux's venous anastomosis was to the common iliac vein, and the arterial anastomosis was to the external iliac artery.

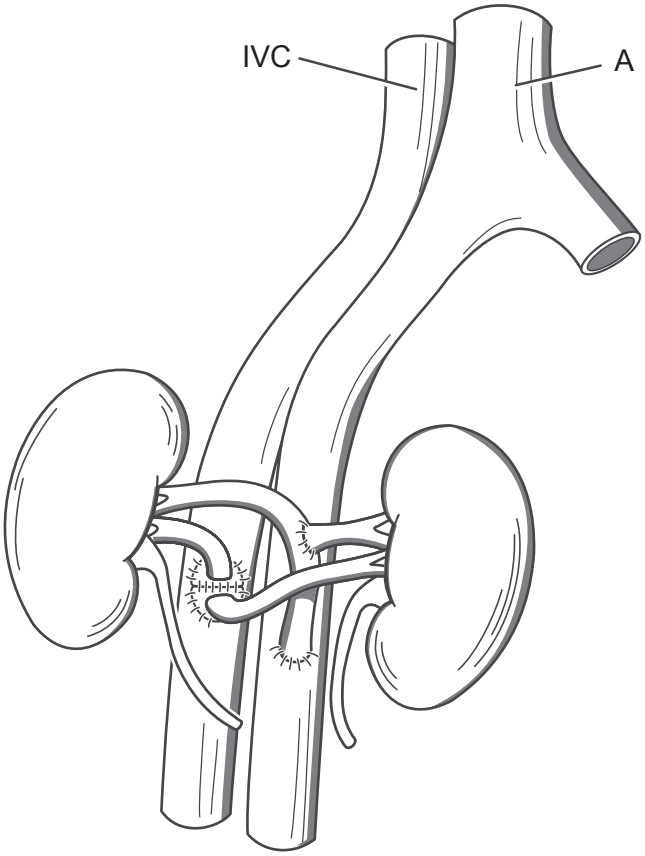


FIGURE 4 Unilateral placement with patch anastomoses

Both groups sutured the two ureters together and then performed a single ureteroneocystostomy.

3.6 | Patient survival—comparison between the techniques

Analysis of these data is limited by the disparate reporting across the studies—not all studies reported both patient and graft survival, and where they were reported, they were not always reported at the same time point. With this limitation in mind, the overall 1-year patient survival within the bilateral group was 97.3%. Within the unilateral placement with separate anastomoses studies, overall 2-year patient survival was 96.7%. It should be noted that Timsit's paper included two groups of patients with different surgical techniques for whom survival was not separately calculated and was 98.1% overall at 1 year.[14] One of the two studies of patients with unilateral placement and patch anastomoses reported patient survival (100% at 1 year and 90% at 1 years—Veroux).[20] There was one clear outlier in terms of 1-year survival: Lee reported 70% patient survival, due to the death of three patients from pneumonia, congestive heart failure, and complications of diabetes.[15]

3.7 | Graft survival—comparison between the techniques

The overall graft survival within the bilateral group at 1 year was 93%; within the unilateral with separate anastomoses was 91.3%; and for

the unilateral with patch anastomoses groups was 96% at 1 year in Ngheim's study and 90% at 1 years in Veroux's study.[19,20] Specific surgical complications leading to graft loss are discussed below.

3.8 | Operative time, graft function, and surgical complications

Once again, analysis of the operative time across the different techniques is limited by lack of information provided within some studies. The results of each study are outlined in Tables 1-3 below. The overall mean operative time for bilateral placement was 315 minutes (with a range of mean operating times of 275-371 minutes). The overall mean operative time for unilateral placement with separate anastomoses was 245 minutes (with the studies having a mean time from 192 to 260 minutes).

Unilateral placement with patch anastomoses took an overall mean time of 171 minutes. The back table time for unilateral placement with patch anastomoses was reported as 60 minutes by Ngheim, while Veroux reported it resulted in a "longer bench time" but did not state exactly how long.[19,20]

Despite the difference between these operative times, the incidence of delayed graft function (defined as a need for post-operative dialysis) was broadly similar across the groups (see Tables 1-3). The overall delayed graft function rate was 20.7% in the bilateral placement group, 24.8% in the unilateral with separate anastomoses group and 23.8% in the unilateral patch anastomosis group. The graft thrombosis rates were 5.5% in the bilateral placement group, 2.8% in the unilateral separate anastomoses group, and 2.27% in the unilateral patch anastomosis group.

