THE USE OF PRE-IMPLANTATION PERFUSION TECHNIQUES OF KIDNEY GRAFTS TO INCREASE ORGAN UTILISATION AND THE INVESTIGATION OF REGENERATIVE CELL THERAPY

By

Robert Pearson

MBBS (Newcastle) MRCS (Ed.) MRes (Newcastle)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

Institute of Cardiovascular and Medical Sciences

College of Medicine, Veterinary & Life Sciences, University of Glasgow

March 2024

ABSTRACT

Despite the advancements in solid organ transplantation, including an ever-increasing living donation programme, there remains a significant shortfall of utilisable grafts to meet the demand. In the United Kingdom, NHS Blood and Transplant released a mission statement entitled “2030: Meeting the Need," highlighting key areas to focus efforts on and tailor technologies. Within this statement, organ utilisation was highlighted as a critical area for improvement and development.

There have been significant developments in recent years with regard to deceased donation in how organs are assessed, transported and prepared for transplantation with organ utilisation in mind. Despite these advancements, the rate of organ decline remains high, and tools and technologies that allow clinicians to assess organs, providing more confidence to accept and utilise offered organs, are vital to improving our utilisation rates. Furthermore, pre-implantation therapeutics may improve grafts from those deemed unsuitable for transplantation to those considered utilisable in certain circumstances.

Ex-vivo normothermic perfusion (EVNP) is one such technology that delivers warmed, oxygenated blood with added nutrients to a kidney graft before implantation, allowing for a period of assessment, and potentially mitigates against some of the critical issues within deceased donation, such as ischaemic reperfusion injury.

The implementation of a clinical EVNP service is then described. Herein we describe the mechanisms by which the technology was introduced as a clinical service to assess the viability of marginal grafts, including the requisite training and trust approval. Obstacles and pitfalls are also discussed, including the sourcing of blood products, staffing considerations and the perfusion of grafts with variant anatomy.

This thesis first outlines the risks evident when high-risk donor kidneys and recipients combine. Retrospective analysis demonstrated that although graft survival and function are satisfactory, this high-risk combination is associated with delayed graft function, prolonged

length of stay in hospital and increased use of secondary care within the first 90 days post-transplant.

This is followed by a report of the successful implantation of seven kidneys as a result of this novel assessment tool. This includes grafts in the deceased donor setting with severe acute kidney injury, poor perfusion and in the context of dual kidney transplantation. Furthermore, the use of this technology to assess arterial reconstruction in two cases of living donor nephrectomy for renal artery stenosis is detailed. To our knowledge, this series includes examples of world-firsts in the use of this technology to expand the donor pool of utilisable grafts.

A systematic review is then conducted which sets the scene for a study in which adipose-derived regenerative cells (ADRC), harvested from donor peri-renal tissue, were delivered via EVNP technology to discarded human kidneys. Perfusion characteristics and urine output were unchanged with ADRC treatment, as were histological features of acute tubular injury. RNA sequencing, however, highlighted differential gene expression to guide further mechanistic evaluation. Of note, no adverse events were found with the delivery of this regenerative therapy.

In summary, EVNP is a valuable assessment tool in transplantation decision-making, with the potential to improve organ utilisation. Furthermore, it provides an opportunity to deliver therapeutic agents, such as regenerative cell populations, as described herein.

TABLE OF CONTENTS

Abstract

List of Tables

Lift of Figures

Dedication

Acknowledgements

Publications

List of Abbreviations

Bibliography

Appendices

TABLE OF CONTENTS

Chapter 1: Introduction *20*

1.1 Established Renal Failure **20**

1.2 Kidney Transplantation **21**

1.3 Ischaemia Reperfusion Injury **22**

1.4 Perfusion Techniques **25**

1.4.1 In-Situ Perfusion Techniques 26

1.4.2 Ex-Situ Perfusion Techniques 27

1.4.3 Clinical Use of Ex-Vivo Normothermic Perfusion in Kidney 29

1.5 Treatment Opportunities During Organ Perfusion **33**

1.5.1 Regenerative Cell Therapies 33

1.5.2 Delivery of Autologous Cells 35

1.5.3 Sources of Autologous Regenerative Cells 36

1.5.4 Bedside Production of ADRCs 38

1.5.5 Cellular Makeup and Properties of ADRC 39

1.6 Aims and Hypothesis **40**

Chapter 2: General Methods: The set Up of a Clinical Perfusion Service in an Acute Tertiary Hospital *41*

2.1 Introduction **41**

2.1.1 The Business Case for EVNP 41

2.1.2 Interventional Procedure Policy 43

2.1.3 Perfusion Training 44

2.1.4 RINTAG Application 44

2.1.5 Departmental Communication 46

2.1.6 Perfusion Protocol 47

2.2 Obstacles and issues **48**

2.2.1 Haematology and Obtaining Blood Products 48

2.2.2 Arterial Cannulation and Grafts with Multiple Vessels 50

2.2.3 Kidney Tray 54

2.2.4 Medtronic Pack Design, Delays and Urgent Recall 58

2.2.5 Navigating the Use of a Tertiary Hospital's Theatre Facilities 59

2.2.6 COVID Pandemic 60

2.2.7 Impact of COVID Pandemic on Renal Trials at a National Level 61

2.3 Conclusions **67**

Chapter 3: Marginal Kidneys and Marginal Recipients – A High-Risk Combination *69*

3.1 Introduction **69**

3.2 Methods **71**

3.3 Results **74**

3.3.1 Recipient and Donor Risk Combination in this Cohort 75

3.3.2 Graft and Patient Survival 76

3.3.3 Delayed Graft Function 78

3.3.4 Short- and Medium-Term Function 78

3.3.5 Index Admission Length 81

3.3.6 Total Inpatient Days and Day Case Use 81

3.3.7 Cost 83

3.3.8 Current Waiting List 85

3.4 Discussion **86**

Chapter 4: Clinical Experience of EVNP As a Viability Assessment Tool *89*

4.1 Overview of Cases **89**

4.2 Rhabdomyolysis / Donor Acute Kidney Injury 89
4.2.1 Introduction 89
4.2.2 Case Detail 91
4.2.3 Discussion 94

4.3 Renal Artery Stenosis 96
4.3.1 Introduction 96
4.3.2 Case Detail – First Perfusion of Kidney with Renal Artery Stenosis 96
4.3.3 Case Detail – Second Perfusion of Kidney with Renal Artery Stenosis 102
4.3.4 Further Perfusion of Kidneys Removed Due to Renal Artery Stenosis Unsuitable for Transplantation 104
4.3.5 Discussion 106

4.4 The Use of EVNP to Facilitate Dual Kidney Transplant 109
4.4.1 Introduction 109
4.4.2 Case Detail 110
4.4.3 Discussion 112

4.5 Prolonged Cold Ischaemic Time / Unwell recipients 114
4.5.1 Introduction 114
4.5.2 Case Detail 115
4.5.3 Discussion 117

4.6 Patient Testimony 119

4.7 Next Steps 119
4.7.1 Funding Application via NSD for Perfusion Nurse 119
4.7.2 Staffing 121
4.7.3 Arterial Cannulation with Complex Anatomy 121

Chapter 5: Therapeutic Efficacy of Mesenchymal StROMAL Cell Population Delivered via Isolated Organ Perfusion: A Systematic Review 122

5.1 Introduction 122

5.2 Methods 123

5.3 Results 123
5.3.1 Kidney 127
5.3.2 Liver 131

5.4 Discussion 135
5.4.1 Anti-Inflammatory 135
5.4.2 Anti-oxidative Stress 136

5.5 Conclusion 137

Chapter 6: Delivery of Adipose-Derived Regenerative Cell Therapy During Ex-Vivo Normothermic Perfusion 139

6.1 Introduction 139
6.1.1 Aims and Objectives 141

6.2 Materials and Methods 142
6.2.1 Study Sponsors, Application and IRAS Submission 142
6.2.2 Ethical Approval 142
6.2.3 Funding 142
6.2.4 Application for Research Discarded Kidney Grafts 142
6.2.5 Transfer of Organs to the Recipient Unit 143
6.2.6 Eligibility Criteria 144
6.2.7 Randomisation of Intervention 145
6.2.8 Harvest of Adipose-Derived Regenerative Cells 145
6.2.9 Schedule of Events 146
6.2.10 Perfusion Protocol 147
6.2.11 Record Sheet for Perfusion Parameters 147
6.2.12 Tissue Preparation and Sample Analysis 148
6.2.13 Histological Analysis 148

6.2.14 RNA Extraction and Sequencing 149
6.3 Results 150
6.3.1 Research Kidney Demographics 150
6.3.2 ADRC Harvest 152
6.3.3 Perfusion Parameters 152
6.3.4 EVNP Assessment Score 158
6.3.5 Perfusate Markers of Kidney Injury 161
6.3.6 Histology 161
6.3.7 RNA Sequencing 166
6.4 Discussion 170
6.5 Conclusions 176
Chapter 7: Conclusions 177

LIST OF TABLES

Table 1-1: EVNP Assessment Score (Adapted from Hosgood, Barlow, Dormer, et al., 2015) 32

Table 3-1:Patient Factors Used to Stratify Donor and Recipient Risk Indices as Per Validation Dataset by NHS Blood and Transplant. (*Kidney Donor Profile Index (KDPI) Guide for Clinicians - OPTN*, accessed March 2024). 71

Table 3-2: Recipient Demographics 74

Table 3-3: Total cost of transplant for different recipient and donor types. 84

Table 4-1: Donor and recipient demographics, including details of procurement and cold ischaemic time.....91

Table 4-2: Detail of staffing request as part of the application to the National Services Division for trained perfusion nursing staff to provide EVNP service during a two-year pilot. 120

Table 5-1: Summary of articles in which mesenchymal stromal cell populations are delivered via isolated organ perfusion in kidney and liver. 125

Table 6-1: Schedule of events detailing the process of research kidney acceptance to post-perfusion tissue storage.....146

Table 6-2: Demographics of the research kidneys.....151

Table 6-3: EVNP Assessment score with pictures of kidneys on perfusion.....159

Table 6-4: Histology results of acute and chronic injury pre- and post-perfusion 162

Table 6-5: Histology photographs of all research kidneys pre- and post-perfusion 164

Table 6-6: Differentially expressed genes between ADRC treatment and control. 167

LIST OF FIGURES

Figure 1-1: Overview of the graft tissue environment between organ procurement and implantation (T. B. Smith et al., 2021) 23

Figure 2-1: EVNP implementation plan as agreed by the Trust approved New Interventional Procedure Policy....43

Figure 2-2: Image of before (top) and after (bottom) perfusion of discarded grafts for education and training purposes as part of the formalised training in ex vivo normothermic perfusion....45

Figure 2-3: Images of before (top) and after (bottom) perfusion of discarded grafts for education and training purposes as part of the formalised training in ex vivo normothermic perfusion....46

Figure 2-4: Blood products return form to the haematology department post perfusion. ...49

Figure 2-5: Cannulation options for EVNP arterial connection. 51

Figure 2-6: A kidney undergoing normothermic perfusion in a case of two renal arteries with separate cannulation with 16G Venflon and vascular cannula. The ureter can be seen cannulated with a nelation catheter in the centre of the image. 52

Figure 2-7: Steel Kidney Tray manufactured by Complete Stainless Ltd., amended from previous iterations used by collaborating research groups. 54

Figure 2-8: Thermal image of a kidney on perfusion in the exposed kidney tray 55

Figure 2-9: Engineering drawings of the proposed 'kidney house' made in collaboration with the University of Glasgow Engineering Department.....56

Figure 2-10: Prototype of 'Kidney House' built in collaboration with the University of Glasgow Engineering Department....57

Figure 2-11: Heatmap demonstrating the starting time of renal transplants within our emergency shared emergency theatres during the period April 2015 – April 2021. 59

Figure 2-12: Bar chart demonstrating the respondents' redeployment status back to clinic duty during the COVID pandemic. 63

Figure 2-13: Confidence levels of responders on whether they felt they would complete their study. 0-100, with 100 being the most confident.....64

Figure 3-1: The NHS Blood and Transplant provided formula for stratifying donors and recipients (Kidney Offering Scheme, accessed March 2024).....72

Figure 3-2: Proportion of Deceased Transplants in Recipient Rick Index Categories and Donor Types Received....75

Figure 3-3: Kaplan-Meier Curves for Patient (top) and Graft (bottom) Survival in the Recipient R4 Cohort....77

Figure 3-4: One-Year Function for all Transplant for Each Recipient Category. 79

Figure 3-5: Estimated Glomerular Filtration Rate for Each Donor Risk Category Over Time. 80

Figure 3-6: First 90 Days by Donor Category. 82

Figure 3-7: Waffle plot of the current waiting list of the West of Scotland catchment area stratified by recipient risk indices. 85

Figure 4-1: Pre- (above) and post-perfusion (below) image of graft with acute kidney injury. 92
Figure 4-2: Serum creatinine (μmol/l) over time for Patient 1, Patient 2, and the Donor kidney. 93
Figure 4-3: Coronal image of pre-operative computed tomography scan (left) and intraoperative imaging demonstrating the neovascular collaterals evident (right). 98
Figure 4-4: Image of backbench reconstruction of the explanted graft (left) with diagram to depict anatomical configuration (right). 98
Figure 4-5: The explanted graft demonstrated excellent global perfusion while being perfused via ex vivo normothermic perfusion. 99
Figure 4-6: CT angiogram images 6 weeks post-transplant demonstrating excellent global graft perfusion. 100
Figure 4-7: Multiplanar volume reconstruction images of the post-transplant CT angiogram demonstrating the two branches emerging from the common stem. 101
Figure 4-8: Line chart demonstrating a change in creatinine levels over time pre- and post-transplant. 104
Figure 4-9: Flowchart detailing the steps following nephrectomy utilising ex vivo normothermic perfusion as an assessment tool. 105
Figure 4-10: Intraoperative post-reperfusion image of the left kidney which had undergone 90 minutes of EVNP prior to implantation 111
Figure 4-11: Recipient creatinine levels in the months prior to and post-dual kidney transplants. All units in μmol/L 111
Figure 4-12: Image of kidney on ex vivo normothermic perfusion for 60 minutes. (EVNP assessment score = 1) 117
Figure 5-1: Search Strategy Flow Diagram; Adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram 124
Figure 6-1: Renal arterial flow (L/min/100g) of the kidneys during EVNP 153
Figure 6-2: Renal blood flow (L/min/100g) over time at 60, 120 and 240 minutes of perfusion. 153
Figure 6-3: Potassium concentrations (mmol/L) in the perfusate of the kidneys during EVNP 154
Figure 6-4: Lactate concentrations (mmol/L) in the perfusate of the kidneys during EVNP 155
Figure 6-5: Urine output of the kidneys during EVNP by treatment group 156
Figure 6-6: Urine output of the kidneys during EVNP separated by EVNP Assessment Score 156
Figure 6-7: Urine output (ml) over time at 60, 120 and 240 minutes of perfusion 157
Figure 6-8: EVNP Assessment Score of all research kidneys 158
Figure 6-9: Post-perfusion Remuzzi score of all research kidneys 163
Figure 6-10: Post-perfusion Acute Tubular Injury (ATI) score of all research kidneys 163
Figure 6-11: Heatmap of top 24 differentially expressed genes 168

Figure 6-12: Heatmap demonstrating statistically significant differential gene expression. 169
Figure 6-13: Gene ontology analysis demonstrating top 40 biological processes by way of clustering differentially expressed genes 169

DEDICATION

To my wife, Sally – I could not have pursued my ambitions to the fullest without your unwavering support. I will be forever grateful.

To my parents – thank you for providing me with the foundations in life to follow my interests. I would not be where I am today without your support and guidance.

ACKNOWLEDGEMENTS

Mr Marc Clancy – Thank you for allowing me to undertake this higher degree. Your knowledge, oversight and connections with other units were instrumental in the completion of this project.

Professor Patrick Mark – Thank you for your guidance. Your expertise and experience were crucial in ensuring this project progressed to its completion.

Mr Ryan Ghita – For setting the scene for this project with your hard work that went before and your advice, guidance and friendship.

Mr William Norton – For your help delivering the perfusion technology.

Dr Rachanchai Chawangwongsanukun – For your assistance with the scientific aspects of this project, knowledge and friendship.

Dr Sarah Hosgood – Your expertise, training and guidance provided this project with the foundation to succeed.

Mr David Kingsmore	Renal Transplantation Department, Queen Elizabeth University Hospital, Glasgow
Dr Rashida Lathan	Institute of Cardiovascular and Medical Sciences, University of Glasgow
Ms Emma Aitken	Renal Transplantation Department, Queen Elizabeth University Hospital, Glasgow
Mr John Asher	Renal Transplantation Department, Queen Elizabeth University Hospital, Glasgow
Ms Karen Stevenson	Renal Transplantation Department, Queen Elizabeth University Hospital, Glasgow
Dr David Kipgen	Pathology Department, Queen Elizabeth University Hospital, Glasgow
Dr Amir Fard	University of Glasgow, UK

DECLARATION

My research degree began in 2019 and ran until 2022. The research was based across two sites, the Institute of Cardiovascular & Medical Sciences, University of Glasgow and the Renal Transplant Department, Queen Elizabeth University Hospital, Glasgow.

I was trained on the perfusion technology by colleagues in Cambridge. I was privileged to use human discarded kidney grafts provided by NHS Blood and Transplant following application to the Research, Innovation and Novel Technologies Advisory Group (RINTAG). Due to the perfusion technology requiring more than one person, it was necessary to use ad hoc help from my fellow clinicians within the transplant unit.

I declare the work presented in this thesis was conducted by myself unless otherwise stated below:

Dr David Kipgen assisted in the immunohistochemistry and analysis of the histology samples.

Dr Amir Fard was the second reviewer of the systematic review on perfusate composition, and the manuscript was co-authored.

Dr Rachanchai Chawangwongsanukun was the second reviewer of the systematic review on regenerative cell therapeutic delivery via perfusion technologies.

GeneWiz, Azenta Life Sciences performed the RNA sequencing analysis and performed the bioinformatic analysis of the results.

University of Glasgow Engineering Department for providing collaboration in the design and creation of the bespoke kidney housing.

Robert Pearson, Glasgow, November 2023

PUBLICATIONS

Pearson R, Murray E, Thomson PC, Mark PB, Clancy M, Asher J (2021). The New UK National Kidney Allocation Scheme with Maximized "R4-D4" Kidney Transplants: Better Patient-to-Graft Longevity Matching May Be at the Cost of More Resources. Exp Clin Transplant. 2021 Nov;19(11):1133-1141. doi: 10.6002/ect.2021.0129.

Pearson R, Geddes G, Mark P, Clancy M, Asher J (2021). Transplantation of Kidneys after Normothermic Perfusion: A Single Center Experience. Clin Transplant. 2021 Oct;35(10):e14431. doi: 10.1111/ctr.14431. Epub 2021 Aug 3.

Pearson R, Asher J, Jackson A, Mark PB, Shumeyko V, Clancy M (2021). Viability assessment and utilization of declined donor kidneys with rhabdomyolysis using ex vivo normothermic perfusion without preimplantation biopsy. Am J Transplant. 2021 Mar;21(3):1317-1321.doi: 10.1111/ajt.16329.

Pearson R, Wubetu J, Jackson A, Kingsmore DB (2021). Living donor kidney transplant following nephrectomy for renal artery stenosis with arterial reconstruction and viability assessment using ex vivo normothermic perfusion BMJ Case Reports 2021 Nov 17;14(11):e245273. doi: 10.1136/bcr-2021-245273.

Fard A, Pearson R, Lathan R, Mark P, Clancy M (2022). Perfusate Composition and Duration of Ex-Vivo Normothermic Perfusion in Kidney Transplantation: A Systematic Review Transplant International 2022 2022 May 11;35:10236. doi: 10.3389/ti.2022.10236.

Norton WG, Pearson R, Devlin J, Nicholson ML, Hosgood SA (2022). Normothermic Machine Perfusion in Renal Transplantation Current Transplantation Reports (2022) 9:308–317 https://doi.org/10.1007/s40472-022-00378-x.

COMPLETED MANUSCRIPTS READY FOR SUBMISSION

Pearson R, Chawangwongsanukun R, Clancy M, Mark P (2023) Therapeutic Efficacy of Mesenchymal Stromal Cell Population Delivered via Isolated Organ Perfusion - A Systematic Review.

NATIONAL AND INTERNATIONAL ORAL AND POSTER PRESENTATIONS

Normothermic Regional Perfusion of Kidneys: A Single Non-Retrieval Centre Experience, Oral Presentation, American Transplant Congress, 2019.

Normothermic Regional Perfusion of Kidneys: A Single Non-Retrieval Centre Experience, Oral Presentation, British Transplant Society, 2020.

Domino kidney transplant following nephrectomy for renal artery stenosis with arterial reconstruction and viability assessment using ex vivo normothermic perfusion: A Case Series, Oral Presentation, TTS, Argentina 2022.

Domino kidney transplant following nephrectomy for renal artery stenosis with arterial reconstruction and viability assessment using ex vivo normothermic perfusion: A Case Series, Poster Presentation, British Transplant Society, Belfast 2022.

The use of ex vivo normothermic perfusion to 'pause' cold ischaemic time to allow for third recipient to be selected and undergo kidney transplant, Poster presentation, British Transplant Society, Belfast, 2022.

LIST OF ABBREVIATIONS

ABCA4	ATP binding cassette subfamily A member 4
ABCA13	ATP binding cassette subfamily A member 13
ADRC	Adipose-Derived Regenerative Cells
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ANCA	Antineutrophil Cytoplasmic Antibodies
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
anti-GBM	Anti-glomerular basement membrane diseases
API	Application Programming Interface
ASTRAL	Angioplasty and Stenting for Renal Artery Lesions
ATG	Anti-Thymocyte Globulin
ATP	Adenosine Triphosphate
BAME	Black and Minority Ethnic
BMI	Body Mass Index
BM-MSC	Bone Marrow-derived Mesenchymal Stem Cells
CAPN6	Caplain 6
CI	Confidence Interval
CIT	Cold Ischaemic Time
CK19	Cytokeratin-19
CO	Carbon Monoxide
CoPP	Cobalt Protoporphyrin
cRF	Calculated Reaction Frequency
CRP	C-Reactive Protein
CT	Computed Tomography
CVVH	Continuous Veno-Venous Hemofiltration
DBD	Donor of Brainstem Death
DCD	Donor of Circulatory Death
DDIT4L	DNA Damage Inducible Transcript 4 Like
DGF	Delayed Graft Function
dUTP	2′-Deoxyuridine, 5′-Triphosphate
ECD	Extended Criteria Donor
ECMO	Extracorporeal Membrane Oxygenation

EPO	Erythropoietin
ESM1	Endothelial cell-specific molecule 1
ESP	Eurotransplant Senior Program
ERF	Established Renal Failure
FMD	Fibromuscular Dysplasia
GDPR	General Data Protection Regulation
GFR	Glomerular Filtration Rate
GO	Gene Ontology
HAS2	Human hyaluronan synthase 2
HBOC	Haemoglobin-Based Oxygen Carrier
HLA	Human Leukocyte Antigen
hMSC	Human Mesenchymal Stem Cells
HO-1	Haem Oxygenase 1
HOT-2	Heam Oxygenase-1 in renal transplantation
HMP	Hypothermic Machine Perfusion
HSP	Heat shock protein
IL	Interleukin
IPP	Interventional Procedure Protocol
IRI	Ischaemic Reperfusion Injury
ISCT	International Society for Cell & Gene Therapy
ISRCTN	International Standard Randomised Controlled Trial Number
KAS	Kidney Allocation Scheme
KDPI	Kidney Donor Profile Index
KDRI	Kidney Donor Risk Index
MAPC	Multi-potent Adult Progenitor Cells
MDT	Multidisciplinary Team
MePEP	Molecular Pathology Evaluation Panel
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stromal Cells
mTOR	Mammalian Target of Rapamycin
MTP	Mitochondrial Permeability Transition
NCT	National Clinical Trials
NEVKP	Normothermic Ex-vivo Kidney Perfusion
NKAS	National Kidney Allocation Scheme

NORS	National Organ Retrieval Service
NRP	Normothermic Regional Perfusion
NSD	National Services Division
PGE2	Prostaglandin E2
Php	Pyridoxalated haemoglobin-polyoxyethylene
PITHIA	Pre-Implantation Trial of Histopathology in Renal Allografts
pMSC	Porcine Mesenchymal Stem Cells
PNF	Primary Non-Function
pRBC	Packed Red Blood Cells
PRES	Posterior Reversible Encephalopathy Syndrome
PTA	Percutaneous Therapeutic Angioplasty
RAS	Renal Artery Stenosis
RIFLE	Risk, Injury, Failure, Loss of kidney function, and End-Stage Kidney Disease
RINTAG	Research, Innovation and Novel Technologies Advisory Group
RT-PCR	Real Time Polymerase Chain Reaction
SaBTO	Safety of Blood, Tissues and Organs
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCS	Static Cold Storage
SERPR	Strathclyde Electronic Renal Patient Record
SNMP	Sub-Normothermic Kidney Perfusion
SNOAR	Specialist Nurse in Organ Assessment and Reconditioning
SOP	Standard Operating Procedure
SPECT DMSA	Dimercaptosuccinic acid renal scintigraphy
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SVF	Stromal Vascular Fraction
TGF-B	Transforming Growth Factor-β
TLR	Toll-like Receptors
Tukey HSD	Tukey's Honestly Significant Difference
UW	University of Wisconsin solution
WIT	Warm Ischaemic Time

CHAPTER 1: INTRODUCTION

A proportion of this introduction was published in Clinical Transplantation (Pearson, Geddes, et al., 2021) and Experimental and Clinical Transplantation (Pearson, Murray, et al., 2021). Permission for the inclusion in this thesis was granted by the respective editorial teams.

1.1 Established Renal Failure

The primary function of the kidney is to filter the blood and excrete waste products and excess fluid from the body. When kidney function deteriorates, leading to an inability to perform these essential functions, patients reach a critical stage known as established renal failure (ERF). Most commonly caused by diabetes and hypertension, ERF is characterised by a gradual and irreversible decline for which there are no curative treatment options. Ultimately, with further deterioration in function, patients require renal replacement therapy (RRT) in the form of dialysis or transplantation. The pre-disposing chronic kidney disease (CKD) is estimated to affect approximately 10% of the global population. Although this disease process can be slowed down or halted with lifestyle modification and medical therapy if detected early, a significant proportion of these patients will progress to ERF (Sundström et al., 2022). Other common causes of ERF include glomerular disease, cystic kidney disease, vascular disease and congenital diseases, amongst others.

In the United Kingdom, there are approximately 30000 adults and children currently dependent on dialysis, a number which is predicted to double by 2030 (Iwagami et al., 2017). This carries not only a significant financial burden, costing approximately £34000 per patient per year but also a significant health burden to the patient (Roberts et al., 2022). Quantifying this risk, patients receiving haemodialysis are reported to have a 6 to 7 times higher all-cause mortality rate compared to the normal population, principally due to cardiovascular disease, infection and treatment withdrawal (Ferreira et al., 2020).

For eligible patients, kidney transplantation provides a method of providing the essential functions of the kidney without the need for RRT. Although the operation and required immunosuppression carry risks of their own, transplantation has been shown to improve both survival (Chaudhry et al., 2024) and quality of life (Wang et al., 2021). There are also financial benefits, with an estimated saving of approximately £25000 per annum for each year of a functioning graft (i.e., no requirement for dialysis) (Rodger & Venter, 2023). As a result, kidney transplantation is considered the gold standard treatment for ERF.

1.2 Kidney Transplantation

In the UK, with over 5000 patients currently active on the waiting list, the average time from listing to kidney transplantation is 2-3 years. To address this need, approximately 3000 kidney transplants are performed annually in the UK, with current graft survival rates (i.e., years of functioning kidney) estimated to be between 15-20 years for deceased kidneys. With this drive for transplantation, the challenge is how best to address the discrepancy between the ever-increasing demand of those in need of a kidney transplant and the utilisable supply of grafts. Notably, although significant progress had been evident with a steady decline, there has been a recent uptick in the number of patients on the kidney transplant waiting list (between 2021 and 2023), which necessitates additional effort to increase the utilisable pool of donor kidneys ('Annual Activity Report', accessed March 2024).

The supply of kidneys for transplantation comes from two sources: living or deceased donation. Furthermore, deceased donation comes in two forms, depending on the nature of the donor's demise: Firstly, donor/donation after circulatory death (DCD) describes the irreversible cessation of circulatory and respiratory function, and secondly, donor/donation after brainstem death (DBD) in which the donor meets a neurological criterion for death, whilst the organs maintain an oxygenated blood supply. Historically, DBD was the primary source of deceased donation, given the well-documented favourable outcomes of graft survival and avoidance of delayed graft function (DGF). More recent data, however, has shown satisfactory survival rates and function of DCD grafts, highlighting the potentially underused resource (Summers et al., 2015).

To increase the pool from which to obtain donor organs, the UK and Europe lead the way in transplanting DCD grafts. Inherent in the nature of DCD procurement, there is a phase of suboptimal perfusion during the preceding hypotensive agonal phase followed by a period where the organs no longer receive oxygenated blood (i.e., warm ischaemia time). This hypoxic insult creates the environment for the pathophysiological process known as ischaemia reperfusion injury (IRI) (Oniscu et al., 2014; Peris et al., 2018; Shapey & Muiesan, 2013). Therefore, organs procured in this setting are obtained promptly via rapid laparotomy following circulatory arrest, and in-situ cold perfusion fluid is circulated through the grafts before being transported via static cold storage (SCS), i.e., on ice. This period of warm ischaemia followed by hypothermia during cold storage (so-called 'cold ischaemic time' (CIT)) is thought to be the principal driving factor behind IRI leading to increased rates of primary non-function (PNF), DGF and poorer long-term graft survival rates (Demiselle et al., 2016).

DBD procurement, by contrast, is performed in a less time-pressured manner as the oxygenation to the organs is maintained. The organs are retrieved, cold flushed and then transported in the same manner via SCS. While there is less warm ischaemia time in this method, these kidneys still undergo cold ischaemia during transportation and remain susceptible, albeit to a lesser extent, to the effects of IRI (Wadei et al., 2013).

DCD usage has increased year on year and now represents nearly 50% of deceased donor transplants in the UK. The use of extended criteria donors (ECD), i.e., donors over 65 years of age, has increased the potential pool from which to obtain donation even further, but this carries inherent challenges with graft survival and rates of DGF. Nevertheless, the proportion of ECD donations here in the UK now represents an increasing share, with 35% of deceased donations provided by donors over 60 years of age ('Transplant Activity Report', accessed March 2024).

NHS Blood and Transplant (NHS BT) has consistently highlighted organ utilisation as one of the critical areas in our efforts to meet the increasing demands in solid organ transplantation. The two most recent strategy documents, 'Taking Organ Transplantation to 2020' and 'Transplantation 2030: Meeting the Need', gave particular attention to organ utilisation, with the former outlining strategies to increase transplant numbers by 50%. Part of these multi-faceted strategy documents was the recognition that novel technologies, such as organ perfusion techniques, are likely to have an increasingly important role in the assessment and potential reconditioning of marginal donor organs.

1.3 Ischaemia Reperfusion Injury

As introduced above, IRI describes the inflammatory and immunological response that results from the process a solid organ undergoes from procurement to the early period post-implantation. The foundations of this process begin during, or even before, organ procurement and continue through cold storage before eventual reperfusion in the recipient, where there is rapid rewarming. The cessation of blood flow, followed by rapid re-warming and oxygenation upon reperfusion, leads to an inflammatory cascade including reactive oxygen species (ROS), cytokines and leucocyte activation (Malek & Nematbakhsh, 2015). In addition, this inflammatory process is alongside a potential immunological response as the recipient reacts to the donor cell surface antigens. This complex multi-factorial phenomenon is well documented, and individual aspects of this pathophysiological process are targets for research to mitigate its deleterious effects.

The severity of IRI is known to impact transplant outcomes. This is seen in the immediate post-operative phase with delayed graft function, where the kidney's function is delayed, but is also evident long-term with poorer graft survival and rate of acute rejection (Ponticelli, 2014; Salvadori et al., 2015a). These longer-term effects are thought to be secondary to increased allogenicity (i.e., heightened response to donor tissue) and nephron and tubular injury, leading to a lower reserve (Eltzschig & Eckle, 2011).

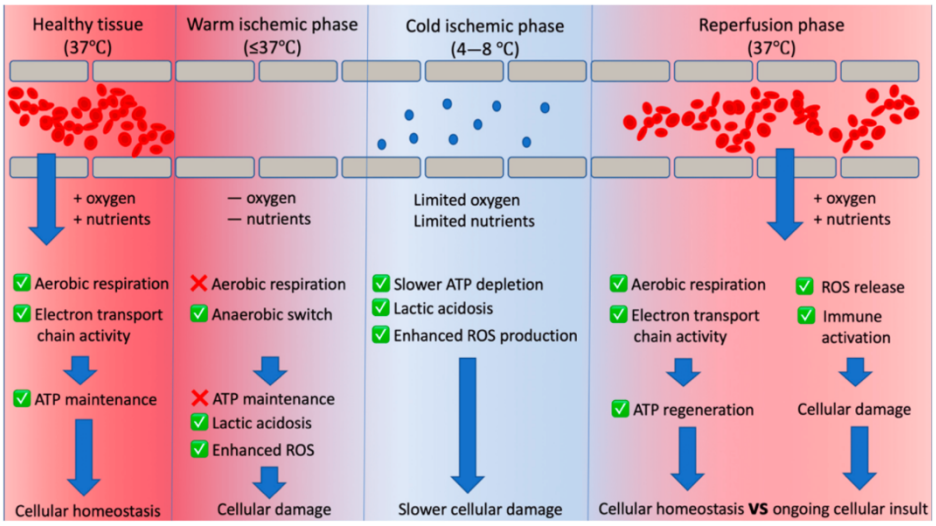


Figure 1-1: Overview of the graft tissue environment between organ procurement and implantation (T. B. Smith et al., 2021)

In healthy tissue pre-donation, there is sufficient oxygenation and nutrient delivery to the tissues. This leads to a homeostatic balance of the energy substrates, including adenosine triphosphate (ATP). The cessation of oxygenation and nutrient delivery at the time of procurement (i.e., warm ischaemia time) results in anaerobic respiration, leading to the accumulation of ROS, lactic acid, and the depletion of ATP (see Figure 1-1 by T. B. Smith et al., 2021). Cooling an organ to hypothermic conditions (4-8°C) attempts to limit this process and reduce the energy demand of the graft. ATP depletion, however, continues throughout this period (Ravaioli et al., 2018). During the implantation of a graft, there is oxygenation and the delivery of nutrients, with re-warming of the tissues; however, the backdrop of inflammatory mediate (e.g., ROS) and ATP depletion is now accompanied by immunological activation.

Due to the complexity of the interlinked processes of IRI, the exact mechanisms are not fully understood. However, understanding the pathways is critical in order to design therapies or

technologies to minimise or reverse the harmful effects. As mentioned, the switch to anaerobic metabolism during ischaemia leads to ATP depletion and lactate formation. At a cellular level, this destabilises the lysosome and cytoskeleton with disruption of the cell surface Na^+/K^+ ATPase membrane protein (Kako et al., 1988). This ATPase dysfunction leads to the intracellular accumulation of water and sodium, causing cell oedema and the build-up of calcium. The accumulation of intracellular calcium leads to the activation of inflammatory proteases, such as caplains. Although, in acidotic conditions, caplains remain inactive, the restoration of normal pH at reperfusion causes caplain-induced cellular injury and death (Chatterjee et al., 2001).

The rise in intracellular calcium also impacts mitochondrial activity, an important source of ATP production. The mitochondria attempt to restore balance by absorbing excess intra-cellular calcium, but once a threshold is reached the mitochondria undergo a process known as mitochondria permeability transition (MPT). This entails a channel within the mitochondrial membrane known as the MPT pore opening, which leads to necroptosis, a form of cell death, and the production of ROS (Strubbe-Rivera et al., 2021).

Upon reperfusion, the already injured cells are re-exposed to oxygen. With the restoration of normal pH, the aforementioned caplains become active, causing cell injury (Salvadori et al., 2015b). In addition, normoxia and the resultant production of ATP leads to significant ROS production. The oxidative stress that ensues with ROS accumulation is thought to be secondary to numerous damaging pathways, including injury to cell signalling, damage to DNA, and further mitochondrial insult (Martin et al., 2019). Consequently, there is release of cytochrome C, succinate and mitochondrial DNA, which are implicated in further cell death and are thought to play an important role in the activation of both the innate and adaptive immune systems (Kang et al., 2015).

Although transplant recipients receive pre-operative immunosuppression (e.g., steroids and IL-2 receptor blockers), there is a second hit by both the innate and adaptive immune response upon reperfusion, dependent on the degree of genetic mismatch between donor and recipient. The pathophysiology of this is out of the scope of this thesis; however, the key processes include complement activation, inflammatory cytokine production, and the activation of Toll-like receptors (TLR). This, in turn, leads to the activation of macrophages, natural killer cells, dendritic cells, CD4+ T cells and B-cells.

Ultimately, IRI results in cell death, endothelial dysfunction, and vasoconstriction. This manifests as a reduction in functional renal mass, graft vascular injury and a pre-disposition

to fibrosis, which in turn impacts early graft function (Smith et al., 2021). Part of the decision-making process in organ offering is the clinician's assessment of whether the donor's kidney is thought to be able to withstand this pathophysiological process. Treatments which successfully minimise the adverse effects of IRI will, therefore, widen the acceptance criteria for donor organs.

1.4 Perfusion Techniques

To mitigate the pathophysiology of organ procurement, there is increasing interest in perfusion techniques, both at the time of organ retrieval and following explantation, i.e., ex vivo perfusion. The optimal timing, duration, temperature and contents of the perfusate are all independent areas of ever-emerging research. Such techniques aim to address the modifiable factors during the periods of ischaemia that occur during procurement and transplantation.

The earliest known concepts for machine perfusion of solid organs date back to the 1930s when Carrel and Lindbergh designed a pulsatile, normothermic oxygenation device (Malinin, 1996). Despite this early innovation, it wasn't until the 1970s that the 'Gambro' machine was shown to preserve human kidneys for up to 36 hours with an albumin solution at hypothermic conditions (5°C). Twenty kidneys were transplanted following a period of machine perfusion, with six discarded due to poor perfusion characteristics – an early demonstration of the ability to assess organs with such techniques. Seventeen of these kidneys were reported to have achieved satisfactory renal function post-transplant (Stephenson et al., 1973).

The advent of modern preservation solutions such as Euro-Collins and University of Wisconsin (UW), however, and the demonstration at the time of their superiority compared to the aforementioned pulsatile machine perfusion, led to the transportation of organs on ice (i.e., SCS) becoming the mainstay of organ preservation (Opelz & Terasaki, 1982). This procurement and transportation method remains essentially unchanged to this day (Balfoussia et al., 2012). The expansion of the donor pool, i.e., the use of organs that would previously have been deemed unsuitable for transplantation, has reinvigorated the exploration of new organ preservation and perfusion techniques.

1.4.1 In-Situ Perfusion Techniques

Normothermic regional perfusion (NRP) is a method of in-situ perfusion of the infra-coeliac viscera in DCD organ procurement with warmed (37°C) oxygenated perfusate using extra-corporeal membrane oxygenation (ECMO). This in-situ method works in three main ways: Firstly, it allows restoration of blood flow following the confirmation of death and before the organ recovery, thus minimising warm ischaemia time (Hessheimer et al., 2018). Furthermore, once the perfusion circuit is established, NRP changes a rapid organ recovery technique inherent in DCD donation to a controlled semi-elective procedure, with the potential benefit of reducing organ damage. Secondly, at a cellular level, the perfusion enables a period of rehabilitation with the restoration of mitochondrial and ATP stores, which have been shown to deplete during both warm and cold ischaemic periods (Amador et al., 2007; García-Valdecasas et al., 1998; Net et al., 2005). Thirdly, this time on the perfusion circuit allows for viability assessment of the graft, theoretically enhancing the transplantation decision-making process (Shapey & Muiesan, 2013).

Demonstrating the benefits of this technique, Minanbres et al. (2017) reported NRP to reverse the poor results seen in controlled DCD liver transplantation. They reported comparable DGF and kidney graft survival rates with DCD kidneys to that of a matched group of DBD transplants (Miñambres et al., 2017). Regarding kidney transplantation, the UK report on the experience of NRP added to this evidence with favourable rates of DGF, however of concern, demonstrated a higher-than-expected graft loss (12%) within 30 days (Oniscu et al., 2014).

As a result, this technology has become commonplace in DCD procurement in Spain, and the multi-centre, nationwide study by Padilla and colleagues reported on over 800 kidneys procured following NRP. Although this study demonstrated no difference in graft primary non-function or patient survival, the DGF and 1-year graft loss rates were significantly increased with standard procurement compared to NRP (Padilla et al., 2021).

The nature of NRP, in which all infra-coeliac organs are perfused, theoretically provides a multi-organ benefit with a single intervention, as opposed to isolated post-procurement techniques. Although more work is necessary, there is increasing evidence that NRP provides a multi-organ benefit by lessening the effects of ischaemia reperfusion injury, offers a viability assessment opportunity and converts the DCD procurement from rapid laparotomy into a more controlled procedure (Carlis et al., 2023).

1.4.2 Ex-Situ Perfusion Techniques

Ex-situ perfusion includes any machine perfusion technique performed after organ procurement to an isolated solid organ. Unlike static cold storage, machine perfusion allows the continuous flow of a perfusate, theoretically providing nutrients and oxygen, if desired, whilst also removing toxic metabolites (Balfoussia et al., 2012).

1.4.2.1 Hypothermic Machine Perfusion

The two main subtypes of ex-situ machine perfusion are hypothermic (4°C) and physiological normothermic (37°C). The intent of a hypothermic state is reduced metabolic activity and preservation of an organ in the absence of oxygenation. First described over 50 years ago, Belzer successfully preserved kidneys in this manner for 17 hours prior to transplantation (Belzer et al., 1968). Early machines were cumbersome to transport and, as aforementioned, were largely replaced by static cold storage techniques once preservation solutions, such as Euro-Collins, had been developed (Collins et al., 1969).

Numerous hypothermic machine perfusion (HMP) devices have been developed in recent years, differing with varying temperatures (4-10°C), flow rates and the presence of oxygenation. The above-mentioned Gambro device is one such example. The most widely used hypothermic machine perfusion devices are the LifePort® (*Organ Recovery Systems*) and the KidneyAssist® (*OrganAssist*). The Cochrane Review by Tingle and colleagues, published in 2019, concluded that hypothermic machine perfusion was superior to static cold storage for both DCD and DBD kidneys, with a reduction in DGF of approximately 23% with the technology (Tingle et al., 2020). Most of the devices in clinical practice currently do not provide oxygenation during the period of perfusion, as it is thought the graft has limited aerobic requirement in the state of hypothermia. There are, however, pilot studies of hypothermic machine devices that deliver supplemental oxygenation, such as the AirDrive™ HMP system (Houtzager et al., 2021).

A key benefit of modern hypothermic devices (e.g., LifePort®) is the safety of such a technique. If, during transportation, the cannulation of the graft was to dislodge and continuous perfusion was to stop, the system would default to an organ environment akin to static cold storage and not threaten the graft. If, by contrast, a normothermic technology were to fail in this manner, the result would be warm ischaemia, which would be significantly more detrimental to the graft.

1.4.2.2 Normothermic Machine Perfusion

There is increasing interest in post-procurement normothermic perfusion techniques (so-called Ex-vivo normothermic perfusion (EVNP). Of note, normothermic machine perfusion (NMP) is an analogous term and is interchangeable with EVNP. These technologies deliver a warmed oxygenated perfusate at physiological temperatures (37°C), most commonly with an oxygen carrier such as blood, a base solution and supplemental additives. The transition from experimental work to clinical practice in this area has gathered significant momentum in recent years following the demonstration of ATP restoration during a brief period of normothermic perfusion in the 1980s (Van Der Wijk et al., 1980).

To date, EVNP has been demonstrated in clinical transplantation in the heart (Ardehali et al., 2015), lung (Cypel et al., 2012), liver (Nasralla et al., 2018) and more recently in kidney solid organ transplantation (Hosgood et al., 2018). Establishing the oxygenated normothermic circuit at the recipient centre allows the following opportunities: 1) reconditioning of the kidney to lessen the effects of IRI and the repletion of energy substrates, 2) viability assessment to aid the transplant decision-making process in marginal grafts and 3) a potential mode for therapeutic intervention.

The recent and accelerated translation into clinical research was grounded on pre-clinical work in animal models. Early work by pioneers in this field, Dr Hosgood and colleagues, demonstrated the re-conditioning effects of EVNP with porcine kidneys exposed to either 24 hours of SCS or 23 hours followed by one hour of EVNP. The EVNP-treated kidneys demonstrated lower intra-renal resistance and had improved metabolic function with evidence of less tubular injury (Hosgood et al., 2013). No difference was found in the expression of inflammatory cytokines (interleukin [IL]-1beta, IL-8, tumour necrosis factor-alpha) or renal function, although IL-6 and heat shock protein 70 were found to be upregulated and postulated to play a crucial role in renal reconditioning.

The group in Toronto, led by Selzner et al., with the development of a porcine model, have also published extensively on the effects of normothermic machine perfusion on kidneys. Early work by this group successfully perfused porcine kidneys for 10 hours with a normothermic oxygenated perfusate consisting of blood, Steen solution and Ringer's lactate (Kaths et al., 2015). This led to the demonstration of an auto-transplantation model in which porcine kidneys were subjected to 30 minutes of warm ischaemia and preserved via SCS or treated with 8 hours of EVNP. The treatment group demonstrated reduced intra-renal resistance, lower lactate levels, and reduced cellular injury markers (e.g., lactate

dehydrogenase (LDH) and aspartate aminotransferase). Following re-implantation to the animal, the kidneys treated with EVNP had lower serum creatinine levels on days 1 to 7 and improved creatinine clearance on day 4 (Kaths et al., 2017).

The same group then investigated the impact of the duration of EVNP on porcine grafts, concluding that periods of 8 or 16 hours were beneficial with regard to serum creatinine levels and tubular injury measures compared to shorter durations (Jm et al., 2017). This finding was confirmed in their recent work, in which 16 hours of EVNP was compared with hypothermic machine perfusion, either with supplemental oxygen or without. The normothermic technique was superior with regard to post-transplant renal function, and more rapid recovery following the procurement process. Tubular injury scores were, however, similar in all groups. Furthermore, the addition of oxygen to the hypothermic perfusion circuit did not have a demonstrable impact on the renal function or markers of tubular injury (Mazilescu et al., 2021).

Of clinical and practical relevance, the Toronto group recently investigated whether there is an additive benefit in the combination of normothermic techniques following a period of hypothermic machine perfusion. In other words, if organs are transported via a hypothermic device such as the LifePort® (*Organ Recovery Systems*) and then undergo a short period of EVNP. The group reported that 16 hours of continuous EVNP was superior to oxygenated hypothermic machine perfusion followed by 3 hours of EVNP, specifically regarding early post-implant function. However, they concluded that the short period of EVNP after hypothermic machine perfusion was comparable to prolonged continuous EVNP and superior to hypothermic perfusion alone (Mazilescu et al., 2024). This combination of strategies is of clinical relevance given the challenges mentioned above in the maintenance of normothermic perfusion techniques during transportation, owing to the risks of warm ischaemia if the connection to the device were to be lost.

1.4.3 Clinical Use of Ex-Vivo Normothermic Perfusion in Kidney

Professor Nicholson and Dr Hosgood, with their team in Cambridge, have been the principal drivers of the translation of EVNP in clinical renal transplantation following the first successful post-EVNP transplantation in 2011 (Hosgood & Nicholson, 2011). The subsequent clinical implementation thus far has gained momentum in the viability assessment of marginal grafts, particularly in DCD kidneys.

Post-procurement organ decline is a significant issue in clinical transplantation and is a crucial area of research aiming to increase the potential pool of usable donor organs. During organ procurement or upon assessment at the recipient centre, if the kidney is seen to inadequately perfuse when flushed with in situ cold flush (typically UW solution), the terms 'inadequate in situ perfusion' or 'poor perfusion' are often used. As many as 18% of kidneys from DCD donors are deemed unsuitable for transplant, with poor perfusion one of the most common reasons cited (Callaghan et al., 2014). The rationale for declining such organs is that the poor perfusion indicates incomplete cooling (i.e., warm ischaemia) and clearance of blood and raises concern regarding primary non-function or poor function post-transplantation. The aetiology of poor perfusion is multifactorial, and potential causes include donor atherosclerosis, technical difficulties at procurement, complex anatomy, intravascular thrombosis, and vasospasm (Hosgood et al., 2016).

Hosgood eloquently described how EVNP could provide viability assessment information by describing that the technology "not only allows a measure of injury but also provides an evaluation of recovery" (Hosgood & Nicholson, 2017). Through a large body of work in kidneys declined by recipient centres, their team have devised a quality assessment score in which kidneys are graded from 1 (least injured) to 5 (the most severe injury) based on three parameters: 1) macroscopic appearance 2) renal blood flow and 3) urine output (see Table 1-1). Notably, a score of 1-4 was deemed potentially transplantable. (Hosgood, Barlow, Hunter, et al., 2015). This work highlighted the potential underutilisation of our current offering system, i.e., kidneys that are declined may be suitable for use if additional tools such as EVNP were adopted.

The perfusion of kidneys with EVNP, which were initially discarded for poor perfusion, was demonstrated initially in an experimental model in which 22 kidneys were observed during EVNP for appearance, creatinine clearance, renal blood flow, and urine output. This study highlighted that many of these discarded kidneys may have been suitable for transplantation (Hosgood, Barlow, Dormer, et al., 2015). This finding led to the successful implantation of two kidneys, which were initially declined due to 'poor perfusion', having demonstrated favourable characteristics during 1 hour of EVNP (Hosgood et al., 2016). This showed two key strengths of this technology: the resuscitative capacity and the opportunity to assess the viability of a graft to aid decision-making, therefore having the potential to expand the pool of usable grafts. A clinical pilot then followed, utilising EVNP in marginal ECD kidneys prior to transplantation, which were deemed viable but were considered suboptimal and prone to the effects of IRI. This work demonstrated that perfusion of these grafts reduced

the rate of DGF from 36% in standard ECD (i.e., static cold storage only) to 6% in EVNP-treated ECD grafts, with no detrimental effects on graft or patient survival at one year (Nicholson & Hosgood, 2013).

This large body of work led to a randomised control trial to assess EVNP vs static cold storage in DCD donors (Hosgood et al., 2017). This multi-centre study included 277 kidneys randomised to either SCS alone (i.e., stored on ice) or SCS plus one hour of EVNP, with delayed graft function as the primary endpoint. The DGF rate in the EVNP group was 60.7% compared to 58.5% in the SCS group (p=0.6); concluding that EVNP did not reduce the rate of DGF in these kidneys. This failure to demonstrate the benefit of the technology has raised questions about its universal utility in DCD donor grafts; however, the DGF rate of approximately 60% in all grafts was unexpected and not in keeping with previous data on DCD kidneys in the UK. There are limitations with the use of DGF as the primary endpoint. Different centres have varying thresholds for dialysis in the post-operative period, and the decision to dialyse is often made by nephrologists depending on serum potassium results and fluid status of the recipient in the days following surgery. This is inherently subjective. Furthermore, grafts that have a period of delayed function often recover and achieve excellent long term graft survival and dialysis independence for the patients.

The nature of donor death (i.e., DCD vs DBD) is also thought to have an impact on the underlying mechanism of DGF where it occurs (Avlonitis et al., 2005). For example, DCD kidneys are thought to be particularly susceptible to reperfusion injury due to the ischaemic hit inherent in DCD graft procurement. The process of brainstem death, by contrast, has been shown in animal models to cause haemodynamic and immunogenic changes which may lead to altered immunogenic environment in the graft contributing to the susceptibility of grafts to DGF (Takada et al., 1998). Further work is required to identify the grafts that will benefit most from the assessment and reconditioning that EVNP is thought to provide.

Table 1-1: EVNP Assessment Score (Adapted from Hosgood, Barlow, Dormer, et al., 2015)

EVNP Score	
Macroscopic Appearance	**Points**
Excellent perfusion with global and even pink appearance	1 point
Moderate perfusion with some areas of patchy or mottled perfusion	2 points
Poor perfusion with a globally mottled and purple appearance	3 points
Renal Blood Flow	**Points**
Mean renal blood flow <50mL/min/100g	1 point
Mean renal blood flow >50mL/min/100g	0 point
Urine output	**Points**
Total urine output (mL) <43mL/hour	1 point
Total urine output (mL) >43mL/hour	0 point
Scores for macroscopic appearance, renal blood flow and urine output will be added to yield an overall assessment score ranging from 1 (the highest quality) to 5 (the lowest quality).	

There are two currently recruiting studies investigating normothermic perfusion techniques in clinical transplantation. APOLLO (*Clinicaltrials.gov ID: NCT04882254*) - due to be completed in 2025 - is a phase II trial from the Erasmus Medical Centre, Netherlands, assessing the efficacy of a two-hour period of EVNP in extended criteria DBD and DCD kidneys compared to hypothermic machine perfusion. Another study based in Berlin, Germany (*Clinicaltrials.gov ID: NCT05031052*) is also due to be completed in 2025 and aims to assess the role of EVNP in DBD kidneys with a 4-hour period of perfusion compared to SCS.

1.5 Treatment Opportunities During Organ Perfusion

One of the key benefits of an EVNP circuit is the ability to deliver therapeutics to the solid organ whilst being perfused, thus allowing localised treatment to the organ whilst circumventing the systemic delivery of the agent to the transplant recipient. Numerous studies have investigated the potential benefit of agents added to the perfusion circuit in animal models, including regenerative cells, gene therapies and nanoparticles.

1.5.1 Regenerative Cell Therapies

Stem cells have the unique property of pluripotency (i.e., the ability to differentiate into various cell types) whilst demonstrating regenerative properties in numerous in vitro models (Baraniak & McDevitt, 2010; Morigi et al., 2004, 2006; Peired et al., 2016). More specifically, mesenchymal stromal cells (MSC), which differ slightly from a true 'stem cell', are a group of cells that can be derived from bone marrow, adipose tissue, placental tissue or amniotic fluid, and have been shown to secrete anti-inflammatory cytokines, growth factors, and immunoregulatory mediators via paracrine actions.

First identified in the 1960s (Friedenstein et al., 1987), MSCs are stromal cells which have been extensively investigated for their regenerative properties. The cells are thought to locate to the site of injury, where they engraft and stimulate repair pathways through anti-apoptotic, anti-oxidant and vasculotropic factors (Block et al., 2009; Togel et al., 2005). As a result of these promising mechanistic actions, this cell group have been evaluated in over 1500 human clinical studies (Phinney et al., 2023).

The definition of MSC, according to The International Society for Cell & Gene Therapy (ISCT®), is based on certain characteristics and cell-surface markers. To satisfy this criterion, the cells must be plastic-adherent, positive for the cell-surface markers CD105, CD73, CD90 and negative for CD45, CD34, CD14, CD11b, CD79, CD19 or human leukocyte antigen class II (Horwitz et al., 2005). This is the minimum criteria, as it is evident that certain subtypes of this cell group may exhibit additional cell surface markers. For example, bone marrow-derived MSC are known to exhibit the cell surface marker STRO-1; and adipose-derived stromal cells are positive for CD44, CD29, CD105, CD13, CD34, CD166, CD10, CD49e and CD59 in addition to the above minimum criterion (Mildmay-White & Khan, 2017).

As aforementioned, MSC differ somewhat from a true 'stem cell'. The term MSC encompasses a heterogenous group of stromal cells that, although they have some overlapping functional abilities to stem cells, do not satisfy the full definition of a stem cell, i.e., one that exhibits plastic adherence, progenitor cell functionality and has both self-renewal capacity and true multipotency. The functional properties of MSC, by contrast, are defined as secretory, homing and immunomodulatory, along with the ability to differentiate in vitro into adipocytes, chondrocytes and osteoblasts. They are not thought, however, to have the ability of extended self-renewal (Soliman et al., 2021; Viswanathan et al., 2019).

Adding complexity to this, the group of cells, or 'bulk population', described by MSC may, however, contain a number of true stem cells within its population. This may, in part, explain why MSCs display stem-cell-like properties, as well as their well-documented paracrine effects (Phinney et al., 2023). Of relevance to this thesis, the terms stem cell and stromal cell are not interchangeable and, as stated, have subtle yet important differences.

The application of the regenerative properties of MSCs has been shown in renal models, where they have been shown to improve medullary inflammation and fibrosis in atherosclerotic renal artery stenosis, and preserve renal function and prevent fibrosis in a porcine transplant model (Baulier et al., 2014). Furthermore, MSCs have shown the tendency to localise in sites of inflammation (Horwitz et al., 2002; Mahmood et al., 2003; Tolar et al., 2010) and migrate to renal tissue, which has been subject to injury (Herrera et al., 2004; Ittrich et al., 2007)(see Figure 1.7). In addition, the systematic review and meta-analysis on the role of MSC therapy in small animal models of kidney injury, based on a total of 21 studies, concluded that MSC therapy was able to improve impaired renal function, and proposed that intra-arterial delivery was the most optimal delivery mechanism (Wang et al., 2013).

More specifically, there is an increasing body of evidence to support the role of MSC in the mitigation of IRI. Having demonstrated favourable properties, e.g., anti-oxidative stress (Pacienza et al., 2019), the role of this regenerative cell population has been evaluated in numerous experimental transplant models (Hu & Zou, 2017; Li et al., 2021; Mordant et al., 2016). For example, MSC-derived extracellular vesicles have been shown to be protective against renal tubular fibrosis by reducing levels of ROS and pro-apoptotic molecules such as caspase-3 and malonaldehyde (Y. Zhou et al., 2013). In addition, a group from China administered bone marrow-derived MSC at the time of reperfusion or two weeks after transplant, compared to controls, in the context of 159 living donor transplants. The study reported lower biopsy-confirmed acute rejection and improved renal function at one year in

the MSC treatment groups (Tan, 2012). These two illustrative examples are part of a large body of evidence which supports their role in the protection of solid organs against the deleterious effects of IRI (Kanazawa et al., 2011; Xie et al., 2022).

The difficulty, however, is the clinical application of such a therapy. Studies have demonstrated that when delivered intravenously, the cells are short-lived and invariably fail to migrate beyond the pulmonary circulation (Eggenhofer et al., 2014; Fischer et al., 2009). Circumventing this, in a porcine model of IRI, work by Sierra-Parraga et al. demonstrated that the administration of MSCs directly into the renal artery facilitates the localisation and accumulation of the regenerative cells, predominantly in the renal cortex, adjacent to the glomeruli, capillary networks and tubules (Sierra-Parraga et al., 2017).

Isolated organ perfusion, as described above, provides an alternative and novel mechanism to deliver such therapies. The feasibility of this has been demonstrated by Pool et al., in which bone-marrow-derived MSCs were administered via a normothermic perfusion circuit, and the group reported the regenerative cells to have clustered in the glomeruli (M. Pool et al., 2019). Brasile et al. also demonstrated the delivery of MSC via isolated perfusion with positive results. Although differing in the type of perfusate used, i.e. non-blood-based, the group demonstrated the delivery of MSC increased ATP synthesis and dampened the inflammatory response (Brasile et al., 2019). More recently, Thompson and colleagues in the UK, demonstrated MSC delivery via isolated perfusion of discarded human kidney grafts, demonstrating improved urine output, reduced intra-vascular resistance and downregulation of pro-inflammatory cytokines (Thompson et al., 2020).

There are reports, however, of significant cell death, with only 10% remaining viable during the period of perfusion (M. Pool et al., 2019). Adding to this, the conditions required for normothermic perfusion have also been shown to reduce the viability of human MSC and impair their ability to adhere to endothelial cells, particularly cells that undergo a freeze-thaw process for preparation (Sierra Parraga et al., 2019). Further work is required, therefore, to confirm whether perfusion techniques are able to deliver these regenerative cell therapies with clinically relevant efficacy.

1.5.2 Delivery of Autologous Cells

When injected by any route, cell therapies, by their nature, face a different physiological response compared to soluble molecules. Once sequestered within a target organ, these cells

will induce a response from both the innate and potentially adaptive immune responses (Lipowsky et al., 2018). Such cells do not need to bind to target receptors like other molecular therapies and achieve physiological effects by paracrine, endocrine and direct cell-to-cell contact mechanisms. Some authors have suggested a degree of immunological privilege with MSC or even that certain cell types are "immunologically null"; however, the evidence for that is not clear (Q. Zhao et al., 2016). All such cell therapies have a degree of cell attrition or death, often significant, even under optimal culture conditions. Furthermore, this has the potential to be worsened by the stress of injection into the bloodstream or parenchyma after engagement with the target tissue.

Most cell therapies rely on large-scale ex-vivo culture and expansion of a specified cell type. These large-scale cell cultures are associated with various problems relating to the genetic stability of cell populations maintained over numerous passages. This requirement for culture rarely allows for anything other than an allogeneic cell therapy, and given the unpredictable timing of solid organ transplants from deceased donors, there is a limited possibility for sourcing autologous cells from the traditional depots of peripheral blood or bone marrow. For these reasons, a cell therapy that is intrinsically "self" is theoretically more desirable for therapeutic purposes. Still, the problem of obtaining sufficient numbers of cells with rehabilitative effects remains. Abundant cell numbers have, however, been demonstrated in the harvest of MSC from adipose tissue, with so-called adipose-derived regenerative cells (Kol et al., 2013).

1.5.3 Sources of Autologous Regenerative Cells

In the context of protecting or reconditioning a solid organ in the peri-transplant phase, therefore, a theoretically optimal regenerative cell therapy would be autologous. In addition, the therapy would be obtainable in sufficient cell numbers to effectively provide a therapeutic effect without the need for costly and prolonged culture expansion. Such a therapy would depend on several key factors:

- The availability of redundant source tissue (which can be safely removed from a patient)
- The safety of accessing or excising the source tissue
- The quantity of cells which can be obtained per gram of source tissue

- The reproducibility and efficiency of the target cell(s) isolation process
- The efficacy of the cell population extracted

For regenerative therapy, the aforementioned MSCs can be constructively isolated from peripheral blood, bone marrow, adipose tissue (Banfi et al., 2000; De Ugarte et al., 2003), and even umbilical cord blood (Erices et al., 2000). Many other tissues have been shown to contain MSCs. However, removal and isolation are not safely possible for many of these vital organs. Bone marrow is a rich source of MSCs, but bone marrow sampling is poorly tolerated, and levels of MSCs isolated are generally too low to provide therapeutic benefit without further expansion through culture (Kramann et al., 2011; Zuk et al., 2002).

Adipose tissue, however, has been shown to contain higher levels of MSCs per tissue weight within its stromal vascular fraction (SVF). Comparative analysis indicates MSC levels are several hundred times higher in adipose SVF compared to human bone marrow (Fraser et al., 2006a). Additionally, adipose stromal vascular fraction contains multiple further nucleated cell subtypes which exhibit beneficial protective/regenerative effects, including macrophages, leucocytes, pericytes and endothelial progenitor cells (Gentile et al., 2012; Riordan et al., 2009). The higher MSC yield, combined with additional bioactive cellular content, has meant that point-of-care isolation of SVF from adipose tissue – also termed Adipose-Derived Regenerative cells (ADRC) - has proved effective for multiple therapeutic indications (Kol et al., 2013).

Given the trends in body mass observed throughout the developed world in recent decades, body fat stores represent one source of autologous tissue that is not in short supply for most patients. Fat can be removed from a suitably accessible subcutaneous location via liposuction or an open surgical site, potentially allowing access to both the subcutaneous body fat and the visceral fat surrounding organs, such as peri-nephric fat. Furthermore, in the context of deceased donor organ transplantation, the utilisation of donor body fat as a source of ADRCs remains another largely unexplored option. The safety and practicalities of this approach have been and continue to be tested in a variety of clinical situations, including fat grafting/breast reconstruction, Crohn's disease (Garcia-Olmo et al., 2008), diabetic foot ulcer healing and intra-coronary artery injection following acute myocardial infarction (van Dijk et al., 2011).

1.5.4 Bedside Production of ADRCs

It was demonstrated over 20 years ago that regenerative cells could be obtained from the SVF of human adipose tissue (Zuk et al., 2001, 2002). Further work demonstrated the presence of these cells and an abundance in cell numbers compared to bone marrow-derived populations (Fraser et al., 2006b; Oedayrajsingh-Varma et al., 2006). ADRCs, therefore, do not require *ex vivo* culture expansion to obtain sufficient cell numbers, whilst this process is necessary for other commonly used sources of regenerative cells (e.g., mesenchymal stromal cells). This circumvents clinical-grade cell culture, which is both timely and expensive (Lin et al., 2008). The underlying mechanism of these additional cells is poorly understood; however, the population used as a whole has been used in both preclinical and clinical studies in myocardial disease (Houtgraaf et al., 2012), bone (Pak, 2012) and peripheral ulcer healing (Cervelli et al., 2011) with the demonstration of regenerative properties.

The avoidance of culture expansion, therefore, allows adipose tissue to be obtained, the enzymatic process completed, and regenerative cell-based therapy to be administered in the same time frame – a point-of-care therapy. The Celution System® (Celution® 800/CRS System) is an approved medical device, which was manufactured in order to standardise the extraction process of ADRC from adipose tissue in a safe and consistent manner. This automated and sterile process can be used at the patient's bedside and reliably produces an ADRC population which can be used as a therapeutic agent. Adipose tissue is obtained from a consenting donor by way of a minimally invasive liposuction (approximately 100-150ml) and inserted into the Celution System using the proprietary single-use sterile set. The tissue is then processed, excess fluid removed, and weighed to estimate the amount of enzyme reagent (Celase®) required. The tissue undergoes enzymatic digestion and, once complete, undergoes centrifugation, and the resultant 5ml volume of cell product is produced (Fraser et al., 2014). The average cell yield is reported to be 3.6×10^5 cells per gram of tissue with viability of approximately 85% (Lin et al., 2008). The produced cell product requires no further steps and can delivered directly to the desired target.

If immediate use is not feasible or desired, the cells can be cryopreserved. Feng et al. demonstrated that ADRCs from Fisher 344 rats isolated by enzymatic disruption with collagenase (Sigma-Aldrich) and Intravase™ (Cytori Therapeutics) can be safely frozen in rat serum gradually to -90°C, then stored in liquid nitrogen (Feng et al., 2010b). As described above, the cell population was pleomorphic with CD45+ leucocytes, endothelial cells, and many other cell types. In an IRI model of acute kidney injury, the use of this previously frozen ADRC reduced mortality and accelerated renal functional recovery. The therapeutic

effect was similar in both the fresh and cryopreserved ADRC populations, including a reduction in cast formation and severity of acute tubular necrosis in the ADRC-treated animals. There was also downregulation of CXCL-2 and IL-6 at 2- and 24-hours post-AKI. This study demonstrated that the cryopreservation of ADRC did not reduce the therapeutic impact in their model and highlighted the potential for cryogenic storage of ADRC for future use. It has been shown that both the number of endothelial progenitor cells and therapeutic potency reduces with age, and this demonstrates the feasibility of procuring these regenerative cells in advance of the need for therapeutic use (Baxter et al., 2004; Walter & Dimmeler, 2002).

1.5.5 Cellular Makeup and Properties of ADRC

Adipose tissue from which ADRCs are extracted is a complex tissue that contains progenitor cells and genes which aid in its storage and mobilisation functions (Merrick et al., 2019). Intact body adipose tissue in its normal anatomical location can, as well as being a key body energy store, act as a protective barrier surrounding organs or tissues and is deemed "healthy". Alternatively, it can be of an inflammatory nature and secrete inflammatory factors such as TGF-beta and, therefore, be deemed unhealthy (Rosen & Spiegelman, 2014).

Adipose tissue is thought to comprise two main classes of cells: adipocytes and stromal cells. Once dissociated and the stromal fraction preserved, an enzymatic and centrifugation process can produce a pleomorphic cell suspension with multiple potential active subsets (Miyazaki T, 2005). The cell populations within this suspension are thought to include stem cells, endothelial cells, and vascular smooth muscle cells (Lin et al., 2008). ADRC describes this uncultured cell suspension, the definition of which, as described above, is based on the presence of certain cell markers (e.g., CD73, CD090, CD44, CD29, CD105, CD13, CD34, CD166, CD10, CD49e and CD59) and negative for others (e.g., CD14, CD31, CD34, and CD45). Although similar to MSC in many regards, it is the abundance and ease with which this cell population can be obtained which is an important practical advantage.

This regenerative pleomorphic cell population can be administered during EVNP without negotiating the pulmonary circuit, with direct access to the target organ during a period of perfusion in a closed circuit. To our knowledge, there are no published studies to date demonstrating the delivery of adipose-derived regenerative cells to human kidneys via an ex vivo normothermic perfusion circuit to assess their role in the amelioration of ischaemia-reperfusion injury with human kidneys following a period of static cold storage.

1.6 Aims and Hypothesis

To summarise, the aims of this thesis are as follows:

- To explore and identify specific types of kidney grafts that could benefit from the assessment period and reconditioning effects of normothermic perfusion technologies
- To describe how a normothermic perfusion service was set up in an acute tertiary hospital with discussion of the obstacles encountered
- To outline novel ways in which EVNP can be used to facilitate renal transplantation
- To describe the delivery of a regenerative cell population, harvested from donor peri-renal fat, via EVNP to human kidney grafts and examine the safety and potential treatment effects

Our hypothesis is that EVNP is a safe clinical tool with the ability to aid decision making in clinical transplantation. Furthermore, the technology offers the advantage of being a vehicle for specific treatment opportunities to target the pathophysiology of ischaemic reperfusion injury.

CHAPTER 2: GENERAL METHODS: THE SET UP OF A CLINICAL PERFUSION SERVICE IN AN ACUTE TERTIARY HOSPITAL

2.1 Introduction

NHS BT consistently highlight organ utilisation as one of the critical areas in our efforts to meet the increasing demands in solid organ transplantation. The two most recent strategy documents, 'Taking Organ Transplantation to 2020' and 'Transplantation 2030: Meeting the Need', both endorsed by the Scottish government, gave particular attention to organ utilisation, with the former outlining strategies which aimed to increase transplants by 50%. This aligned with a locally held finding that a significant proportion of kidneys were being declined, particularly within the higher-risk donor groups, which may be suitable for transplantation if additional assessment tools and strategies were available. A local audit in 2016-2017 assessing all donor offers found that 35 of 220 (15.9%) declined due to perceived risk of poor function, and 23 (10.5%) declined as deemed unsuitable for the named recipient.

As part of this retrospective work, performed before the period of this research, further examination of the declined deceased donor offers highlighted 20 offers which were thought to have potentially led to transplant if a favourable EVNP assessment score was provided during the decision-making process (Hosgood, Barlow, Dormer, et al., 2015).

2.1.1 The Business Case for EVNP

Given the publication and presentation of the possibilities with emerging perfusion technologies such as EVNP, our clinical team embarked on a business proposal to set up a perfusion service to improve organ utilisation (see Appendix A). If the increase in transplants as per national strategy was to be provided, it was thought that this would be most likely through the use of kidneys from higher-risk donors, which may be declined due to the anticipation of poor post-transplant function.

The aims of this business case, therefore, were threefold:

i) Viability assessment of high-risk grafts

a. Assessment of recovery of the most marginal kidneys which are not currently being used

b. Evaluation of 'poorly perfused' kidneys, i.e., incomplete clearance of blood

c. Assessment of the integrity of ureteric blood supply for kidneys received with stripped ureters

ii) Reconditioning of kidneys

a. Acceptance of kidneys with long cold ischaemic times

b. Local use of kidneys for backup recipients, e.g., after positive crossmatch for the index recipient

iii) Future therapeutic possibilities for research purposes

a. Administration of agents to ameliorate ischaemia-reperfusion injury

b. Administration of anti-viral agents, e.g., to allow safer use of organs from hepatitis C-positive donors

The anticipated costs included both capital investment in the hardware required and the recurring costs of the consumables (tubing sets and perfusate materials). The capital costs included the pump console (Medtronic Bio Console 560), the Hico Variotherm 555 Warming Unit and other required hardware components. The capital cost was estimated at £49461, and £582.74 for the consumables for each kidney. This business case was successful with the National Service Division (NSD), Scotland, providing funding for the capital investment and NHS Greater Glasgow & Clyde providing the funding for ongoing consumables.

2.1.2 Interventional Procedure Policy

The research period for this thesis coincided with the acquisition of the perfusion hardware following the successful business case. The task from this point, primarily, was to take this hardware and develop a clinical perfusion service using this novel technology as a viability tool. Within the local trust, the use of novel technologies for interventional use fell within the remit of the New Interventional Procedure Policy (IPP), which provides the governance structure to authorise and review the introduction of new clinical technologies. An application to this body was submitted, outlining the framework by which requisite training would be attained from leading experts and detailing how the introduction of the technology to patient care would be subject to review on an annual basis.

The trust approved the use of EVNP as a novel technology in October 2018 based on an agreed implementation plan (see Figure 2-1). This IPP application was supported by the extensive work performed elsewhere in the UK, primarily in Cambridge, demonstrating the safety and feasibility of EVNP. The trust approval reflected on this body of evidence, and permission was granted on the proviso that grafts would only be used for transplant if they performed satisfactorily during the period of perfusion, thus providing confidence to proceed transplantation. To seek additional funding for consumables, an additional application to a local research grant was submitted; the NHS Greater Glasgow & Clyde Research Endowment Fund kindly granted £15k to partly fund the consumables to introduce this technology.

	Provisional date	Date completed	Comments
Interventional procedure policy approval	October 2018		
Training including certificate of completion	End of September 2018		
First use of EVNP	Mid October 2018		
Review of patient data following initial 5 cases	End of October 2018		
Embark of full data collection	End of October 2018		

Figure 2-1: EVNP implementation plan as agreed by the Trust approved New Interventional Procedure Policy

2.1.3 Perfusion Training

In-person training was provided by the principal drivers of this technology, Dr Hosgood and Professor Nicholson, in Cambridge, UK. In addition, a visit from colleagues in Newcastle, who had gained experience with the perfusion device, also provided in-house training for our clinical consultant and registrar team to provide familiarity with the technology. The hands-on training provided an opportunity to learn how grafts were safely connected via arterial cannulation, to set up and deliver the perfusate composition, and how to perform the testing throughout perfusion.

2.1.4 RINTAG Application

Once the above training was completed, an application for 'Education and Training' purposes was submitted to the Research, Innovation and Novel Technologies Advisory Group (RINTAG). This approval body, run by NHSBT, provides access to donated tissue deemed unsuitable for transplant to be offered to approved research projects across the UK. The first application submitted was, as stated, for education and training purposes in order to gain sufficient expertise with between 5-10 kidney grafts. This application was successful, and these grafts provided the experience to set up perfusion devices, prepare the perfusate, and practice safe cannulation and disconnection of the grafts. Each graft was scored per the EVNP Assessment Score (Hosgood, Barlow, Dormer, et al., 2015), urine output was measured, and arterial blood gases were sought to guide perfusate maintenance (see Figure 2-2 and Figure 2-3). All grafts were perfused for 60 minutes and discarded as per trust protocol. Following successful perfusions with six grafts, a meeting was held locally with the clinical team and IPP representatives and approval was gained for the use of EVNP as a clinical tool. These grafts had average cold ischaemic times of 34 hours, owing to the cumulative time of the initial offer to transplant units, the subsequent decline, the offer to various research units and the organisation of transportation thereafter. Although these grafts were not formally analysed for consideration for transplant, two of the grafts scored either a '1' or '2' on the EVNP Assessment Score, having demonstrated fair or good global perfusion, adequate urine output and did not demonstrate high vascular resistance.

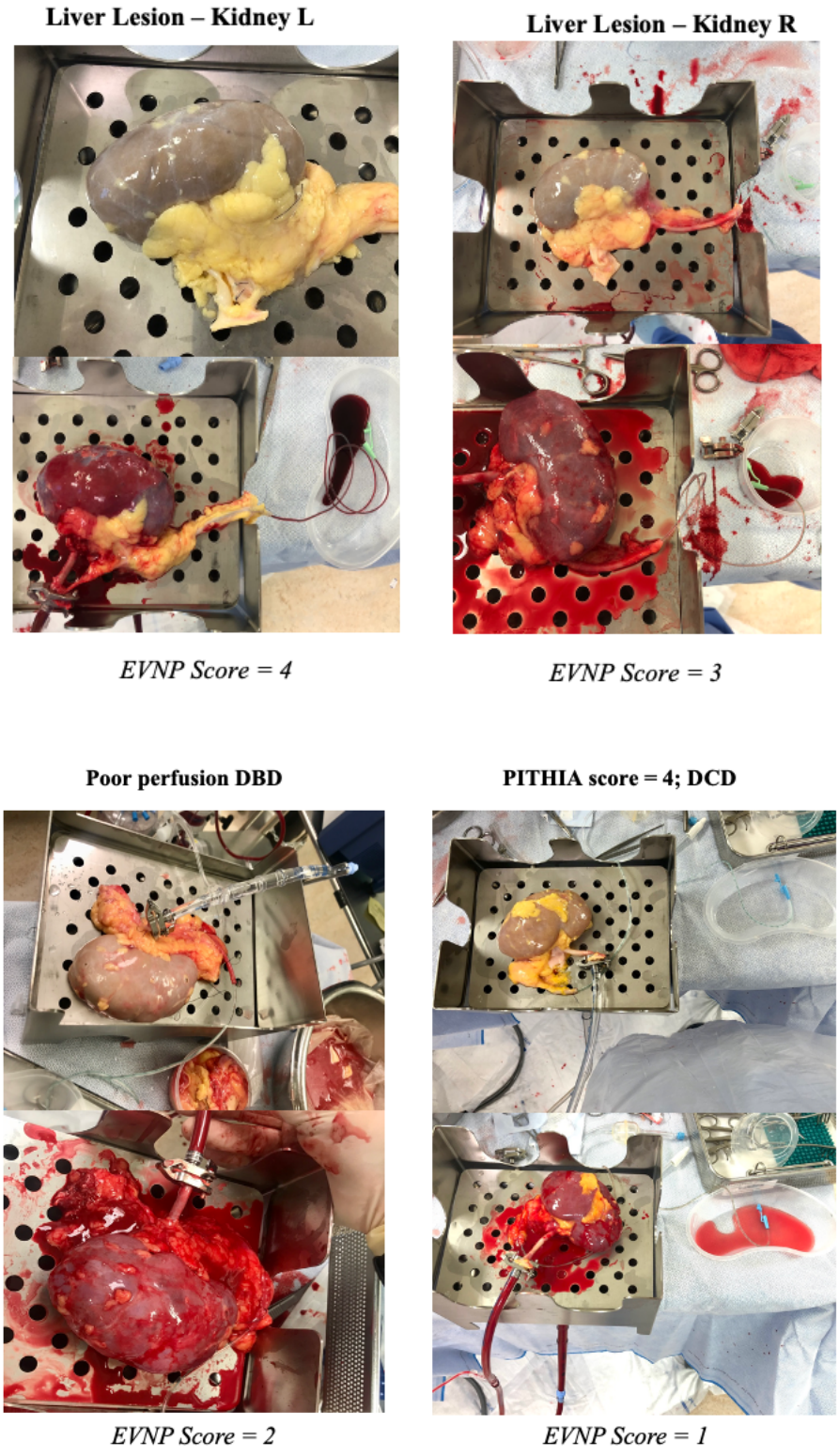


Figure 2-2: Image of before (top) and after (bottom) perfusion of discarded grafts for education and training purposes as part of the formalised training in ex vivo normothermic perfusion.

Abbreviations: DBD: Donor after brainstem death**; EVNP:** Ex-vivo normothermic perfusion**; L:** left**; PITHIA:** Pre-implantation Trial of Histopathology in Renal Transplant Allografts**; R:** right**.**

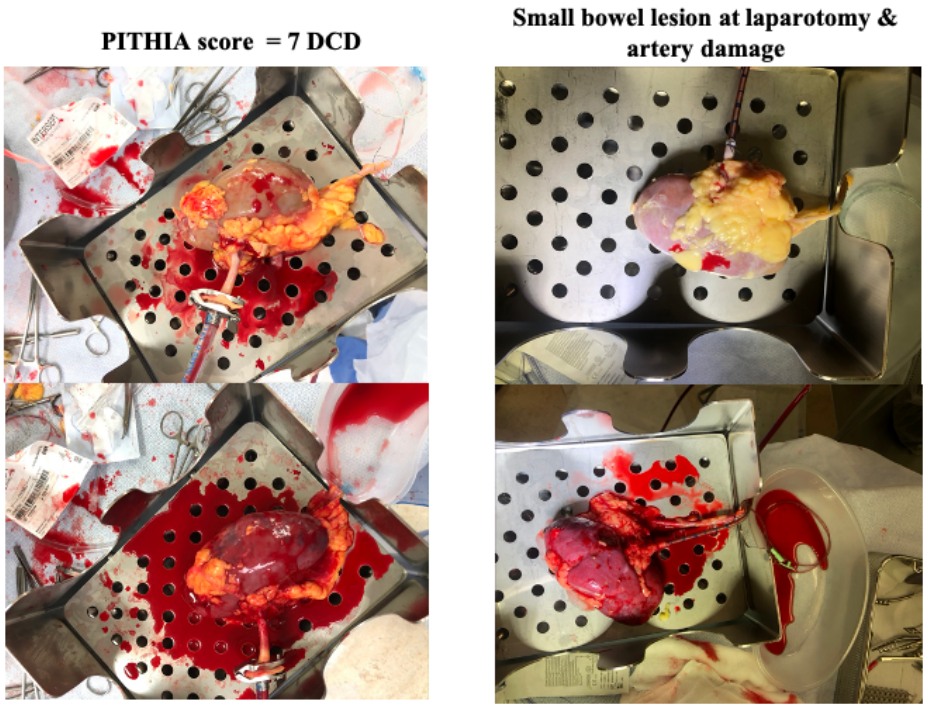


Figure 2-3: Images of before (top) and after (bottom) perfusion of discarded grafts for education and training purposes as part of the formalised training in ex vivo normothermic perfusion.

Abbreviations: DCD: Donor after circulatory death**; EVNP:** Ex-vivo normothermic perfusion**; PITHIA:** Pre-implantation Trial of Histopathology in Renal Transplant Allografts**.**

2.1.5 Departmental Communication

Once the equipment was available and training completed, in-house communication was circulated to all consultant transplant surgeons and transplant coordinators, i.e., all staff receiving offers of kidneys for consideration for transplant. The following scenarios were proposed as cases that may be considered suitable for EVNP: Marginal kidneys, which may be declined without further assessment providing reassuring information; borderline Remuzzi scores; and 'poor perfusion' found during organ procurement. It was explained that following the period of 60-minute perfusion, an EVNP assessment score could be provided, with 1-3 considered transplantable and 4-5 considered unsuitable for transplant. The decision would ultimately be with the implanting surgeon, and a decision would be honoured if clinical concern remained despite a favourable EVNP assessment score. An 'On Call Guide to EVNP' was created and circulated detailing the process of informing the research registrar, providing time to source the unit of packed red cells and set up in the equipment to minimise any delays.

2.1.6 Perfusion Protocol

Given that the trust approval for EVNP use as a novel clinical technology was based on the safety data by other UK groups, the protocol was unchanged from this work (Hosgood et al., 2017). This protocol was for a perfusion duration of 60 minutes and utilised the following materials in the perfusate: Ringer's solution, Prostacyclin, 5 % Dextrose, Synthamin 17, Cernevit multivitamin, Insulin, Sodium Bicarbonate, Mannitol, Dexamethasone, Heparin, Soltran and one unit of O-negative packed red cells. Temperature was maintained at 37.4°C, non-pulsatile pressure was maintained at approximately 75mmHg, and 95% O2 / 5% CO2 gaseous mix was set to 0.1L/min. Pre-cannulation arterial blood gas analysis was taken, and a pH of 7.3-7.5 was maintained with additional Sodium bicarbonate as required. Arterial gas sampling was conducted every 30 minutes. Following the 60-minute perfusion, the grafts were disconnected and flushed with 1L of cooled Soltran to return the graft to hypothermic condition and placed on ice (See Appendix B for Clinical Perfusion Checklist).

2.2 Obstacles and issues

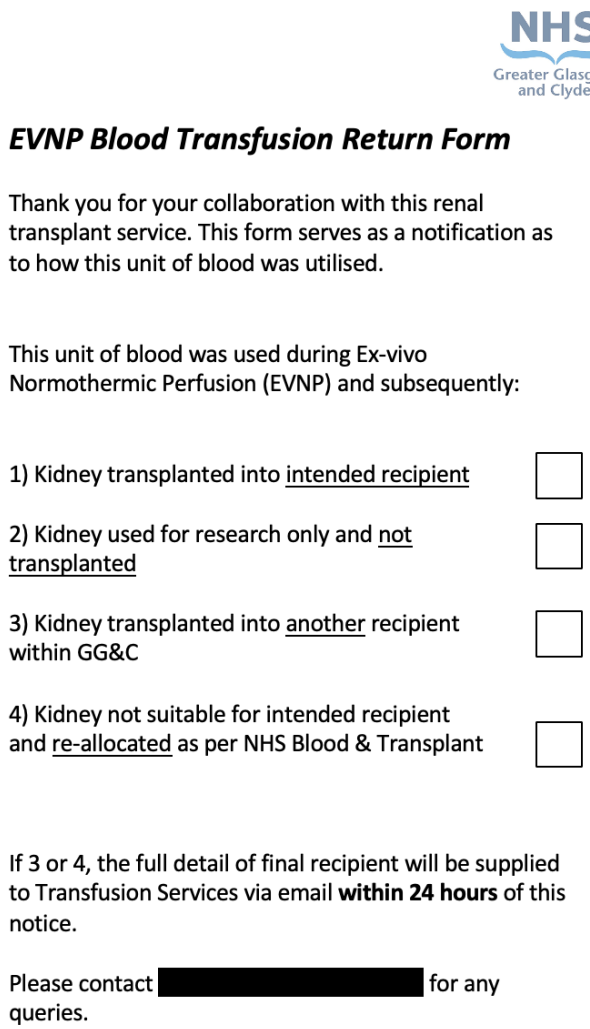
2.2.1 Haematology and Obtaining Blood Products

The perfusion of a kidney graft requires, in principle, a perfusate with an oxygen carrier. There are emerging alternatives to the use of packed red cells; however, as stated above, the protocol in this work utilised a single unit of O-negative or group-specific packed red cells warmed to normothermic conditions. Discussions with other units outlined various mechanisms to access suitable packed red cells, including applications for research supply of blood products. Given the complexity and cost inherent in this process, meetings were held with our local haematology department with a view to using local supply. A framework was discussed with the requirement for blood products, which was as follows: a) O-negative or group-specific packed red cells for the initial RINTAG grafts for education and training (5-10 units); these units could be recently expired units, no older than two weeks old; b) O-negative or group-specific packed red cells for the use in clinical EVNP, estimated at approximately 20 units over three years. The number of units used at the end of the first 12 months to be reviewed to ensure numbers remained sustainable; c) O-negative or group-specific packed red cells for research use, estimated at a maximum of 12 units to be required.

On this basis, the local haematology department agreed to supply blood products for this work. Multiple educational events for the haematology staff were conducted to provide a backdrop to the research, including an explanation that the requirement of these units may be out-of-hours. Following this, a standard operating procedure (SOP) was created and approved outlining how the products would be formally requested, communicated with haematology staff, transported to the theatre suite and documentation of how the unit was used to be returned to the laboratory.

Of importance, with regards to the use of blood products for clinical EVNP use, it was noted that a kidney could be considered for transplantation with the ex vivo perfusion assessment tool. Following this period of assessment, in which the unit of blood was used as the basis of the perfusate, the graft could take the following pathway: a) considered suitable for transplant and transplanted into the named recipient; b) considered unsuitable for transplant and discarded; and c) considered unsuitable for transplant and the graft re-offered as per NHSBT to other units, and therefore offered to another named recipient. Although the grafts are cold-flushed with a unit of Soltran at the end of the perfusion period, the small, possible microscopic remainder of the packed red cells could feasibly be given via a transplanted graft to a second recipient than initially planned. Given O-negative or group-specific, this was not thought to represent clinical concern, but the ultimate destination of these products

required formal documentation. Although this outcome was understood to be unlikely, the ultimate destination of the blood products was to be confirmed in all cases to ensure records were kept as per SOP (see Figure 2-4).



NHS
Greater Glasgow and Clyde

EVNP Blood Transfusion Return Form

Thank you for your collaboration with this renal transplant service. This form serves as a notification as to how this unit of blood was utilised.

This unit of blood was used during Ex-vivo Normothermic Perfusion (EVNP) and subsequently:

1) Kidney transplanted into intended recipient ☐

2) Kidney used for research only and not transplanted ☐

3) Kidney transplanted into another recipient within GG&C ☐

4) Kidney not suitable for intended recipient and re-allocated as per NHS Blood & Transplant ☐

If 3 or 4, the full detail of final recipient will be supplied to Transfusion Services via email **within 24 hours** of this notice.

Please contact [redacted] for any queries.

Figure 2-4: Blood products return form to the haematology department post perfusion.

2.2.2 Arterial Cannulation and Grafts with Multiple Vessels

In the early iterations of EVNP, the artery and vein were individually cannulated to create the closed circuit. The technology evolved to its current clinical form in which the artery is attached to the circuit, but the vein drains passively into a collecting system, which is then connected to the closed circuit for re-circulation. Depending on vessel calibre, positioning and number, there are various ways to connect the renal artery to the circuit. It is important to note that most pre-clinical work with perfusion technology is based on porcine grafts, which exhibit minimal vessel variance, i.e., the vast majority of grafts have a single vein and artery. Human kidneys, however, are much more likely to demonstrate variance in this regard, with an incidence of approximately 30% (Pradhay et al. 2021). Multiple renal arteries, present in approximately 20%, are one form of anatomical variance that can pose challenges with connection to the perfusion circuit.

Any tool that facilitates transplantation, such as EVNP, must augment the implantation process, not just the decision to transplant. At the very least, the process of perfusing cannot make the implantation more difficult. How the circuit attaches to the artery is of great importance, as any damage or significant shortening of vessels for eventual anastomosis at implantation would be of concern.

The currently available and widely used arterial devices include vascular cannulas, which are inserted directly into the vessel and secured with a ligature, or a Water's clamp, that connects to the aortic patch preserved at the time of retrieval to provide connection to all vessels which exit the patch within the clamp (see Figure 2-5). The connection to the vessel, with either method, requires a tight seal to prevent leakage/bleeding and inherent in this seal is resultant pressure damage to the vessel. The Water's clamp is often used where multiple renal arteries exit the aorta in close proximity, i.e., within the size of the clamp. If there is a distance (>2cm) between where the arteries exit the aorta, there are limited options for cannulating such grafts.

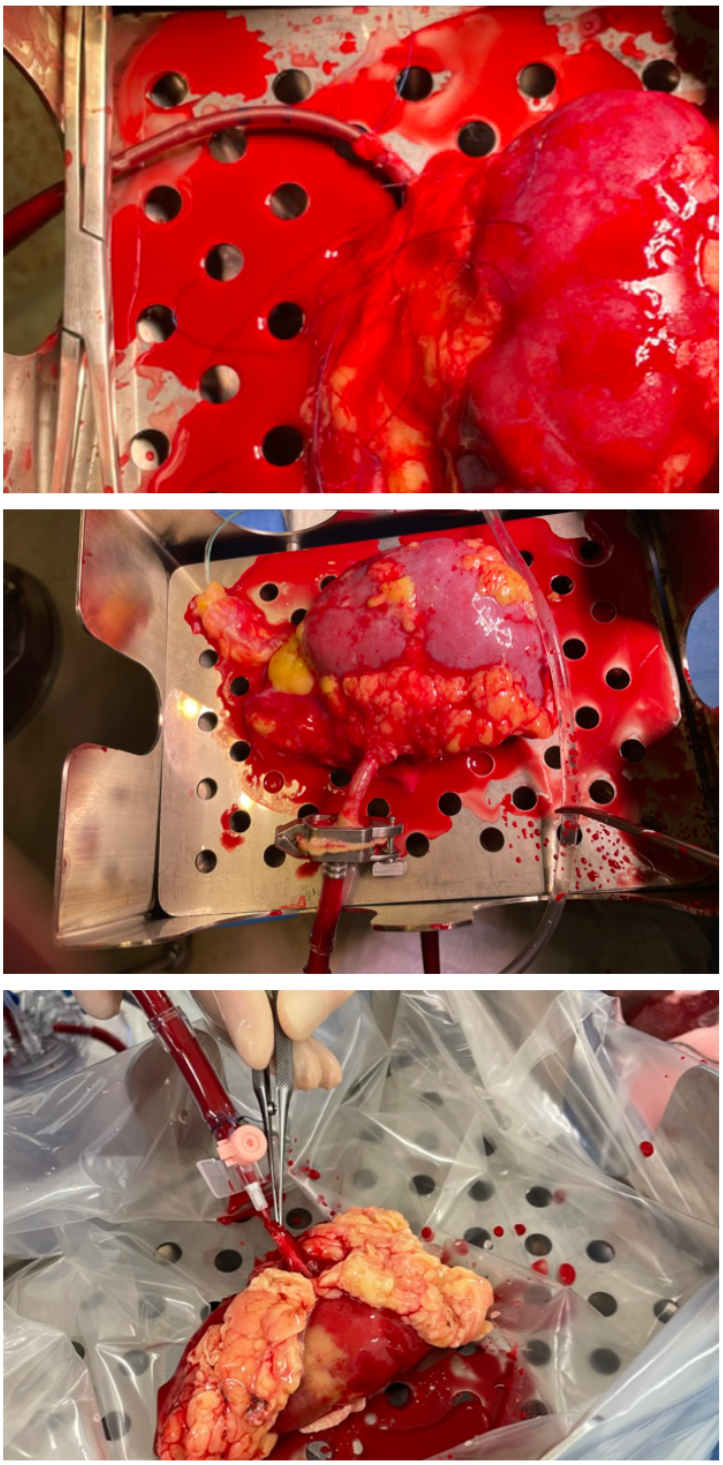


Figure 2-5: Cannulation options for EVNP arterial connection.

(Top) Artery connected via arterial cannula and secured with a ligature; (middle) Water's clamp securing vessel using the aortic patch as pictured; a (bottom) example of a vessel too small to accept venous catheter successfully perfused with 20G Venflon.

The ligature used in the vascular cannula will typically require the artery to be shortened just beyond the ligature for implantation due to the pressure injury caused. Similarly, the aortic patch with the Water's clamp is usually trimmed down to exclude the tissue which has been pressurised by the seal of the clamp. Furthermore, the vascular cannula will only facilitate connection to a single vessel unless a Y-connector is used to split the tubing into two streams to allow for multiple vascular cannulas to be attached (see Figure 2-6). Although pressure is monitored throughout perfusion and aimed to be approximately 75mmHg, it is postulated, according to Poiseuille's law, to be limited by the smallest calibre cannula if two limbs are on the circuit. Our experience in this circumstance is that macroscopic global perfusion is achievable; however, more work is required to explore the effect multiple cannulations have on graft perfusion.

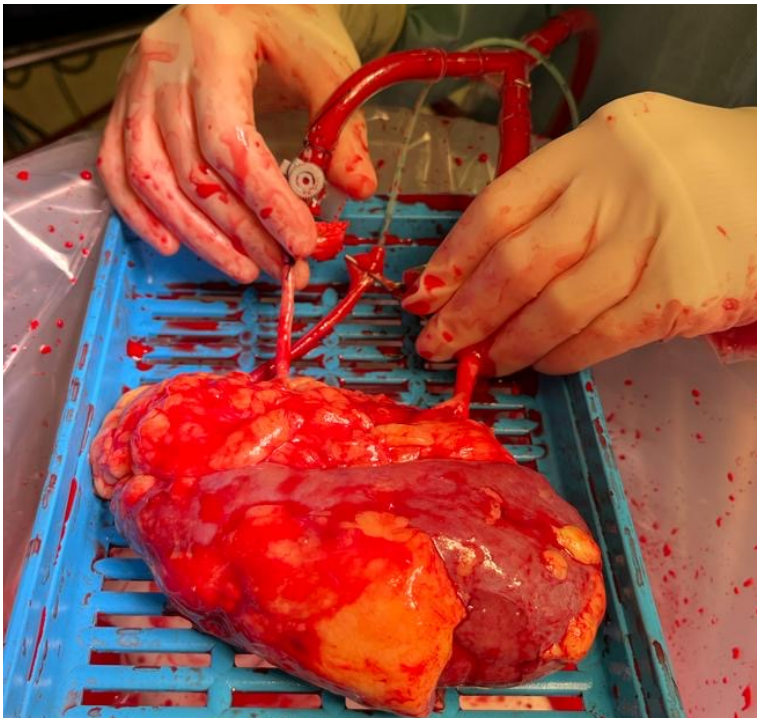


Figure 2-6: A kidney undergoing normothermic perfusion in a case of two renal arteries with separate cannulation with 16G Venflon and vascular cannula. The ureter can be seen cannulated with a nelation catheter in the centre of the image.