A number of patients lost a single graft but did not require dialysis: This was particularly striking in Timsit's paper, as in the unilateral placement group 5 grafts of a total of 28 were explanted due to thrombosis, but none of those patients required a return to dialysis.[14] In the bilateral placement group within that paper, four of 82 grafts were lost due to thrombosis, but again no one required a return to dialysis.[10] One patient in Ngheim's series of performing a patch anastomosis had a thrombosis of the renal vein of the medial kidney, and the authors felt that this was likely because the vein had been left too long.[19] None of the patients in the other patch anastomosis study—Veroux—had thrombosis.[20] Apart from graft thrombosis (with or without graft loss), the most commonly reported complications were wound dehiscense, hematoma, urinary fistula, and lymphocoele.

3.9 | Decision to perform dual renal transplant (DKT)

All but one of the centers only performed dual renal transplant (rather than single transplant) when the organs were from an extended criteria donor, and most of the organs had been rejected for use as a single transplant by other centers. Two of the 10 donors whose kidneys were used in a DKT in Lee's study did not fit an ECD definition.[15] Andres, Remuzzi, Johnson, D'Arcy, Veroux M, Gaber, Moore, and Veroux P all used a biopsy score to determine dual allocation.[5,9,11-13,17,18,20] Ekser[4] used a biopsy score if the patient had elevated creatinine, hypertension, or diabetes as well as being over 60. Timsit[14] used the donor's terminal eGFR to determine allocation as single, dual, or discard. De Serres[3] transplanted all kidneys from patients >75 as dual kidneys, and all of the kidneys from <75 year old donors had been rejected by all other centers for single transplant. Lee, Rigotti, and Ngheim all seem to have performed a dual transplant based on ECD status alone.[10,16,19]

4 | DISCUSSION

The results of this systematic review support the view that DKT is a safe and feasible option for expanded organ donation criteria. The small number of patients in all of these studies, but especially in the studies examining outcomes in patients who had transplanted kidneys placed unilaterally, makes it difficult to draw conclusions regarding the superiority of one technique. Unfortunately, a recently published study which included 200 patients could not be included as neither patient nor graft survival was reported according to the type of surgical technique used, and an attempt to gain further information from the corresponding author was unsuccessful.[21]

All three techniques appear to have broadly similar patient and graft survival, but as noted above, the lack of consistent outcome reporting has made it difficult to draw any definite conclusions. The obvious difference between the techniques is the operative time: Unilateral placement is approximately 1 hour shorter than bilateral placement. The initial assumption might be that this shortening of operative time was offset by a longer back table preparation—and therefore a longer cold ischemic time—but this does not appear to be the case, although inconsistency of data reporting in this regard again made interpretation difficult. Intuitively, the shorter operating time would also lead to improved patient and graft outcomes, but this is not reflected in the current data.

There are a few advantages and disadvantages of bilateral vs unilateral placement. If kidneys are placed bilaterally, then both sides are scarred and access for re-operation becomes more difficult. On the other hand, if the patient has a narrow pelvis, bilateral placement may appear advantageous because it is easier to fit both kidneys in the space available. At the University Hospital in Madrid, unit practice is to place the grafts bilaterally so that it is easy to access the vessels; there is enough space; and if a surgical complication does occur, it does not impact on the other graft.[22] (Medina-Polo [josemedinapolo@movistar.es], email, July 26, 2016). By way of contrast, in Rigotti's recently published review of 200 dual kidney transplants, it is stated that a major impetus behind the move from bilateral placement to unilateral placement with separate anastomoses was driven by reduced operating time, faster patient recovery, and the ability to leave the contralateral iliac fossa untouched in case of a need for retransplant.[21]

While a number of different methods of anastomosis were presented, there is one possible method which has not been discussed: to perform an en bloc dual transplant using the IVC and aorta, as is commonly performed in pediatrics. No cohort studies or case series using this technique have been reported in adults. Our experience is

that the aorta of extended criteria donors is often heavily calcified and may have dissected, which makes the arterial anastomosis technically difficult and increases the risk of graft thrombosis.

The focus of this study was on the technical aspects of performing dual kidney transplants. The excellent results, in terms of both graft and patient survival, raise questions as to whether these organs could have been transplanted singly (and thereby increased the available organ pool), rather than having been allocated to a single patient. This was especially obvious when the number of grafts lost is compared to the number of patients requiring return to dialysis—most patients who lost a single graft did not return to dialysis, so presumably they only required one (rather than two) kidneys.

From this analysis, the three differing techniques have equivalent delayed graft function as well as patient and graft survival rates, although operative time appears shorter when a unilateral incision is employed. This is an encouraging result as it means that the surgeon can choose to use the technique which is most appropriate for their own skills and for the patient.

This study has a number of limitations. The first is that the data provided in the individual studies were sometimes incomplete or absent, and conclusions could only be drawn from the data that were provided. Not all surgical complications (for example, graft thrombosis, surgical site infection, and lymphocoele) were reported by all centers, and outcomes such as patient and graft survival were not recorded at uniform time points. Likewise, it was not always clear why a particular technique of transplant was utilized, and therefore, it was not possible to draw conclusions regarding the superiority of one technique over another for a particular patient population (atherosclerosis, high BMI, and so forth). The second is that the number of studies (and therefore patients) evaluating the three techniques was different, with far more patients have bilateral placement rather than either technique of unilateral placement, and this made it difficult to draw conclusions regarding possible superiority of the less commonly utilized techniques.