The vascular cannulas are available in various sizes, but our experience is that some vessels are too small in calibre to accept the cannula, and therefore, other methods are required, such as using a peripheral cannula (Venflon) commonly used for venous cannulation (see Figure 2-5). Securing this in order to leave the graft to perfuse independently, in our experience, was found to be challenging, and in these circumstances, it was believed that someone with adequate surgical experience, i.e., able to deal with unexpected bleeding, was required at the graft side for the entirety of perfusion.

To widen the use of normothermic perfusion across either retrieval centres or with regional perfusion centres would require consideration of how best to attach kidneys with multiple renal arteries. Staffing of such centres would also require consideration of the requisite expertise to troubleshoot this issue and manage potential complications. Following on from the experience of this work, we believe there to be insufficient options in the case of multiple renal arteries, which are present in approximately a fifth of all renal grafts.

2.2.3 Kidney Tray

2.2.3.1 Initial Design and Production

The collaboration from both Cambridge and Newcastle was instrumental in this project. During set up, information was shared with regards to a design for a 'kidney tray' which housed the graft during the period of perfusion. The tray was sterilisable, and its key property was to collect venous drainage through a fenestrated stage and connect to ¼ inch tubing to allow recirculation of the perfusate. The tray used by the aforementioned research groups was amended slightly to remove a bar which had been designed to hold cannulated vessels in place, which had been advantageous in previous perfusion iterations where the vein was also cannulated. As only the artery was cannulated for this perfusion, thus protecting the vein from shortening or ligature injury, the design was amended by our group, and the bar was removed. A steel manufacturer in Glasgow, Complete Stainless Ltd, manufactured two of the bespoke products at our request (see Figure 2-7).

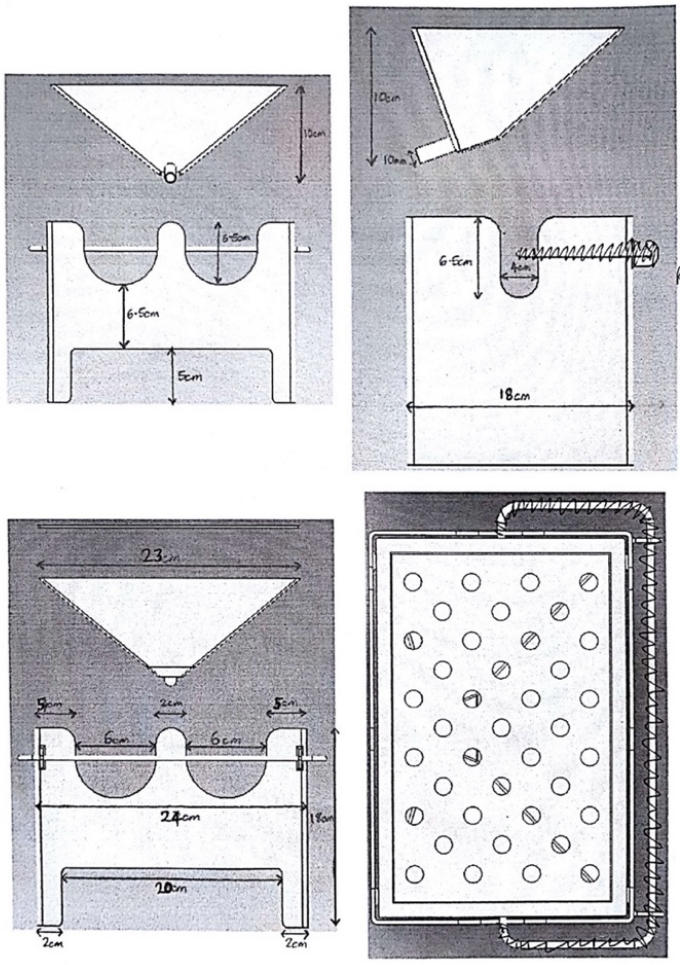


Figure 2-7: Steel Kidney Tray manufactured by Complete Stainless Ltd., amended from previous iterations used by collaborating research groups.

2.2.3.2 Kidney Box Redesign

Limitations of this original device became evident through experience, with particular regard to loss of humidity and temperature with the open design (see Figure 2-8). A collaboration with the University of Glasgow Engineering Department was sought, and the team worked with post-graduate engineers to design a new bespoke housing which could be used alongside this kidney tray to reduce humidity loss.

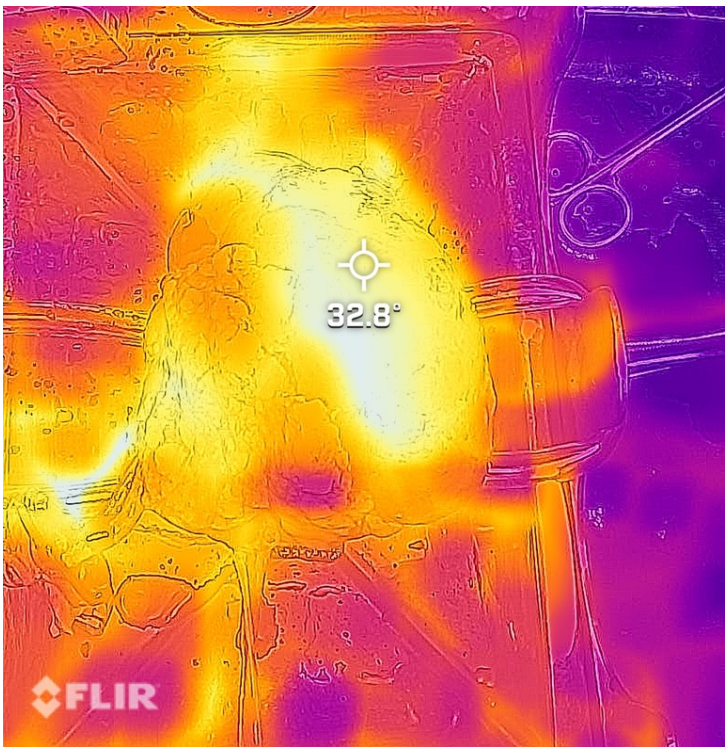


Figure 2-8: Thermal image of a kidney on perfusion in the exposed kidney tray

Image taken with Flir One (Gen 2) smartphone thermal imaging camera. Surface temperature as seen at 32.8°C with heat dissipation evident onto the metallic surface of the tray.

The collaboration aimed to create a graft housing that could be used within our current hardware with the following properties:

- Housing to sit within the current steel kidney tray
- Reduce heat and humidity loss
- Transparent to allow good views of the graft
- Easy access to vessels should adjustment be required or bleeding encountered
- Sterilisable

We were aware of other research devices and augmentations to the circuit, such as surrounding the whole steel tray with plastic housing or the use of a ‘bowel bag’ to reduce humidity loss. Both, however, reduce access to the graft and obscure vision for review of the graft appearance during perfusion. The device went through numerous iterations before a prototype was designed (See Figure 2-9) and built (see Figure 2-10).

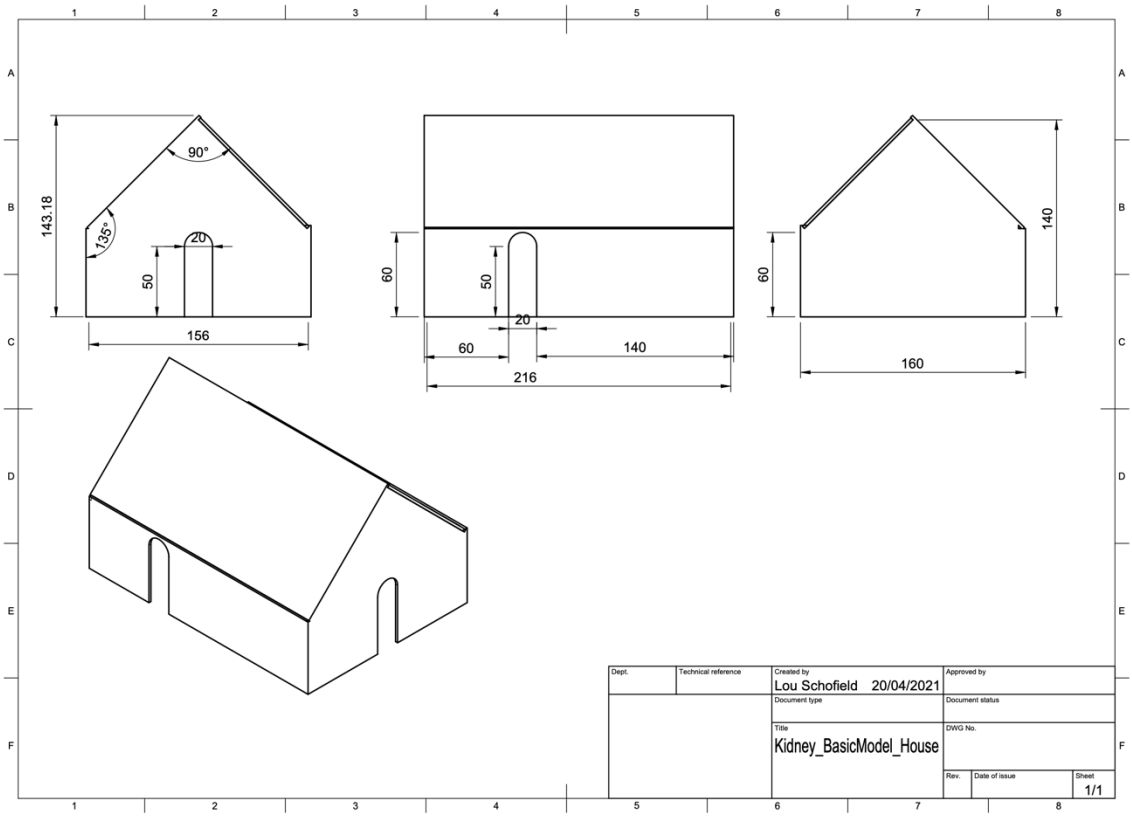


Figure 2-9: Engineering drawings of the proposed ‘kidney house’ made in collaboration with the University of Glasgow Engineering Department.

The prototype was built with 5mm thick clear cast acrylic, which would be sterilisable with the ethylene oxide sterilisation process to avoid the product dismantling with the heat of an autoclave.

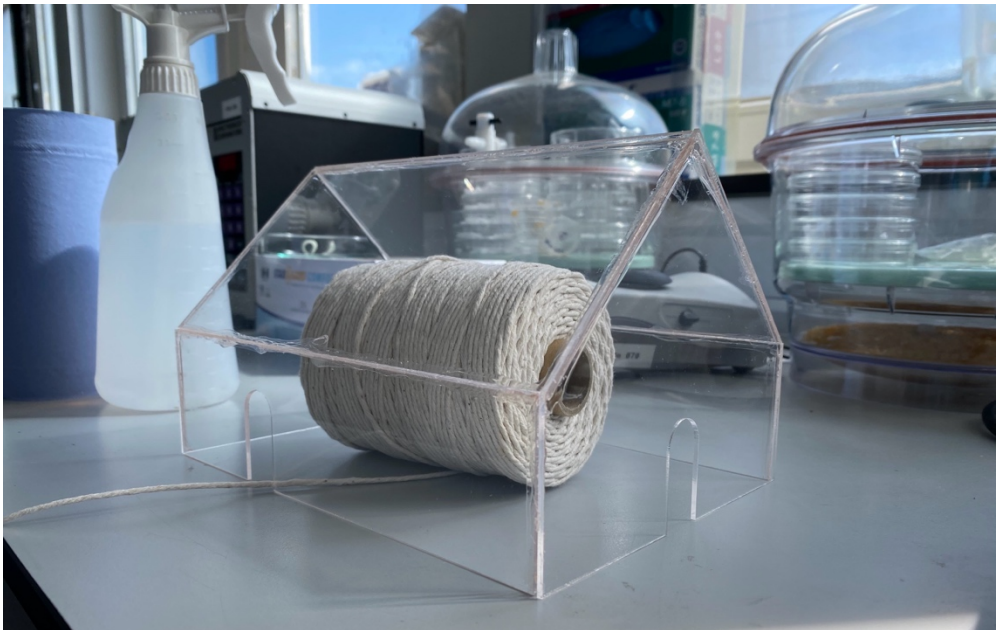


Figure 2-10: Prototype of 'Kidney House' built in collaboration with the University of Glasgow Engineering Department

2.2.4 Medtronic Pack Design, Delays and Urgent Recall

2.2.4.1 Design

The hardware system purchased was the Medtronic BioConsole 560, which had subtle differences from the now-discontinued BioConsole 550 used in Newcastle and Cambridge. This iteration of the device was created following the discontinuation of the prior model; the principal differences included a new pump head and the use of a paediatric flow transducer 'TX50P', both of which necessitated a change to the bespoke tubing packs used in other collaborating research units.

Following consultation with Medtronic representatives, and discussion with collaborators, we made the requisite changes to the pump head ('*AFFINITY PUMP'*) and the flow probe ('*BIO-PROBE'*) to match the paediatric flow transducer. In addition, amendments were made to tubing lengths, and vented taps were switched to non-vented three-way taps, having listened to the troubleshooting and experience from other units. The tubing pack iteration M448036A was created specifically for our unit and ordered (see Appendix I). The lead time for these orders, as they are made individually for each unit, was a minimum of 16 weeks. A challenge in this process was the constraints of our budget and not knowing exactly how many tubing kits would be required, given the unpredictability of the clinical perfusions. The regularity of perfusions and predictability improved with time once research perfusions had begun.

2.2.4.1 Recall

In February 2021, just as we began clinically perfusing kidneys, there was an 'Urgent Field Safety Notice' released by Medtronic to urgently recall all packs containing the Affinity Pixie™ Oxygenator. The Medtronic BioConsole 560 used for this perfusion was originally and is still principally used for paediatric ECMO, and there were laboratory concerns at a factory level that the oxygenators may have had bacterial contamination. The tubing systems, therefore, were deemed unsafe for clinical use and required urgent worldwide recall. The oxygenator is a key component of the tubing system, the main consumable required for each perfusion, both in a research and clinical setting. Each tubing system iteration is individual to our requirements, and as a result, 8 packs had to be returned to Medtronic, and an urgent order for new tubing packs was submitted with a lead time of 16

weeks. This caused significant delays in preparedness for future perfusion opportunities. There was a planned clinical EVNP for a nephrectomised graft shortly after this recall and the Cambridge team kindly offered us a pack, albeit slightly different tubing design, to facilitate this perfusion.

2.2.5 Navigating the Use of a Tertiary Hospital's Theatre Facilities

The Queen Elizabeth University Hospital (QEUH), Greater Glasgow & Clyde, is a large (>1600 bed) acute facility - the 2nd largest in the UK - and performs more emergency laparotomies than any other acute hospital in Scotland (*First National Report of the Emergency Laparoscopic and Laparotomy Scottish Audit | Turas | Learn*, accessed March 2024). In the absence of a dedicated clinical area to deliver EVNP or a separate transplant theatre facility, the acute theatre facilities are shared with all other specialities within the hospital. A retrospective review of the transplant team's use of the theatre facilities over a six-year period demonstrated that the use of the emergency theatre was significant, and of interest, increasingly evenly spread across the 24-hour period, with a growing number of transplants being performed in the early hours (00:00 – 06:00) compared with previous years (see Figure 2-11). Thus, demonstrating a shift from predominantly day and evening implantation, to a more even spread throughout the 24-hour period.

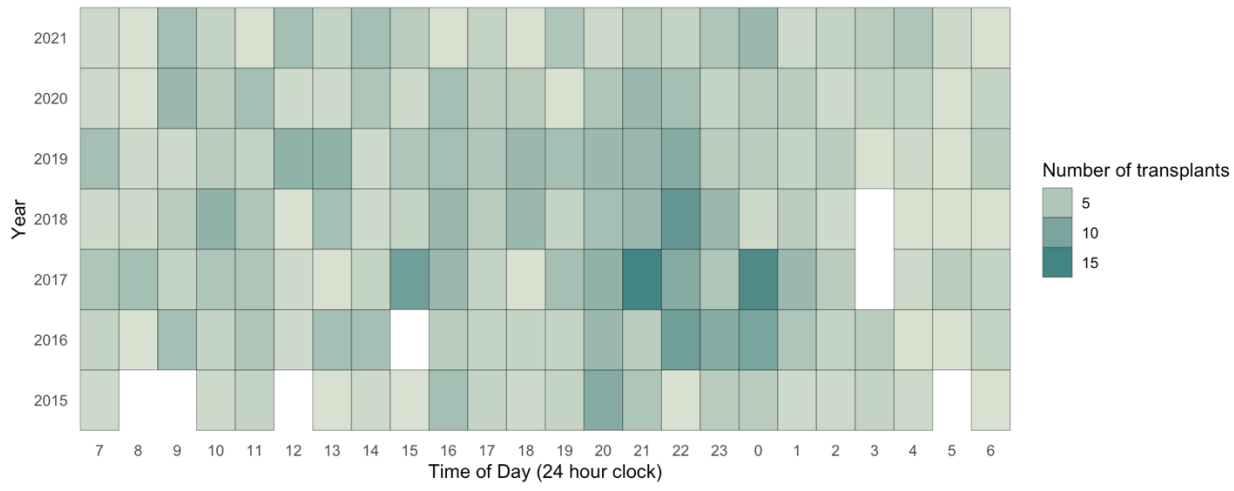


Figure 2-11: Heatmap demonstrating the starting time of renal transplants within our emergency shared emergency theatres during the period April 2015 – April 2021.

The resultant challenge, therefore, is how best to co-ordinate the additional use of theatre space which the delivery of EVNP requires. To address this issue, several meetings were held with senior theatre coordinators explaining the practicalities of the delivery of this technology, including when we would expect this to take place, and how long for. The proposed result of this was, when available, an adjacent empty theatre space would be used to prepare, or 'back-bench', the graft for transplantation, and this would facilitate space for EVNP should it be required. In addition to this, auxiliary staffing would be provided, if available. The delivery of this technology would not have been possible without the goodwill of the theatre staff at the QEUH.

2.2.6 COVID Pandemic

The novel coronavirus SARS-Cov-2 and the resulting 'COVID-19' pandemic caused significant disruptions to healthcare systems across the world. There were specific challenges and dilemmas in the delivery of clinical solid organ transplantation in this environment, and as a result, the research described herein and the work of many other research groups were directly impacted. It was reported at the time that COVID-19 infection carried a mortality of 18-32% in transplant recipients who are immunosuppressed and often co-morbid (Cravedi et al., 2020). However, these apparent risks had to be precariously balanced against the morbidity associated with delaying transplantation and a similarly alarming mortality rate among dialysis patients (Hilbrands et al., 2020). As a result, transplant programs were severely impacted worldwide. In the United Kingdom, renal deceased donor transplantation decreased by 68% in 2020 compared to 2019 in the months of March to May, during the height of the first wave, corresponding to an estimated loss of up to 1,672 transplant opportunities (Manara et al., 2020; Sharma et al., 2020). The corollary of this reduced activity was reduced organ offers, in part due to reduced intensive care capacity to consider donation and, therefore, reduced opportunities for clinical perfusion.

A significant secondary impact was the reduced capacity for a novel technology in this environment. Clinical EVNP was in its infancy in our trust when the pandemic broke, and the surgical service was stretched. EVNP was delivered within the shared emergency theatres and the pandemic posed new issues with regards to theatre turnaround time, infection control and staffing levels, to name a few. In addition to this, with the heightened risk of solid organ transplantation in this challenging environment, the willingness of the clinical transplanting team to utilise a new technology was understandably affected. This

improved with time as the pandemic eased and risks were better understood. Although the transplant unit only closed for transplantation for two weeks, the impact was long-lasting and, in some ways, remains present today. As capacity in the theatre and hospital in general eased, clinical perfusion regained momentum, particularly in a research capacity.

2.2.7 Impact of COVID Pandemic on Renal Trials at a National Level

Clinical and basic science research are essential components of modern healthcare systems, striving to ensure best practices and discover new treatments and technologies. In the UK, the pandemic forced health boards to concentrate resources and staff on the management of COVID-19 and as a result, many hospitals and research facilities suspended and/or modified research activity (Sathian et al., 2020; Tuttle, 2020). The extent and impact of this are poorly understood, and during our research interruption, we aimed to reach out to the renal transplant research community, which strives to advance the treatment of a vulnerable patient group, to investigate the impact the COVID-19 pandemic has had on the ability to conduct research in this important clinical area.

2.2.7.1 Methods

A search of all currently recruiting or suspended trials was undertaken on both the ISRCTN registry and Clinicaltrials.gov website (NCT database) to identify studies within renal transplant with the search including ‘renal’ or ‘kidney’ and/or ‘transplant’ in the United Kingdom as of February 2021, including both clinical and pre-clinical research.

A questionnaire was created using Survey Monkey (www.surveymonkey.com) and publicised directly to the primary investigators as per the NCT / ISRCTN database search and additionally publicised via the social media channel ‘X’, formally known as Twitter (www.twitter.com) where researchers in the field of renal transplantation were invited to take part.

To supplement the quantitative data obtained from the above we invited leading researchers to describe their account of how the pandemic has affected their research. The survey aimed to assess not just the impact on the research but also the redeployment of researchers and difficulties with funding; therefore, the survey could be completed by multiple individuals within the same research group. Respondents were given the option to remain anonymous.

2.2.7.2 Results

The NCT / ISRCTN database search highlighted 40 studies within the UK which were currently either ‘active’ or ‘suspended’ as of February 2021. In cases where email addresses were documented, the primary investigators were contacted via email, in line with GDPR, and invited to complete the survey. The survey was completed by 88 respondents and was open for four weeks. This included 15 clinical academics, 36 clinical fellows, 28 research nurses, 5 trainees, three trial managers, and one full-time NHS consultant. 36 responses were from single-centre studies (40.9%), the remainder multi-centre. The majority of respondents (n=50) were involved in pre-clinical research (57.5%). Although many respondents remained anonymous, three of the prominent UK trials (HOT-2, PITHIA and EVNP Cambridge) returned surveys.

Responses were found to be from varying time points within the research process, with 20 in the ‘Design/Set-up’ phase, 43 currently recruiting, 23 in the follow-up phase, and two studies undergoing data analysis. The vast majority of researchers were redeployed to other areas during the pandemic, with as few as 11 (12.7%) stating they were not redeployed. Of those that were (n=76), the amount of time away from research differed: less than one month (n=28, 37%), less than three months (n=30, 40%), less than six months (n=14, 18.6%) and over six months (n=4, 5.3%) (see Figure 2-12).

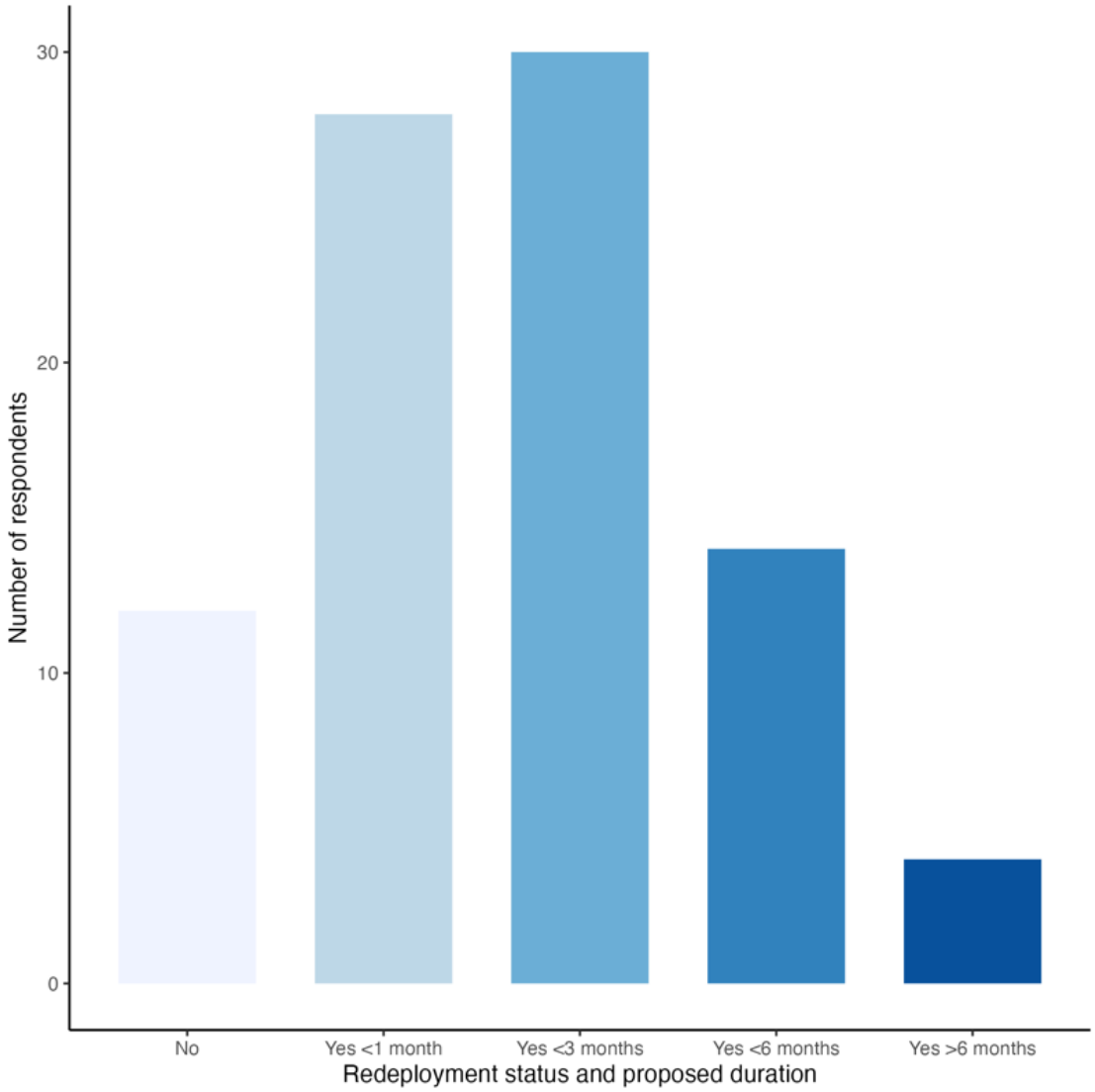


Figure 2-12: Bar chart demonstrating the respondents' redeployment status back to clinic duty during the COVID pandemic.

Eight (9.1%) of studies stated the research was able to continue through the first wave of the pandemic. 21 (23.9%) stated the study was suspended entirely, 37 (42.1%) stated that recruitment was suspended and 22 (25%) stated the study was able to continue 'somewhat, however, adaptations were required.' For those affected, the most common barrier to research continuing was declared to be hospital-wide discontinuation of prospective research (51, 58.0%). Other barriers included personal or staff redeployment (n=34, 38.6%), study ongoing but difficulties in recruitment / obtaining samples (n=25, 28.4%), staffing shortages (n=12, 14.1%), and trial-specific issues (n=5, 5.6%).

With regards to the 'second wave' of the pandemic in the UK relating to November 2020 to January 2021 inclusive, the majority (52, 59.1%) reported that their study had restarted; however, 24 respondents (27.3%) stated their study remained suspended or had stopped.

10 respondents (11.5%) reported no financial implications to their study, 31 (35.6%) stated they had submitted extension requests to funding bodies, and 35 (40.2%) stated they had applied for tuition fee extension (PhD/MD/MPhil, etc.). 11 stated their study was non-funded.

When asked whether they felt confident that the study would be completed, 26 (29.9%) stated they felt they would complete it, 34 (39.1%) stated they felt likely to complete it with an extended deadline, and 22 (25.3%) stated they were likely to complete but with lower recruitment and/or major adaptation. 5 (5.8%) stated they felt unlikely to complete their study. Regarding overall confidence, respondents were asked to rate their overall confidence on a scale of 0 to 100, with 100 being most confident, that their study would complete (see Figure 2-13).

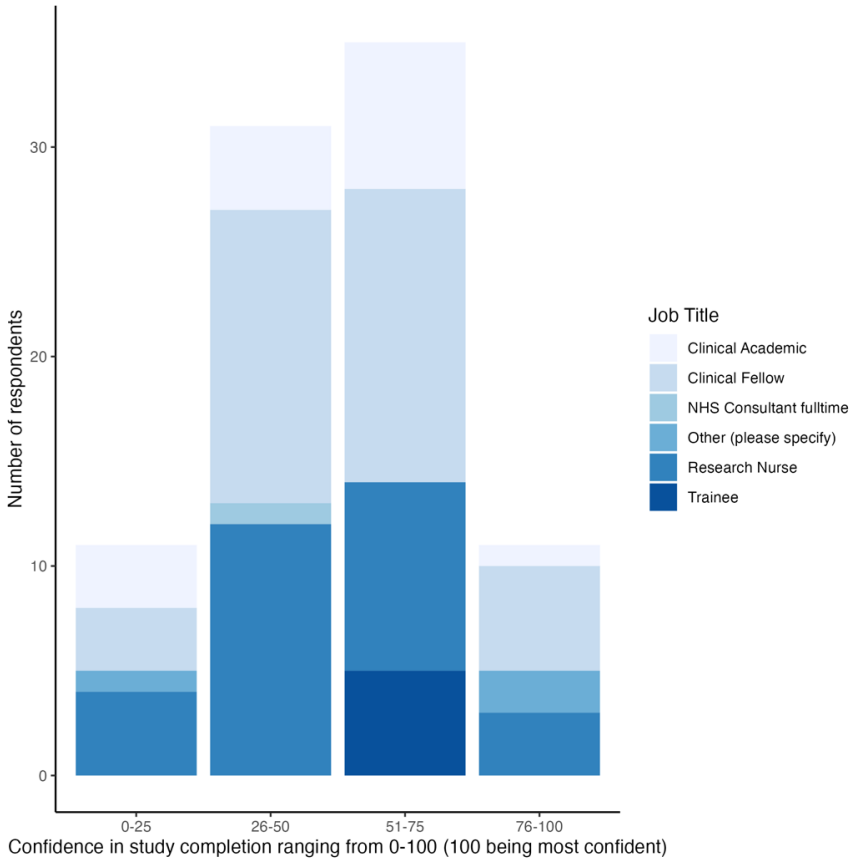


Figure 2-13: Confidence levels of responders on whether they felt they would complete their study. 0-100, with 100 being the most confident.

Allowing for free text space for researchers to comment on additional key impacts to their research highlighted issues with study design, i.e., stepped-wedge cluster randomised trials, which are not designed to be stopped mid-way through recruitment and concern that the "full impact of this on data analysis is not yet known." In addition, trials involving peri-operative care describe "huge disruption", and one study group described the difficulty in finding additional study centres as many sites "temporarily not opening non-COVID trials."

The final survey question asked respondents to indicate their confidence in the completion of their study ranging from 0 'not at all confident' to 100 'very confident.' The mean response was 54 (range 0-100). Split into quartiles, 11 (12%) provided the subjective value of 0-25, 31 (35%) 26-50, 35 (40%) 51-75 and 11 (12%) 76-100. There was no apparent statistical difference in this confidence metric for job title ($p=0.4$), redeployment status ($p>0.9$) or research type ($p>0.9$).

2.2.7.3 Discussion

The COVID-19 pandemic resulted in wide-ranging impacts on healthcare systems across the world. Although the delivery of healthcare during this challenging period is of paramount importance, there has undoubtedly been a substantial impact on the completion of clinical and pre-clinical research. Although far from exhaustive, the data presented here provides a snapshot of some of the issues with funding, redeployment and confidence with trial completion.

Chen et al. (2021) conducted interviews with clinical researchers which highlighted that the pandemic has led to deviations from study protocols, higher loss to follow-up and additional workload required for COVID-19 screening (Z. Chen et al., 2021). Furthermore, the work discussed that although slowly improving, the additional workload required, alongside the anxiety of patient subjects, may take considerable time to return to pre-pandemic levels. Participant attrition was also identified as a factor which has the potential to threaten trial completion (Shiely et al., 2021).

Although many non-COVID trials have been impacted or suspended, there have been many success stories with the rapid implementation and streamlining of COVID-19 studies evident in the production of effective vaccines (van Dorn, 2020). Herein we report that despite

significant rates of redeployment and funding issues to overcome, nearly 95% of respondents reported that they were likely to complete their work, albeit with an extended deadline, reduced recruitment or major adaptation.

2.3 Conclusions

Herein, we describe the process and logistical challenges in the introduction of a new technology to a busy tertiary acute hospital. Although there is growing evidence for the role of normothermic perfusion techniques in transplantation, these tools remain in their relative infancy and local support at a trust level was critical in the introduction of EVNP in this setting.

Improving organ utilisation was an objective at a departmental level, which reflected national strategies such as the NHSBT release entitled 'Transplantation 2030: Meeting the Need'. This led to a submission and successful business case for a viability assessment tool, which provided some base funding to purchase the hardware. Education and training then followed, provided by supportive collaborating groups in Cambridge and Newcastle. 10 declined kidneys were kindly provided by RINTAG to establish further experience with the complexities of the technology before clinical introduction.

Notable challenges included overcoming the unpredictability of how many perfusions would take place. As momentum grew, more opportunities for perfusions were considered and, therefore, performed. Yet, the pandemic fell shortly after the introduction of this work, which significantly halted progress and led to difficulties in the re-introduction of EVNP. This unpredictability and outside influence, which significantly impacted clinical transplantation, created challenges in ordering consumables. Many of the consumables had expiry dates, and the Medtronic tubing packs specifically had significant lead times of up to 16 weeks for delivery. Delivering this technology locally at a recipient centre, in our opinion, is likely to have challenges in this regard, as opposed to how a regional perfusion centre may be able to better predict and provide a service.

Blood products were kindly donated by our local haematology team. This worked well for both research and clinical perfusions, and the work to formalise standard operating procedures in the use of this resource was critical. There were challenges, however, as there were occasions where we placed significant demand on this resource and then periods of time where no resource was required for long periods. There is increasing evidence that longer periods of perfusion, as discussed in later chapters in this thesis, are likely to benefit from non-blood-based perfusates. If the assessment period of 1 hour was to be demonstrated to be analogous with non-blood-based products, such as Steen or artificial oxygen carriers, this would circumvent a significant obstacle in the clinical delivery of EVNP. More work is required to demonstrate equivalence.

As described above, our experience with this technology highlighted the issues with variant arterial anatomy, which, as stated, is present in up to 20% of grafts (Pradhay et al., 2021). Although it is possible to cannulate such grafts with the currently available connection types (e.g., Water's clamp, arterial cannula), in our experience, arteries which were small in calibre or in cases where there was distance between the multiple arteries provided difficulties. Although most issues were successfully overcome, e.g., using venflons and splitting the tubing with Y-connectors, more work is required to assess how workarounds such as these affect perfusion pressure and flow rates through the graft. Furthermore, in some cases, surgical reconstruction of the vessel was required to establish a secure connection with the arterial circuit.

In contrast to the above statement, where the positives of regional perfusion centres were considered, complex arterial anatomy, and particularly the need for arterial reconstruction, would require expert surgical staffing of such units. The surgical expertise of staffing at regional perfusion centres is an important consideration if such facilities are considered. Furthermore, the staffing would require a considerable amount of out-of-hours working and would carry significant expense.

CHAPTER 3: MARGINAL KIDNEYS AND MARGINAL RECIPIENTS – A HIGH-RISK COMBINATION

This chapter is based on a publication in Experimental and Clinical Transplantation in 2021 (Pearson, Murray, et al., 2021). Permission to include this work was granted by the editorial team.

3.1 Introduction

With over 5000 patients on the United Kingdom waiting list for a transplant, there remains a significant shortfall between the current availability of kidneys and those in need of a transplant (*Statistics about Organ Donation - NHS Organ Donation Register | Organ Donation - English*, accessed March 2024). Recent strategies attempting to address this have focused on expanding the use of extended-criteria donors, all whilst maintaining efforts to increase awareness and availability of the living-donor sharing scheme. There have been positive results, with a 21% decrease in waiting list numbers in ten years, albeit with a recent uptick. However, this achievement was enabled by a 115% increase in DCD use and the use of 'higher risk kidneys' which have increased by 119% over the same time period (Gridelli & Remuzzi, 2000; Rao et al., 2009).

Furthermore, the prevalence of diabetes as a cause of kidney failure continues to rise, and there is an increasing number of older adults with co-morbid conditions requiring dialysis and/or kidney transplantation as a treatment for ERF (Andre et al., 2014; Levitt, 2015; D. A. Wu et al., 2019). Another critical priority is maximising the functioning lifespan of a kidney graft, which is achievable through better matching of patient and graft life expectancy. The previous UK national kidney allocation scheme, in place since 2006, successfully reduced the waiting times for those most difficult to match and improved equity for Black, Asian, and minority ethnic (BAME) patients but conversely has seen an increase in median waiting time, as well as an increase in the number of discarded grafts (Johnson et al., 2014).

In March 2018, NHS Blood and Transplant published a proposed kidney matching scheme implemented in September 2019 with the following principal aims: to better match patient and graft expected survival, improve waiting times for difficult-to-match patients, and decrease offer decline rates. Furthermore, DBD and DCD kidneys are combined into a single

allocation scheme. There remains a tiered system where the most difficult to match or those with a waiting list duration over seven years are considered ‘Tier A’, with the rest in ‘Tier B’ subject to the revised scoring system. The scheme utilises new donor and recipient risk indices which rank donors from one to four (D1-D4) and recipients (R1-R4), with the aim of matching recipient and donor, i.e., increasing the proportion of R4 recipients receiving a D4 graft, for example. Furthermore, targets for age difference between donor and recipient were proposed, with fewer than 8% receiving a kidney of >25 years age difference, and 20% for 15-25 year difference (*Kidney Offering Scheme*, accessed March 2024).

Whilst accepting that five-year patient and graft survival may be impacted by the proposed scheme, the aim of better matching patient and graft survival is deemed necessary with the theoretically reduced rate of re-transplantation as a corollary. There are unanswered questions, however, as to how this new scheme will impact already overstretched clinical transplantation units. Furthermore, the revised allocation scheme was formulated using modelling data from a historical cohort of transplants performed during a period with a degree of centre-based recipient selection (i.e., choosing a suitable recipient for the offered organ) and, therefore, may not appropriately account for this clinician discretion and uncaptured bias. Matching the most vulnerable patient group with the poorest quality donor grafts may pose particular challenges with prolonged admissions, higher rates of delayed graft function and higher need for re-intervention in the early post-transplant period. This work aims to evaluate a retrospective cohort, re-stratified as per the proposed risk index, to address this question.

3.2 Methods

A retrospective analysis was performed on prospectively acquired data on all deceased donor transplants between January 2015 and December 2016 at Queen Elizabeth University Hospital, Glasgow – a unit that provides transplantation services to a population of approximately 2.5 million. Living donation kidneys were excluded from the analysis. Both recipients and donors were re-classified using the new quartiles (i.e., D1-4; R1-4) (*Kidney Donor Profile Index (KDPI) Guide for Clinicians - OPTN*, accessed March 2024). The donor risk index took into account donor age, height, length of hospital stay, CMV status, eGFR, female sex and past medical history of hypertension (see Table 3-1 and Figure 3-1). Recipients were stratified as per age, diabetes, and dialysis status and duration. All factors were differentially weighted as per the computed algorithm. Due to the retrospective nature of this study, the institutional review board did not require research ethics approval and was completed in accordance with the 'Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)' guidelines.

Table 3-1:Patient Factors Used to Stratify Donor and Recipient Risk Indices as Per Validation Dataset by NHS Blood and Transplant. (*Kidney Donor Profile Index (KDPI) Guide for Clinicians - OPTN*, accessed March 2024).

Donor Factor	Hazard Ratio	p Value	Recipient Factor	Hazard ratio	p Value
Age	1.02	<0.0001	Age (<=25)	1	0.9
Height	0.86	0.0005	Age (>25)	1.02	<0.001
Hospital Stay	1.02	0.006	Dialysis Status	1.43	<0.001
CMV	1.2	0.02	Diabetic	1.32	0.003
eGFR	0.98	0.02	Time on dialysis (years)	1.03	0.004
Female	0.83	0.04			
Hypertension	1.15	0.1			

Donor-recipient risk index combinations

A donor risk score (DRI) is calculated for each donor on offer using 7 risk factors. A donor is then categorised in to one of 4 groups based on the risk score and by pre-determined cut-off values. D1 (lowest risk), D2, D3 and D4 (highest risk).

DRI = exp { 0.023 x (donor age-50) +
-0.152 x ([donor height-170]/10) +
0.149 x (history of hypertension) +
-0.184 x (female donor) +
0.190 x (CMV +ve donor) +
-0.023 x ([offer eGFR-90]/10) +
0.015 x (days in hospital) }

D1	≤ 0.79
D2	0.79 – 1.12
D3	1.12 – 1.50
D4	≥1.50

A recipient risk score (RRI) is calculated, for each eligible patient using 4 risk factors. A recipient is then categorised in to one of 4 groups based on the risk score and by pre-determined cut-off values. R1 (lowest risk), R2, R3 and R4 (highest risk).

RRI = exp { 0 x (recipient age≤25)-50) +
0.016 x ((recipient age>25)-50) +
0.361 x (recipient on dialysis at registration) +
0.033 x ([waiting time from dialysis-950]/365.25) +
0.252 x (Diabetic recipient) }

R1	≤ 0.74
R2	0.74 - 0.94
R3	0.94 – 1.20
R4	≥1.20

Figure 3-1: The NHS Blood and Transplant provided formula for stratifying donors and recipients (Kidney Offering Scheme, accessed March 2024).

The following patient outcomes were collected: length of stay, readmission, frequency of radiological investigation (e.g., computed tomography and transplant ultrasound scan), and patient and graft survival. Inpatient days were calculated as the index admission length, in addition to any readmission days within the two-year follow-up period. In order to accommodate censored data (i.e., adjusted for censoring events of death or graft loss), we calculated hospitalisation as days per 1000 patient days (1000/pd), providing data on inpatient bed use that reflects only time with functioning transplant.

The use of day-case facilities was defined as a pre-arranged outpatient attendance to a facility adjacent to our transplant ward, which did not require an overnight stay in the hospital, such as for routine ureteric stent removal; these were not included in inpatient day analyses. Data was obtained from the prospectively maintained Strathclyde Electronic Renal Patient Record (SERPR, Vitalpulse, UK).

The cost analysis was performed using The National Schedule of Reference Costs, 2016-17 - NHS trusts and NHS foundation trusts (*Archived Reference Costs | NHS Improvement*, accessed March 2024). Individual healthcare item costs as per the National Schedule of Reference costs and values stated are the average unit cost to the NHS of providing the secondary healthcare item to patients.

The definition of DGF was the need for dialysis within seven days post-transplant, excluding a single session of dialysis within the first 24 hours for hyperkalaemia. The total follow-up period is from the date of the first transplant in the series (January 2015) up to and including December 2019; admission days are only reported for the first two years post-transplant. The current and active waiting list for the West of Scotland was also accessed via local transplant coordinators and re-stratified as per the aforementioned recipient risk indices.

Statistical analysis was performed using R version 4.1 with *survival, KMsurv, car* and *dplyr* and *ggplot2* packages (Therneau et al., 2023). Difference between groups was assessed with Pearson's Chi squared test. Comparison between donor and recipient types for non-parametric data was assessed with Kruskal-Wallis with Dunn's post-hoc test. Graft function was analysed using Tukey's HSD (honestly significant difference) test. The log-rank test was used for patient and graft survival analysis. Cost differences were assessed using the Wilcoxon rank sum test with continuity correction.

3.3 Results

There were 195 deceased donor transplants performed during this period, 82 (42.1%) were DCD and 113 (57.9%) were DBD. Male to female ratio was 1.1:1. Mean donor and recipient age was 51 and 51.7 years, respectively. Median waiting time from dialysis to transplantation was 681 days. Mean recipient body mass index was 27.1 (m^2/kg). Two percent had an age difference between donor and recipient of >25 years, and 12.8% of 15-25 years, well within the NHSBT proposed figures of 7.8% and 20.4% respectively. Regarding inpatient bed days, median index admission was 8 days including all transplants, and total inpatient days was 15 days (median, mean 26.8 days, range 5 – 158 days) over a 2-year follow up period (see Table 3-2).

Table 3-2: Recipient Demographics

Recipient Type		R1	R2	R3	R4
Age at Transplant	(mean, years)	33.4	35.2	42.2	56.1
	SD	2.4	11.5	12.1	10.2
Waiting time on dialysis	(median, days)	0, 0-560	62	425	1068
Prior Transplant	(n, %)	2 (40%)	2 (14.3%)	7 (21.8%)	21 (14.6%)
Proportion receiving D4 graft	(n, %)	1 (20%)	2 (14.2%)	8 (25%)	55 (38.1%)
Proportion receiving DCD	(n, %)	5 (0%)	6 (42.8%)	10 (31.2%)	66 (45.8%)
Recipient diabetes	(n, %)	0	0	1 (3.1%)	15 (10.4%)
Index Admission Length (days)	Mean	7.8	8	6.5	9
	Median (range)	7 (6-14)	8 (5-139)	6.5 (6-64)	9 (5-158)
	IQR	3	18.5	11.25	29.25
Total	(n)	5	14	32	144

Abbreviations: **DCD**: Donor of circulatory Death; **IQR**: Interquartile Range; **R**: Recipient risk indices; **SD**: Standard deviation.

3.3.1 Recipient and Donor Risk Combination in this Cohort

Using the revised allocation scheme donor risk index, 39 of 195 (20.0%) would have been considered D1, 54 D2 (27.6%), 36 D3 (18.5%) and 66 D4 (33.8%). The DBD:DCD ratio for each donor category was as follows: D1 2.25:1, D2 1.35:1, D3 1.57:1 and D4 1:1. The proportion, therefore, of DCD was higher in the D4 cohort (50% vs 38.0% in D1-D3), although this was not statistically significant, Chi Squared = 3.9, p = 0.11. By contrast, in this cohort, the recipient risk index was unevenly distributed, with 5 of 195 (2.6%) R1, 14 (7.2%) R2, 32 (16.4%) R3, and 144 (73.8%) R4, see Figure 3-2. In this cohort, R4 recipients received a relatively equal allocation of donor risk categories, with 24 of 144 (16.6%) receiving a D1 graft, 39 (27.1%) D2, 26 (18.1%) D3 and 55 (38.1%) a D4. The 'R4/D4' combination, therefore, made up 28.2% of all transplants, n = 55.

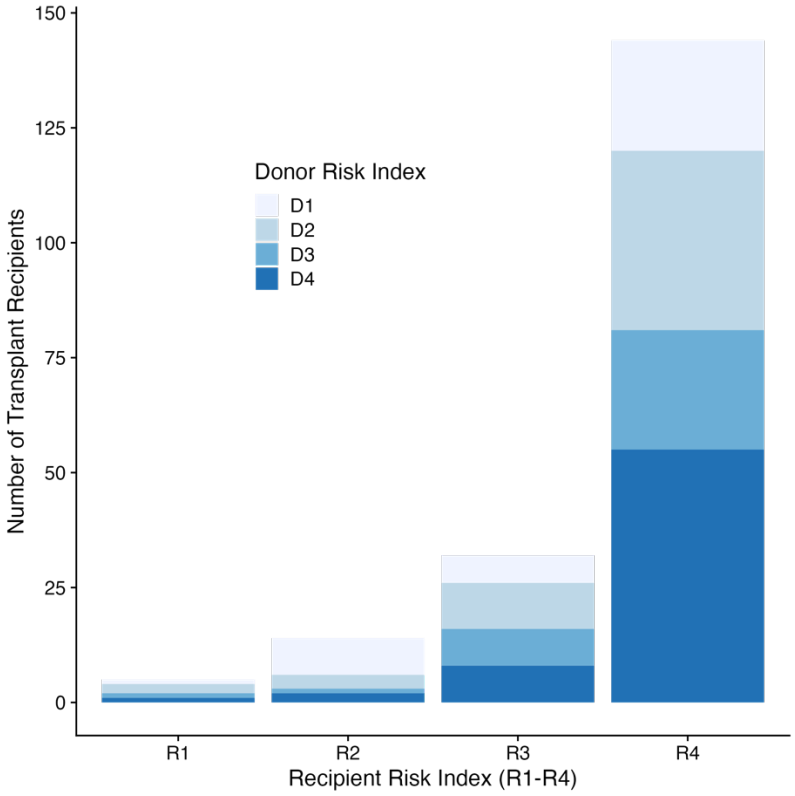


Figure 3-2: Proportion of Deceased Transplants in Recipient Rick Index Categories and Donor Types Received

3.3.2 Graft and Patient Survival

Recipient survival was 100% for R1, R2, and R3 groups at four years. One-year survival in the R4 cohort was 97.2% (four deaths) and 93.8% (9 deaths) at four years. Analysing the R4 cohort alone, there was no difference in patient survival dependent on donor category: 4-year survival was 95.8%, 94.9%, 96.2% and 90.9% for D1, D2, D3 and D4, respectively (see Figure 3.3).

Graft survival in the D4 cohort at 1 year was 87.9%, compared to 97.4% for D1, and D2 and D3 were both 94.4%; differences were not statistically significant (log-rank test, $p = 0.2$). Overall graft survival at 4 years was 88% (all donor types). Assessing the R4 cohort alone, there was no difference in graft survival between donor risk categories, 83.6% at 4 years with D4 graft (87.5%, 87.2%, 88.5%, for D1, D2 and D3, respectively) (see Figure 3-3).

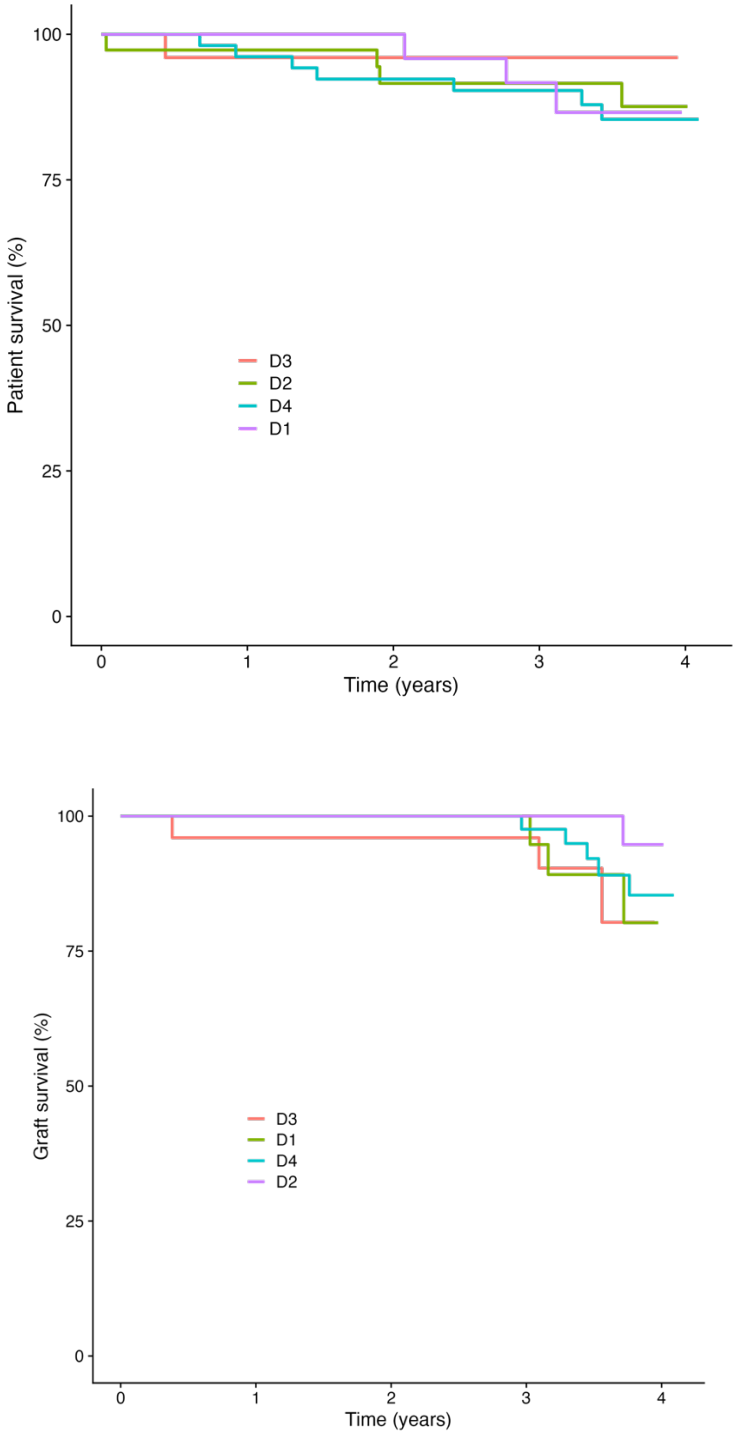


Figure 3-3: Kaplan-Meier Curves for Patient (top) and Graft (bottom) Survival in the Recipient R4 Cohort

3.3.3 Delayed Graft Function

The overall incidence of DGF was 26.8% for all transplants. The rate of DGF was highest in the D4 group at 39.1%, compared to 21.1% in D1-3 grafts, p = 0.014. Within the R4 cohort, the overall DGF rate was 33.0%; a combination of R4/D4 demonstrated a rate of 44.4%, compared to 26.4% with D1-3 grafts in R4 recipients, p = 0.003. The mean duration of DGF was 4.0 days in R4/D4 group with a maximum of 28 days, compared to 1.9 days for D1, 2.4 days for D2 and 1.5 days for D3 in R4 recipients; there was no statistical difference evident between individual groups. The incidence of DGF demonstrated a relationship with kidney function at 1 year: among R4 recipients, median eGFR at one year was 28.5 ml/min/1.73m² in recipients with DGF, compared to 52.9 ml/min/1.73m² in patients without.

The effect of donor type (i.e., DCD versus DBD) was assessed. D1-3 DBD grafts had a DGF rate of 17.5% (14 of 80) compared to 25.8% (8 of 31) in the D4 DBD graft group; this difference was not statistically significant, p=0.47. D1-3 DCD grafts had a DGF rate of 27% (14 of 48) compared to 51.5% (17 of 33) in the D4 DCD graft group; this difference was not statistically significant, p=0.072.

3.3.4 Short- and Medium-Term Function

Function at seven days was lower with a D4 graft with a median eGFR of 11.85 mL/min/1.73m² (mean 21.1), compared to 36.9, 28.2 and 30.9, with D1, D2 and D3 grafts, respectively. Observed differences only reached statistical significance with D4 vs D1 ($p<0.001$). The linear pattern of inferior function with D1 to D4 grafts remained at 14 days, with mean eGFR ranging from 51.3 (D1) to 31.0 (D4). Excretory renal function at one year was worse if in receipt of a D4 graft; median eGFR 36.5, in contrast to 72.7, 53.7 and 55.0 for D1, D2 and D3, respectively (all units in mL/min/1.73m²; $p < 0.05$ between all groups) (see Figure 3-4).

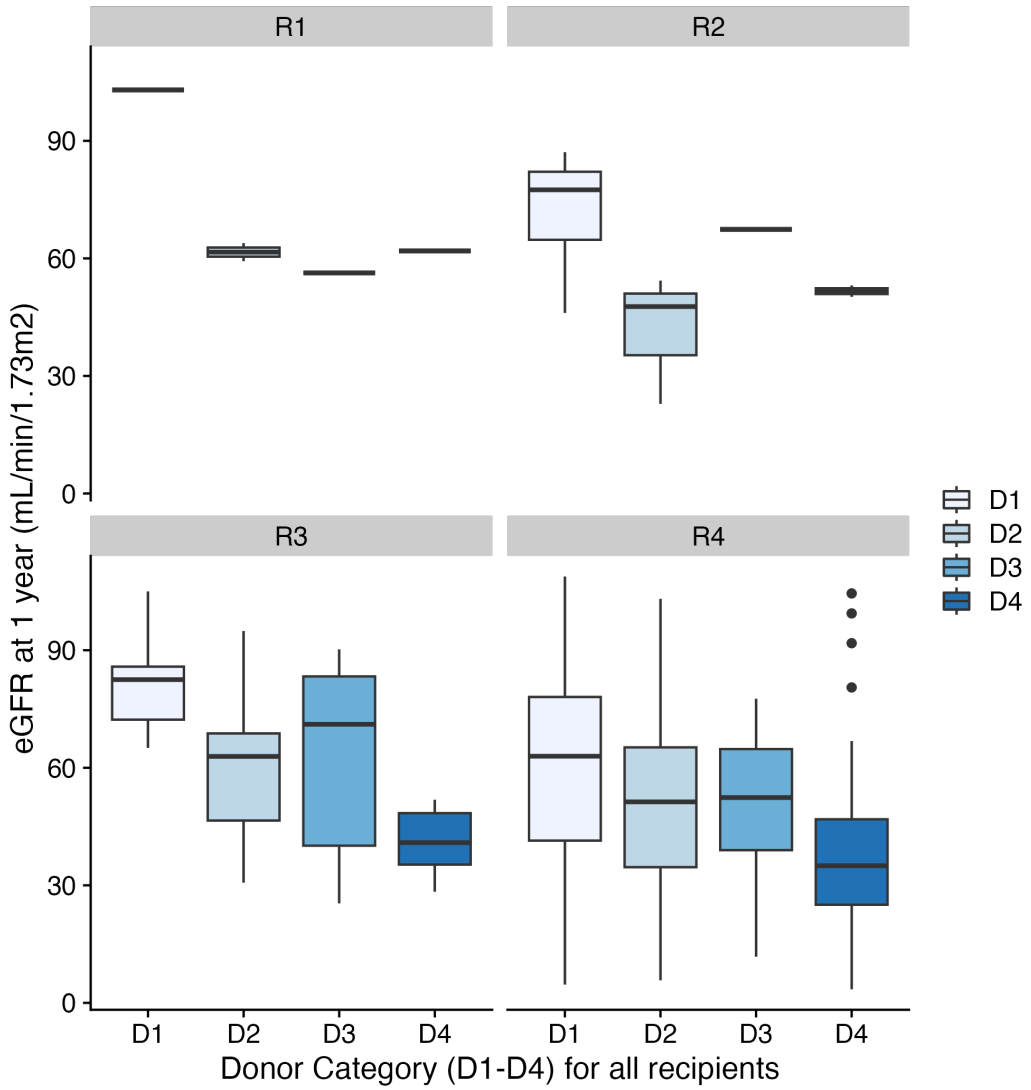


Figure 3-4: One-Year Function for all Transplant for Each Recipient Category.

Abbreviations: D: Donor**; eGFR:** estimated glomerular filtration rate**; R:** Recipient. *Excretory renal function at one year was worse if in receipt of a D4 compared to D1, D2 or D3; $p < 0.05$ between all groups).*

Within the R4 cohort, function at all time points was lower with a D4 graft compared to all other donor risk categories. Median function with an R4/D4 combination at 7 days was 10.6 (mean, 17.5), improving to 22.3 at 14 days, 35.0 at 1 year and 36.7 at 2 years. A D4 graft achieved a higher median eGFR if given to an R1-3 recipient (48.4) compared to an R4 recipient (35.0), all units stated in mL/min/1.73m², see Figure 3-5.

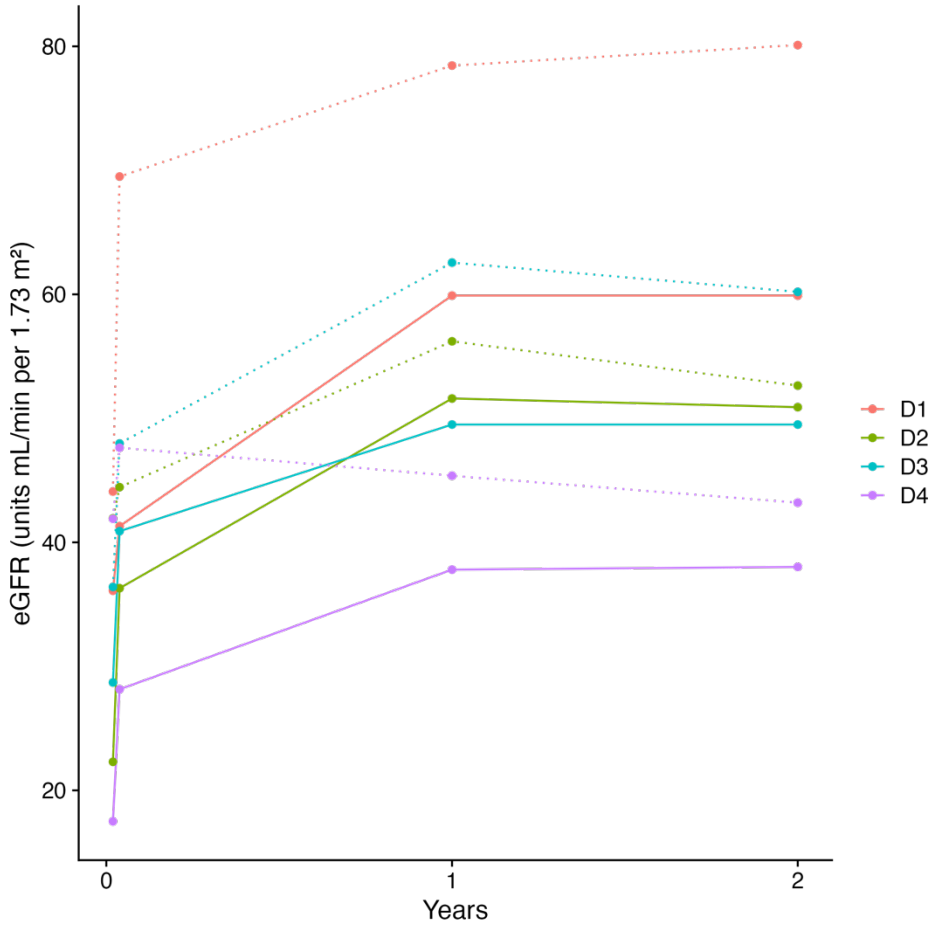


Figure 3-5: Estimated Glomerular Filtration Rate for Each Donor Risk Category Over Time.

Abbreviations: D: Donor**; eGFR:** estimated glomerular filtration rate. Dashed lines represent grafts transplanted to R1-R3 recipients.

3.3.5 Index Admission Length

The mean index admission length for all transplants was 11.3 days (median 8, range 5 - 158). For R4 recipients with all donor types, the mean index admission length was longer (12.4 vs 8.1 for R1-3, p=0.002). Within the R4 group, index admission was the longest with a D4 graft (mean 13.5, median 11 days). For D1, D2 and D3 median index admission length was 8 days (mean values 11.9, 12.6 and 10.2 days, respectively); D4 vs D3 (p=0.038) D4 vs D1 (p=0.047); D4 vs D2 non-significant (p=0.07), Furthermore, for R1-3 recipient receiving a D4 graft had a mean index admission length of 7.9 days (median 7.0) vs 13.5 days with R4 (p=0.001).

Assessing DBD transplants alone in all recipient types, median index admission was longer with a D4 graft vs D1-3 (median 9 vs. 7 days, mean 9.6 vs. 11.2 days, p=0.014). In the R4 DBD cohort, this difference was also present, with a median index admission of 7 days for D1-3 compared to 10 days for the D4 graft (mean 10.8 vs. 12.4 days, p=0.014).

3.3.6 Total Inpatient Days and Day Case Use

Considering that poorer quality kidneys (D4) and higher risk recipients (R4) may be independently or mutually associated with rates of hospital admission, both factors were analysed. At 90 days, recipient types R1-3 (including all donor categories, censored for graft loss/death) accrued a median of 8 inpatient days, compared to a median of 10.3 for R4 (mean 11.1 vs 14.1, P= 0.013, Mann-Whitney test). The donor categories, D1-3 (including all recipient categories), had a median of 9.2 days vs. 11.2 for D4 (mean 13.5 vs. 16.9, P=0.005). By two years, only the donor category retained statistical significance as a discriminator in regards to inpatient days: R4 recipients (of all donor types) demonstrated a median of 14.7 days compared to 12.0 for R1-3 recipients (mean 22.2 vs. 19.9, P= 0.185, Mann-Whitney test); D4 accruing a median 16.5 days vs. D1-3 12.6 days (mean 24.1 vs. 20.3, P=0.022). Within the R4 group, the R4/D4 demonstrated additional inpatient days compared to R4 recipients receiving D1-3 grafts in the 90-day post-operative period (median 13.6 vs. 8.9 days, P=0.006 Mann-Whitney).

During the early postoperative period (first 90 days), the rate of hospital admission days per 1000 patient days for R1-3 was 122.9, vs 185.7 for R4 (P=0.0001 Chi^2); D1-3 was 154.1, vs D4 196.7 (P= 0.014). By two years follow-up, rates were comparable between categories; R1-3 27.4 and R4 38.7/1000 patient days (P=0.198). At both 90 days and at two years, the

R4D4 combination had approximately twice the rate of hospital inpatient days than R-1-3 recipients (223.8 R4D4 vs. 122.9 R1-3 at 90 days, Chi ^{2} P<0.0001; 47.1 vs. 27.4 at 2 years, P=0.027, all data per 1000 patient days), see Figure 3-6.

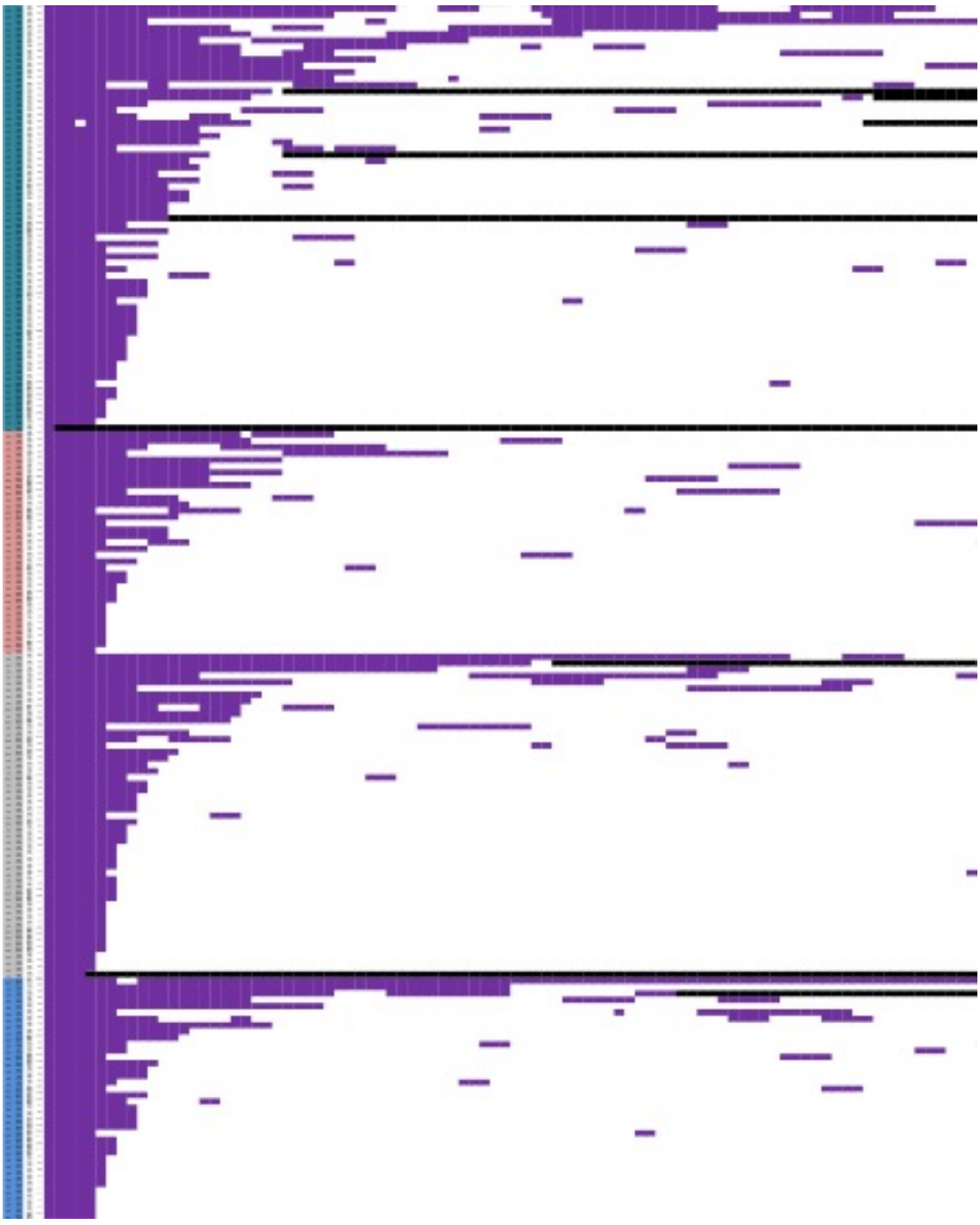


Figure 3-6: First 90 Days by Donor Category.

D1, blue; D2, grey; D3, orange; D4, teal. Each recipient is represented by a line on the y-axis, and each inpatient day is represented by a square in purple on the x-axis. Black is censored data (death or graft loss).

Regarding day case attendances, the mean for all transplants was 5.6 attendances (range 0-28). This value was higher with R3 recipients (6.2 attendances, vs. 1.8 for R1, 5.1 for R2 and 5.7 for R4); observed differences were not statistically significant (p = 0.27). The R4/D4 combination use day case facilities an average of 5.2 times, compared to 5.8 in the R4/D3 group and 6.7 in the R4/D2 group; differences are non-significant (p=0.8).

Regarding the use of ultrasound facilities, D4 grafts of all recipient types had the highest use at 5.6 scans during the follow-up period, with the highest use in R4/D4 at 5.9 scans. Ultrasound use was lowest with D1 grafts (3.8 scans). However, the differences observed were not statistically significant. The mean use of computed tomography (CT) was 0.9 scans per patient. There were no apparent differences observed between donor or recipient risk types.

3.3.7 Cost

The estimated cost of a deceased donor kidney transplant is £12,167 and £12,888 for DCD and DBD, respectively (*Archived Reference Costs | NHS Improvement*, accessed March 2024). An excess bed day beyond an expected admission length (8 days for DBD, 9 for DCD), is costed at £510 for DCD and £549 for DBD transplants. The total cost of an R4 transplant was £28754 compared to £21619 for an R1-3 transplant, p = 0.001. The R4/D4 transplant cost the most, at £31213, at the time of writing, in comparison to R4/D1 £29104, R4/D2 £27677 and R4/D3 £24845; however, due to significant variance within groups, the observed difference was not statistically significant (p=0.1), see Table 3-3. As previously mentioned, index admission length with R4/D4 transplant was longer compared to other donor types, with a mean of 13.5 days, in comparison to 11.7 days for R4 grafts with D1-3 grafts (p=0.001). This additional inpatient stay of 1.8 days (mean) equates to a £918-988 (DCD and DBD) increase in index admission cost per transplant, not including the cost of investigations or interventions during admission. Assessing D4 grafts alone, the mean excess bed days (beyond average index admission) was 0.8 when transplanted to R1-3 recipients and 5.3 for R4 recipients, with a mean associated cost of £435.0 compared to £2772.1 (see Appendix H).

Table 3-3: Total cost of transplant for different recipient and donor types.

Transplant Type		Total Cost Including Transplant (£, mean)
R1-3 (all donor types)		21619.3
R4 (all donor types)		28754.2*
R4	**D1**	29104.1
	D2	27677.4
	D3	24845.2
	D4	31213.0

Abbreviations: **R1-3**: recipient risk indices groups 1 to 3; **R4**: recipient risk indices group 4. **D1**: donor risk indices group 1. **D2**: donor risk indices group 2. **D3**: donor risk indices group 3. **D4**: donor risk indices group 4. Total costs include cost of transplant and length of index admission with excess bed days. * = Difference between R4 and R1-3 was statistically significant p=0.001. Differences between subgroups of R4 were not found to be statistically significant.

3.3.8 Current Waiting List

A time of this report, there were approximately 450 patients on the West of Scotland renal transplant waiting list, 239 of which were currently active. Average age is 53.5 years, BMI 27.7kg/m², 1.4:1 M:F ratio. As per the new allocation scheme those considered 'Tier A' (i.e., calculated reaction frequency (cRF) 100%, waiting time over 7 years or matchability score of 10) are not stratified using the recipient risk indices (26 patients). Using the recipient risk criteria as previously described, 140 of 213 (65.7%) potential recipients are deemed R4, 37 (17.4%) R3, 27 (12.7%) R2, and 9 (4.2%) R1, see Figure 3-7. Regarding the cRF, 31 of Tier B patients (14.5%) had a cRF over 85% and are therefore considered highly sensitised.

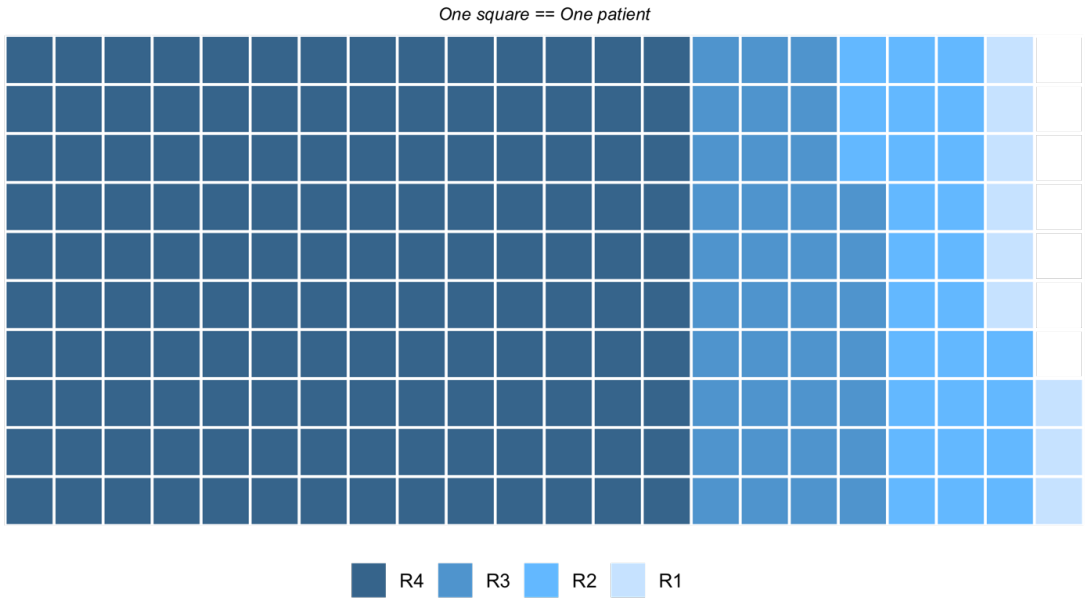


Figure 3-7: Waffle plot of the current waiting list of the West of Scotland catchment area stratified by recipient risk indices.

Each square represents one patient on the waiting list; 65% of the waiting list is in the highest risk 'R4' category.

3.4 Discussion

The new UK kidney transplant allocation scheme came into effect in September 2019, having remained unchanged - excluding minor amendments - since 2006 (D. A. Wu et al., 2017). Underpinning the donor/recipient risk stratification was a simulation based on over 7000 patients in a cohort spanning ten years from 2006 to 2016. The revised scheme aimed to better match patient and graft age, principally to reduce the discrepancy between patient and graft life expectancy, but in part to minimise offer decline rates, which ranged from 24 to 69% in the UK in 2016/2017. Historically, advancing age has been a barrier to renal transplantation, with a quoted 30% reduction in transplant access with each additional decade over 55 (Huang et al., 2009). Although survival and quality of life benefits accompanying transplantation in elderly recipients cannot match younger populations (Humar et al., 2003; Shah et al., 2008; Wolfe et al., 1999), there is a necessary drive to make access to transplant more equitable, particularly given the advancing age of the recipient population. Due to the shortage of donor organs, there will always be difficult and conflicting prioritisation considering those who could benefit the most whilst creating fair, equitable access to all recipients.

The European Seniors Programme (ESP) initiative rolled out in 2001 was the first kidney allocation scheme to give a higher priority to matching donor and recipient ages. Donor grafts over 65 years were allocated to recipients over 65 years, regardless of HLA matching, with consideration of proximity to minimise cold ischaemic time. Five year patient and graft survival was reported at 60% and 47%, respectively, which was lower than older recipients receiving younger grafts in a matched cohort (Frei et al., 2008). Due to the omission of HLA matching in the model, the rates of acute rejection and immunosuppression load were increased (Dreyer et al., 2015). Furthermore, subsequent studies reported the rate of surgical complications (47%) and return to theatre (28%) were significantly higher with the ESP group (Bentas et al., 2008). Thus, highlighting that there may be an additional impact beyond crude patient and graft survival rates.

To facilitate the estimation of graft survival, the kidney donor risk profile (KDRI) is a tool based on donor characteristics to aid the decision-making process in the renal transplantation (Rao et al., 2009; Schamberger et al., 2018). In the United States, the Kidney Allocation Scheme (KAS), utilising the KDRI and released in 2014, aimed to both improve equity of access to all recipients and "maximize the potential survival of every transplanted kidney" (Friedewald et al., 2013). Furthermore, the information provided by this risk stratification tool aimed to reduce graft discard (Aubert et al., 2019). Moving away from the binary

standard criteria vs. extended criteria donor (SCD/ECD), the allocation scheme implemented the concept of the kidney donor profile index (KDPI) based on the KDRI and not dissimilar to risk stratification used in the aforementioned UK scheme. There was recognition that this scheme, whilst improving utility with improved transplant rates for the highly sensitised and also maximising life years gained, would negatively impact access to transplantation for those over 50 years (D. A. Wu et al., 2017). Furthermore, reflecting on this scheme, Butler et al. (2019) recently demonstrated an increase in two-year mortality with post- versus pre-KAS implementation (6.31% vs 5.91%, respectively). Although overall graft loss appeared unaltered, the study identified that two subgroups (46-55 and 56-65 years) may disproportionately carry an increased risk of mortality and graft loss (Butler et al., 2019).

Two-thirds of the current active waiting list in our centre would be deemed the highest risk category, 'R4'. It is unclear whether this patient cohort is representative of the national waiting list as a whole. Additionally, this skew to high-risk donors may reflect the evolving composition of the ERF patient group, which due to the time passed is not comparable to the data used to create the model. Unsurprisingly, this study demonstrates that R4 recipients (receiving all donor types) have a longer index admission, higher rate of DGF and higher total inpatient days (including re-admissions). Of particular interest is that all of these metrics worsen with the combination of a D4 graft with an R4 recipient, a so-called R4/D4 transplant. Patient and graft survival at four years in the R4/D4 group, however, was unaffected at 90.9% and 83.6%, respectively.

The difficulty in generalisability of the findings presented here, owing to the potential difference in recipient cohorts between transplant units, is a limitation of this work. Furthermore, given the skewed distribution of the R4 cohort, the study size is comparatively small within R1 and R2. The impact of donor type (DCD vs. DBD) is important to note. The higher rate of DGF with DCD compared to DBD was not statistically significant; furthermore, the rate of DGF in the D4 group (in both DCD and DBD subgroups) was increased compared to D1-3, demonstrating the additional impact of these factors within the risk indices. In addition, index admission length and readmission rates may be influenced by factors beyond the risk indices documented here, e.g., frailty, and socio-economic status, which are not accounted for in these data.

With particular regard to our transplant unit, the simulation publicised by NHSBT stated that approximately 70% of R4 recipients would be allocated D4 grafts, and therefore potentially 45% of all transplants, as is predicted, would be an R4/D4 combination. The sequelae of this would create significant additional strain on healthcare, particularly as the burden of resource

use is front-loaded in the first few months post-transplant. The additional resource strain may be offset by a larger proportion of R1-3 patients receiving better-quality grafts; however, this is yet to be proven. Furthermore, whilst acknowledging that this new scheme may facilitate more transplants and ultimately be cost-effective by removing R4 recipients from dialysis, finances may need to be redirected to the services that will shoulder the strain of this resource shift.

Emerging perfusion techniques such as NRP and EVNP used to pre-condition, assess, and mitigate the effects of ischaemia reperfusion injury, may prove crucial in order to improve early function, reduce rates of DGF and dampen the impact of allocating the poorest quality grafts to the most at-risk recipients. Notably, the largest randomised trial to date failed to show a demonstrable reduction in delayed graft function when the technology was used for all DCD patient and kidney combinations. Specifically utilising such technologies in high-risk donor/recipient combinations such as the R4/D4 combination may target the grafts most susceptible to poor early graft function and warrants further assessment. The perfusion technology could potentially be combined with the emerging therapeutic interventions such as cytokine depletion, gene therapies and regenerative cell populations to further improve the immunogenic environment within the transplanted graft.

CHAPTER 4: CLINICAL EXPERIENCE OF EVNP AS A VIABILITY ASSESSMENT TOOL

This chapter includes data and sections of two manuscripts which were published in 2021, in the American Journal of Transplant (Pearson, Asher, et al., 2021) and BMJ Case Reports (Pearson, Wubetu, et al., 2021). Permission to include this work within this thesis was granted by the respective editorial teams.

4.1 Overview of Cases

In December 2020, the first clinical perfusion of a kidney graft was performed in Glasgow. Following on from this, despite having to navigate the COVID pandemic, a total of seven kidneys were implanted as a result of EVNP. It is believed that none of these grafts would have been considered suitable for transplant without the period of assessment provided by EVNP. At the time of writing, all of the recipients in receipt of the documented grafts remain off dialysis with functioning grafts. Full detail of all clinic use of EVNP is found below.

4.2 Rhabdomyolysis / Donor Acute Kidney Injury

4.2.1 Introduction

This clinical perfusion, described herein, was from a graft with acute kidney injury secondary to donor rhabdomyolysis. The increasing use of 'extended-criteria' donors (ECD) has been one strategy to increase the availability of grafts. Although it has been widely shown that kidneys from ECDs have inferior outcomes (Querard et al., 2016), the proportional impact of donor terminal creatinine is less certain. Terminal creatinine is a predictive component of the US-based kidney donor profile index (KDPI, formerly KDRI) (Dahmen et al., 2019); however, it is not part of the more recently implemented UK allocation donor risk indices (Watson et al., 2020).

Despite the aforementioned uncertainty, terminal donor creatinine has long been thought of as a negative indicator of post-transplant function. Studies have shown higher rates of DGF and prolonged index hospital admission (Domagala et al., 2019; Farney et al., 2013; Kayler et al., 2009; Zheng et al., 2018). These studies have, however, used varying absolute serum creatinine levels to stratify between the presence and severity of AKI. Most studies adopt

the commonly used stratification tools RIFLE criteria or the more recent Acute Kidney Injury Network (AKIN) criteria (Lopes & Jorge, 2013), both of which are designed for the assessment and treatment of native kidney injury and, although relevant, are not designed for use in real-time transplant decision making. A retrospective analysis of the UK transplant registry assessed transplant outcomes in relation to the AKIN criteria and, similarly, that donors with AKI were associated with higher rates of DGF but also reported increased graft loss and primary non-function (PNF) with AKIN stage 3 kidneys. The recommendations were that careful selection of AKIN stage 1 and 2 is safe, but caution is advised with AKIN stage 3 donors (Boffa et al., 2017).

As a result, donor acute kidney injury (AKI) is commonly cited as a reason to decline a donor organ. A retrospective study by Kayler in 2009 found that where terminal creatinine exceeded 2.0mg/dL (equivalent to 176 μmol/L), neither kidney was retrieved on 44% of occasions, in contrast to 2.3% when levels were less than 1.99mg/dL or lower, and those >2.0mg/dL were associated with a seven-fold increased risk of organ decline post-retrieval compared to grafts with terminal creatinine <1.5mg/dL (132 μmol/L)(Kayler et al., 2009). Corroborating this, a European group previously reported donor creatinine to be the second most common reason for organ decline (Dahmane et al., 2006).

Rhabdomyolysis is a well-known cause of AKI, which is characterised by a syndrome of muscle injury, myoglobinuria and electrolyte imbalance. Myoglobin is thought to be the primary toxin responsible for the resultant renal tubular injury by way of tubular obstruction, inflammation, and vasoconstriction. The aetiology of rhabdomyolysis is commonly trauma with or without crush injuries; however, it is also well described in illicit drug use and with medication side effects (Bajema & Rotmans, 2018). Kidneys from donors with severe AKI secondary to rhabdomyolysis have been transplanted with varying results. There is limited data on the outcomes of kidneys transplanted from deceased donors with AKI due to rhabdomyolysis; although DGF is common, long-term graft outcomes have been reported to be acceptable (C.-B. Chen et al., 2017; Santos et al., 2019). Pre-implantation histological analysis may aid the decision-making process, but in many transplant centres, the resources are not in place to routinely perform pre-implantation analysis. In this case, EVNP provided an alternative method of assessment of such grafts.

4.2.2 Case Detail

A pair of kidneys from a 37-year-old male DCD were offered to our transplant unit. Past medical history included recreational drug use only. Serum creatinine on admission to intensive care was 185μmol/L and increased to 282μmol/L, 442μmol/L, and 742μmol/L over the subsequent days prior to the donation offer. Historic serum creatinine prior to admission was 79μmol/L. A clinical diagnosis of rhabdomyolysis was made based on serum creatinine kinase (peak value 36,284 U/L) and urine colour. At the time of offer, terminal serum creatinine was 901μmol/L.

Both intended recipients were counselled and consented to the use of these kidneys on the proviso that the kidneys had an acceptable viability assessment with EVNP. The first kidney was accepted for a 61-year-old with end-stage renal failure treated with peritoneal dialysis (see Table 4-1. for donor and recipient demographics).

Table 4-1: Donor and recipient demographics, including details of procurement and cold ischaemic time

	Age	Primary Renal Disease	Renal Replacement Therapy	Past medical history	WIT (mins)	CIT (Hours: mins)	KDPI / KDRI	UK Donor /Rec Risk Indices	EPTS score
Donor	37			Nil**			KDRI 56% - 1.06*	D2	-
Recipient 1	61	Lithium toxicity	Peritoneal Dialysis	Ischaemic heart disease	9	10:20		R3	30%
Recipient 2	64	IgA Nephropathy	Haemodialysis	Ischaemic heart disease	23 (9 + 14)	13:40		R3	48%

*KDPI creatinine level capped at 8mg/dL, so unable to enter the accurate value of 10.2mg/dL

**Cause of death – Intracranial haemorrhage

WIT – Warm Ischaemic time

CIT – Cold Ischaemic time

EPTS score - Estimated Post Transplant Survival

EVNP was conducted as per the previously described Nicholson protocol; pressure was maintained at 75mmHg, with a single unit of 0 negative packed red cells suspended in a crystalloid solution with multivitamins, sodium bicarbonate, dextrose and dexamethasone administered, warmed to a temperature of 37°C with no additional filtration of the perfusion circuit (Figure 4-1).

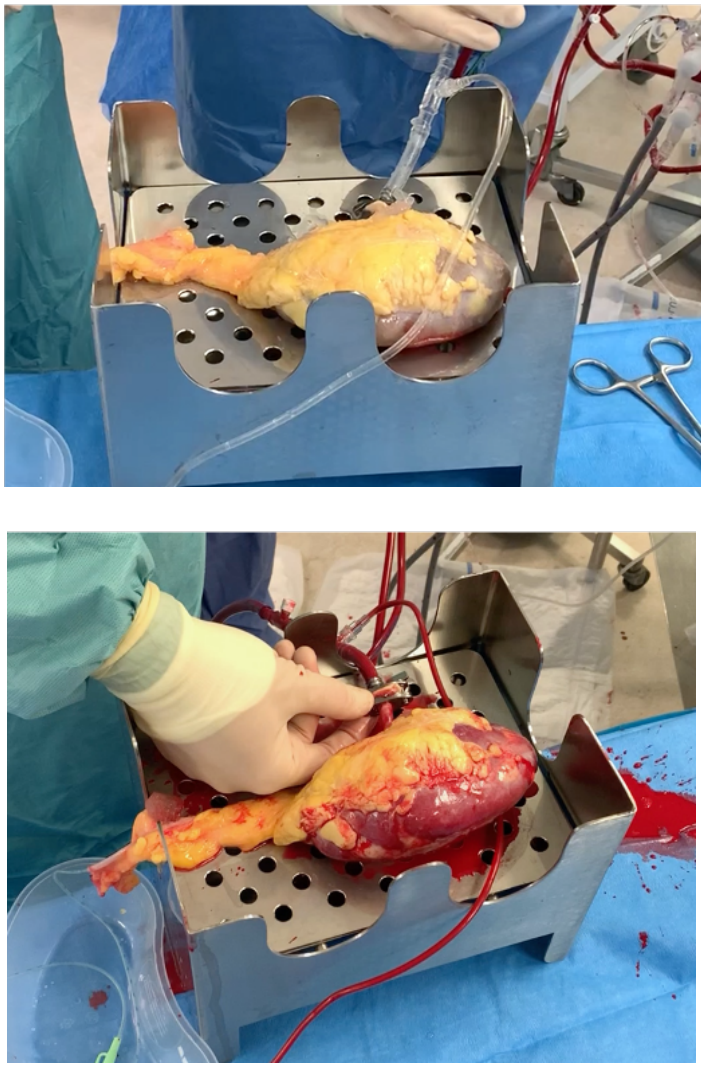


Figure 4-1: Pre- (above) and post-perfusion (below) image of graft with acute kidney injury.

The kidney maintained a good appearance throughout the 75 minutes of perfusion. Mean renal blood flow was 110ml/min/100g and produced 85mls of urine (EVNP quality assessment score of 2: Moderate appearance (2 points); Mean renal blood flow >50ml/min/100g (0 points), Total urine output (ml) >43mL/hour (0 points)). Urine was clear and demonstrated a normal appearance; however, it was not tested for myoglobin. Blood gas analysis from the arterial limb of the perfusion circuit demonstrated potassium and lactate

within normal levels. To minimise overall ischaemic time, at 20 minutes of perfusion, the decision was made to proceed with the operation for the planned recipient. The kidney was removed from the perfusion device at 75 minutes, flushed with 2L of cold hyperosmolar citrate perfusion solution and maintained on ice until required. The operation was performed without complication.

There were 9 minutes of warm ischemic time at organ procurement and a total of 10 hours and 20 minutes of cold ischemic time, 45 minutes of which followed EVNP when placed back on ice prior to implantation. Whilst acknowledging that pairs of kidneys do not always perform in an identical manner, the implanting surgeon for the second kidney was given confidence by the period of assessment provided by EVNP of the first kidney. With this additional information and wishing to minimise further cold ischemic time, the decision was made to implant the second graft without EVNP. The recipient was a 64-year-old haemodialysis-dependent patient. Unfortunately, on reperfusion of the kidney, there was evidence of hilar bleeding, which resulted in an additional 14 minutes of warm ischaemic time whilst control was established and kidney perfusion recommenced. The patient required dialysis in the immediate postoperative period due to hyperkalaemia, and delayed graft function persisted for 7 days. Both grafts were functioning well at two months post-transplant, with a serum creatinine of 62µmol/L and 122µmol/L for Patient 1 and Patient 2, respectively (corresponding to an eGFR of 88.5 and 55.3mL/min/1.73m^2, see Figure 4-2).

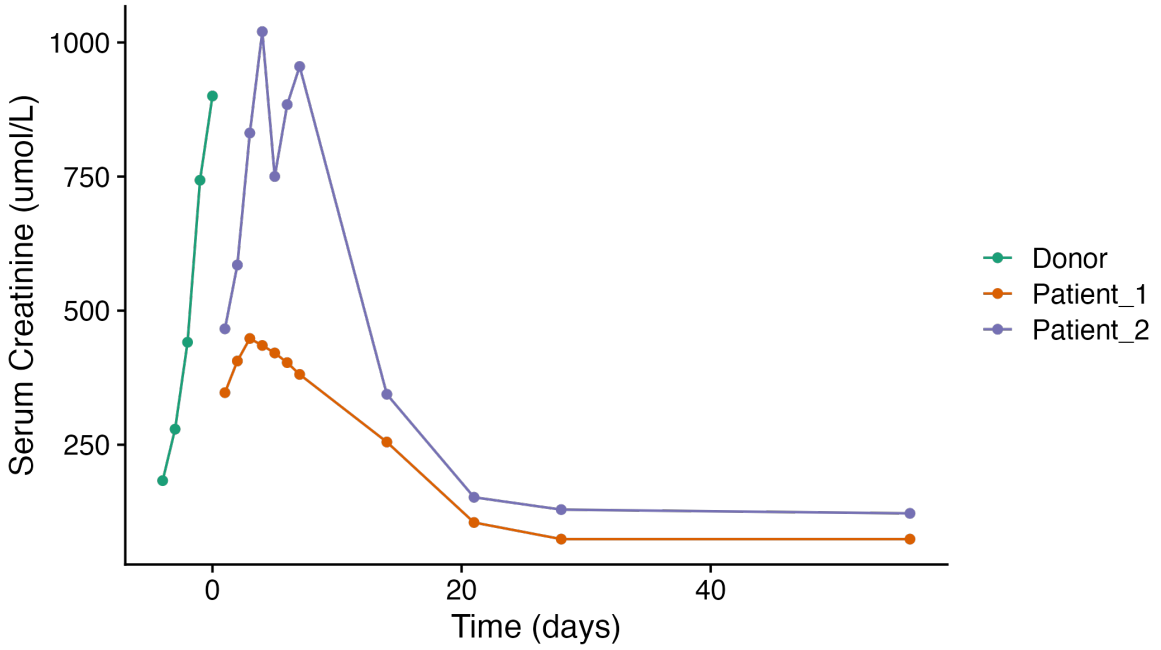


Figure 4-2: Serum creatinine (µmol/l) over time for Patient 1, Patient 2, and the Donor kidney.

4.2.3 Discussion

Kidneys are commonly declined due to donor AKI, particularly when classified as severe (AKIN 3 criteria), as the literature strongly advises caution with such grafts. Pre-implantation biopsies may offer additional information in cases with otherwise favourable donor characteristics and a reversible cause of the apparent AKI. The availability of real-time biopsy information in many transplant centres, however, is limited. The provision of a 24-hour service is complex and costly, and evidence for the ability of pre-implantation histology to improve kidney utilization remains lacking. Similarly, the overall cost-effectiveness of this approach remains in question. A UK national study, PITHIA, has been instituted to address this question in DCD kidneys, but kidneys from younger donors with AKI, such as those described in this report, fall outside its scope (Ayorinde et al., 2019). Neither kidney in this report was biopsied prior to implantation.

Ex-vivo normothermic perfusion offers an alternative method of assessing such kidneys providing real-time information on the appearance whilst on perfusion, intra-renal vascular resistance via pump parameters, and urine output. This approach has the potential to expand the pool of utilisable grafts through the implantation of currently discarded kidneys, as in this case. Furthermore, in this case, where the pair of kidneys was offered, the viability assessment of the first kidney whilst on perfusion was deemed sufficient for the paired kidney to be considered transplantable without EVNP. It is important to note that the cost of perfusing a kidney *ex vivo* as per established protocols is approximately £1000 and requires a considerable amount of hardware and expertise. In circumstances where there is uncertainty regarding graft suitability, delivering EVNP to one of the paired grafts could provide clinicians with helpful information on both grafts, even if destined for a different transplant unit.

An important further consideration is the reconditioning potential of EVNP, a benefit now demonstrated in pre-clinical studies (Hameed et al., 2019), and under evaluation in an ongoing randomized control trial comparing EVNP to static cold storage (Hosgood et al., 2017). Importantly, this report documents the use of EVNP with only a single pair of DCD grafts and therefore, the generalisability of these findings is limited. Furthermore, in this case, due to the surgical complication evident during the implantation with Patient 2, it is not possible to draw any conclusions from the apparent differences between the two grafts, much less the therapeutic/reconditioning effect of EVNP. Moreover, there were many recipient differences between these two cases. Pre- and post-EVNP biopsies were not taken in this instance, which represents a further limitation of this report. Histological evidence of

‘washout’ of the obstructing casts seen in rhabdomyolysis-induced kidney injury, for example, may have contributed to the understanding of this perfusion technique’s therapeutic utility.

It is our conclusion, however, that this report illustrates the ability of EVNP to assess grafts at the extremes of common graft acceptance, where a favourable evaluation would provide clinicians with sufficient information to proceed with transplantation. The new National Kidney Allocation Scheme (commenced September 2019) in the UK offers paired kidneys to transplantation units where donors are categorized as the marginal (so-called ‘D4’) and the donor age exceeds 70 years. As a result, more units will be offered pairs of kidneys in this manner, and therefore, assessment of one kidney may be sufficient to provide assurance to transplant both grafts.

4.3 Renal Artery Stenosis

4.3.1 Introduction

Herein, we describe the perfusion of four kidneys, which were removed from patients with refractory hypertension secondary to renal artery stenosis (RAS). RAS is an anatomical description of various disease processes that result in the narrowing of the renal artery and reduced renal perfusion (Weber & Dieter, 2014). The consequent reduction of the functional glomerular mass may be of little clinical consequence if the contralateral kidney is normal. However, impaired renal perfusion can lead to increased renin production and subsequent drug-resistant hypertension. Approximately 90% of RAS is attributable to atherosclerosis, the remaining 10% non-atherosclerotic, of which the majority is secondary to fibromuscular dysplasia (FMD) or congenital stenosis (*Renal Artery Stenosis | NIDDK*, accessed March 2024). The congenital subtypes can be either idiopathic or related to congenital malformation syndromes, both of which are more commonly identified in younger individuals with RAS (Robinson et al., 1991).