AUTHORS' CONTRIBUTIONS

Annelise Cocco: Performed research design, performance of the research, primary writer of the manuscript, and data analysis; Sara Shahrestani: Second author of the manuscript, performed data analysis; Nicholas Cocco: Third author of the manuscript, performed analytic tools; Ahmer Hameed and Henry Pleass: Performed data analysis and research design; Lawrence Yuen, Wayne Hawthorne, and Brendan Ryan: Performed data analysis; Vincent Lam: Performed research design.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Wen CP, Matsushita K, Coresh J, et al. Chronic Kidney Disease Prognosis: relative risks of chronic kidney disease for mortality and end-stage renal disease across races are similar. *Kidney Int*. 2014;86:819-827.
2. Shafran D, Kodish E, Tzakis A. Organ shortage: the greatest challenge facing transplant medicine. *World J Surg*. 2014;38:1650-1657.
3. De Serres SA, Caumartin Y, Noel R, et al. Dual-kidney transplants as an alternative for very marginal donors: long-term follow-up in 63 patients. *Transplantation*. 2010;90:1125-1130.
4. Ekser B, Furian L, Broggiato A, et al. Technical aspects of unilateral dual kidney transplantation from expanded criteria donors: experience of 100 patients. *Am J Transplant*. 2010;10:2000-2007.
5. Moore PS, Farney AC, Sundberg AK, et al. Dual kidney transplantation: a case-control comparison with single kidney transplantation from standard and expanded criteria donors. *Transplantation*. 2007;83:1551-1556.
6. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000;283:2008-2012.
7. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions. http://cochrane-handbook.org. Published 2008 [updated September 2009]. Accessed June 14, 2015.
8. Wells G, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Published 2011. Accessed July 5, 2016.
9. Andres A, Morales JM, Herrero JC, et al. Double versus single renal allografts from aged donors. *Transplantation*. 2000;69:2060-2066.
10. Lee CM, Carter JT, Weinstein RJ, et al. Dual kidney transplantation: older donors for older recipients. *J Am Coll Surg*. 1999;189:82-91; discussion 91-82.
11. Johnson LB, Kuo PC, Dafoe DC, et al. Double adult renal allografts: a technique for expansion of the cadaveric kidney donor pool. *Surgery*. 1996;120:580-584.
12. Remuzzi G, Grinyo J, Ruggenenti P, et al. Early experience with dual kidney transplantation in adults using expanded donor criteria. *J Am Soc Nephrol*. 1999;10:2591-2598.
13. D'Arcy FT, O'Connor KM, Shields W, et al. Dual kidney transplantation with organs from extended criteria cadaveric donors. *J Urol*. 2009;182:1477-1481.
14. Timsit MO, Rabant M, Snanoudj R, et al. Single graft loss in dual renal transplant recipients: impact of graft placement of recipient outcomes. *Transpl Int*. 2011;24:51-57.
15. Lee RS, Miller E, Marsh CL, et al. Intermediate outcomes of dual renal allografts: the University of Washington experience. *J Urol*. 2003;169:855-858.
16. Rigotti P, Cadrobbi R, Furian L, et al. Short-term outcome of dual kidney transplantation at a single center. *Transplant Proc*. 2001;33:3771-3773.
17. Veroux M, Corona D, Gagliano M, et al. Monolateral dual kidney transplantation from marginal donors. *Transplant Proc*. 2007;39:1800-1802.
18. Gaber AO, Shokouh-Amiri H, Nezakatgoo N, et al. Ipsilateral placement in double-kidney transplantation. *Transplantation*. 2007;84:929-931.
19. Ngheim DD. Simultaneous double adult kidney transplantation using single arterial and venous anastomoses. *Urology*. 2006;67:1076-1078.
20. Veroux P, Giuffrida G, Cappellani A, et al. Two-as-one monolateral dual kidney transplantation. *Urology*. 2011;77:227-230.
21. Rigotti P, Capovilla G, Di Bella C, et al. A single-center experience with 200 dual kidney transplantations. *Clin Transplant*. 2014;28:1433-1440.
22. Medina-Polo P, Pamplona-Casamayor M, Miranda-Utrera N, et al. Dual kidney transplantation involving organs from expanded criteria donors: a review of our series and an update on current indications. *Transplant Proc*. 2014;46:3412-3415.

How to cite this article: Cocco A, Shahrestani S, Cocco N, et al. Dual kidney transplant techniques: A systematic review. *Clin Transplant*. 2017;31:e13016. https://doi.org/10.1111/ctr.13016