The management of drug-resistant hypertension due to RAS is based on the treatment of hypertension and the restoration of renal perfusion, primarily with percutaneous angioplasty (PTA) with/without stenting (ASTRAL Investigators et al., 2009). Second-line management where endovascular interventions are not successful may involve a variety of approaches, including open revascularisation, auto-transplantation or nephrectomy (Ham et al., 2010; Mhaske et al., 2019; Raman et al., 2016). Of critical importance is predicting the improvement in renal function from the restoration of circulation to a chronically ischemic kidney. Currently, this has been based on crude parameters of pre-operative isotope scanning and anatomical measurements.

4.3.2 Case Detail – First Perfusion of Kidney with Renal Artery Stenosis

A 19-year-old male (Patient A) presented with poorly controlled hypertension on four antihypertensive agents (Ramipril, Amlodipine, Doxasozin, and Spironolactone). Renin concentration was elevated at 363.1 (mIU/L) with normal aldosterone (22pmol/L) and normal CRP. Cross-sectional imaging demonstrated unilateral renal artery stenosis, thought to be of congenital aetiology, with normal contralateral kidney and no other arterial abnormalities. Perfusion was delayed and provided via an extensive network of collaterals originating from the splenic arterial supply.

Planar and SPECT DMSA scans demonstrated the left kidney to be small, without any areas of focal cortical scarring. The differential function was 62% and 38% between the right and left kidneys, respectively. The absolute GFR was 115.9ml/min. Preoperative blood pressure was 151/62mmHg. No urinary protein was evident. Complement C3/C4 and ANCA vasculitis screen were both normal.

A multidisciplinary team meeting comprising nephrologists, interventional radiologists, and vascular and transplant surgical colleagues reviewed all details. PTA was deemed futile and potentially hazardous due to the length of the lesion, the termination in small hilar branches and the likely fibrotic nature of the occlusion. Given that the contralateral kidney was of excellent function, the young age and poorly controlled hypertension, the patient elected for surgical intervention rather than medical management. Additionally, the patient did not deem the uncertain return of renal function worth the additional risks and uncertainty inherent in a restorative procedure (bypass or autotransplant) and elected for nephrectomy alone. They expressed a wish at this stage to donate the kidney, if possible, to the transplant waiting list. After lengthy local discussions, the necessary tissue typing and workup with our living donor transplant team was therefore performed.

Following the NHSBT matching run, a 64-year-old female (Patient B) with established renal failure was identified. The patient was approaching dialysis secondary to IgA nephropathy with an eGFR of 10.3ml/min with no living-donor options; the mismatch was 220MM. The patient was informed of the offer, and a long discussion with the patient and family took place. They were made aware that the donor vessels may be unsuitable for implantation, and also consented regarding the uncertainty of the primary disease process affecting the donor vasculature and that the long-term function was difficult to predict, albeit with favourable donor age.

A hand-assisted laparoscopic nephrectomy was performed with care taken to preserve the extensive neovascular collaterals (see Figure 4-3). The kidney was removed, and cold perfusion commenced (Soltran, *Baxter Healthcare*). Although accepting the flow of perfusion fluid via the renal artery, less than one-third of the graft was cleared of blood. Following this, cannulation of the collateral vessel was performed, and the flow of perfusion fluid improved. However, the overall kidney appearance remained poor, with incomplete clearance within the graft and a patchy macroscopic appearance. The primary renal artery was spatulated to open the evidently stenosed segment. An arterial reconstruction was then formed with the spatulated renal artery patched with a segment of the collateral vessel to create a common stem (see Figure 4-3). This newly formed common stem tolerated the flow

of cold perfusion fluid; however, the global appearance remained patchy with incomplete clearance of blood.

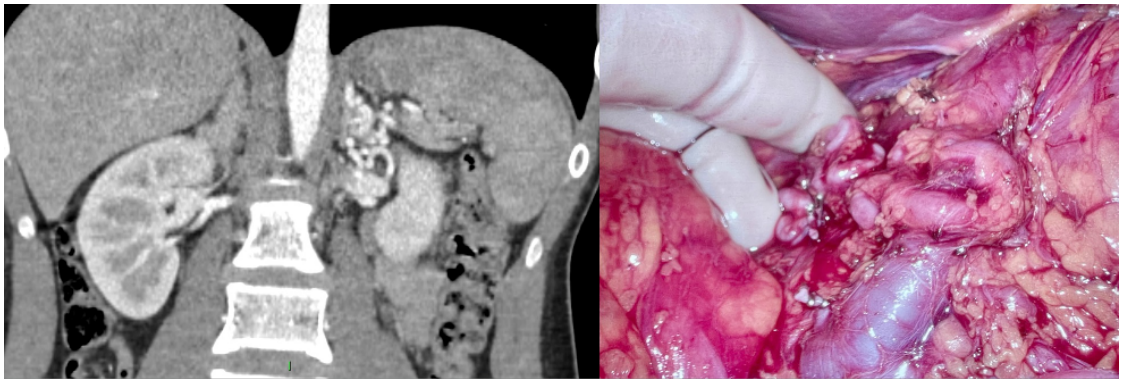


Figure 4-3: Coronal image of pre-operative computed tomography scan (left) and intraoperative imaging demonstrating the neovascular collaterals evident (right).

Both images demonstrating presence of venous collaterals overlying the renal vein with the stenosed renal artery underneath the vein.

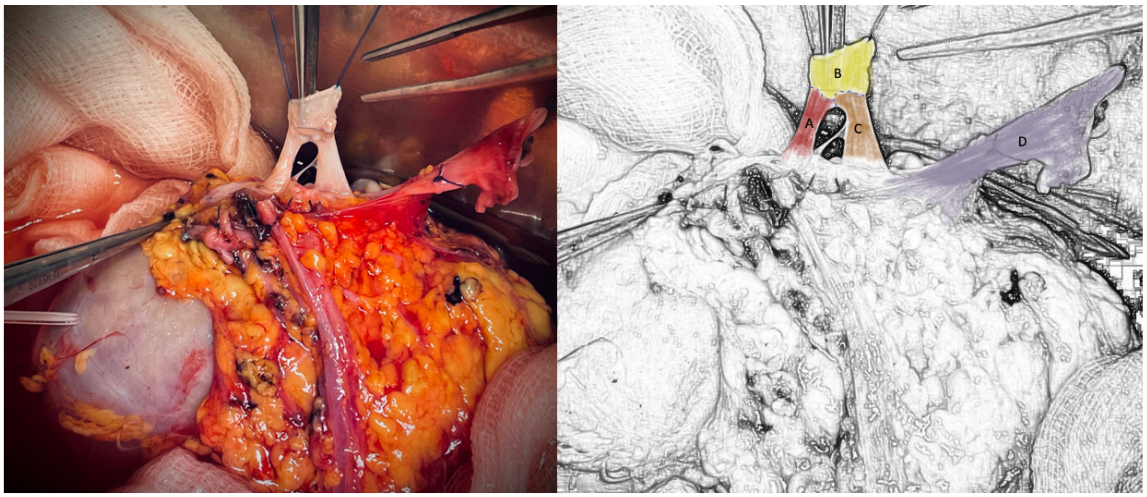


Figure 4-4: Image of backbench reconstruction of the explanted graft (left) with diagram to depict anatomical configuration (right).

The explanted graft was assessed on the 'back bench' and found to have incomplete clearance of blood with cold perfusion flush via both renal artery and large collateral. An arterial reconstruction was created between the spatulated renal artery and collateral vessel, creating a common stem. (Image right) Line drawing depiction of the arterial reconstruction where A = main donor renal artery following spatulation; B = segment of collateral vessel creating common stem; C = main collateral vessel; and D = renal vein.

At this point, the graft was deemed unusable as it could not be ascertained whether adequate perfusion could be achieved via the common arterial stem. The clinical team made the decision to assess the graft perfusion by utilising EVNP as a viability tool. A unit of 0-negative blood and Ringer's lactate were used to form the basis of a perfusate with additives as per the aforementioned protocol commonly used in the UK. A pressure of 75mmHg and a temperature of 37°C were maintained, and upon perfusion, the graft demonstrated excellent global perfusion (see Figure 4-5). Within a few minutes, the graft was found to produce urine. Specific information regarding adequacy perfusion via the arterial reconstruction had been provided, and therefore, the graft was only perfused for 20 minutes. At this point, the decision was made to implant the graft to the recipient. The transplant was performed without complication with a primary warm ischaemic time of 6 minutes and a cold ischaemic time of 5 hours and 18 minutes. The graft achieved primary function, and the recipient was discharged on day 6 with a creatinine of 87μmol/L. The patient remains well and at three years post-transplant with a stable eGFR of 58ml/min.

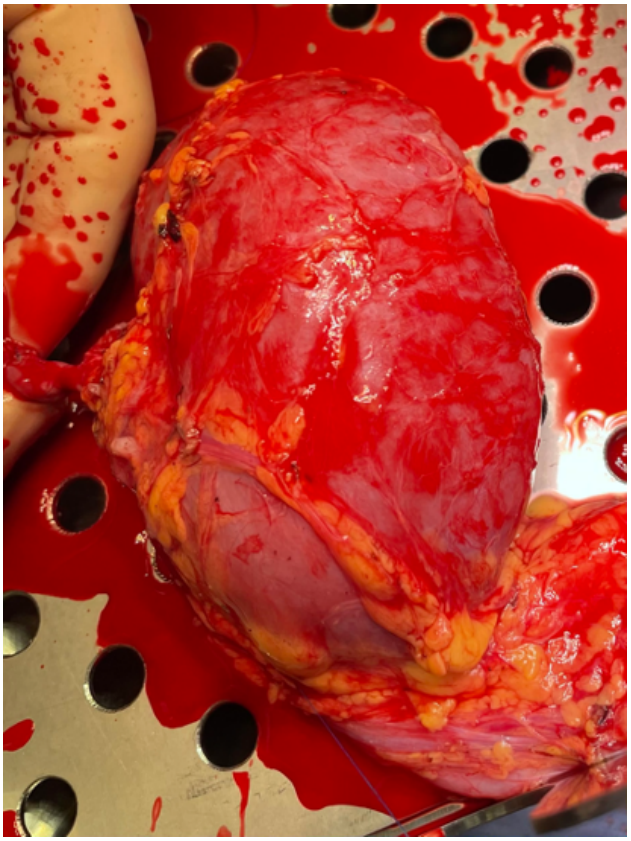


Figure 4-5: The explanted graft demonstrated excellent global perfusion while being perfused via ex vivo normothermic perfusion.

The donor recovered well from the nephrectomy and was discharged on day 1 with no anti-hypertensive medication. Serum creatinine measured 94μmol/L on day of discharge, and remains well with an eGFR>60ml/min. All anti-hypertensive medications remain withheld, and the most recent blood pressure reading is 131/65mmHg.

A CT angiogram was performed two months post-transplant to evaluate the arterial supply to the transplanted kidney (see Figure 4-6 and Figure 4-7). The images demonstrated no significant stenosis of the transplant renal artery with excellent global perfusion of the graft; in addition, serial ultrasound scans have shown excellent global perfusion and patent renal artery.

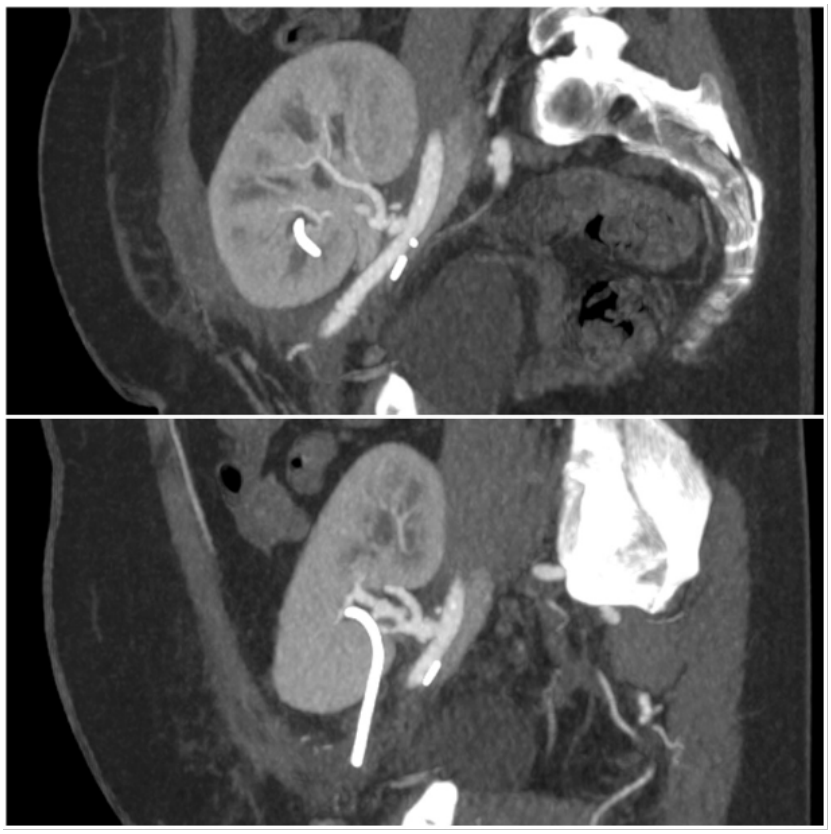


Figure 4-6: CT angiogram images 6 weeks post-transplant demonstrating excellent global graft perfusion.

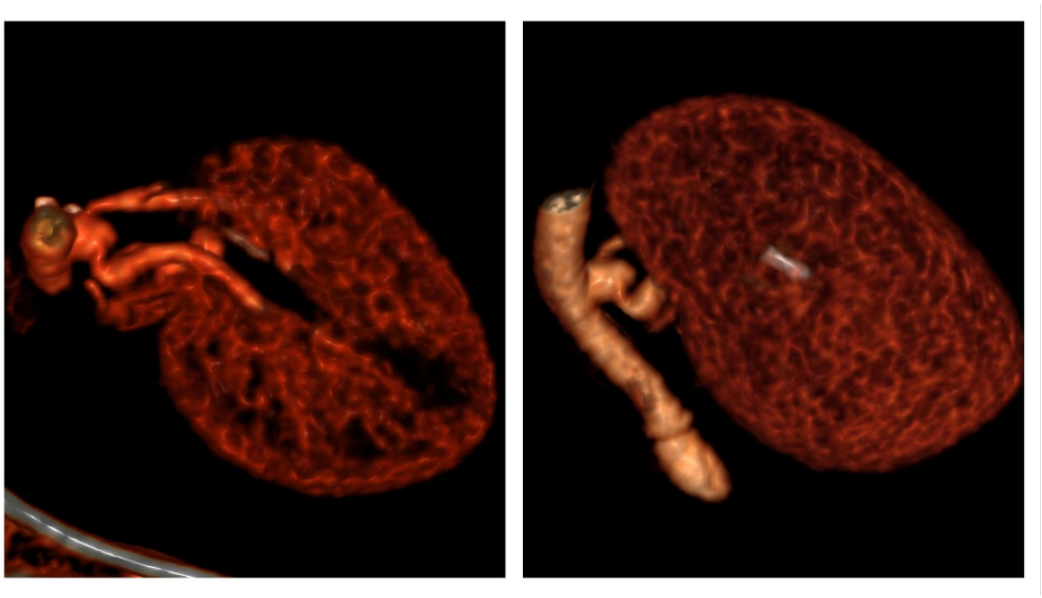


Figure 4-7: Multiplanar volume reconstruction images of the post-transplant CT angiogram demonstrating the two branches emerging from the common stem.

4.3.3 Case Detail – Second Perfusion of Kidney with Renal Artery Stenosis

A 29-year-old female (Patient C) was under the care of renal physicians with refractory hypertension secondary to isolated renal artery stenosis. The underlying diagnosis was thought to be FMD, and despite multi-agent anti-hypertensive therapy (Indapamide, Ramipril and Atenolol), the patient's systolic blood pressure was 190mmHg. Due to longstanding hypertension, there was evidence of systemic sequelae and end-organ damage, including chronic kidney disease (serum creatinine 553micromoles/L; eGFR 7.2ml/min/1.73m^2), left ventricular hypertrophy, retinal changes, a history of cerebrovascular disease with previous lacunar infarct and MRI-confirmed posterior reversible encephalopathy syndrome (PRES) and right-sided pons infarct.

Imaging of the affected kidney by way of DMSA renogram and magnetic resonance angiography (MRA) demonstrated 57%/43% split function; the left renal vasculature was normal; however, the right renal artery was occluded beyond its ostium, with a hypertrophied early branch supplying the capsule, which turned back to fill two arteries supplying the upper pole. Further collaterals were identified, which reconstituted at the hilum in an antegrade fashion, branching to supply both the upper and lower poles. The case was discussed in our regional reno-vascular MDT and the decision was to offer right-sided therapeutic nephrectomy.

In the wake of the previous domino transplant in a nephrectomised kidney with RAS, this patient was similarly counselled about the potential assessment of the graft using EVNP and its possible use for a recipient on the waiting list. The patient declined autotransplant; however, was in agreement the graft could be used for another recipient if it was thought the graft could be of benefit to someone. The requisite tissue typing was performed by NHSBT and a potential suitable recipient was identified.

The recipient (Recipient D) was a 63-year-old female on hospital haemodialysis with precarious access issues; currently dependent on a central access line due to previous failed fistula attempts. The primary renal disease was histologically proven anti-GBM Goodpasture's disease, and past medical history included ANCA-negative systemic vasculitis. The mismatch was 110, and flow T and B-cell crossmatches were negative; no donor-specific antibodies were detected.

The elective laparoscopic hand-assisted nephrectomy was performed in the usual fashion without complication. There were no intra-operative issues. The kidney itself appeared well-perfused prior to removal, and significant collateral vessels were noted and spared. The

kidney was removed with a warm ischaemic time of five minutes. It was promptly perfused on the back bench with cooled Soltran (Baxter, UK) and displayed 'reasonable' perfusion, with clearance of blood only possible via cannulation of the main collateral vessels. With the donor's consent, a small portion of the great saphenous vein was harvested for arterial reconstruction, creating a common stem between the main collateral and the original renal artery, which was dissected and spatulated.

EVNP was set up as previously described with a unit of group-specific packed red cells and warmed to normothermia. The newly created common stem was relatively short, and we did not want to threaten the reconstruction with a ligature to secure the stem to an arterial cannula, so the cannula was held in position manually by the surgical team. The desired 75mmHg of pressure was achieved, and excellent global perfusion of the kidney was demonstrated with early urine production. Renal resistance was stable throughout, and the EVNP assessment score = 1. The perfusion duration was cut short due to the challenges with the arterial cannulation at 25 minutes. It was felt as a clinical team that EVNP had provided us with the information required to proceed to transplantation, specifically whether the arterial reconstruction provided global supply to the graft and whether haemostasis was achieved. The graft was then flushed with Soltran (Baxter, UK) and placed on ice.

Transplantation proceeded with the intended recipient, and the implantation was successful with good macroscopic global perfusion of the graft evident on reperfusion. Anastomosis time was 21 minutes, and total cold ischaemic time was 5 hours 38 minutes. Immediate post-operative bedside ultrasound scanning demonstrated widespread graft perfusion, and the graft achieved primary function. The recipient was discharged on day 7 with a serum creatinine of 99 micromoles/L (eGFR 61.1 ml/min/1.73m^2) (see Figure 4-8). Most recent laboratory tests reveal a serum creatinine of 79 micromoles/L (eGFR >60 ml/min/1.73m^2) over two years post-transplant.

The donor was discharged on day 3 with a single anti-hypertensive agent (Atenolol) with a systolic blood pressure of 130mmHg. Clinical review at one-year post-nephrectomy demonstrated a blood pressure of 141/110mmHg and is currently on no regular anti-hypertensive medications.

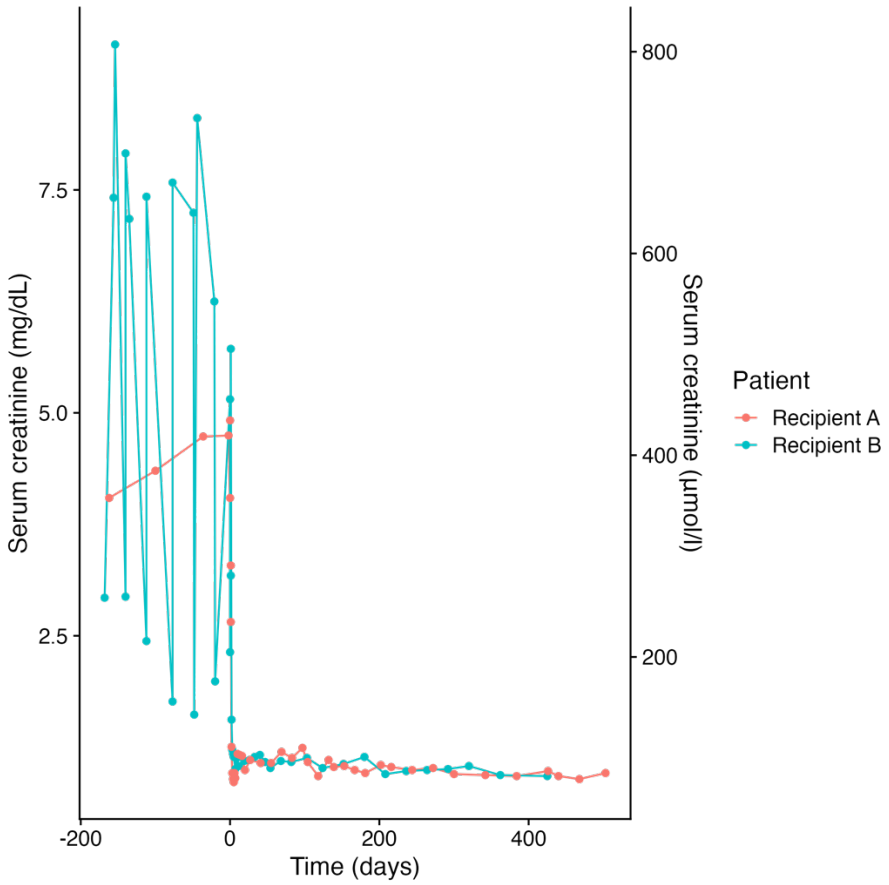


Figure 4-8: Line chart demonstrating a change in creatinine levels over time pre- and post-transplant.

Time 0 indicates the day of transplant; both Recipient A and Recipient B achieved primary function, which was sustained at one-year post-transplant.

4.3.4 Further Perfusion of Kidneys Removed Due to Renal Artery Stenosis Unsuitable for Transplantation

At the time of writing, two further nephrectomies for patients with refractory hypertension have been performed and subsequently perfused via EVNP. These kidneys were perfused for a period of one hour using the aforementioned clinical protocol with a unit of packed red cells.

The first was a kidney that had MRA-confirmed atherosclerotic occlusion to the solitary renal artery. The chronic occlusion had led to the patient's hypertension, requiring four anti-hypertensive agents; however, blood pressure remained poorly controlled. Planar and SPECT DMSA scans demonstrated the right kidney to be small, and differential function was 1% and 99% between the right and left kidneys, respectively. It was felt that the

demonstration of only 1% split function and significant chronicity were too significant to consider implantation, and therefore, tissue typing was not performed.

The kidney was assessed on the back table, the renal artery was spatulated, and there was enough distal renal artery to connect to an arterial cannula with a securing ligature. EVNP was performed for one hour, and a score of 4 was achieved: patchy perfusion (2), high resistance (1) and reduced urine output (1). The assessment period confirmed our pre-perfusion perception of this kidney that it was not suitable for transplantation.

The second kidney was from a recipient who had longstanding hypertension, which had not resolved despite an attempt at renal artery endovascular stenting. The underlying aetiology was atherosclerotic disease, and the most recent imaging demonstrated stent occlusion. Split function was shown to be 6% in the affected kidney. The nephrectomy was performed, and the kidney was assessed on the back table. The renal artery was spatulated, the stent removed, and an arterial cannular secured with a ligature to provide connection to the EVNP circuit. Similar to the previous case, a score of 4 was achieved: patchy perfusion (2), high resistance (1) and reduced urine output (1) (see Figure 4-9).

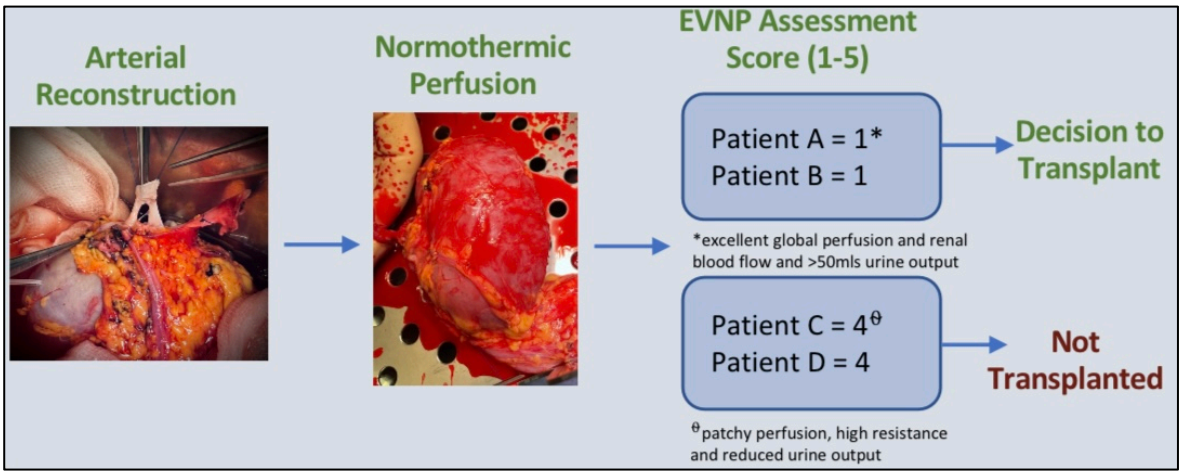


Figure 4-9: Flowchart detailing the steps following nephrectomy utilising ex vivo normothermic perfusion as an assessment tool.

4.3.5 Discussion

Living donor transplantation of a graft removed from a hypertensive patient with renal artery stenosis is uncommon. Furthermore, the detail of the report above, to our knowledge, is the first instance in which a perfusion circuit was utilised to assess the adequacy of the renal artery reconstruction, the perfusion quality, viability and, therefore, suitability for transplantation. Without the improved perfusion demonstrated by EVNP, these grafts would not have been transplanted.

Endovascular treatment is considered a first-line intervention for hypertension refractory to medical therapy in RAS to relieve the mechanical obstruction and reduce the ischemic stimulus that drives the renin production (Raman et al., 2016). Failure of PTA may mandate surgical review with revascularisation, nephrectomy, or autotransplantation as treatment options. This decision is tailored to the renal needs of the patients, body habitus and risks of short-term procedure and long-term function. Whilst initially attractive, autotransplantation itself carries significant risks with reported transplant failure of 10.7% and morbidity of 46.2% (Moghadamyeghaneh et al., 2017; Tran et al., 2015). In these cases, partly because of these risks, the donor and family elected for nephrectomy alone without reimplantation.

The potential use of a kidney removed due to renal artery stenosis is contentious, and RAS is often regarded as a contraindication to transplant. This is the first report of utilising novel technology to demonstrate the viability of two kidneys that would not have been otherwise transplanted. Given that the function of the organ may have been impacted by non-reversible ischaemic nephropathy, the demonstration of both adequate perfusion (satisfactory reconstruction) and excellent function with the early production of urine, EVNP allowed these organs to be transplanted without the need for pathological assessment.

There are case reports in the literature that detail the transplantation of grafts from living donors with RAS without this back bench assessment: various arterial reconstructions are described, including utilizing internal iliac artery (Matsushita et al., 2020), vein harvested from the recipient (Pfeiffer et al., 2002), and cryopreserved iliac artery graft from a deceased donor (Nguefouet Momo et al., 2020); all of which report satisfactory transplant outcomes. Regarding the deceased donor transplantation, satisfactory results have also been reported following resection of the diseased portion and reconstruction, which was evident in the assessment of the graft at the recipient centre (Kim et al., 2019).

The requirement for long-term follow-up of both donor and recipient is evident. Timing of disease recurrence is unpredictable, with groups reporting the progression of disease and return of hypertension beyond one year post-transplant (Parasuraman et al., 2004). Despite the unpredictability, long-term outcomes of renal artery reconstruction for FMD are reported as satisfactory, with one analysis reporting 88.5% graft survival at 5 years and similar function at one year compared to non-FMD affected grafts (Sagban et al., 2014).

As many as 10% of all transplants require a degree of reconstruction to the donor renal artery prior to implantation, and although adding a degree of complexity, such transplants demonstrate satisfactory graft survival (84.3% at 5 years) (Ta et al., 2014). In the first case report, a side-to-side arterial reconstruction was formed with the large collateral vessel, which had arisen from the donor's coeliac axis. Although it was possible to administer cold perfusion fluid via the revised common stem, it was not possible to sufficiently clear the graft, and at this point, there remained insufficient confidence to transplant the graft.

EVNP has been shown to be an effective point-of-care tool for the assessment and potential reconditioning of marginal deceased donor grafts prior to transplantation. Depending on perfusion parameters, macroscopic appearance, and urine output, the kidney can be evaluated following vascular refashioning (EVNP quality assessment score 1–5), and with a score of 3 or less, be considered safe for transplantation (Hosgood, Barlow, Hunter, et al., 2015). The assessment tool has been demonstrated with declined donor after circulatory death (DCD) kidneys due to "poor perfusion", whereby a favourable quality assessment score facilitated subsequent successful transplantation (Hosgood et al., 2018).

It was important to demonstrate that EVNP findings corroborated with the pre-nephrectomy functional assessments of the two further kidneys, not considered for implantation, that were removed for refractory hypertension. These two kidneys had poor split function (1% and 6%), and it was thought they were unlikely to provide benefit to a recipient. The EVNP scores (both 4) demonstrated that EVNP was sensitive to this poor function and chronic damage and, therefore, an effective assessment tool in this scenario.

The potential of EVNP to assess and facilitate *ex vivo* interventions is often discussed. In the context of liver transplantation, the use of ex vivo perfusion to assess the adequacy and quality of arterial reconstruction was recently reported by Nasralla and colleagues whereby the group presented the ex vivo reconstruction of five livers with anatomical arterial

variations (Nasralla et al., 2020). Once complete, the subsequent period of normothermic perfusion allowed functional evaluation of the anastomotic patency and macroscopic appearance of the graft, similar to this case. To our knowledge, this report is the first to have used EVNP to evaluate complex arterial reconstruction in two kidneys to facilitate transplantation. This may offer an avenue for expansion of the donor pool in cases of complex anatomy or damage at procurement.

To conclude, this case demonstrates the use of EVNP as an assessment tool to evaluate arterial reconstruction in two cases of unrelated living donation in which the donors had unilateral renal artery stenosis and hypertension. The key principles outlined are careful selection of donors, considerate consent processes with the potential recipient, expert arterial reconstruction - with or without viability assessment tool - and vigilant long-term follow-up for both donor and recipient.

4.4 The Use of EVNP to Facilitate Dual Kidney Transplant

4.4.1 Introduction

The limited supply of organs in the context of the increasing demand for utilisable grafts continues to strain the transplant service. In recent years there has been a considerable shift, particularly in parts of Europe, to use more 'marginal' or 'extended criteria' grafts to expand the donor pool (Echterdiek et al., 2019). The term 'marginal graft' is yet to be conclusively defined, yet the increase in the use of DCD grafts and grafts from older donor age is self-evident (Summers et al., 2015).

It is often reported that kidneys from older donors have shorter graft lifespans and are more susceptible to the effects of ischaemic reperfusion injury and, therefore, DGF (Gerbase-DeLima et al., 2020). At present, risk stratification to facilitate real-time decision-making rests on donor demographic data provided to the clinical team. Donor risk tools provide additional information, such as the KDRI (Rao et al., 2009) in the United States and the UK's recently revised national kidney allocation scheme (NKAS), which subcategorises donors from 1 to 4 (with 4 being the highest risk) (Watson et al., 2020). The assessment of the donor graft is then carefully considered with reference to the specific recipient in receipt of the offer.

Acknowledging some of the factors with donors of advanced age, the aforementioned UK NKAS offers grafts from the highest risk group, D4, where donor age exceeds 70 years, as a pair for dual transplant, allowing the recipient centre to decide whether to use one or both grafts (*Kidney Allocation System - Professional Education - OPTN*, accessed March 2024). Dual kidney transplants (DKT) have been shown to be a viable option in select recipients, offering satisfactory medium-term survival (Stratta et al., 2016). Apprehension of the use of a single graft from this category has been a factor contributing to organ decline rates, which is also somewhat addressed with dual kidney transplantation by maximising potential functional nephron mass (Khalil et al., 2017).

EVNP has emerged as a potential viability assessment tool which provides a pseudo-physiological state of normothermic oxygenation. The perfusion allows for a period of observation prior to implantation, therefore bolstering the decision-making process (Hosgood, Barlow, Hunter, et al., 2015). In this report, we describe the use of EVNP in a case of dual kidney transplant in which the perfusion technology reduced cold ischaemic time and provided additional information regarding the perfusion quality of the second graft.

4.4.2 Case Detail

A kidney pair was offered from a 71-year-old 'D4' DBD for a 66yo highly-sensitised patient who previously had a bilateral lung transplant and was struggling on haemodialysis due to difficulties with access (problematic left-sided fistula with central vein stenosis; at the time of offer was dependent on central access). The graft was well matched (000MM), and terminal creatinine was 117μmol/L and eGFR 61.7ml/min. Discussion was held between the surgical team and nephrologists – given the recipient's access issues and comorbidity, it was felt dual transplantation offered the best potential outcome in terms of function and long-term avoidance of dialysis. The kidneys were accepted and arrived at the recipient unit with nine hours of cold ischaemia.

Both kidneys were inspected and prepared for transplantation. The right kidney appeared well-perfused and was implanted directly into the patient's right side. The left kidney, however, demonstrated patchy perfusion with evidence of incomplete clearance of blood at procurement. Whilst the operation was in progress, the second kidney was placed on EVNP with a red cell-based perfusate for 90 minutes, at 37°C and pressure maintained at 75mmHg. Following the 90-minute period of perfusion, the graft demonstrated moderate global perfusion, 110mls urine output, and satisfactory renal blood flow (>50ml/min/100g); EVNP assessment score = 2. The graft was then cold perfused with Soltran (Baxter, UK).

The CIT for the first implanted kidney (right) was 12h35mins. The second kidney (right) was being perfused ex vivo prior to in situ reperfusion of the first. CIT was, therefore, 11h5mins, plus an additional 1h15mins following EVNP (total 12h20mins). Both kidneys reperfused with good global perfusion (see Figure 4.10). The patient received a short session of haemodialysis post-operatively for hyperkalaemia (6.0mmol/L) and made an excellent post-operative recovery. Beyond this initial dialysis immediately post-op, the kidneys achieved primary function with a day 7 serum creatinine of 85μmol/L and eGFR 61.7ml/min. The patient was discharged on day 12- and 3-month creatinine was 80μmol/L (see Figure 4-11).

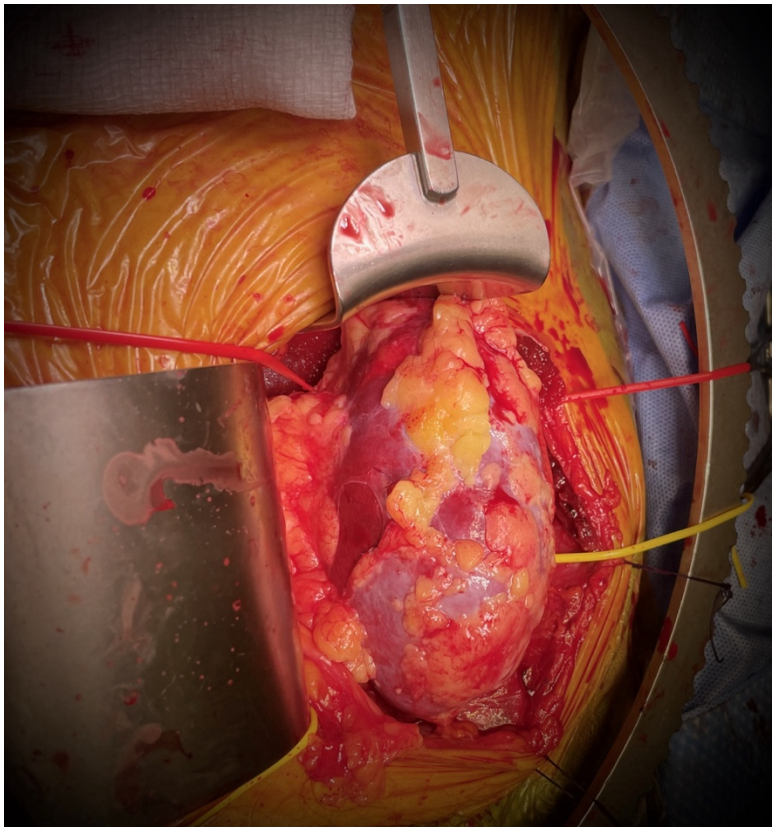


Figure 4-10: Intraoperative post-reperfusion image of the left kidney which had undergone 90 minutes of EVNP prior to implantation

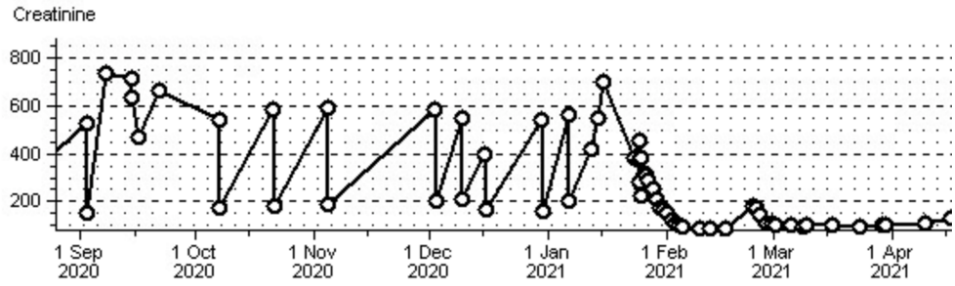


Figure 4-11: Recipient creatinine levels in the months prior to and post-dual kidney transplants. All units in µmol/L

The recipient developed COVID-19 four months post-transplant, requiring intensive care with high-flow nasal oxygen supplementation. Throughout this illness, her renal function remained stable (eGFR>55ml/min); however, the immunosuppression was reduced during her concurrent illness. One month later, creatinine was found to have risen to 150µmol/L,

and a biopsy of the left kidney demonstrated mild interstitial inflammation and tubulitis consistent with acute cell-mediated rejection (Banff category 3), which was treated with methylprednisolone, in addition to low levels of detectable BK virus. Renal function is now continuing to improve, and the most recent eGFR is 31ml/min.

4.4.3 Discussion

The use of donors from advanced age groups is ever-increasing. Following the introduction of the revised UK kidney allocation scheme, donors from the highest risk category (D4) where the donor age exceeds 70 years will be offered as a pair at the recipient centres' discretion. Dual kidney transplantation (DKT) has been shown to be a potential mechanism to maximise the utility of kidneys from extended criteria donors whilst decreasing organ decline rates (Gallinat et al., 2011; C. M. Lee et al., 1996; Mallon et al., 2015; Mendel et al., 2018; Remuzzi et al., 2006; Rigotti et al., 2014). Lee et al. (2020) reported on 15 DKTs from donors older than 70 years; CIT was quoted at under five hours (+/- 109 minutes), considerably less than the average UK deceased donor programme CIT of 13.1hours (April 2020 to March 2021). The reported rate of delayed graft function with DKT was 20%, and it is of interest the group utilised donor demographics (e.g. age, serum creatinine and eGFR) rather than the more commonly used Remuzzi pre-implantation histology grading system due to lack of access to this service (K. W. Lee et al., 2020).

Providing more granular data on the CIT of each kidney, Mendel et al. (2018) published on 39 DKTs, where CIT was 18h18 minutes for the first kidney and 19h20 for the second implanted kidney (mean value, +/- 4h02 and 4h17, respectively). There was a 49% (19/39) rate of surgical revision, and 5 of 39 (12.8%) had a single graft removed due to venous complication. Despite the above complications, however, the group reported superior renal function with DKT as opposed to matched single kidney transplants with no difference in graft survival at one year (Mendel et al., 2018). The meta-analysis by Montero et al. (2018) with over 9000 participants corroborated the above with a higher rate of surgical complication with DKT, yet found superior graft function at one year and better patient survival and 5 years (Montero et al., 2018). In addition, the UK registry demonstrated similar 5-year graft survival with DKT compared to single transplants, with adjustment for confounding variables (Ibrahim et al., 2020).

Tanriover and colleagues reported the use of a donor risk scoring system (Kidney Donor Profile Index, or KDPI), in which a KDPI > 90% where there is a higher rate of organ

decline, to retrospectively assess decisions surrounding DKT. The group found DKT with KDPI>90% was associated with lower graft failure and improved patient survival compared to single transplants within the same donor risk stratification (Tanriover et al., 2014).

Recipient team discretion has been the mainstay in decision-making of dual vs single kidney transplantation, whether that be utilising information via pre-implantation biopsy or donor risk scoring tools. With the new UK allocation system, alongside the currently recruiting PITHIA study, both of these methods can be used in conjunction. More specifically, the Remuzzi score of <5 suggests single graft implantation, 5-6 as dual and 7 or more suggests organ decline.

Regarding the use of perfusion techniques, Navarro et al. utilised hypothermic machine perfusion parameters (e.g., pressure flow index and concentration of glutathione transferase) to guide dual vs single transplant. To date, this has not been demonstrated with normothermic perfusion techniques (Navarro et al., 2008). In this case, the use of EVNP was not solely for graft assessment, and its primary rationale was to reduce cold ischaemic time in a graft which was felt to be particularly susceptible to this insult. However, EVNP did provide reassuring metrics during its perfusion, including moderate global perfusion (subjectively improved appearance to that of time of graft preparation on initial receipt of organ), good urine output and satisfactory renal blood flow (i.e., not high resistance).

The use of two grafts in a single patient to maximise nephron mass must be weighed against the possibility of each of these grafts having the potential to provide enough function for two recipients. In other words, DKT must not reduce the organ pool but be used in such grafts, which, when considered alone, would not be considered sufficient when transplanted as a single graft (i.e., at the extremes of acceptance). It is not possible to speculate the impact of the reduction in cold ischaemic time provided by EVNP in this case. From the clinical team's perspective, it was an attempt to improve variables which are within our control to maximise functional nephron mass in a vulnerable high-risk patient.

4.5 Prolonged Cold Ischaemic Time / Unwell recipients

4.5.1 Introduction

In the UK, the National Kidney Allocation Scheme, facilitated by the centralised NHS BT, provides named offers to a transplant centre for a specific patient. On receipt of the offer, it is common practice for the graft to be accepted and the proposed recipient to be contacted and brought to the hospital for pre-operative assessment, discussion and consent purposes. During this pre-operative assessment, it is not uncommon for unforeseen issues to be identified, resulting in the recipient being deemed unsuitable for the offered graft (e.g., acute illness, flare of chronic health issues, recipient decline). In this scenario, the graft is offered back to NHS BT for re-allocation. Given the logistic and time constraints of subsequent graft transportation to another unit, the graft may be offered again locally. In this scenario, the recipient centre is then provided with a matching run whereby the clinical team can select an appropriate local recipient. This method minimises cold ischaemic time by reducing further travel time with graft relocation.

The COVID-19 pandemic added complexity to the pre-operative assessment of proposed recipients. Obtaining a negative RT-PCR swab result for SARS-CoV-2 has been made routine in most transplant centres. This process, at the time of this report, could take anywhere between two and 12 hours. Recipients were, therefore, invited to the unit as early as possible to minimise delays in this pre-operative assessment which could delay transplantation. If the proposed recipient were to be deemed unsuitable, contacting and admitting a second recipient at short notice can lengthen, sometimes beyond reasonable limits, the cold ischaemic time of a graft.

There is growing evidence for the role of EVNP as a viability assessment tool to provide additional information to guide decision-making. The period of normothermic perfusion has also been shown, principally in the pre-clinical setting, to have potential reconditioning benefits with restoration of ATP and mitigation of IRI. It is commonly considered that once a graft is established in this pseudo-physiological state, cold ischaemic time is effectively ‘paused’.

4.5.2 Case Detail

A 66-year-old male DCD graft was offered to our transplant unit. The initial offer was made to Patient A, a peritoneal dialysis diabetic patient, who was contacted and duly attended the transplant unit. Bloods were drawn, and a swab for SARS-CoV-2 was obtained and sent to virology. At 08:00, upon consultant review, it became evident that the patient had an infected lower limb ulcer requiring antibiotic therapy and precluded transplantation.

The NHS BT hub was informed, and the decision was made to reallocate the graft to a local recipient, to prevent graft relocation. A second recipient (Patient B) was identified using the provided matching run. Patient B was a haemodialysis patient who had previously undergone a thoracoabdominal aortic graft. On admission, routine blood tests demonstrated elevated inflammatory markers (specifically raised C-Reactive Protein), and there was concern there may be subclinical infection of the graft, which was also deemed to preclude potential transplantation. The CIT of the graft at this point was 11 hours.

The graft arrived at the recipient unit at 21:00 and was promptly taken to the theatre and prepared for transplantation at the back table. The graft was a right kidney with single vessel anatomy; the appearance of the graft, however, was described as 'patchy perfusion' (i.e., incomplete clearance of blood). Cold perfusion fluid (Soltran, Baxter Healthcare) was administered with only partial improvement in the global appearance.

For an additional time, the matching run was referred to, and a third patient (Patient C) was considered. The third potential recipient was a 53-year-old female (blood group A) who was on pre-dialysis (eGFR of 8ml/min) and was a current inpatient in another acute renal ward following the elective insertion of a peritoneal dialysis catheter as they approached the need for renal replacement therapy. The primary renal disease was diabetic nephropathy alongside cystic disease. The mismatch was 111MM (0% cRF). At the time this patient was identified as a suitable candidate, there were 18 hours of cold ischaemic on the graft.

At this point, our unit had a DCD graft with increasing cold ischaemic time and a perfusion quality described as 'patchy'. Allowing time for consideration, discussion, and awaiting the result of SARS-Cov-2 RT-PCR it was felt the cold ischaemic time would increase beyond acceptable limits without an intervention such as EVNP. Furthermore, given the suboptimal graft appearance, EVNP was thought to offer additional information on perfusion characteristics and, therefore, aid the decision-making process.

EVNP was commenced at 23:30, utilising the previously published Nicholson protocol with a perfusate based on a unit of packed red blood cells, Ringer's lactate, nutritional additives, dexamethasone and heparin. Non-pulsatile pressure was maintained at 75mmHg and temperature at 37.5°C.

Following one hour of perfusion, good global perfusion was achieved and 160ml urine output was produced. Slightly high flow resistance was evident at the start of perfusion; however, this improved, and >50ml/min/100g of blood flow was achieved by the end of the hour (see Figure 4-12). The graft was deemed to have an EVNP quality assessment score of 2: Grade 1 Macroscopic appearance [1 point], renal blood flow < but 50ml/min/100g [1 point] and urine output >50mls [0 points]. Of note, given the improvement of resistance by the end of the perfusion duration, the graft could have arguably scored 0 points for flow - it was a clinical decision to be cautious in this regard. At one hour, the kidney was removed from the EVNP device and flushed with cold perfusion fluid before being placed back on the ice until implantation.

During this time, the patient's negative RT-PCR had returned, and the patient was prepared for theatre. The implantation began at 01:10 and in situ reperfusion occurred at 03:30, resulting in a total cold ischaemic time of 23 hours 52 minutes. Procurement warm ischaemic time was 15 minutes. Anastomosis time was 29 minutes. The kidney was seen to be producing urine before the ureteric anastomosis was formed. The procedure was complicated by a bleed from the renal vein (500mls blood loss) thought to be secondary to a slipped Ligaclip, which was controlled with a single additional suture; despite this, initial reperfusion was described as ‘good’, and Patient C recovered well post-operatively. There was no requirement for post-operative dialysis; creatinine levels fell from day two onwards, and primary function was achieved. Day one creatinine measured at 561 micromoles/L (eGFR 7 ml/min/1.73m^2). She had a routine post-operative course and was medically fit for discharge home on day 6 post-transplantation. Creatinine on discharge was 306 micromoles/L (eGFR 14 ml/min/1.73m^2). Following discharge, the recipient has continued to improve, achieving a creatinine of 166 micromoles/L (eGFR28 ml/min/1.73m2) at one-month post-transplantation. Renal function at one-year post-transplant remains largely static with creatinine ranging between 160 and 200 micromoles/L.

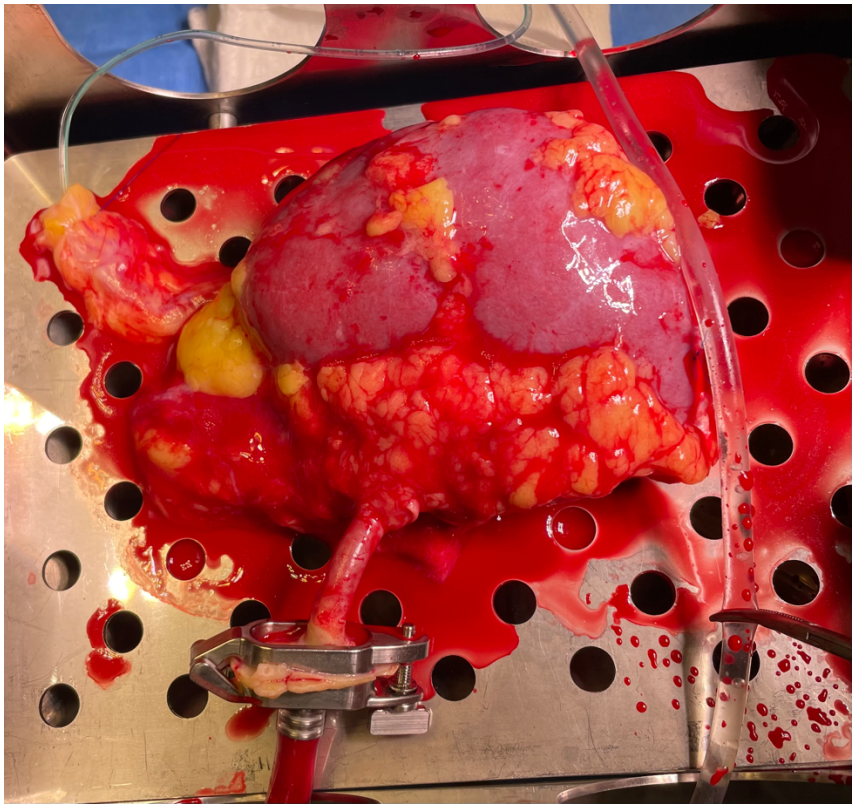


Figure 4-12: Image of kidney on ex vivo normothermic perfusion for 60 minutes. (EVNP assessment score = 1)

4.5.3 Discussion

As a clinical team, we do not believe this graft would have been implanted without the period of normothermic perfusion. This report outlines a clinical example, which is relatively unusual, in which multiple recipients were deemed unsuitable for transplantation, leading to increasing cold ischaemic times of a graft, potentially rendering the graft unsuitable for transplantation. EVNP has been shown to be a feasible tool in the assessment of grafts (Hosgood et al., 2018), particularly marginal grafts of uncertain suitability for transplantation. Increased cold ischaemic time in a US registry study was cited as the reason for the decline in 9% of all declined grafts (Lum et al., 2022). The reason for this hesitance to use organs with increased cold ischaemic time is well documented: the rates of delayed graft function and primary non-function (PNF) increase with an increase in cold ischaemic times (Summers et al., 2013). The aforementioned US study, however, found that when looking at graft function at one year and graft survival at 10 years, rather than primary function, the grafts with prolonged ischaemic time and a favourable risk index (KDPI<85%) were statistically indistinguishable to grafts with favourable ischaemic times and poor graft risk indices (KDPI>85%). Thus concluding that ischaemic time alone, in higher quality

organs, may not be a suitable reason for organ decline, as these grafts may provide years of dialysis independence (Lum et al., 2022).

KDPI and the UK ‘R4D4’ risk indices are based on recipient demographic data and act as aids for the transplant clinicians weighing up the offer of a graft for a named recipient. Other tools used to aid this complex decision-making process, such as preimplantation histology of grafts by way of retrieval biopsies, do not yet have a role in UK practice. The UK trial PITHIA, which aims to evaluate the impact of urgent preimplantation kidney biopsies, has completed recruitment; however, results remain pending (Ayorinde et al., 2019). The logistical challenges remain, however, in the complexity and financial implications of establishing a national, 24-hour, digital histopathology service. Furthermore, in the US, where pre-implantation biopsies are used more frequently, this tool has not conclusively yielded an increase in organ utilisation. Point-of-care tools, such as perfusion technologies, provide alternative means to assess marginal grafts.

The increase of cold ischaemic time is known to deplete the stores of ATP within a graft, which is thought to be a considerable factor in reperfusion injury and detrimental to early graft function. Although yet to be conclusively demonstrated in clinical trials, EVNP has been shown in experimental models to replenish ATP stores (Hosgood et al., 2013), hence why this technology is thought to have reconditioning benefits to organs. Marginal organs, therefore, with advancing cold ischaemic times, could be assessed during the period of perfusion for intra-vascular resistance, urine production and macroscopic appearance whilst also theoretically replenishing ATP stores, which have been diminished by the cold ischaemic time. Thus, providing more point-of-care information for the transplant decisions but also ameliorating some of the adverse effects of cold storage with the aim of improving early graft function.

This case outlines the role of EVNP in assessing a marginal organ and effectively pausing cold ischaemic time, which facilitated our transplantation unit to identify, prepare and prepare a recipient for transplantation and prevent organ decline.

4.6 Patient Testimony

Patient experience was sought from one of the patients who received a kidney transplant from a graft which was deemed suitable for transplant following a period of assessment with EVNP.

"I got the call exactly a week before Christmas to say there was a potential donor. I was told that the donor had problems with his blood vessels to one of his kidneys, and that they would operate on him and try to repair and if they could the kidney would possibly be donated to me. The surgery on the donor would take place the next day. I was told I would know by early afternoon. It was a few minutes after 16:00 when the door opened and the surgeon told me that there was a risk of blood clotting and asked if I wanted to go ahead. There was no doubt in my mind and I was happy to go ahead. I have total faith in my surgeon. I was taken down promptly and had a big grin under my mask. I did not feel nervous, only happy at this chance. Things went well and it was the best Christmas present. The kidney is doing well and I feel totally blessed and so grateful to donor."

4.7 Next Steps

4.7.1 Funding Application via NSD for Perfusion Nurse

The initial business case predicted that a minimum of 10 extra transplants would be generated in the first year. As detailed above, despite the significant impacts of the COVID pandemic, 7 additional transplants were performed, which would not have been performed without the use of EVNP. This business case to set up the EVNP hardware and service detailed (See Appendix A) that 10 functioning transplants would equate to a cost-benefit of £241k, approximately £24k per transplant per year of functioning graft, owing to the reduction in dialysis costs, as per NHSBT.

As a result of this work detailed above and our experience in the delivery of this technology, an application was submitted to the commissioning group in Scotland, National Services Division (NSD), for a two-year pilot of EVNP. The detail of this application was to progress from the current model, whereby the technology was delivered by a single research registrar, to a trained perfusion team, able to provide round the clock cover to deliver this viability assessment tool. The application requested two band 6 nurses who would be trained to be specialist nurses in organ assessment and reconditioning ('SNOAR') (see Table 4-2). The

business case primarily proposed support for the salary costs required to deliver this service in addition to the consumable cost of the perfusion kit.

In addition, retrospective analysis of our own donor offers added evidence to the application to demonstrate potential cost savings. There were 64 declined offers during the period of analysis, and reflective application of the SaBTO (Safety of Blood, Tissues and Organs) guidelines on donor disease and terminal creatinine excluded 29 of these offers from being suitable for transplantation. The remaining 35, however, were retrospectively considered, in principle, suitable for viability assessment by EVNP. The work in Cambridge on perfusion of discarded kidneys (Hosgood et al., 2018) and previous work in which histological analysis of discarded kidneys was performed (Callaghan et al., 2014) postulated that 50% and 45% of the discarded grafts in their series, respectively, were potentially suitable for transplant. A conservative value of 17% was used for the purpose of this funding application, and therefore, it was estimated an additional 6 transplants per annum would be facilitated, with a cost saving of £147,600 ongoing per year of function grafts, which offset the cost of the staffing requested.

Table 4-2: Detail of staffing request as part of the application to the National Services Division for trained perfusion nursing staff to provide EVNP service during a two-year pilot.

Staffing requirements and Salary costs
Year 1
• 2 wte. specialist nurses in organ assessment and reconditioning ('SNOAR')
• 2 x band 6 nurse salary @ £51,548
• Year 1 total cost £103,096
Year 2 – dependent on achievement of program milestones
• 2 *further* band 6 nurse salary @ £51,548
• 1 upgrade of salary band 6 to band 7 for identified team lead/clinical managent role @ £60,757
• Year 2 total cost £215,401

In April 2022, the Scottish Government approved this application and granted the funding for two band 6 perfusion nurses and 10 perfusion consumables for the financial year of 2022/23 with a total of £109,400 – this was on the NSD recommendation of a 2-year basis and subject to the outcome at the end of that period.

Despite the funding being allocated for these roles, the trust was not able to successfully appoint. The reasons for this, anecdotally, were twofold: 1) there was no one within our

current pool of nursing staff with the requisite skillset, and 2) external candidates who had previous experience were reluctant to take on a new role with novel technology with the risk that entails. Although anecdotal, this highlights potential issues other units may have in the progression from implementation to maintenance of a novel technology such as this.

4.7.2 Staffing

Given the difficulties in recruitment to the roles, as outlined above, the staffing of normothermic perfusion technologies requires significant consideration. The implementation of EVNP outlined in this thesis was dependent on a single research registrar being available 24 hours a day on a volunteer basis. Clearly, this is not a sustainable model in the ongoing delivery of such a technology moving forward.

Standard office hours in the UK are 9-5, with the remuneration of work beyond these hours routinely priced at a higher rate. With 16 hours each working day and 24 hours each weekend day, over 75% of the hours of a 24-hour working week would be considered out-of-hours and, therefore, very expensive to staff appropriately. Particularly given the level of expertise required to appropriately staff EVNP. This cost could, however, be offset by potential additional transplants, but only if there were sufficient numbers of perfusions taking place. The unpredictability of when and how often EVNP will be required adds to these logistical challenges in a single-unit service, as described herein.

4.7.3 Arterial Cannulation with Complex Anatomy

It is our belief that if ex vivo perfusion techniques were to become commonplace, particularly in the context of regional perfusion centres, issues with complex and varied arterial anatomy would come to the fore. Work is ongoing by our research team to design and develop a bespoke cannulation device which secures internally (akin to an endotracheal tube) with graduated calibre. This would allow a wider range of vessels to be safely cannulated without the need for ligature and, therefore, shortening of the vessel. In addition, work to evaluate the impact of multiple arterial connections on perfusion pressures (i.e. two cannulas, or cannula along Venflon) is critical to ensure predictable and reproducible perfusion to the grafts.

CHAPTER 5: THERAPEUTIC EFFICACY OF MESENCHYMAL STROMAL CELL POPULATION DELIVERED VIA ISOLATED ORGAN PERFUSION: A SYSTEMATIC REVIEW

5.1 Introduction

The prevalence of end-stage renal failure and subsequent requirements for renal replacement therapy continues to rise across the Western world. Renal transplantation is the gold standard treatment; however, the shortage of donor organs remains a key barrier in addressing the increasing recipient demand (*Statistics about Organ Donation - NHS Organ Donation Register | Organ Donation - English*, accessed March 2024). The proportional increase in the use of extended-criteria donors and DCD grafts is an attempt to address this shortage. It is well documented that such grafts are less resilient to the effects of IRI and, therefore, have been shown to demonstrate higher rates of DGF, PNF and reduced graft lifespan (Saidi RF, 2014; H. Zhao et al., 2018). IRI is a multifactorial phenomenon associated with a myriad of factors, including the duration of cold ischaemia, donor graft characteristics, and the recipient immunogenic environment (Carden & Granger, 2000). The result is a protective, yet non-specific, immunological reaction with activation of ROS and resultant damage to local tissue with both short- and long-term effects (Chouchani et al., 2014). Numerous studies have shown that this process diminishes nephron mass and predisposes it to hypoxic injury and graft immunogenicity, ultimately contributing to graft failure (M.-Y. Wu et al., 2018). Amelioration, or ideally prevention, of organ injury in this critical early phase is of particular research interest.

There is emerging evidence and interest in pre-implantation perfusion techniques both at the time of procurement and at the recipient transplantation unit. When delivered at the recipient centre, the graft is perfused ex-vivo for a duration in which the graft can be assessed (using the EVNP Quality Assessment Score), providing useful pre-implantation information. This can be particularly useful when grafts are considered marginal, or there are post-procurement concerns such as ‘poor perfusion’, a term often used for the description where there is incomplete clearance of blood from the graft (Hosgood, Barlow, Hunter, et al., 2015). The ex vivo perfusion can be of varying temperatures (most commonly normothermic vs hypothermic) and for a specific duration. The perfusate is circulated in a closed loop, which provides a unique opportunity to deliver a therapeutic agent directly to the isolated kidney, circumventing the drawbacks to systemic patient administration, such as pulmonary

sequestration and systemic side effects. Many therapeutic agents are being considered, including nanoparticles (NPs), gene therapies and stem cell therapies (DiRito et al., 2018), which will be discussed in greater detail for this review.

Amelioration, or ideally prevention, of organ injury in this critical early phase is of particular research interest. Regenerative cell therapies have arisen as potential therapies for both ischaemia-reperfusion, immunosuppression modulation and long-term graft protection (Torres Crigna et al., 2018). Furthermore, regenerative cells (or stem cells) are unique in their ability to differentiate into varying cell lineages from a common precursor. In particular, mesenchymal stromal cells (MSCs) can be harvested from a variety of sources, including bone marrow, umbilical cord, adipose tissue and blood. They have shown promise in renal therapy in animal models with the ability to be anti-apoptotic, regenerative and protective against acute kidney injury. This review will detail research conducted thus far in which regenerative cells are delivered directly to an isolated kidney or liver graft.

5.2 Methods

This systematic review was conducted in alignment with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRIMSA) Statement. A precursor literature review was conducted to identify suitable keywords. An extensive literature review was then conducted using the following databases: Embase (Ovid), Ovid Medline, Scopus, and BIOSIS Previews. The keywords used were as follows: ‘perfusate’ or ‘perfusion’ and ‘kidney’ or ‘liver’ or ‘transplant’ or ‘organ’ and ‘stem’ or ‘regenerative’ or ‘mesenchymal’ (see Figure 5-1). The search field includes all years and studies in all languages. The eligibility criteria were based on the following research question: what is the efficacy of regenerative cell delivery during isolated perfusion of kidney grafts?

5.3 Results

2852 articles were identified. Duplicates were removed, resulting in 977 unique entries. Two independent reviewers were used and identified 25 articles for inclusion, 15 kidney and 10 liver (see Table 5-1). An independent third reviewer was used to resolve 28 conflicts.

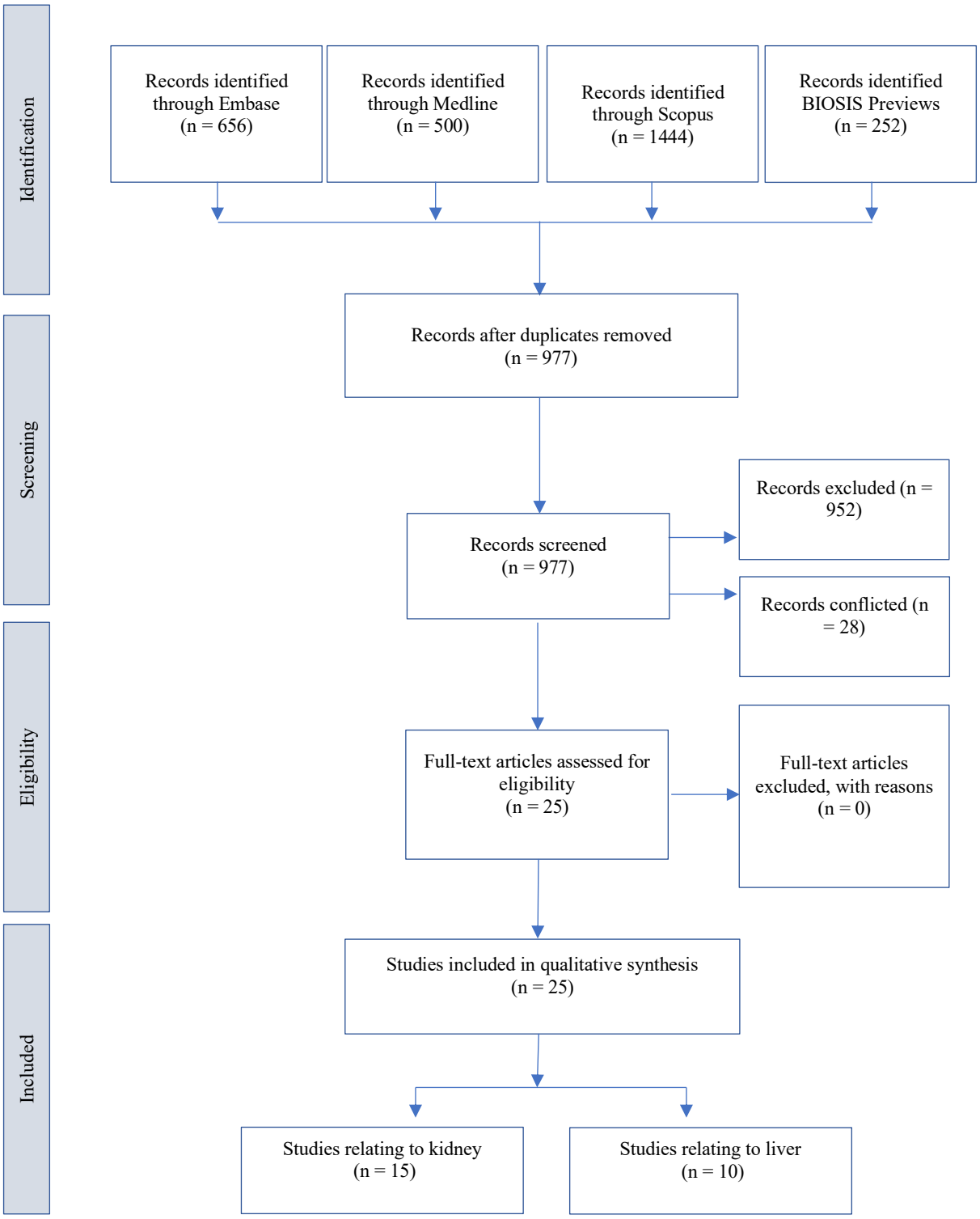


Figure 5-1: Search Strategy Flow Diagram; Adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram

Table 5-1: Summary of articles in which mesenchymal stromal cell populations are delivered via isolated organ perfusion in kidney and liver.

Theme	Study	Design	Subject Model	Objectives	Main Outcome Measures	Key Findings
Kidney	Rozenburg K et all. 2019	Oral presentation, currently unpublished; pre-clinical	Porcine	3 hours of hypothermic perfusion following by 7 hours of normothermic perfusion +/- adipose-derived porcine MSC	Renal blood flow, renal resistance, urine output. Cytokine production	Delivery of adipose-derived MSC feasible to porcine kidneys. Demonstrated improved renal blood flow, MSC located within glomeruli (dose dependent).
	Gregorini M et al. 2017	Published; preclinical	Rodent F344	Assess role of MSC-derived extra-cellular vesicles during 4 hours of hypothermic perfusion	Degree of ischaemic damage; Lactate production, glucose consumption,	MSC derived extracellular vesicles were protective against IRI by preserving enzymatic processes
	Sierra-Parraga JM et al. 2019	Published; preclinical	Human and porcine MSC	Assess the impact of normothermic perfusion conditions on peri-renal adipose-derived MSC + difference between human and porcine MSC	MSC viability, ROS production, ability to adhere to endothelial cells	Human MSC more resistant to perfusate suspension and freeze-thawing process. Ability for the MSC to adhere to endothelial cells reduced by perfusate conditions. Secretory profile maintained, but increase in pro-inflammatory cytokines with perfusate compared to culture medium.
	Pool et al. 2019	Published; preclinical	Porcine model	Feasibility of delivering MSCs during 7 hours of normothermic perfusion	Localization of MSC, cell viability	MSC were successfully delivered via normothermic perfusion and were found to localize to glomerular capillaries in a dose-dependent manner. Viability reduced to approximately 10% following perfusion.
	Pool et al. 2020	Published; preclinical	Porcine	Assess impact of MSC delivery during 7 hours of normothermic perfusion.	Lactate production, NGAL, cytokine production, renal blood flow	No different in functional outcomes (i.e. blood flow, urine output) with MSC delivery. Reduced levels of lactate, NGAL and NAG suggesting protection against renal tubular injury compared to control.
	Vallant et al. 2017	Unpublished thesis; preclinical	Porcine	Delivery of human MSCs to porcine kidneys during hypothermic perfusion (4 hours) followed by period of normothermic perfusion (2 hours)	Localization of MSC post-perfusion, functional parameters including blood flow, markers for IRI	MSCs were located within the kidney graft post-perfusion. NGAL levels higher with MSC treatment. Expression of TNF-a and EDN-1 also elevated with MSC treatment. Unable to conclude whether positive or negative in relation to protection against IRI.
	Eertman et al. 2017	Unpublished data; preclinical	Porcine	Delivery of pre-labelled MSC during 7 hours of perfusion to assess localization	Histological analysis for localization, perfusate analysis	MSC localized to cortex and glomeruli. MSCs found within perfusate after first pass through graft.
	Rocca et al. 2015	Abstract publication; preclinical	Rodent	Delivery of MSC via hypothermic perfusion for 4 hours.	Inflammatory mediate, markers for IRI	Increased ATP synthesis within treatment group. Reduced markers of oxidate stress (MDA). Concluded MSC protective against IRI.
	Renner et al. 2012	Oral presentation; preclinical	Murine	Labelled MSC delivered via hypothermic perfusion for 2 hours.	MSC localization and cell viability	Perfusate reduced viability of MSC compared to culture medium. MSC localized to glomerular structures by 30 minutes into perfusion.
	Thompson et al. 2020	Published; preclinical	Human	Bone marrow derived MSC delivered to kidney grafts deemed untransplantable perfused for 7 hours	Functional parameters, markers for IRI	Improved urine output following MSC delivery. NGAL reduced, but no difference evident in KIM-1. Improved medullary blood flow. Anti-inflammatory IL-10 increased in MSC group. MSC localized to glomeruli.
	Brasile et al. 2019	Published; preclinical	Human	Delivery of MSC via exsanguinous metabolic support (non-RBC based perfusion) for 24 hours	Cytokine production, histological analysis, functional parameters	Increased ATP synthesis and reduction in IL-6 and IL-10 with MSC treatment, increase in markers of mitosis. No change in functional parameters.

Liver	Rigo et al. 2018	Published; Preclinical	Murine model	Human liver stem cell-derived extracellular vesicles (EV) delivered via normothermic perfusion for four hours.	Bile production, oxygen consumption and transaminase levels. Histological evidence of ischaemic injury.	Reduced evidence of necrosis and apoptosis (reduced Suzuki score); although elevated in all groups, AST and ALT less elevated in EV treatment group. No difference in bile production. Protective against hypoxic injury.
	Laing et al. 2020	Published; Preclinical	Human	Delivery of multi-potent adult progenitor cells (MAPC) during normothermic perfusion for 6 hours via portal vein or hepatic artery	Functional parameters, organ viability, lactate levels, cytokine production.	No difference in flow parameters. 3 of 6 reached graft viability criteria. MAPC delivery upregulated cytokines including IL-4, IL-5, Il-6, IL10 and MCP-1. Delivery via hepatic artery appeared to improve delivery of cells to vascular endothelium compared to portal vein delivery. Concluded MAPC anti-inflammatory and protective against ischaemic injury.
	Verstegen et al. 2020	Published: Preclinical	Porcine	Delivery of hMSC via hypothermic perfusion following by period of normothermic perfusion for 4 hours	Histological analysis, localization of MSC	MSC found in patchy distribution throughout liver. No difference found between arterial or venous delivery. Il=6 and IL-8 elevated following MSC delivery.
	Yang et al. 2020	Published: Preclinical	Murine model	Delivery of BM-MSC to murine livers via normothermic perfusion for up to 8 hours	Histological analysis, functional parameters	Histology revealed less vacuolar degeneration, sinusoid congestion and inflammatory cell infiltrate with MSC treatment. Reduced mitochondrial damage, inhibition of macrophage activation with MSC treatment, which was thought to be protective against endothelial damage. Improved bile production and lactate clearance in treatment group.
	Yang et al. 2020	Published, preclinical	Murine model	Delivery of BM-MSC to murine livers via normothermic perfusion for up to 8 hours with corroboration of findings against IAR20 in vitro model	Downstream mechanism analysis, histological analysis.	MSC group demonstrated reduced ROS production, mitochondrial damage and increased the mitochondrial membrane potential. Mechanism of protection against IRI thought to be in part relative to JNK and NF-κB activation. The group concluded that the BM-MSCs downregulated the activation of JNK-NF-kappa-B and upregulated AMPK activation.
	Cao et al. 2020	Published: preclinical	Rodent model	BM-MSC transduced with HO-1 gene delivered via normothermic perfusion model	Histological analysis, transaminase levels, cytokine profile	Livers treated with HO-1 BM-MSC demonstrated improved Suzuki histological score (measure of ischaemic injury), reduced bile duct injury (using CK19 as surrogate marker) and reduced pro-inflammatory cytokines IL-6, IL-1B and TNF-a. Reduced expression of TLR-4 and its pro-inflammatory ligand HMGB-1 reported as evidence of anti-inflammatory properties of HO-1 BM-MSC.

5.3.1 Kidney

There are numerous widely used acronyms for the currently available perfusion techniques; for the purpose of this report, we will describe their temperature and device type rather than the often-interchangeable nomenclature. In summary, ex-situ or ex-vivo normothermic perfusion (EVNP), i.e., perfusion of a solid organ graft, is interchangeable with the also commonly used normothermic machine perfusion (NMP). Cold techniques utilising hypothermic conditions principally fall under the umbrella of hypothermic machine perfusion (HMP). Normothermic regional perfusion is an in-situ technique performed at the time of graft procurement, and studies relating to this technique are not included in this review.

A collaborative research group with colleagues in Oxford and Rotterdam presented data authored by Rozenberg et al. on porcine kidneys which underwent three hours of hypothermic machine perfusion (HMP) followed by a period of seven hours of NMP using an oxygenated, autologous blood-based solution containing albumin. At 1 hour, the kidneys were administered with the vehicle or a dose adjustment of porcine adipose-derived MSCs. The group found no difference in renal blood flow, renal resistance or urine output. They were able to demonstrate the predominant localisation of MSCs to the glomeruli but also in the capillary networks adjacent to the capillaries. The amount of which present in the glomeruli was dose-dependent. Over time, all groups were found to have an increase in prostaglandin E2 (PGE2). The group concluded from their work that the delivery of MSC via hypothermic perfusion, followed by normothermic machine perfusion, was safe and feasible (Rozenberg & Al, 2020).

An Italian group led by Gregorini (2017) assessed the role of MSC-derived extracellular vesicles delivered during hypothermic machine perfusion in a Fisher F344 rodent DCD model. Rat kidneys were initially cold perfused for four hours with Belzer solution with or without MSCs, which were isolated from EGFP transgenic SD rats' bone marrow, or extracellular vesicles (EV), which were obtained from the supernatant. The MSC and EV-treated kidneys demonstrated less global ischaemic damage; the effluent fluid demonstrated significantly lower levels of lactate, LDH and glucose, postulating a larger use of energy substrates. The group concluded that MSC with EV preserved the enzymatic machinery, thus protecting the kidneys from reperfusion injury (Gregorini et al., 2017).

Sierra-Parraga's group in Rotterdam have published extensively on the role of MSC delivered via normothermic perfusion utilising porcine models and has formed the international consortium Molecular Pathology Evaluation Panel (MePEP) to facilitate the

translation of this emerging therapy to human clinical trials (Sierra-Parraga et al., 2017). In 2019, the group published work on cryopreserved human MSC (hMSC), derived from perirenal adipose tissue, and porcine MSC (pMSC), derived from subcutaneous tissue. The group demonstrated that suspension in normothermic perfusate reduced the viability of pMSC by 40% and hMSC by 15%. Furthermore, they found that the freeze-thawing process impaired the survival of MSC, and also increased levels of reactive oxygen species and, as a result, reduced mitochondrial activity. The group also found that human MSCs behaved differently than the porcine equivalent (Sierra Parraga et al., 2019).

The key findings included the reduction in cell viability of MSC when suspended in perfusion fluid with hMSC more resistant to suspension conditions compared to the porcine counterparts; more specifically, they found impaired adhesion to endothelial cells, particularly when cells were subjected to the freeze-thawing process, particularly in perfusate compared to culture medium. The freeze-thawing process also increased levels of reactive oxygen species, particularly in the first hour after the thawing process. Of note, the secretory profile of angiogenic factors of MSC was unaffected by suspension within the perfusion fluid (VEGF, PDGF, ANG-1 and HGF); inflammatory cytokines (IL-6 and MCP-1), however, were increased in perfusion fluid compared to the culture medium.(Sierra Parraga et al., 2019)

Later in 2019, the same group published data on the feasibility of delivering MSCs to kidneys during machine perfusion. The porcine kidneys were perfused at 37 degrees for 7 hours, and at the one-hour point, various doses of human adipose-derived MSCs were added. MSCs were found to localise in the lumen of glomerular capillaries but only in the highest dose group. Flow cytometry found that the infusion process reduced cell number dramatically to 10% during perfusion, and only a small amount reached the glomeruli (M. Pool et al., 2019). The use of human cells in the porcine model was a recognised limitation, given the potential xenograft effect.

A recent publication by Pool et al. utilised 2-3 hours of hypothermia machine perfusion prior to a 7-hour period of normothermic machine perfusion at 37 degrees, during which human adipose-derived or bone marrow-derived MSCs were administered compared to control. Of clinical relevance, the porcine grafts used in this model had sustained ischaemic injury, mimicking the real-life transplant process. They demonstrated the kidneys perfused with MSCs had lower lactate levels and neutrophil gelatinase-associated lipocalcin (NGAL) levels for both adipose- and bone marrow-derived MSC compared to the control. Pro-inflammatory cytokines IL-6 and IL-8 were also found to be elevated in the treatment group.

There was no difference demonstrated in perfusion flow rate or urine output between groups. Creatinine clearance appeared greater in the adipose MSC group. However, this did not reach statistical significance, and it was concluded there was no difference between groups for functional markers. N-acetyl-beta-d-glucosaminidase (NAG), a marker of tubular injury, was significantly lower in the adipose-derived MSC group compared to the control, as well as NGAL and LDH, suggesting a lesser degree of renal injury in the treatment groups (M. B. F. Pool et al., 2020).

This led to the work using a porcine autotransplantation model in 2021, whereby adipose-derived mesenchymal stem cells were delivered via a normothermic perfusion circuit following a period of hypothermia. The group concluded the treatment was safe and feasible, yet failed to demonstrate an improvement in post-transplant levels of serum creatinine and glomerular filtration rate. Histology markers of kidney injury were unchanged, as were the levels of NGAL (Lohmann et al., 2021).

Utilising the aforementioned protocol by Pool and colleagues, in which grafts sustained 20 mins warm ischaemia, followed by 3-4 hours of oxygenated hypothermic perfusion and then 7 hours of normothermic perfusion. Pre-labelled adipose-derived MSCW were delivered, and the authors describe the distribution of the regenerative cells after 6 hours of perfusion, with the majority localising within the cortex and homing to the glomeruli. They also noted that MSCs were present within perfusate effluent, and therefore, not all cells are filtered out by the kidney in the first pass through the graft (M. Pool et al., 2019).

Vallant et al. (2017) perfused paired porcine kidneys for four hours at 4 degrees Celsius with or without administration of human MSCs and subsequently perfused at normothermic temperature for two hours. The MSCs were located within the kidney post-perfusion. The expression of TNF-alpha, NGAL and EDN-1 was higher in the kidneys treated with MSCs. No difference was found in functional parameters. The group were unable to determine whether the effect of MSCs was positive or negative in relation to the IRI (Vallant, 2018).

An abstract publication by Rocca in 2015 documented a rodent model of 4 hours of hypothermic perfusion (4 degrees) with Belzer UW solution (control) compared to Belzer solution infused with rodent MSC. The group found upregulation of SRP-dependent co-translational protein, the citric acid cycle and ATP synthesis in the treatment group. A marker of oxidate stress, malondialdehyde (MDA), was also found to be reduced in the MSC treatment group, and they concluded MSC may be protective against IRI by upregulating genes contributing to energy saving (Rocca et al., 2015). The same group also presented data demonstrating MSC localisation to the interstitium, tubules and glomeruli with a reduced

histological damage score using tubular casts as a marker of tubular necrosis in the kidneys perfused with Belzer plus rodent MSC (Pattonieri, 2014).

In 2012 an oral presentation by Renner et al. presented work on the feasibility of MSC delivery in a murine model. CFSE-labelled MSCs were delivered via ex-vivo hypothermic perfusion for two hours. The cells were located in or near glomerular structures at 30 minutes. However, the group noted a significant reduction in cell viability in perfusate (38%) compared to 104% in culture medium (Renner et al., 2012).

Following on from a feasibility study (Thompson et al., 2018), Thompson et al. used human kidneys (retrieved but deemed non-viable) and perfused at normothermia for 7 hours with an oxygenated red cell-based perfusate. Human commercially sourced bone marrow-derived MSCs were labelled (using CellTracker Red CMPTX) and delivered following 1 hour of perfusion. The viability of the cells pre-infusion was 91%. The treatment group exhibited higher urine output compared to control. The biomarker NGAL was reduced in the treatment group. However, no difference was found with KIM-1. The treatment group also exhibited improved restoration of medullary blood flow at the four-hour time point, measured by microflow Doppler imaging. Regarding cytokines, IL-1beta was reduced, whilst the anti-inflammatory IL-10 was increased in the MSC group. The group were able to localise the cells in the glomeruli and peritubular space following the 7-hour perfusion duration. Concluding both functional and biochemical improvement in the MSC treatment group (Thompson et al., 2020).

In the Netherlands, Brasile et al. presented data on paired human kidneys perfused using exsanguinous metabolic support (EMS) at 32 degrees for 24 hours. The MSC treatment group demonstrated a reduction in inflammatory cytokines and an increase in ATP synthesis. Nuclear staining was also found to increase in mitotic figures, corroborated by PCNA and Clusterin staining (Brasile & Stubenitsky, 2017). The publication in 2019 further detailed the EMS system used: an acellular oxygenated medium with a disposable organ chamber. Commercially sourced MSCs were frozen and thawed, and labelled with PKH26 Red Fluorescent Cell Linker Kit. The infusion of MSC did not impact functional parameters such as renal perfusion pressure, vascular flow or oxygen consumption. Post-perfusion histological examination could not identify labelled cells that had travelled into the renal parenchyma, and within this acellular perfusate, the MSC were thought to have remained within the circulating perfusate (>95% of cells found in the perfusate post-perfusion). Despite remaining in the perfusate, the MSC treatment group exhibited increased ATP synthesis and reduced the pro-inflammatory state with a reduction in the inflammatory

cytokines IL-6, IL-10 and IL-1beta. It was postulated the reduced inflammatory state may have impeded endothelial cell adhesion. H&E staining of the grafts demonstrated the MSC treatment group to exhibit a higher number of cells undergoing mitosis. The finding of mitosis, the group postulates, is evidence of actual renal regeneration, which, whilst present in the control group, was enhanced by the MSC treatment (Brasile et al., 2019).

5.3.2 Liver

Hou et al. (2018) utilised an animal model to investigate the protective role of bone marrow-derived MSC on biliary epithelial cells when delivered via an NMP circuit. A DCD rodent model was used as livers were perfused for 4 hours in normothermic conditions. Control groups included in situ perfusion with DMEM bases culture solution with BM-MSCs and SCS group. Survival was improved in the NMP group, and in particular, evidence of biliary epithelial damage was reduced. The biliary progenitor cell marker, cytokeratin 19 (CK19), was found to be reduced following SCS alone and preserved with normothermic conditions. There was less bile duct dilatation and inflammatory cell infiltrate compared with SCS livers, and the group concluded that NMP with BM-MSC treatment was protective against biliary epithelial cell damage following warm ischaemia (Hou et al., 2019).

Assessing the paracrine effect of MSC by evaluating the role of extra-cellular vesicles, Rigo et al. (2018) examined the potential role of human liver stem cell-derived extra-cellular vesicles (HLSC-EV) delivered during normothermic perfusion in a murine animal model. The livers were perfused for four hours. They measured bile production, oxygen consumption and 'cytolysis' parameters such as AST, ALT, and LDH. The engraftment of the extra-cellular vesicles was then quantified by way of immunofluorescence. The perfusion solution contained Williams E Medium, and HLSC-EV were administered 15 minutes into the perfusion. There was evidence of histological damage (necrosis and apoptosis), which was reduced in the treatment group, evident in the reduction of the Suzuki score (Suzuki et al., 1993). Biochemical analysis showed an increase in AST, ALT and LDH levels throughout perfusion in both groups. However, the EV group had lower levels of AST and LDH at three hours. There were no differences in ALT levels between groups nor a difference in bile production. The EVs were evident within hepatocytes on immunofluorescent analysis post-perfusion. Concluding a role in HLSC-EV in protection against hypoxic injury during NMP (Rigo et al., 2018).

More recently, Laing et al. (2020), from the Birmingham group, perfused six discarded livers using the Organ Assist device for six hours. $50x10^6$ multipotent adult progenitor cells

(MAPC) were delivered either via the portal vein (n=3) or the hepatic artery (n=3). The group found no difference in flow parameters with infusion of MAPC by either route. Three of the six livers reached established criteria for organ viability, i.e., metabolised lactate to below 2.5 mmol/L within two hours of perfusion. The MAPC used were bone-marrow-derived, commercially sourced MSC, but differ from standard MSC in their cellular phenotype, specifically, negative for CD140a, CD140b, alkaline phosphatase and expression of the major histocompatibility complex class I at lower levels. A cytokine/chemokine analysis performed using a multiplex analysis identified nine targets which were upregulated following administrated of the MAPC and included IL4, 5, 6, 8, 10, MCP-1, SDF-1 alpha, IL-1 beta, and GM-CSF. The MAPC were identified within the liver grafts using fluorescence microscopy. However, the cells were not found to pass into the left lobe of the liver, perhaps suggesting the cells may have been trapped within the disposable perfusate circuit if they did not engraft on the first pass. Cells delivered via the portal vein appeared to localise within the sinusoidal channels in comparison to the vascular endothelium when delivered via the hepatic artery. Furthermore, given the arterial supply to the bile ducts, the presence of cells adjacent to bile duct endothelial was proposed to be potentially protective against clinically relevant bile duct damage, which often occurs upon organ reperfusion. IL-4 and IL-10 activity was seen to increase following infusion with MAPC, as these two have been shown to be protective in hepatic IRI (Walker et al., 2010). The group concluded the hepatic artery delivery route is potentially beneficial to cellular engraftment, and the MAPC infusion upregulated key markers, which have been previously shown to be anti-inflammatory and immunomodulatory (Laing et al., 2020).

Also analysing the paracrine mechanism of MSCs, Verstegen et al. (2020) published work on the delivery of human MSCs during a 30-minute period of hypothermic oxygenated machine perfusion of 8 porcine liver grafts, which was followed by a period of 4 hours of normothermic oxygenated perfusion. The hMSC, via bioluminescent imaging, were seen in a patchy distribution throughout the liver. There were no apparent differences between arterial or portal veinous delivery. The regenerative cells were found to maintain their paracrine activity after infusion, and levels of IL-6 and IL-8 were elevated. In pig livers that were not infused with hMSC, no IL-6 or IL-8 was detected. Thus, concluding that the evidence of paracrine activity of hMSC after delivery indicates regenerative and immune-modulatory activity during this normothermic perfusion technique (Verstegen et al., 2020).

Yang et al. (2020) also utilised bone marrow-derived MSC in a murine model. The livers were obtained following ligation of the rat aorta and were therefore harvested in a method

akin to DCD donation. The livers were perfused at normothermic conditions for up to 8 hours. The control group was static cold storage, and the two treatment groups were NMP alone or NMP with MSC delivery. The MSCs were harvested from the femur/tibia of the sacrificed rats within the model and were not commercially sourced. Both NMP and NMP with MSC treatment improved liver function and reduced apparent histological damage: The livers subjected to SCS alone demonstrated severe hepatic vacuolar degeneration and hepatic sinusoid congestion; by comparison, the livers treated with MSC had almost no vacuolar degeneration, hepatic sinusoid congestion, or inflammatory cell infiltration, and had less hepatic sinusoid congestion, all findings were superior to the livers treated with NMP alone. Mitochondrial damage was also found to be reduced with NMP and NMP with MSC. Furthermore, MSC delivery, compared to NMP alone, inhibited intra-hepatic macrophage activation, and was protective against endothelial damage and improved microcirculation perfusion. The main difference was thought to be the protection of sinusoidal endothelial injury with MSC by way of macrophage activation inhibition and intercellular adhesion. Post-perfusion histology confirmed the presence of colonisation of the MSC within the hepatic sinusoids. The MSC group demonstrated lower ALT and AST levels than NMP alone, and also significantly higher bile production at each time point. The improvement in microcirculation was thought to be due to the regulation of the powerful vasoconstrictor, ET-1 (Yang, Cao, Sun, Hou, et al., 2020).

Further work by the same group again looked at the same four distinct groups: Control, SCS, NMP and NMP treated with MSC. The work was corroborated by an in vitro model of oxidative stress using rodent liver epithelial cells, known as the IAR20. To induce hypoxic conditions, the cells were stimulated with H202 at various concentrations for 30 minutes. The NMP-treated group demonstrated attenuation of DCD hepatocyte apoptosis, a difference which was more marked in the NMP with MSC treatment group, demonstrating that MSC was superior to NMP alone in this regard. The MSC treated group demonstrated significantly inhibited ROS, with reduced mitochondrial damage and increased mitochondrial membrane potential level. MPO is a peroxidase responsible for the production of oxygen free radicals, which are harmful during IRI, and was used as a marker of oxidative stress. GSH, an antioxidant, was also used as a biomarker for oxidative damage. MPO was lower, and GSH was higher in MSC-treated liver grafts, and the group concluded that the MSC are protective against oxidative damage. This was corroborated in the IAR20 oxidative in vitro model, where treatment with BM-MSCs improved levels of GSH and reduced ROS production. The mechanism of this was thought to be in part due to JNK, a subfamily member of the MAPK, and part of the MAPK cascade (Nakano et al., 2006; Yang, Cao, Sun,

Hou, et al., 2020). JNK is known to respond to various stress stimuli and induce NFKB activation. The JUN N-terminal kinase-nuclear factor kappa B (JNK-NF-kappa-B) signalling pathway in the IAR20 model was also found to be downregulated in the MSC group, suggesting that this may be elucidating the mechanistic effect of MSC. AMPK activation is also regulated by metabolic stress and is affected by ROS activation. AMPK phosphorylation levels were elevated in the treatment group, as was p-ACC, a downstream marker of AMPK activity. The group concluded that the BMMSCs downregulated the activation of JNK-NF-kappa-B and upregulated AMPK activation (Yang, Cao, Sun, Hou, et al., 2020).

Cao and colleagues used modified bone marrow-derived (BM)-MSCs in a DCD rat liver model. The BM-MSCs were cultured and transduced with the HO-1 gene, which is a cytoprotective enzyme which has anti-oxidant and anti-apoptotic properties. The HO-1 co-culture was not shown to alter the morphology of the BM-MSCs, and delivery of the cells via an NMP model demonstrated HO-1 expression within the transplanted liver. The delivery of HO-1 BM-MSCs reduced levels of ALT and AST, improved histological appearances (preserved lobular structure), and reduced Suzuki scores. CK19 is a marker seen in bike duct epithelial cells, and due to the fact that it is reduced in injured bile ducts, levels can be used as a marker of bile duct injuries. Levels of CK19 were higher within the BM-MSC NMP treatment group, and this effect was greater with MSCs which were pre-treated with HO-1. The HO-1-treated BM-MSC also reduced the pro-inflammatory cytokines (IL-6, Il-1B, TNF-a) and reduced the expression of TLR-4 and NF-κB pathway-related molecules. The molecule HMGB-1 was also found to be transiently elevated post-perfusion but reduced post-op day 7 in both MSC and HO-1MSC groups, and it found that HO-1 augmented this reduction in the pro-inflammatory HMGB-1, which is known to be an important ligand of TLR4. The group demonstrate the effect of NMP with BM-MSC but also the potential to augment MSC with pre-treatment to target specific pro-inflammatory pathways (L. Wu et al., 2022).

5.4 Discussion

The work described in this report was thought to fall into two main sub-categories of mechanistic action of the regenerative cell populations.

5.4.1 Anti-Inflammatory

IRI is the result of a cascade of inflammatory processes inherent in the transplantation process (i.e., procurement, storage, and reperfusion). Ameliorating, or mitigating against, some of these inflammatory processes is an important improvable variable in transplantation. MSC populations have been demonstrated to be anti-inflammatory in numerous clinical scenarios, such as inflammatory arthritis, bowel disease, heart disease and ischaemic stroke (Ankrum & Karp, 2010; Ayala-Cuellar et al., 2018). The cytokine storm of IRI upon reperfusion is a key part of the inflammatory process and results in nephron damage.

Pre-clinical studies have demonstrated the potential role stromal-derived MSCs may play in the mitigation of IRI (Gu et al., 2016; Tarng et al., 2016; L. Zhou et al., 2017). The cells have been shown to secrete cytokines/chemokines and various growth factors which modulate the regenerative properties of the renal epithelial cells. The delivery of such treatments, however, has posed practical challenges, including but not exhaustively: Dosing, first-pass metabolism, and side effects, both short- and long-term. Isolated delivery of this cell population via a perfusion technique addresses many of these obstacles, yet is not without consideration. Perfusion of a graft at normothermic conditions without treatment has been shown to increase inflammatory cytokines within the perfusate (Hosgood, Barlow, Hunter, et al., 2015). In an attempt to address this, the Cambridge group have developed a cytokine filter to add to the perfusion circuit, however it does not discriminate pro- from anti-inflammatory cytokines.

Lohmann et al. (2021), in the autotransplant model with the delivery of adipose-derived MSC, demonstrated the potent inflammatory cytokine IL-6 to be increased in all groups, but no difference was found with the delivery of AD-MSCs (Lohmann et al., 2021). In contrast, Brasile et al. (2019) demonstrated a significant reduction in inflammatory cytokines. IL-6, IL-10, TNF-alpha, MCP-3, and IL1-β were all downregulated in the MSC treatment group. The reduction in TNF-alpha was particularly pronounced, leading to the postulation that reduction in this key pro-inflammatory cytokine leads to less ICAM expression on the surface of vascular endothelial cells, thus reducing diapedesis and transfer of immune cells. In addition to this, Brasile's group demonstrated the perfused MSCs were found to be located

principally in the microcirculatory system and increased ATP stores within the medullary tissue, the area thought to be most at risk of ischaemic injury (Brasile et al., 2019).

Pyruvate plays a key role in ischaemic cell energy metabolism, and it is thought pyruvate levels are reduced in ischaemic conditions, and the administration of pyruvate can be protective against ischaemic damage (Salahudeen et al., 1991). The model demonstrated by Gregorini et al. (2017) utilising MSC from rodent bone marrow delivered in an isolated hypothermic perfusion model demonstrated elevated pyruvate levels in the kidneys treated with MSC and proposed this as evidence of the MSC anti-inflammatory effect. Furthermore, renal ischaemic damage at histology was decreased in those kidneys perfused with MSC-EVs, ultimately postulating that the MSC-EVs provided stability to the enzymatic machinery, key for cell viability, and protection against organ injury (Gregorini et al., 2017).

5.4.2 Anti-oxidative Stress

There is increasing data on the protective properties of MSCs against oxidative stress. This has been shown in diabetic kidney injury, fatty liver disease retina, brain and bone, amongst others (Domingues et al., 2019). This is thought to be due to multiple effects, but broadly speaking, either directly via ROS scavenging, suppressing inflammation, mitochondrial donation/transfer, or via indirect pathways by increasing anti-oxidant defences a (Stavely & Nurgali, 2020). The redox state is commonly defined as the balance between oxidants (pro-oxidants and anti-oxidants. During injury, and particularly periods of hypoxia seen in graft ischaemia, the deviation from this redox state leads to a shift towards a pro-oxidant state with increased free radicals, such as ROS (Sies, 2015). This, in turn, causes a cascade of inflammation, ultimately leading to leucocyte activation, cytokine storm and tissue injury. Furthermore, the ROS increase leads to mitochondrial oxidative phosphorylation, which impedes the process in which ATP is produced, the key energy provider within a cell (Malek & Nematbakhsh, 2015). There are many key pathways and molecules within this energy production pathway which are often used as markers of oxidative injury or targets for therapy.

Gregorini (2017) demonstrated the anti-oxidant effects of MSC via isolated perfusion with altered mRNA expression of key markers of oxidative stress in the treatment group. Isocitrate dehydrogenase 2 (idh2), NADH dehydrogenase Fe-S protein 8 (Ndufs8), and pyruvate dehydrogenase beta were all found to be elevated in kidneys which were perfused with MSC and extracellular vesicles compared to the control, which was Belzer solution alone.

Malondialdehyde, another commonly used marker of oxidative stress (Khoubnasabjafari et al., 2015), was down-regulated in the MSC+EV group, along with LDH and lactate levels (Gregorini et al., 2017); similar findings were demonstrated by Rocca et al. 2015 with a decrease in levels of MDA in the treatment group in a hypothermic perfusion model (Rocca et al., 2015). NADPH is crucial in the production of glutathione, a potent anti-oxidant, and the group also found elevated Idh2 (the gene which encodes NADPH) (Liu et al., 2011).

Brasile's group demonstrated a significant increase in the concentrations of ATP within the renal tissue in a normothermic perfusion model with treated MSC, a finding predominantly seen within the medulla. This evident increase in ATP was thought to be crucial in synthetic renal function and the regenerative capacity of the renal epithelium (Brasile et al., 2019).

Adding to the above, Yang et al. (2020) demonstrated by way of a rodent liver DCD model in which MSCs were delivered via a normothermic perfusion model. Where MSC treatment was given alongside an 8-hour period of normothermic perfusion, there was a significant reduction in the level of myeloperoxidase (MPO), MDA and mitochondrial damage, suggesting protection against oxidative stress. NF-κB and AMPK pathways were also maintained in the MSC alongside the NMP treatment group, both of which are linked to oxidative injury (Yang, Cao, Sun, Lin, et al., 2020).

Mitigation against, or at the least amelioration of, oxidative injury in the context of organ transplantation is a key strategy to protect grafts from the process of transplantation. MSCs clearly show promise in this regard and may augment perfusion strategies, particularly for high-risk marginal grafts. The choice of where to source this regenerative cell population is, of course, a key practical decision which requires further work. Commercially sourced cell populations provide reliability but come at a cost and often require culture expansion. Point-of-care cell populations such as adipose-derived MSC may offer advantages in terms of convenience and compatibility at a molecular level, but harvest and processing time are important considerations.

5.5 Conclusion

Improving the quality of the solid organ grafts available for transplantation is a key research interest. Ex vivo or isolated circuits where treatments can be delivered directly to the graft offer a unique opportunity to bypass systemic circulation and, therefore, limit potential side effects to the host.

Data presented herein in this report demonstrates the potential role of mesenchymal stromal cells for the treatment of grafts pre-implantation. The next steps of the research require collaboration and consideration as to how best such treatment technologies can be made deliverable on a wider scale and how the assessment of grafts can become reproducible and communicable between perfusion and implantation centres. Furthermore, comparisons of the subtypes of MSC, dosing studies, optimal storage requirements and preparation of the cell populations alongside long-term safety considerations are important next steps.

CHAPTER 6: DELIVERY OF ADIPOSE-DERIVED REGENERATIVE CELL THERAPY DURING EX-VIVO NORMOTHERMIC PERFUSION

6.1 Introduction

In order to mitigate the pathophysiology of IRI, there is increasing interest in kidney perfusion techniques, both at the time of organ procurement and following explantation, such as ex-vivo perfusion. The optimal timing, duration, temperature and contents of the perfusate are all independent areas of ever-emerging research. Normothermic perfusion of organs at the recipient centre has been demonstrated in a clinical setting in the heart (Ardehali et al., 2015), lung (Cypel et al., 2012), liver (Nasralla et al., 2020) and more recently, as discussed extensively in previous chapters of this thesis, kidney solid organ transplantation. The perfusion circuit allows warmed, oxygenated blood to be circulated through the kidney, re-establishing a pseudo-physiological state, and crucially facilitates the following opportunities: a period of reconditioning by re-establishing oxygenation and altering the mechanism of IRI; viability assessment to aid the transplant decision-making process in marginal grafts; and of particular interest, a potential mode for therapeutic intervention.

The assessment opportunity provided by the period of perfusion has been extensively discussed in prior chapters within this thesis. One of the key benefits, however, of an EVNP circuit is the ability to deliver therapeutics to the solid organ whilst being perfused, thus allowing localised treatment to the organ whilst circumventing the systemic delivery of the agent to the transplant recipient. Numerous studies have investigated the potential benefit of agents added to the perfusion circuit in animal models, including stem cells, gene therapies and nanoparticles.

As discussed previously within this thesis, regenerative cell therapies such as MSCs have been demonstrated to have regenerative properties in many organ systems, including the kidneys. The anti-inflammatory cytokines, growth factors, and immunoregulatory mediators can mitigate against medullary inflammation and fibrosis in atherosclerotic renal injury (Eirin et al., 2015; Nooshabadi et al., 2018), and preserve renal function and prevent fibrosis in a porcine transplant model (Baulier et al., 2014).

The difficulty, however, is the clinical delivery of such a therapy given that such cells invariably fail to migrate beyond the pulmonary circulation and can be short-lived with

significant cell attrition rates with intravenous delivery (Eggenhofer et al., 2014; Fischer et al., 2009). Intra-arterial delivery has been reported in porcine models and is demonstrated to lead to improved localisation and accumulation of the regenerative cells, predominantly within the renal cortex, adjacent to the glomeruli, capillary networks and tubules (Sierra-Parraga et al., 2017).

The delivery of such therapies via normothermic perfusion techniques remains relatively unexplored. In 2019, Pool et al. demonstrated the feasibility of delivering commercially sourced bone-marrow-derived mesenchymal stem cells in a normothermic perfusion circuit and reported the clustering of the regenerative cells in the glomeruli but found a significant cell death rate leaving only 10% of viable cells during the period of perfusion (M. Pool et al., 2019).

In the UK, Thompson and colleagues utilised declined human kidneys (retrieved but considered unsuitable for transplantation) to deliver cell therapies. These kidneys were perfused at normothermia for a duration of 7 hours, and commercially sourced human BM-MSCs were administered after the first hour of perfusion. The grafts receiving MSC treatment demonstrated increased urine output and reduced levels of the biomarker NGAL, often used as a marker of tubular injury. Of note, the research team localised the cells in the glomeruli and peritubular space after the 7-hour perfusion duration (Thompson et al., 2020).

As aforementioned, mesenchymal stromal cells have been shown to be harvested from adipose tissue. Work by our own laboratory at the Institute of Cardiovascular Medicine, affiliated with University of Glasgow, Scotland, has attempted to elucidate the mechanistic effects of adipose-derived regenerative cells in a rodent model of ischaemia reperfusion injury (Lathan et al., 2019). We have demonstrated that following ischaemia, ADRCs appear to improve renal function in the short- and medium-term, which was associated with an upregulation of key immuno-modulatory cytokines.

ADRC can be delivered during EVNP without negotiating the pulmonary circuit, with direct access to the target organ during a period of perfusion within a closed circuit. In addition, due to the cell number that is available from adipose tissue, this cell population does not require culture expansion like many other MSC subtypes. ADRC can be harvested, therefore, and delivered directly to the target organ during a period of perfusion. To our knowledge, there are no published studies to date demonstrating the efficacy of ADRC within an ex vivo normothermic perfusion circuit to assess their role in amelioration of IRI within the human kidney following a period of static cold storage.

6.1.1 Aims and Objectives

This study involved the administration of ADRC directly to the kidney via an ex-vivo perfusion circuit with warmed-oxygenated blood. The utilisation of this circuit provided us with a unique mode of delivering this therapeutic agent. During the period of perfusion, perfusate samples can be taken at various time points and further analysed within our laboratory facilities for recognised markers of renal injury, including TGF-β, IL1- β, and NGAL.

Urine production was measured and analysed. Furthermore, as the kidneys were not subsequently transplanted, histology of the grafts was performed to assess levels of fibrosis and tubular damage. This also provided an opportunity to further assess safety of ADRC delivery as the histology would provide an opportunity to assess the grafts for any evidence of thrombosis.

The aim of this study was to assess whether adipose-derived regenerative cells can be administered during ex-vivo normothermic perfusion of the human kidney and to evaluate the effect this therapy has on the immunogenic environment.

The objectives of this study were to elucidate:

- To examine whether ADRC can be safely delivered to a kidney during ex-vivo normothermic perfusion
    - *Endpoint:* Assess using post-perfusion histology for evidence of glomerular thrombus
- To assess how ADRC treatment affects the immunogenic environment, e.g., cytokine release and inflammatory mediation.
    - *Endpoint:* Assess cytokine levels and markers of renal tubular injury using Luminex assay

Further understanding of the mechanism and role of ADRC modulation of the immunogenic environment and evidence of this treatment's safety could advance this work to a clinical trial in which kidneys are perfused with EVNP, with or without ADRC administration, and then subsequently transplanted. Ultimately with the goal of reducing the deleterious impact of IRI and improving early transplant function.

6.2 Materials and Methods

6.2.1 Study Sponsors, Application and IRAS Submission

A study protocol was prepared and submitted to the NHS Greater Glasgow & Clyde (GG&C) Health Research Authority to request a study sponsor. This application, as aforementioned, outlined the background of perfusion technologies and treatment opportunities in the context of kidney transplantation. A sponsor was granted (GN19RE465), and the study protocol was refined with assistance from experts in the field across the UK (see Appendix C). In September 2019, a final version was ratified and submitted to the Integrated Research Application System (IRAS) for consideration of ethical approval.

6.2.2 Ethical Approval

The IRAS application (Project ID: 269613) was submitted in March 2020, and the study was considered for proportionate review by the London – Westminster Research Ethics Committee. In April 2020, the Proportionate Review Sub-Committee confirmed favourable ethical opinion (see Appendix D) (REC reference: 20/LO/0256). This successful application was then communicated with our local sponsor and the study was granted NHS GG&C Board Approval in September 2020.

6.2.3 Funding

Prior work by our research group has been funded by an endowment fund held by the NHS GG&C Renal Transplantation Unit. For this project, a costed application was submitted to the NHS GG&C Endowment Fellowship Funding Award; a grant of £14,484 was awarded (see Appendix E). This provided finance for the perfusion consumables, transfer of organs and tissue analysis.

6.2.4 Application for Research Discarded Kidney Grafts

Once ethical approval was granted, an application was made to NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group) for the use of discarded research kidneys. The application (see Appendix F), submitted in September 2020,

requested access to untransplantable organs through the national allocation scheme. The document outlined the rationale and aims of the project, confirmed ethical approval, and asserted that the protocol was both externally and institutionally peer-reviewed. We requested access to 12 kidney grafts as part of a basic science study for perfusion and tissue analysis. Importantly, these grafts were not for consideration for implantation if found to be of sufficient graft quality and were for research purposes only. In November 2020, the advisory group RINTAG approved the application (ODT Study No. 107). Successful RINTAG applications are ranked based on study quality and importance, with studies that could ultimately lead to a successful transplant (i.e., organs assessed and could then be used), ranked the highest (from 1-7, with 1 being the highest rank). This study was given an initial ranking of number 2 in the research organ allocation scheme.

6.2.5 Transfer of Organs to the Recipient Unit

The research organ allocation scheme offers grafts to the principal investigator by way of a short message service (SMS) text message. The message details the following:

- Organ detail:
    - DCD or DBD, Left or Right
    - Donor age, blood group
    - Perfusion time (i.e., when in situ cold perfusion was commenced); *This was used to estimate eventual cold ischaemic time once transported to our unit.*
    - Virology status
- Organ location
- Reason for organ decline

Based on this information, the researcher, in receipt of this message, had 45 minutes to contact the centrally-located Organ and Tissue Donation and Transplantation (ODT) Hub by telephone to state interest in the research organ. The hub, then, depending on the ranking of the study, would then authorise acceptance of the grant.

An agreement to transfer material and organs between NHS BT and NHS GG&C was authorised to facilitate the official transfer of this precious commodity. Amvale Medical Transplant Limited is a courier service which holds numerous contracts across the UK transporting clinical tissue, such as solid organs, for transplant. A new service level agreement between Amvale and NHS GG&C was created, which allowed for a 24-hour courier service to facilitate the transfer of an offered research organ. This allowed for the safe and prompt transfer of the grafts to Queen Elizabeth University Hospital, Glasgow, thus minimising cold ischaemic time. Once the graft arrived at the QEUH, Glasgow, all identifiable information travelling with the organ was removed and destroyed in confidential waste.

6.2.6 Eligibility Criteria

All research kidneys offered through the NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group) to the principal investigator were considered eligible for this study. All kidneys were from deceased donors who have research consent and had been refused by transplant units for clinical transplantation and were therefore offered to research units.

Inclusion Criteria

- Deceased donor kidney(s) offered to the principal investigator through the national advisory group NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group).

Exclusion Criteria

- Renal anatomy that precludes successful cannulation and attachment to the ex-vivo perfusion circuit, i.e., multiple arteries without common stem (unable to perfuse entire graft)
- A significant ureteric injury that makes urinalysis and measurement of urine output not possible
- Evidence of extensive malignancy in the graft that would have a structural detrimental effect on perfusion and/or renal blood flow

6.2.7 Randomisation of Intervention

A blocked randomisation list was created numbered 1 to 12, and therefore grafts were pre-randomised to either control (EVNP for 4 hours) or treatment (EVNP for 4 hours plus delivery of ADRC).

6.2.8 Harvest of Adipose-Derived Regenerative Cells

Peri-renal adipose tissue was sourced from the research organs once transported to the Queen Elizabeth University Hospital, Glasgow. Following organ procurement, the kidney was placed on ice (i.e., static cold storage) and transferred as per standard practice in clinical transplantation. Once arrived in our unit, the graft was transferred to a sterile bench, submerged in hypothermic conditions within the operating theatre. The principal researcher then removed peri-renal adipose tissue from the kidney. This process is part of the clinical transplantation process, whereby this adipose tissue is removed in the process of preparing the kidney and its vasculature for implantation to a recipient. As part of this research study, the kidney was prepared in the same fashion in order to connect the graft to the arterial cannula and, therefore, the perfusion circuit.

At this time, the block randomisation was then sought to determine whether the graft would undergo control (EVNP only) or treatment with EVNP plus delivery of ADRC. If the graft was randomised to control, no further preparation of the graft was required, and the organ was stored on ice prior to commencing perfusion. If randomised to the treatment group, the peri-renal adipose tissue was used to harvest ADRC.

The Celution System® (Celution® 800/CRS System) is an approved medical device, which was manufactured in order to standardize the extraction process of ADRC from adipose tissue in a safe and consistent manner. The protocol for this automated and sterile process is published (Fraser et al., 2014). This bedside process was performed according to the manufacturer's instructions, which has been demonstrated to reliably produce an ADRC population which can be used as a therapeutic agent. Approximately 100-150ml of adipose tissue is obtained from the peri-renal tissue, as described above and inserted into the Celution System using the proprietary single-use sterile set. The tissue is then processed, excess fluid removed, and weighed to estimate the amount of enzyme reagent (Celase®) required. The tissue undergoes enzymatic digestion and, once complete, undergoes centrifugation, and the resultant 5ml volume of cell product is produced. The average cell yield is reported to be 3.6 x 10^5 cells per gram of tissue with viability of approximately 85% (Lin et al., 2008). The

produced cell product requires no further steps and can delivered directly to the desired target. Viability of the cells will be assessed using the automated cell counter, Nucleocounter® NC-200™ with a Via1-Cassette, providing both cell count and viability of cells within the sample.

6.2.9 Schedule of Events

The sequence of events from the offer of a research organ to its perfusion and, ultimately, tissue storage for analysis can be seen below in Table 6-1.

Table 6-1: Schedule of events detailing the process of research kidney acceptance to post-perfusion tissue storage

Area of Activity	Specific Activity	Duration (Minutes)	Undertaken by
SMS text	Receive offer of research kidney	10	PI
Phone call	Accept offer and communicate with ODT hub		
Phone call	Arrange courier of graft from donor unit	30	PI
QEUH	Graft arrives at QEUH	-	
QEUH	Randomisation process		PI
QEUH	Organisation of where EVNP can take place at the given time	30	PI
QEUH	Liaise with theatre staff (Staff nurse band 6) re: space in theatres	10	PI
QEUH Theatres	Proceed with EVNP only if suitable space available and no disruption to theatre utility		
QEUH Theatres	Set up EVNP equipment	30	PI
QEUH Theatres	Run EVNP (time dependent on quality of graft)	120-240	PI
QEUH Theatres	Take samples of perfusate during EVNP	-	PI
QEUH Theatres	Administer regenerative cell population through EVNP circuit	-	PI
QEUH Theatres	Stop EVNP	-	PI
QEUH Theatres	Take required tissue biopsies from tissue for laboratory analysis	10	PI
QEUH Theatres	Discard kidney graft as per standard theatre protocol	15	Theatre nursing staff – standard procedure

ICAMS	Transfer samples to ICAMS facility at Glasgow University via Taxi	20	PI
ICAMS	Samples stored for further scientific analysis as per tissue transfer agreement	10	PI

Abbreviations: EVNP: Ex vivo normothermic perfusion; **ICAMS:** The Institute of Cardiovascular & Medical Science; **ODT**: Organ and Tissue Donation and Transplantation; **PI**: Principal Investigator; **QEUH:** Queen Elizabeth University Hospital.

6.2.10 Perfusion Protocol

The perfusion of the graft was performed as per widely used clinical protocol by Hosgood and colleagues in the UK (Hosgood et al., 2017). However, in this study, the duration of perfusion was extended to four hours. Group-specific or O-negative packed red cells were provided by our local clinical haematology service. Kidneys randomised to the treatment group were administered ADRC via the arterial inflow at 60 minutes. Perfusate samples (10ml) were taken at the following time points (minutes): 30, 55, 90, 120, 180, and 240. Perfusate samples were taken from the arterial limb of the perfusion circuit with two functions: 1) For real-time adjustment of the circuit parameters, i.e., to guide the need for bicarbonate to adjust the pH of perfusate, and 2) To sample perfusate for later analysis. The measure of renal blood flow was also taken throughout the period of perfusion (measured by the Medtronic Bio-Console 560 device). Once perfusion was complete, samples taken were transported via taxi and stored in our laboratory facility.

6.2.11 Record Sheet for Perfusion Parameters

A record sheet was prepared to collect the real-time data during the period of perfusion (see Appendix G). The document provided a standardised collection of the following parameters every five minutes:

- Renal blood flow (ml)
- Pump Speed (rpm)
- Mean Arterial Pressure (mmHg)
- Temp (°C)
- pH on arterial blood gas

In addition to the above, urine output was measured every 30 minutes. An EVNP Assessment Score was then declared at the end of the four-hour perfusion period.

6.2.12 Tissue Preparation and Sample Analysis

Perfusion and urine samples were taken and stored on ice for the remainder of perfusion prior to transfer. Core biopsies were taken at hourly intervals (unable to take larger samples due to risk of haemorrhage whilst on perfusion circuit). Wedge biopsies were taken at the four-hour time point, just prior to the disconnection from the perfusion device. All biopsies were placed into 10% formalin containers. Samples were then transferred as per the Tissue Transfer Agreement to our laboratory facility and were prepared for future analysis.

Histology samples, after 3-4 days, were washed with PBS solution and placed in 70% ethanol for storage. Samples were kept in darkness throughout storage and transportation. Post-perfusion kidney tissue was snap-frozen, and separate samples were placed in a lysis buffer. Perfusate samples from the venous outflow were centrifuged at 1000g for 10 minutes at 4°C. The supernatant was then removed and stored at -80°C.

6.2.13 Histological Analysis

Paraffin-embedded 5μm-thick sections of renal tissue were stained with haematoxylin and eosin, and trichrome stains. Analysis was performed by an experienced renal histopathologist, and the Remuzzi score was calculated by assessing the following four variables: glomerular global sclerosis, tubular atrophy, interstitial fibrosis, and arterial and arteriolar narrowing. This produced a score (maximum of 12) and is a reproducible measure, which is commonly used in clinical transplantation in pre-implantation biopsies of marginal kidneys. Guidance on the interpretation of the Remuzzi score states that a kidney with a score of 3 or less should be transplanted singly (i.e., standard transplant), a score of 4-5 to be considered for dual transplant (i.e., both kidneys of a pair to be used for a single recipient), and a score of 6 or more to be discarded (Ayorinde et al., 2019).

In addition, the pathologist scored the samples with a semi-quantitative percentage score of acute tubular injury (ATI). The features of acute tubular injury are one or both of the following: 1) tubular epithelial cell loss of apical brush border and cytoplasmic flattening, or 2) nonisometric tubular epithelial cell cytoplasmic vacuolization. The presence of either

of these was given a score between 0 and 4 as a percentage of the total cortical area of the biopsy (0 = 0–1%; 1= >1– 10%; 2 = >10–25%; 3 = >25–50%; 4 > 50%) (Moeckel, 2018; Tt et al., 2019).

6.2.14 RNA Extraction and Sequencing

RNA extraction was performed on biopsies snap frozen and stored at −80°C. Samples were transferred to GeneWiz, Azenta Life Sciences for sample preparation and analysis. The reference genome used for analysis was Homo sapiens GRCh38 (download link available at: https://uswest.ensembl.org/Homo_sapiens/Info/Index). The RNA sequencing workflow was as follows: ribosomal RNA depletion, RNA fragmentation and random priming, cDNA synthesis and 2′-Deoxyuridine, 5′-Triphosphate (dUTP) incorporation, adenosine tailing and finally adaptor ligation, dUTP strand degradation and PCR amplification.

Data was then analysed by the bioinformatics team, which involved quality assurance steps (evaluation of sequence quality and trim reads) before the reads were mapped with reference to the human genome (GRCh38). Unique gene hit counts that fell within exon regions were then evaluated using the R statistical environment with the programme *featureCounts*, obtained from the Subread package v.1.5.2. Hit counts were generated using the *gene_id* feature and compared between control and ADRC treatment.

Following the identification of unique gene counts, downstream expression analysis was performed using the software *DESeq2.* Log2 fold changes and differential expression was analysed with the Wald test. Genes with differential expression were those with p values less than 0.05 and log fold change over 1. To identify potential biological processes, gene ontology analysis was then performed with the software *GeneSCF v1.1-p2*, and clustered against the *goa_human* ontology dataset to determine statistical significance.

6.3 Results

In total, 8 kidneys were recruited for this study. 5 controls, and 3 treatment kidneys which received ADRC treatment. All kidneys were perfused at normothermia for the four-hour duration as per protocol.

K5 was randomised to the treatment group; the kidney, however, had been declined by a transplant unit for anatomical reasons: the ureter had been skeletonised (i.e., damaged) at the time of procurement, and there were multiple large cysts seen on the renal parenchyma. The kidney was assessed at a transplant unit whilst being considered for use, and the majority of the peri-renal adipose tissue had been removed as part of this assessment. This kidney was, therefore, defaulted to the control group as there was insufficient adipose tissue for ADRC preparation.

6.3.1 Research Kidney Demographics

Six of the kidneys were from DCD donors; two were from DBD donors. Donor age ranged from 41 to 72 years (mean 59.8 years). Cold ischaemic time ranged from 16 hours 36 minutes to 36 hours (mean average of 25 hours 11 minutes). The grafts randomised to ADRC treatment had a mean cold ischaemic time of 22 hours and 36 minutes, compared to 26 hours and 38 minutes for the control group.

There were multiple reasons for organ decline, including poor graft perfusion (i.e., incomplete clearance of blood with cold perfusion at procurement), high pre-implantation PITHIA score, atheromatous vessel disease, and the presence of renal cysts (see Table 6-2).

Table 6-2: Demographics of the research kidneys

Kidney	Group	Donor Type	Donor Age	Reason for decline	CIT
K1	Control	DCD	*55*	Diseased and atherosclerotic aorta and renal artery	24.3
K2	Control	DBD	*61*	Multiple arteries; 2nd artery severed at time of back bench preparation	26.75
K3	ADRC	DCD	*58*	Poor perfusion	24.2
K4	Control	DCD	*65*	Poor perfusion	36
K5	Control	DCD	*72*	Multiple cysts, atheromatous aortic patch	26
K6	ADRC	DBD	*41*	Poor perfusion	27.5
K7	Control	DCD	*56*	Ureter skeletonised; multiple large renal cysts	20.1
K8	ADRC	DBD	*70*	Pre-implantation biopsy – PITHIA = 6	16.6

Abbreviations: CIT: cold ischaemic time; **DCD**: donation after circulatory death; **DBD**: donation after brainstem death; **PITHIA**: Pre-implantation trial of histopathology in renal transplant allografts.

6.3.2 ADRC Harvest

There were four kidneys randomised to ADRC treatment. As discussed, one of these kidneys did not have the required amount of adipose tissue (100-150ml) in the perirenal fat and therefore defaulted to the control group. The three kidneys in which ADRC harvest took place with the Celution® 800 System were K3, K6 and K8. The viability of the cells from the perirenal donor tissue was as follows: 78%, 85% and 81%, respectively. 150ml of adipose tissue was obtained, and resulting cell numbers were $1.4x10^6$, $1.2x10^6$ and $1.5x10^6$, respectively (i.e., between 1.2 and 1.4 million cells with average viability of 81.3%).

6.3.3 Perfusion Parameters

As per the EVNP record sheet (Appendix G), perfusion parameters were measured at specified intervals throughout the period of perfusion, up to 240 minutes (4 hours) for all kidneys.

6.3.3.1 Renal Blood Flow

Non-pulsatile pressure was maintained at 75mmHg as per protocol. The renal blood flow (ml/min) is commonly used as a marker of intra-renal resistance, with higher blood flow found in kidneys with lower intra-renal resistance. All of the kidneys demonstrated a general trend of increased renal blood flow over the duration of the perfusion (see Figure 6-1). The kidneys with initially suboptimal renal blood flow appear to stabilise after 60 minutes of perfusion. There was no apparent difference between the control and treatment groups.

Mean renal blood flow was 0.3L/min/100g in the control and treatment groups at 60 minutes. The flow rates at 240 minutes increased in the control group to 0.4L/min/100g and stayed constant within the ADRC group at 0.3L/min/100g, although this difference was not statistically significant ($p= 0.68$) (see Figure 6-2).

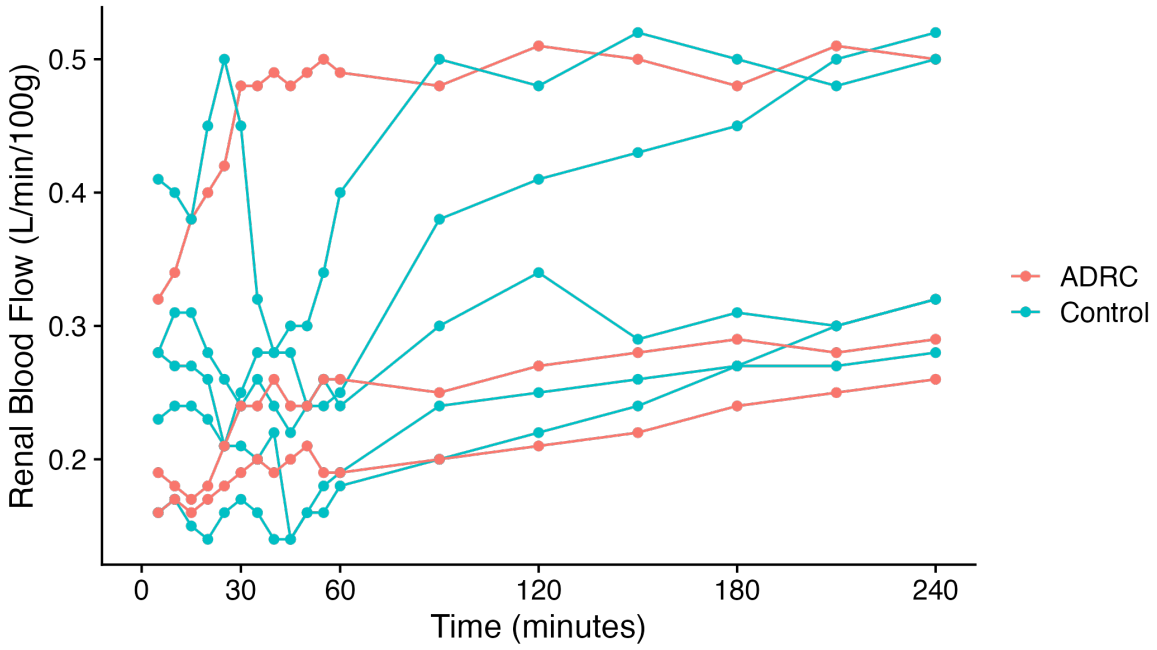


Figure 6-1: Renal arterial flow (L/min/100g) of the kidneys during EVNP

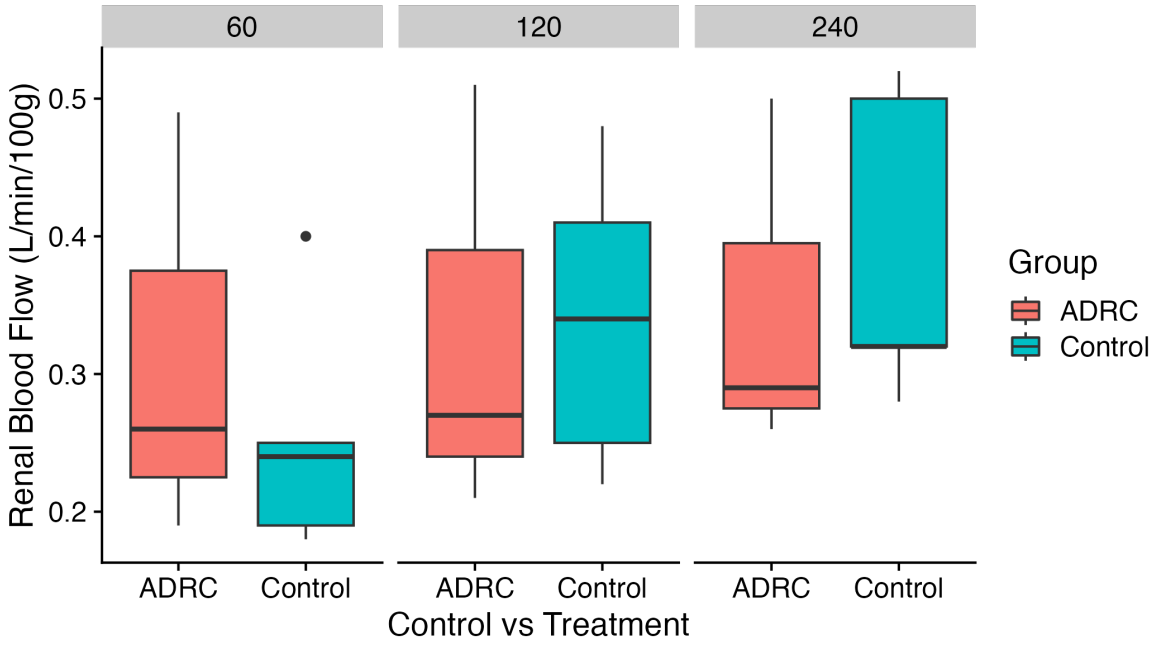


Figure 6-2: Renal blood flow (L/min/100g) over time at 60, 120 and 240 minutes of perfusion.

6.3.3.2 Potassium Concentration

It was anticipated that levels of potassium would be elevated at time zero given the cold ischaemic time of the research grafts. In addition, the packed red cells ranged from 4-8 days old and, therefore, may have contributed to the higher-than-expected pre-perfusion potassium levels.

The general trend was a reduction in potassium levels throughout the perfusion (see Figure 6-3). 6 of the 8 kidneys (K1 and K6, being the exceptions) exhibited an initial spike in potassium levels at 30 or 60 minutes, and then all of the kidneys reduced the potassium concentrations thereafter.

It is unknown whether potassium clearance can be used as a surrogate for nephron function during EVNP. No differences were found in potassium clearance between treatment groups.

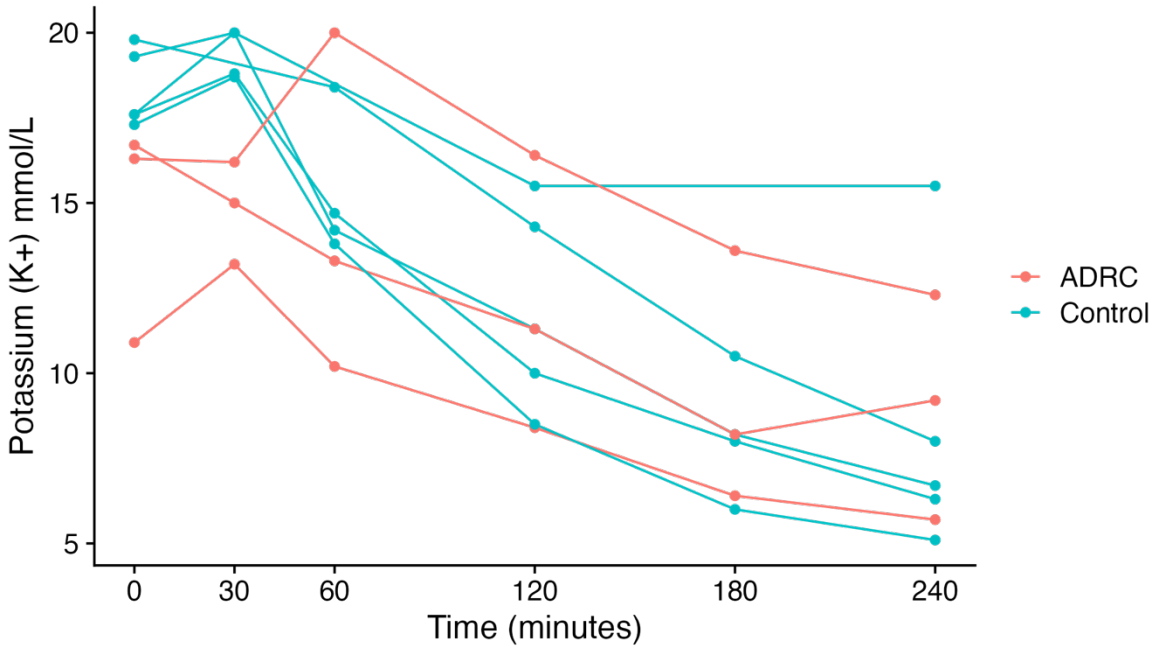


Figure 6-3: Potassium concentrations (mmol/L) in the perfusate of the kidneys during EVNP

6.3.3.3 Lactate Concentration

Lactate levels were found to be elevated, particularly in four of the grafts (three control: K4, K5, and K7; and one treatment kidney: K6). Levels were >20mmol/L for the duration, apart from one from this subset (K4), which appeared to start clearing lactate between 120 and 240 minutes (see Figure 6-4). The clinical significance of this finding is uncertain and may reflect the prolonged cold ischaemia evident in these research grafts.

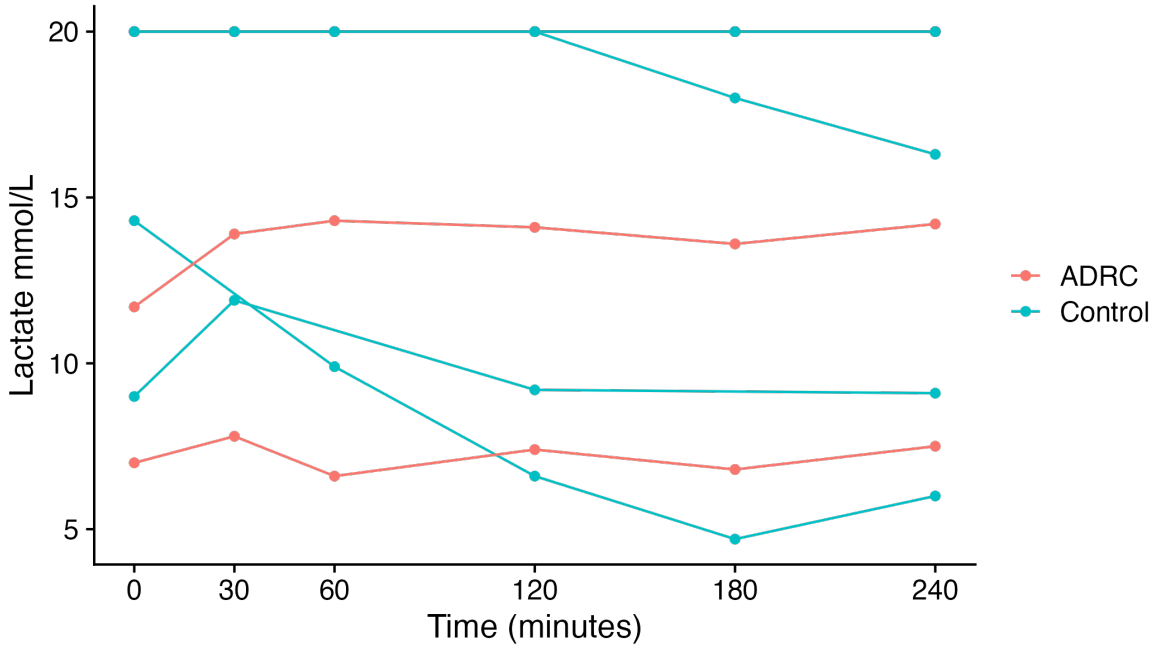


Figure 6-4: Lactate concentrations (mmol/L) in the perfusate of the kidneys during EVNP

6.3.3.4 Urine Output

A third of the EVNP Assessment Score is based on the urine output achieved at 60 minutes. Two of the grafts, K1 and K8, failed to reach the 43ml/hr target. Achieving less than this target gives a score of 1 to be added to the total. The remaining grafts all produced more than 43ml of urine in the first 60 minutes, and all kidneys produced urine consistently throughout the perfusion duration (see Figure 6-5 and Figure 6-6). Urine output in the ADRC group appeared lower than the control group at the earlier time points; however, this difference did not reach statistical significance. This apparent difference narrowed over time, and urine output at 4 hours was equal between groups (see Figure 6-7).

At 60 minutes, the mean urine output was 65mls in the ADRC group, compared to 102mls for the controls (this apparent difference was not statistically significant, p = 0.26). Urine output at 240 minutes was very similar in both groups at 310ml and 314ml for ADRC and control, respectively.

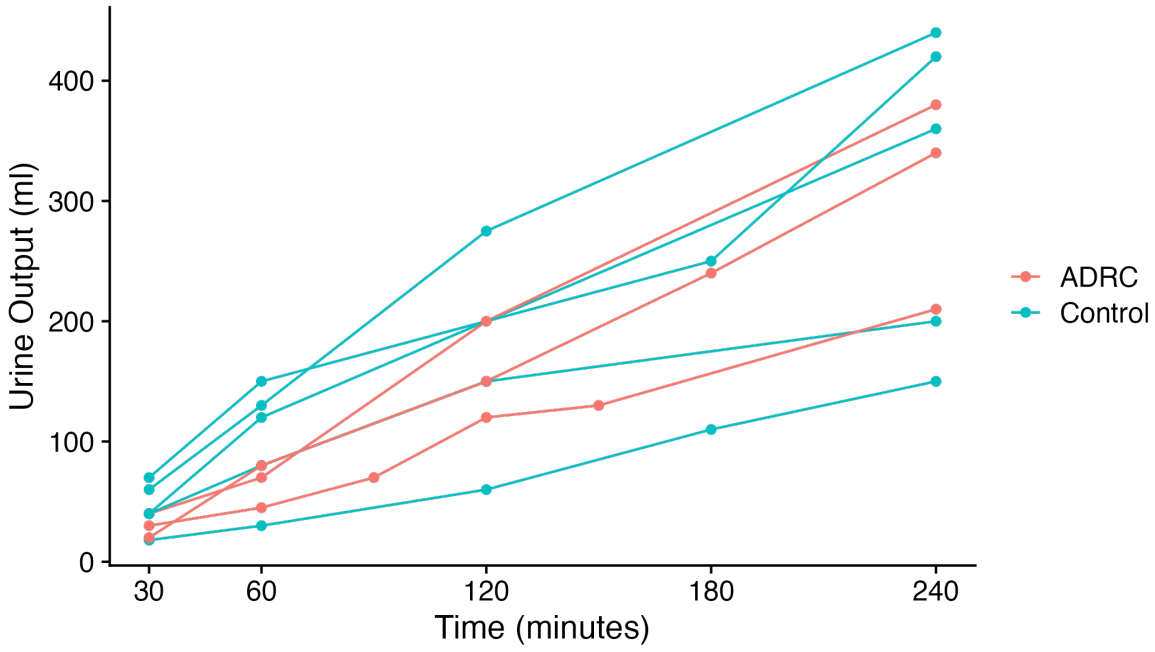


Figure 6-5: Urine output of the kidneys during EVNP by treatment group

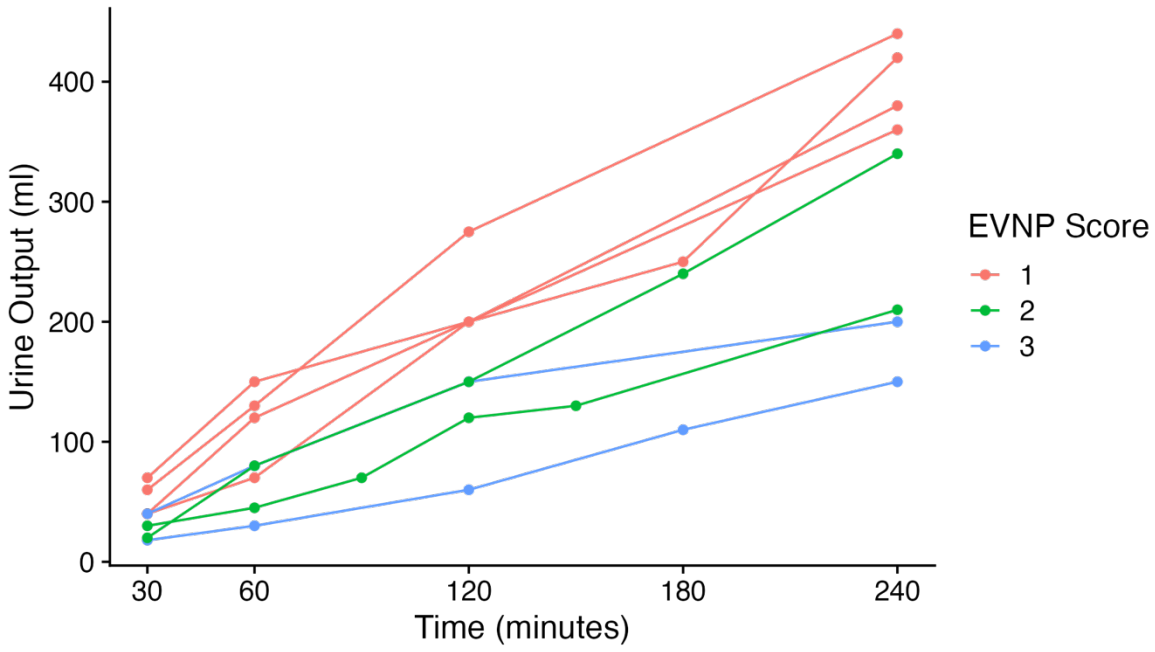


Figure 6-6: Urine output of the kidneys during EVNP separated by EVNP Assessment Score

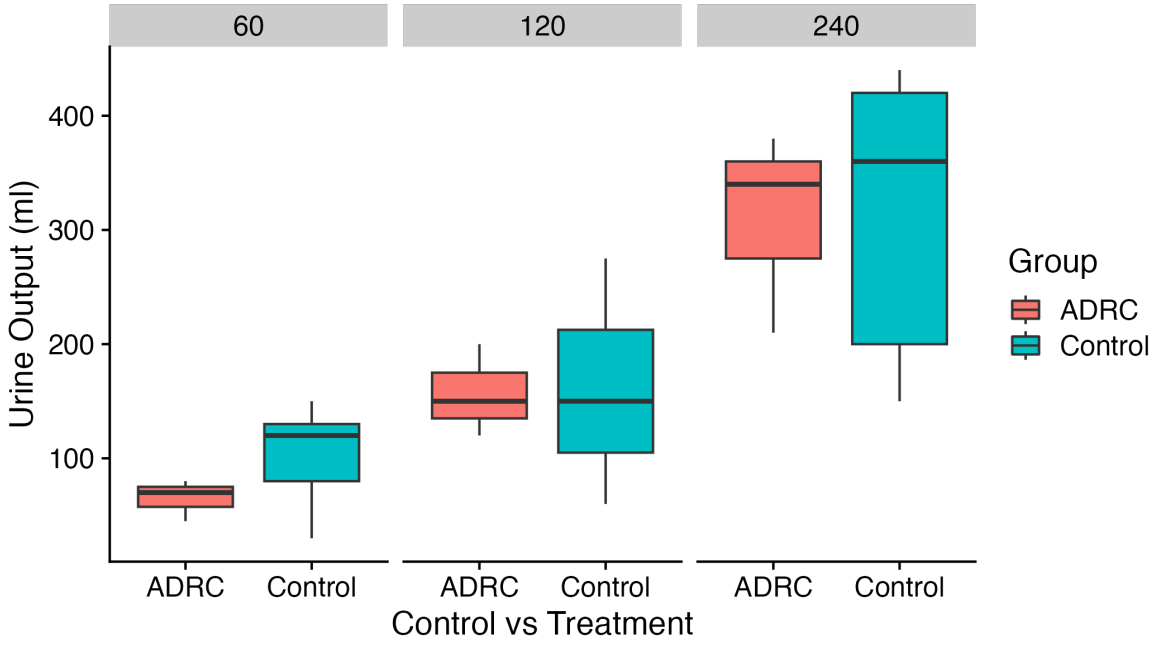


Figure 6-7: Urine output (ml) over time at 60, 120 and 240 minutes of perfusion

6.3.4 EVNP Assessment Score

All of the kidneys scored 3 or less, and therefore, by this metric alone, would be considered suitable for transplantation (see Figure 6-8 and Table 6-3). All of the kidneys achieved the requisite renal blood flow (per 100g of kidney tissue) even when the macroscopic appearance was suboptimal. Two of the kidneys (K1 and K8) failed to produce 43ml of urine in the first hour, yet both of these kidneys progressed and produced more than 43ml/hr thereafter.

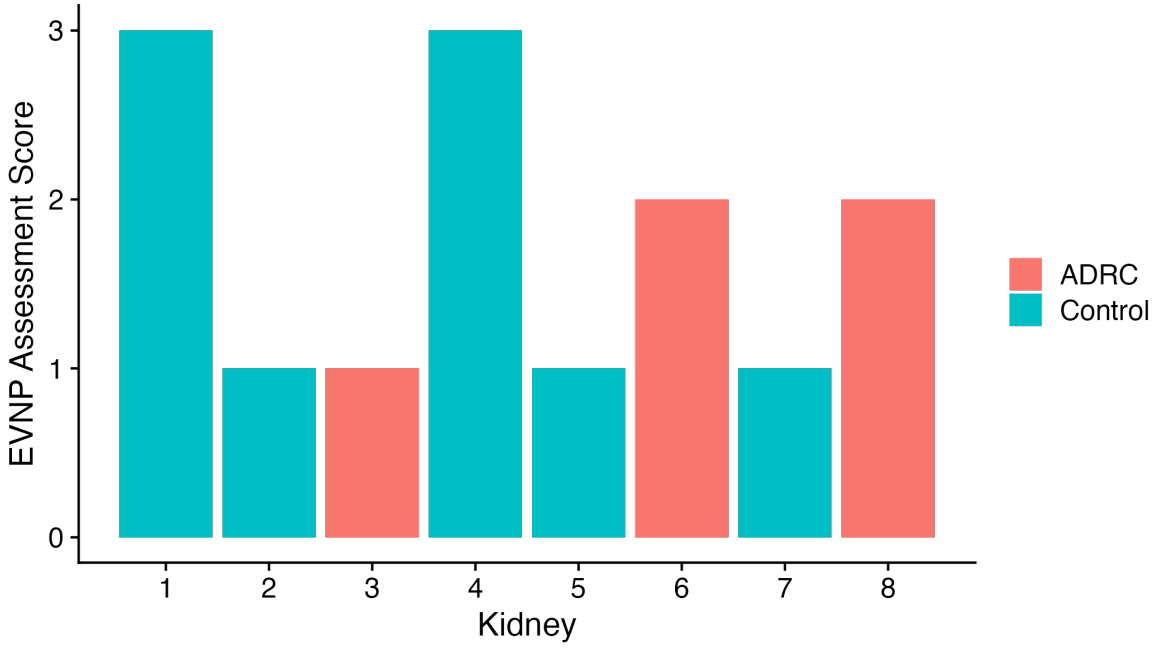
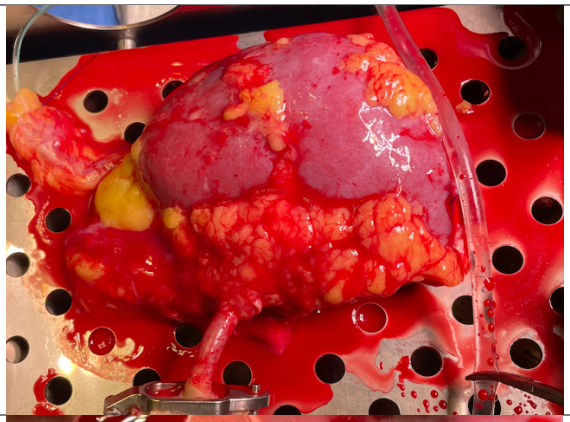
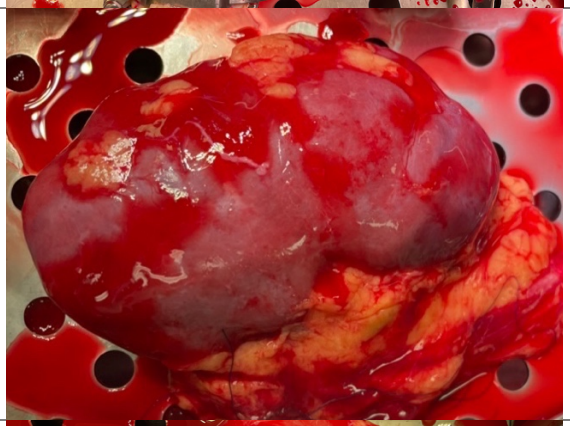
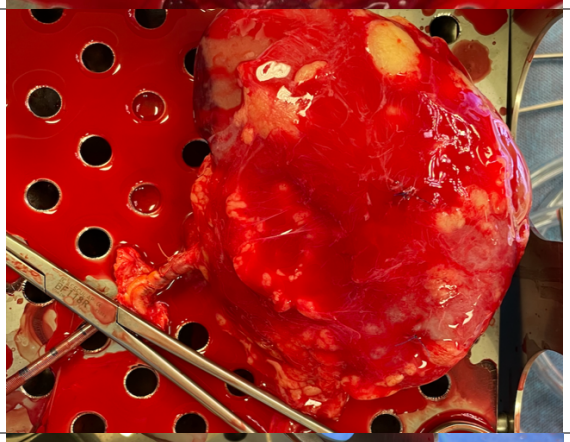
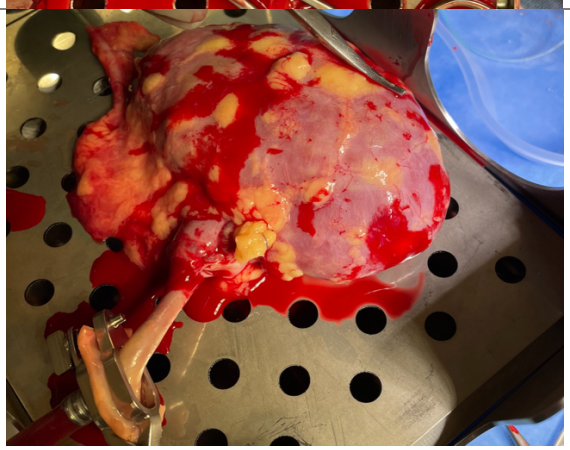
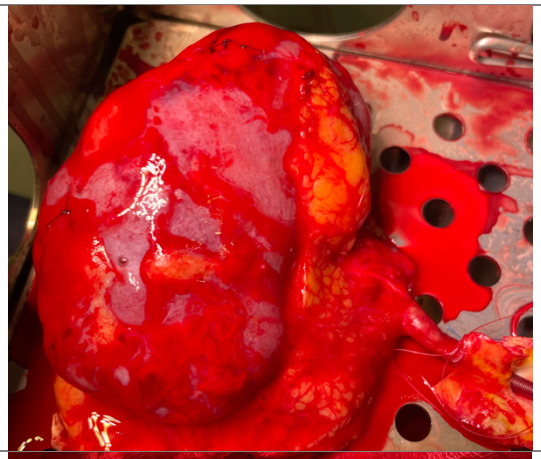
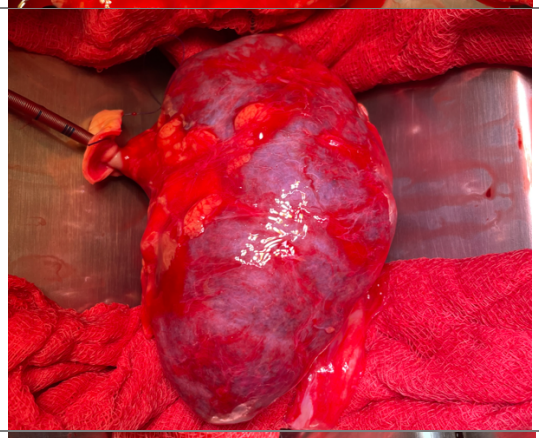
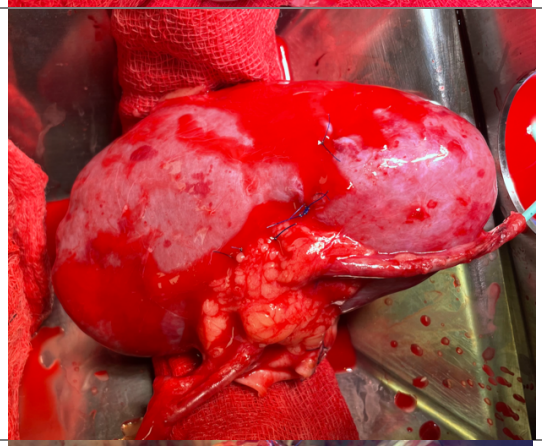
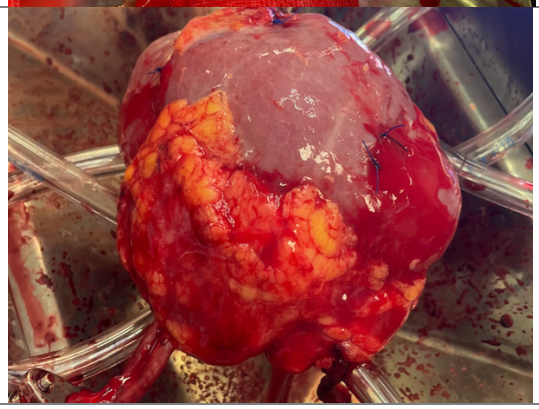


Figure 6-8: EVNP Assessment Score of all research kidneys

Table 6-3: EVNP Assessment score with pictures of kidneys on perfusion

Kidney	Image on Perfusion	Assessment Score Parameters	Points
1		Macroscopic Appearance = Moderate	2
		Renal arterial flow = 73.1	0
		Urine output= 150ml (30mls at 1 hour)	1
		Total	**3**
2		Macroscopic Appearance = Excellent	1
		Renal arterial flow = 79.6	0
		Urine output= 360ml (120ml at 1 hour)	0
		Total	**1**
3 ADRC		Macroscopic Appearance = Excellent	1
		Renal arterial flow = 111.8	0
		Urine output= 380ml (70ml at 1 hour)	0
		Total	**1**
4		Macroscopic Appearance = Poor but improved over time	3
		Renal arterial flow = 97.0	0
		Urine output= 200ml (80ml at 1 hour)	0
		Total	**3**

Kidney	Image on Perfusion	Assessment Score Parameters	Points
5		Macroscopic Appearance = Excellent	1
		Renal arterial flow = 71.1	0
		Urine output= 310mls (150ml at 1 hour)	0
		Total	**1**
6 ADRC		Macroscopic Appearance = Moderate	2
		Renal arterial flow = 82.9	0
		Urine output= 340ml (80ml at 1 hour)	0
		Total	**2**
7		Macroscopic Appearance = Excellent	1
		Renal arterial flow = 69.2	0
		Urine output= 440ml (130ml at 1 hour)	0
		Total	**1**
8 ADRC		Macroscopic Appearance = Excellent	1
		Renal arterial flow = 64.3	0
		Urine output= 210ml (42mls at 1 hour)	1
		Total	**2**

6.3.5 Perfusate Markers of Kidney Injury

Luminex assay was unsuccessful due to insufficient concentrations. Repeat Luminex kidney injury panel to be performed.

6.3.6 Histology

All kidneys had pre- and post-perfusion biopsies. Histology was evaluated independently by an experienced renal histopathologist; as samples were anonymised, the reviewer was blinded to whether the kidneys were in the control or treatment groups. The Remuzzi score was used as a marker of chronic injury, and the acute tubular injury (ATI) score was used as a marker of acute injury.

Only K8 had a Remuzzi score of 4; the rest had a score of 3 or less (see Table 6-4). The guidance on Remuzzi interpretation would advise kidneys with a score of 3 to be used for transplant as a single implant.

The K5 post-perfusion histology sample did not have the requisite number of nephrons within the sample to be scored and was therefore scored as 'inadequate'. For the purpose of analysis, given that the Remuzzi score is a marker of chronic injury, the pre-perfusion score was used.

K7 exhibited evidence of widespread glomeruli thrombus (see Table 6-5). This was a finding seen in both pre- and post-perfusion samples. This was evident despite a favourable Remuzzi score (score = 0) and EVNP assessment score of 2, with a total urine output of 440mls.

The highest Remuzzi score (4) was found in K8, which had been declined for transplantation due to biopsy results which had been taken at the time of procurement (PITHIA score = 6). All other kidneys scored 3 or less (see Figure 6-9). There was no statistical difference found in ADRC vs control.

A score of acute tubular injury was given, which ranged from 0 to 4. All kidneys scored 2 or less, which corresponds to less than 25% of the total area displaying features of acute tubular injury (see Figure 6-10). Of note, K8, which had a chronic injury Remuzzi score of 4, scored 0 (<1% affected) on the measure of acute tubular injury.

Table 6-4: Histology results of acute and chronic injury pre- and post-perfusion

Kidney	Group	Timing of biopsy	Remuzzi Score	ATI Score
K1	Control	Pre	1	0
		Post	1	1
K2	Control	Pre	1	0
		Post	0	1
K3	ADRC	Pre	2	1
		Post	0	2
K4	Control	Pre	1	1
		Post	1	1
K5	Control	Pre	2	1
		Post	Inadequate	0
K6	ADRC	Pre	1	1
		Post	3	1
K7	Control	Pre	0	2
		Post	0	2
K8	ADRC	Pre	4	1
		Post	4	0

Abbreviations: ADRC: adipose-derived regenerative cells; **ATI**: acute tubular injury.

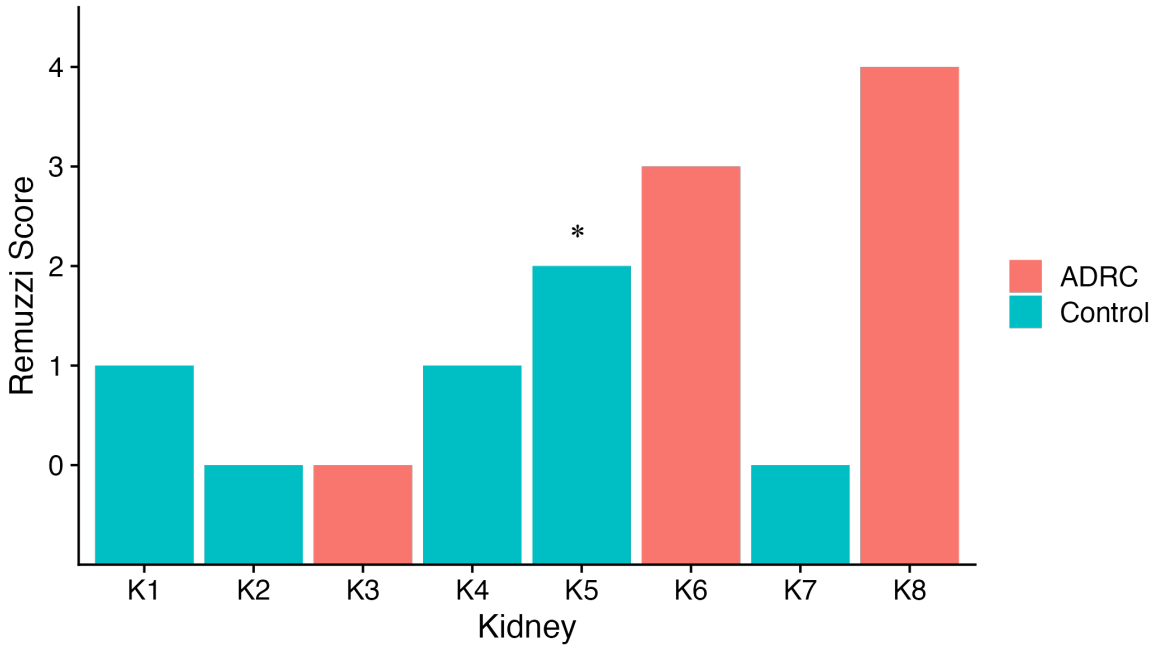


Figure 6-9: Post-perfusion Remuzzi score of all research kidneys

*K5 post-perfusion biopsy was deemed insufficient for Remuzzi scoring; for the purpose of this bar chart, the pre-perfusion score of 2 was used.

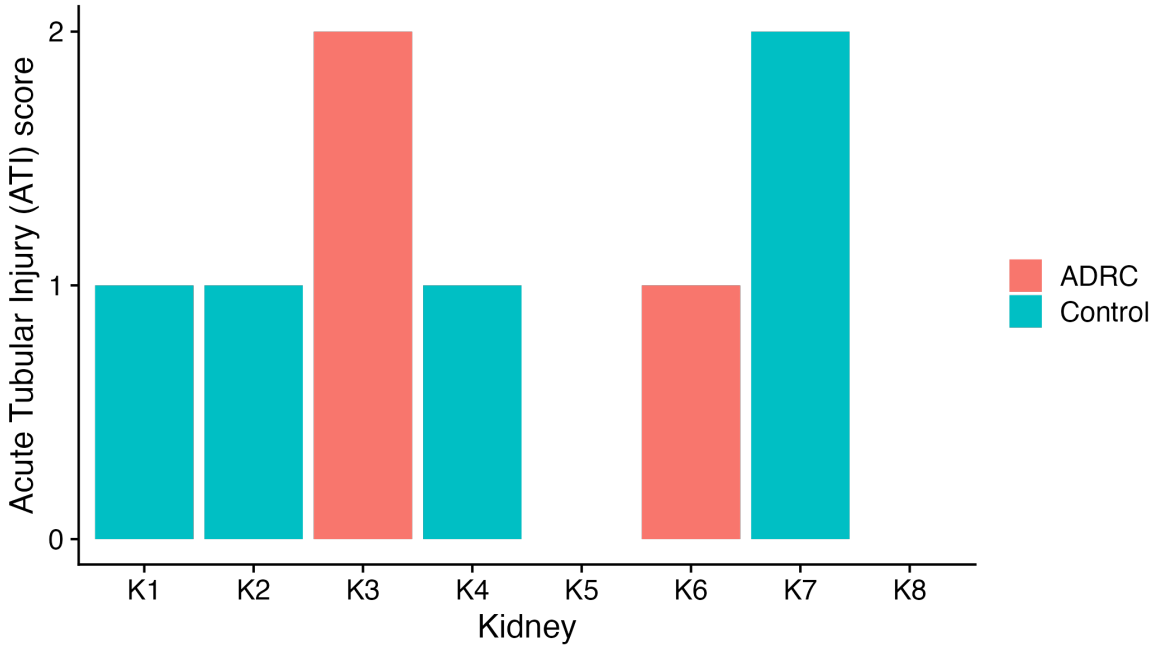
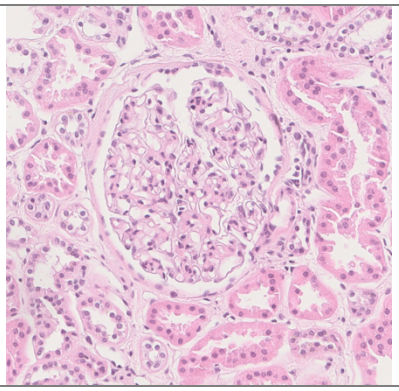
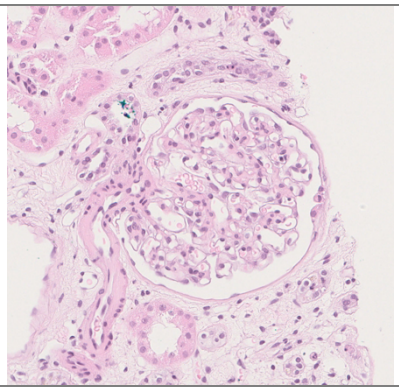
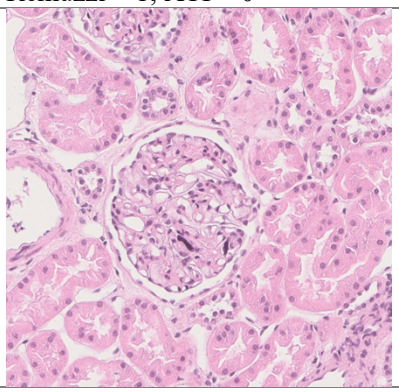
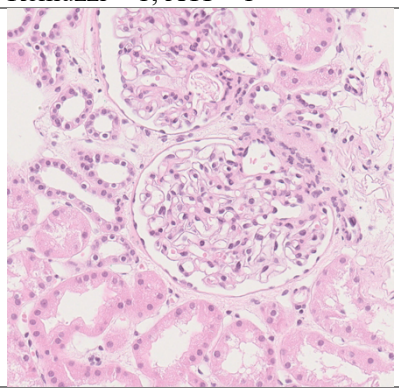
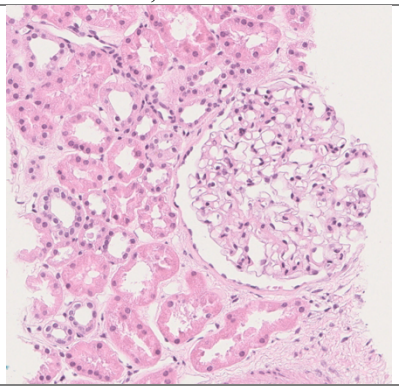
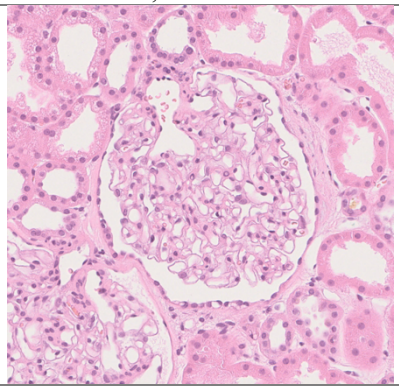
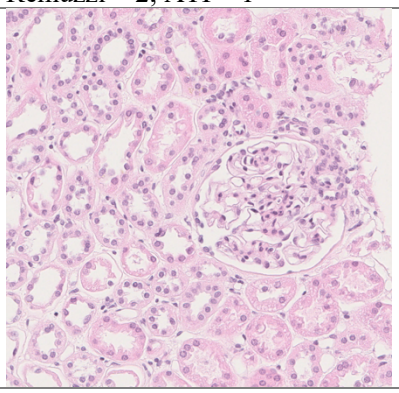
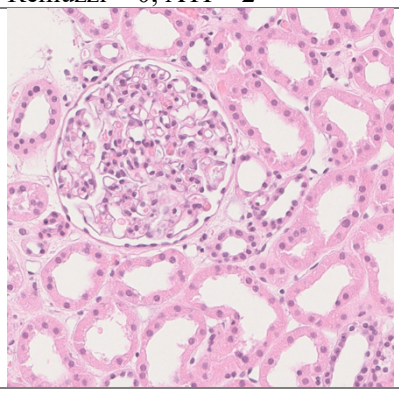
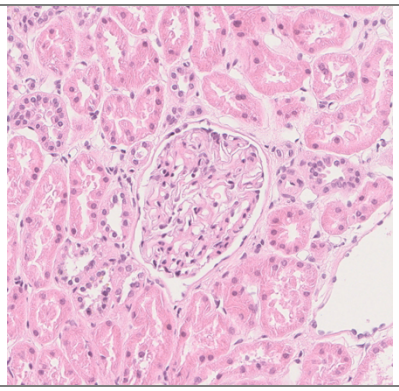
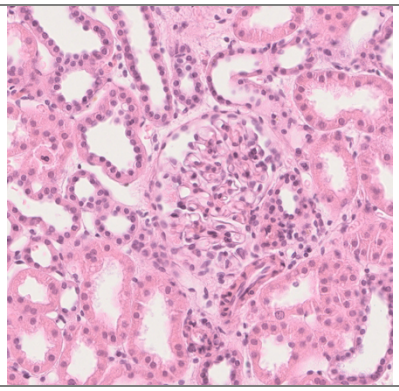
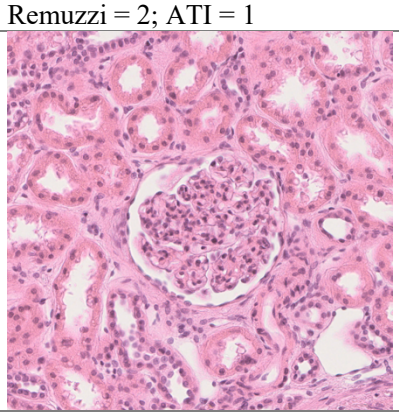
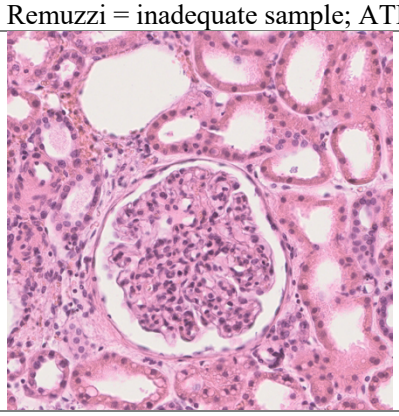
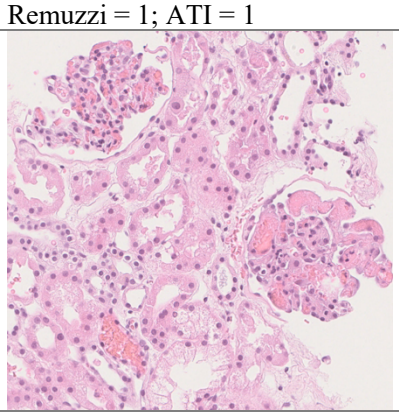
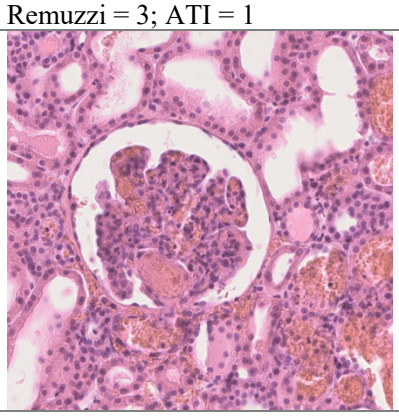
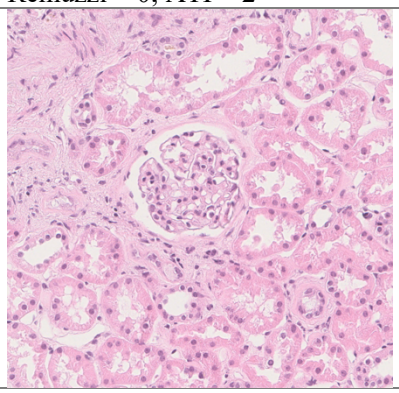
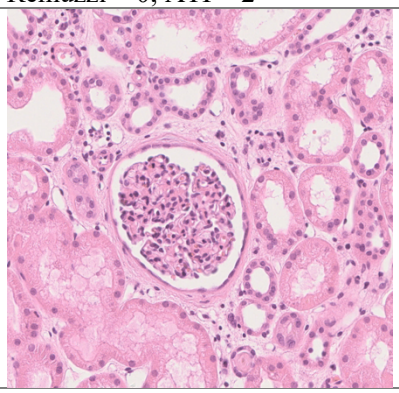


Figure 6-10: Post-perfusion Acute Tubular Injury (ATI) score of all research kidneys

Table 6-5: Histology photographs of all research kidneys pre- and post-perfusion

Kidney	Histology images	
	Pre-perfusion	Post-perfusion
K1		
	Remuzzi = 1; ATI = 0	Remuzzi = 1; ATI = 1
K2		
	Remuzzi = 1; ATI = 0	Remuzzi = 0; ATI = 1
K3		
	Remuzzi = 2; ATI = 1	Remuzzi = 0; ATI = 2
K4		
	Remuzzi = 1; ATI = 1	Remuzzi = 1; ATI = 1

Kidney	Histology images	
	Pre-perfusion	Post-perfusion
K5		
	Remuzzi = 2; ATI = 1	Remuzzi = inadequate sample; ATI = 0
K6		
	Remuzzi = 1; ATI = 1	Remuzzi = 3; ATI = 1
K7		
	Remuzzi = 0; ATI = 2	Remuzzi = 0; ATI = 2
K8		
	Remuzzi = 4; ATI = 1	Remuzzi = 4; ATI = 0

20x magnification of Haematoxylin & Eosin-stained kidney core biopsy samples. K7 demonstrated widespread glomerular thrombi as pictured in both pre- and post-perfusion samples. **Abbreviations**: **ATI**: *Acute Tubular Injury score*

6.3.7 RNA Sequencing

RNA sequencing analysis of all samples demonstrated over 231 million reads with an average read of 28.8 million reads per sample. All samples achieved a percentage score of over 90% of reads with base number over 30. These reads were mapped to the reference genome (Homo sapiens GRCh38).

Differential gene expression analysis revealed 24 differentially expressed genes in the comparison of ADRC treatment vs control (i.e., those that reached statistical significance) (see Table 6-6 and Figure 6-11 and Figure 6-12). Of note, upregulated genes in the ADRC group included SLC14A1, a known urea transporter in the kidney; human hyaluronan synthase 2 (HAS2), which is expressed in proximal tubular cells but has also been demonstrated in renal tubule production in vitro (Soulié et al., 2014). The gene DNA Damage Inducible Transcript 4 Like (DDIT4L), was also upregulated in the ADRC group. This gene has been shown to be increased in various cellular stress conditions (Fattahi et al., 2021), and of interest has been demonstrated to play a role in the regulation and fate of mesenchymal stem cells via mTOR signalling pathways (Gharibi et al., 2016).

Upregulated genes in the control group included the gene which encodes endothelial specific molecule-1 (ESM1), which has been shown to be upregulated in pro-inflammatory states driving leucocyte activation and has been associated with kidney injury in both chronic kidney disease and post-transplant (Holthoff et al., 2022). The gene Caplain 6 (CAPN6) was also upregulated in the control group. This gene is known to play an important role in cell stability and has been shown to demonstrate anti-apoptotic properties (L. Chen et al., 2020). ABCA13 and ABCA4 are both genes which encode ATP-binding cassette transporters, which were also both upregulated in the control group. Neither is well understood, yet ABCA13 has previously been identified in murine kidney models to be important in molecular transfer across cell membranes (Barros et al., 2003).

Gene ontology (GO) analysis was used to identify potential biological processes associated with the identified differentially expressed genes. Numerous pathways were demonstrated to differ in the treatment ADRC group vs control. Identified upregulated biological processes within the ADRC group included: positive regulation of nephron tubule epithelial cell differentiation (GO:2000768), negative regulation of cellular response to growth factor stimulus (GO:0090288), negative regulation of neutrophil chemotaxis, glomerular parietal cell differentiation (GO:0072139), and oxidative reduction process (GO:0055114) (see Figure 6-13).

Table 6-6: Differentially expressed genes between ADRC treatment and control.

Gene ID	Gene Name	Alias	Log2 Fold Change	p value	p adj*
NSG00000279576	ENSG00000279576	Metastasis associated lung adenocarcinoma transcript 1	-2.75	0	0.02
ENSG00000259347	AC087482.1	-	-2.24	0	0.04
ENSG00000204610	TRIM15	Tripartite motif containing 15	-2.16	0	0
ENSG00000164283	ESM1	Endothelial cell specific molecule 1	-2.09	0	0.04
ENSG00000206557	TRIM71	Tripartite motif containing 17	-1.93	0	0.03
ENSG00000187134	AKR1C1	Aldo-Keto reductase family 1 Member C1	-1.91	0	0.04
ENSG00000196876	SCN8A	Sodium voltage-gated channel alpha subunit 8	-1.49	0	0.04
ENSG00000145147	SLIT2	Slit guidance ligand 2	-1.45	0	0
ENSG00000173253	DMRT2	Double sex and mab-3 related transcription factor 2	-1.37	0	0.03
ENSG00000178445	GLDC	Glycine decarboxylase	-1.26	0	0
ENSG00000072682	P4HA2	Prolyl 4-hydroxylase subunit alpha 2	-1.22	0	0.03
ENSG00000225697	SLC26A6	Solute carrier family 26 member 6	-1.05	0	0.03
ENSG00000164128	NPY1R	Neuropeptide Y receptor Y1	1.23	0	0.02
ENSG00000007062	PROM1	Prominin 1	1.38	0	0.04
ENSG00000198691	ABCA4	ATP binding cassette subfamily A member 4	1.73	0	0.03
ENSG00000196616	ADH1B	Alcohol dehydrogenase 1B Class 1, beta polypeptide	1.9	0	0.03
ENSG00000077274	CAPN6	Caplain 6	1.93	0	0.03
ENSG00000082497	SERTAD4	SERTA domain containing 4	1.97	0	0.03
ENSG00000171051	FPR1	Formyl peptide receptor 1	1.98	0	0.04
ENSG00000145358	DDIT4L	DNA damage inducible transcript 4 like	1.99	0	0.04
ENSG00000141469	SLC14A1	Solute carrier family 14 member 1	2.56	0	0.03
ENSG00000170961	HAS2	Hyaluronan synthase 2	2.68	0	0.03
ENSG00000179869	ABCA13	ATP binding cassette subfamily A member 13	3.5	0	0.03
ENSG00000263862	LINC01543	Long intergenic non-protein coding RNA 1543	4.59	0	0.03

Negative Log2 fold change denotes lesser expression in the treatment ADRC group compared to control; Positive Log2 fold change denotes increased expression in the treatment ADRC group; *The Benjamini-Hochberg adjusted p-value.

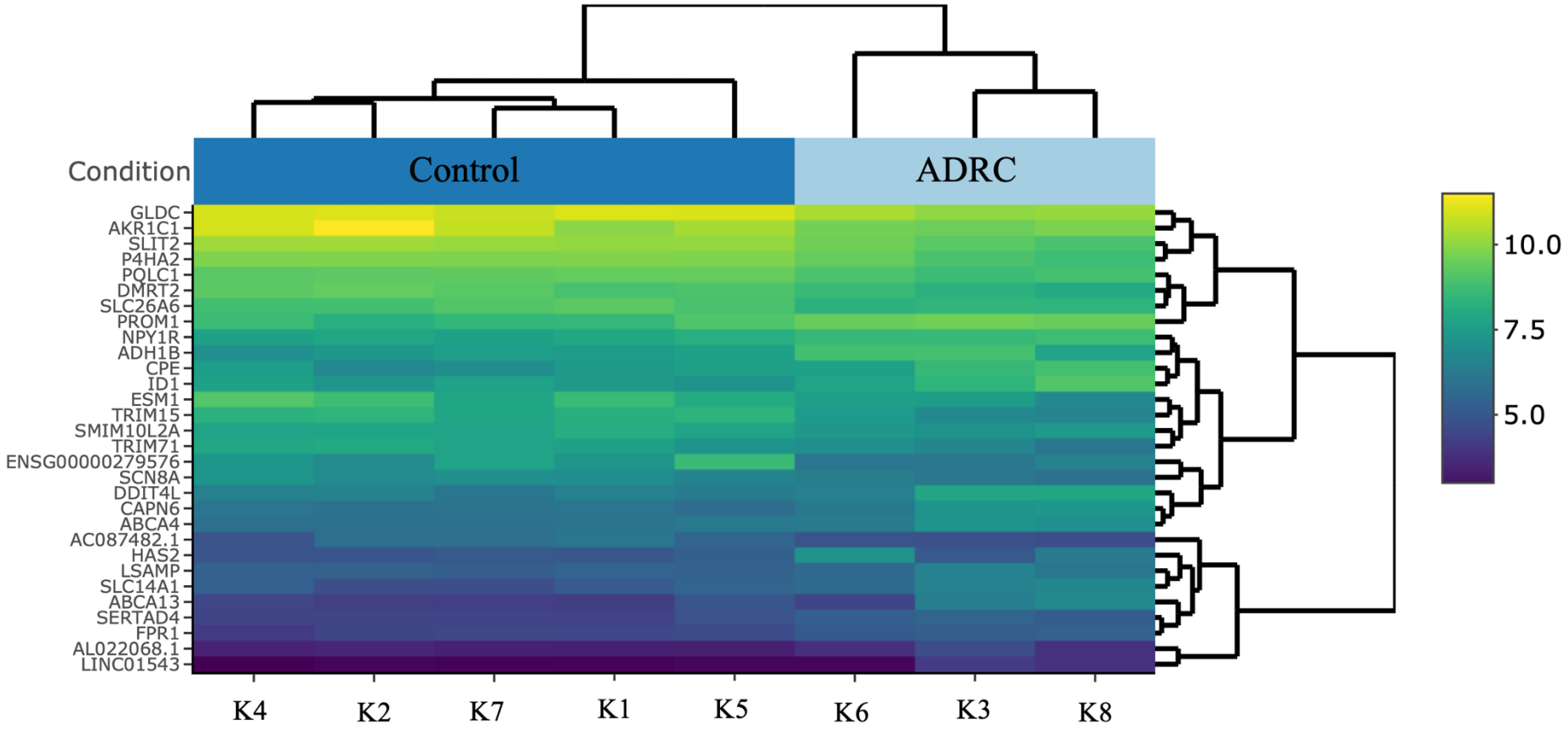


Figure 6-11: Heatmap of top 24 differentially expressed genes

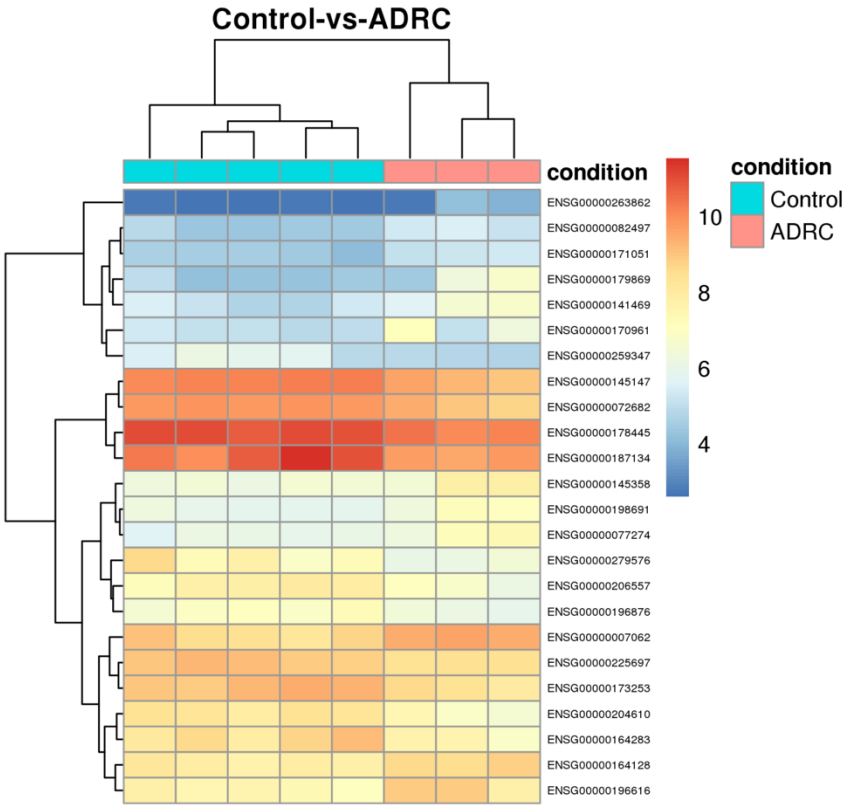


Figure 6-12: Heatmap demonstrating statistically significant differential gene expression.

Condition = Gene ID

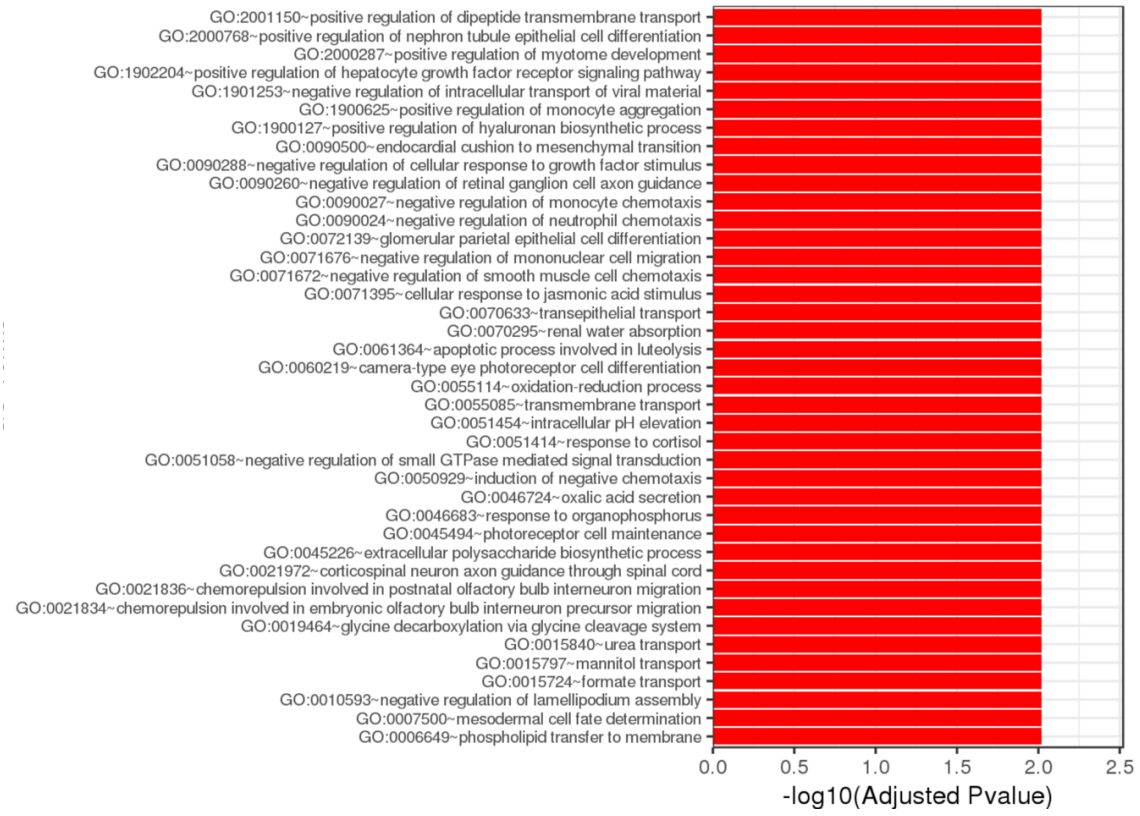


Figure 6-13: Gene ontology analysis demonstrating top 40 biological processes by way of clustering differentially expressed genes

6.4 Discussion

This study outlines the use of eight human kidneys, which were declined for transplant, that were perfused for four hours. These grafts were perfused at normothermia with a unit of oxygenated packed red cells, and the treatment group was treated with a regenerative cell population. The regenerative cells used, known as adipose-derived regenerative cells, were obtained 'at the bedside' from the fat obtained alongside the kidney during its procurement. To our knowledge, this is the first study to demonstrate the administration of donor peri-renal adipose tissue to a kidney during a period of normothermic perfusion.

All of the kidneys achieved an EVNP assessment score of 3 or less, which, using this metric alone, would deem them suitable for transplant. Furthermore, only one of the grafts had a Remuzzi chronic injury score of more than 3 (K8 = score of 4), corroborating that the majority of the grafts (7 of 8) were suitable for implantation as single grafts. Of note, one of the UK transplant centres, which uses a pre-implantation biopsy service routinely, has adopted a modified interpretation of the Remuzzi score and considers a score of 4 as safe to be used individually (Ayorinde et al., 2019). Using this metric alone, this unit, therefore, would have considered all of the grafts herein suitable for transplant as single grafts. Although both the Remuzzi score and EVNP assessment score were favourable, neither of these metrics take into account other factors, such as cold ischaemic time or concern regarding malignancy, which would likely have precluded the use of these grafts for transplant.

It is our interpretation of these grafts following the period of assessment afforded by EVNP that 5 of the 8 could have been used for transplantation if EVNP had been used as part of the viability assessment process. K1, a 55-year-old DCD graft with diseased and atherosclerotic aorta, performed well with good flow rates and urine output. K2 was described as having multiple arteries and concern that one of the arteries had been damaged; the graft perfused well via the remaining artery with less than 10% affected by the damaged artery and demonstrated excellent perfusion. K3, K4 and K6 were all declined for 'poor perfusion', i.e., incomplete clearance of blood at the time of in situ cold perfusion; all three of these grafts performed satisfactorily during this period of perfusion, with good flow rates and urine output. Of note, K4, which initially demonstrated moderate/poor macroscopic perfusion, slowly improved over time and, by the 4-hour point, had a good perfusion appearance. K5 and K7 were declined for large renal cysts, and K8 for a high PITHIA score. We do not believe that EVNP would change an assessment decision of kidneys with these specific concerns. However, of note, the Remuzzi score, performed by our own histopathologist, was

not in concordance with this score and gave K8 a pre- and port-perfusion Remuzzi score of 4, highlighting the subjectiveness of this assessment.

Cold ischaemic time was elevated in the grafts used in this study (mean of 25 hours and 11 minutes) owing to the logistics of obtaining research organs which have been declined for clinical use. Normothermic perfusion techniques, such as EVNP, used at this late-stage post-procurement may yet have a role in widening graft utilisation if the reconditioning effects of this technology are further demonstrated. However, it's role, based on current evidence, would support the use of EVNP at an earlier stage (less cold ischaemic time) if such grafts are to be then reconsidered for implantation.

ADRC were administered to three grafts within this study. This cell population has been demonstrated in numerous systems to have regenerative, anti-inflammatory and immunomodulatory properties, including kidney models and the amelioration of IRI (Feng et al., 2010a). Thompson et al. and Pool et al. recently reported on the delivery of mesenchymal stell cells, of which ADRC is a subtype, in a human discarded kidney model and porcine model, respectively (M. Pool et al., 2019; Thompson et al., 2020). The MSCs used in the human kidney study were commercially sourced, requiring a freeze-thaw process and culture expansion. The MSCs in the work by Pool et al., however, were harvested from porcine adipose tissue and then isolated, culture-expanded and cryopreserved. In addition to the above, Brasile et al. also reported on the use of commercially sourced MSCs delivered via sub-normothermic exsanguinous machine perfusion (EMS) (i.e., non-blood product based) in a human kidney model (Brasile et al., 2019).

With regard to the wider context of MSC delivery to marginal kidneys, there remains considerable obstacles before the translation to clinical use (F. Chen et al., 2023). Although favourable signals have been demonstrated in multiple studies, by way of impacting inflammatory cytokines and markers of tubular injury, the therapy has not yet been shown to demonstrably improve early functional parameters during a period of perfusion. Furthermore, the role of MSC therapy in the developing field of xenotransplantation is currently poorly explored and requires substantial investigation; whether this 'tool' may offer a method of modulating the immune environment in the context of other therapies with a the goal of improved tolerance of inter-species chimerism is of great interest (Xi et al., 2023).

In the design of this study, a critical criterion for the harvest and delivery of regenerative cell therapy was that it should not delay or complicate the process of clinical transplantation. The

use of recipient adipose tissue was considered, however would require the recipient operation to begin to allow the adipose tissue to be harvested, and then prepared and delivered via EVNP, thus delaying implantation (prolonged CIT) and lengthen the duration of the operation. Third party adipose tissue was also considered, yet requires storage, and thus a freezing process. Clinical transplant often occurs out of hours at relatively short notice and obtaining cells with the additional necessary thawing process were thought to cause significant practical challenges. It was thought that obtaining adipose tissue from the peri-renal donor tissue allowed for timely cell preparation with very little or no delay to implantation (i.e., no prolongation of CIT).

Work by our own research group has experience with a point-of-care device known as the Celution System® (Celution® 800/CRS System) manufactured by Lorem Cytori. With an input of 100-150ml of adipose tissue, following an enzymatic process and centrifugation, an output of an ADRC population is produced with a viability of over 80%. In this study, from the three treatment kidneys, an average cell number of 1.3 million cells (range 1.2-1.5 million cells) was obtained with an average viability of 81%. This ADRC population were subsequently administered via the arterial limb during the period of perfusion of these three grafts. The avoidance of culture expansion and allogeneic effects (as the cells are genetically identical to the donor graft) offers considerable practical benefits. The limitations, however, include a reliance on a sufficient amount of peri-renal adipose tissue, as demonstrated in K5 of this study, where the back bench preparation of this kidney removed the adipose tissue from the graft prior to transportation.

From this work and our own prior clinical experience, we believe that the vast majority of grafts procured would have the required 100-150ml of adipose tissue for use with the Celution System®. Furthermore, this work demonstrates the feasibility of obtaining a viable ADRC population from donor peri-renal adipose tissue that has undergone a degree of warm and cold ischaemia. There will be varying amounts of adipose tissue on each graft, however, and of variable quality, which may lead to differing cell count and variability in the output. As there was an abundance of adipose tissue in the three treatment kidneys described above, there was no significant discrepancy in the cell output. If a discrepancy was identified, this would lead to challenges with dosing and reproducibility, particularly in the scientific setting. Furthermore, there are practical challenges in the process of labelling cells for post-perfusion localisation, which is important to further understand the mechanistic effects of regenerative cell therapy delivered via EVNP.

There was no apparent difference in perfusion parameters following the administration of ADRC in this report. Although urine output was found to be lower (although not statistically significant) in the first hour in the ADRC group, this difference was not present at the 4-hour time point. Nor were there any differences evident in histological features of either chronic (Remuzzi) or acute tubular injury scoring.

More work is required to establish the optimal dose of MSC to be delivered during perfusion techniques. This study demonstrates that the peri-renal adipose tissue from donor kidneys maintains satisfactory ADRC viability despite the procurement process (e.g., warm ischaemia followed by a period of cold ischaemia). However, it is clear that more work is required to assess the cell population and properties of ADRC at different levels of cold ischaemic to ascertain if the process of procurement is detrimental to the cell group beyond mere viability and cell number.

A dosing study by Munk et al. demonstrated that high dosing of adipose-derived MSCs delivered via the renal artery (over a hundred million MSCs) led to glomeruli microthrombi and evidence of a blood-mediated inflammatory reaction with reduced renal perfusion and tubular damage (Munk et al., 2022). Although considerably less cell number delivery of ADRC, no microthrombi were evident in the kidneys that received ADRC in this study. Within the control group, one of the grafts (K7) demonstrated widespread glomerular thrombi of uncertain significance. Of clinical concern, none of the EVNP assessment score parameters highlighted an issue with this graft whilst on perfusion, raising concern that these parameters may not be sensitive to this finding, which carries significant clinical concern. Of note, although one of the kidneys in this series demonstrated glomerular thrombi, the finding was present in both pre-and post-perfusion biopsies and was in the control group.

Although reassuring that no glomerular thrombus was present following the delivery of ADRC, it is important to note that unlike the systemic circulation, the perfusate does not contain clotting factors and is also dosed with low molecular weight heparin. Further studies with the addition of clotting factors to the perfusion circuit, or alternatively porcine autotransplant models, may be necessary to further investigate this safety risk with ADRC delivery.

Of consideration, a pitfall with the therapeutic use of pluripotent stem cells is the small but significant risk of teratoma formation or tumorigenesis in the longer term (King, 2014). Although this risk not thought to be present with MSC subtypes (e.g., ADRC) due to the small proportion of true pluripotent stem cells and lack of need for culture expansion (Sato

et al., 2019), this theoretical risk should nevertheless be considered in translation to clinical use. The systematic review by Toyserkani assessed the safety profile of ADRC therapy, and concluded that the treatment is safe, with no reported events of tumorigenesis, however discussed that robust long-term data was lacking (Toyserkani et al., 2017). It is important to note that ADRC treatment of renal grafts, in clinical use will then be implanted into a patient in which the immune system will be dampened by the immunosuppression used in clinical transplantation. If ADRC treatment via EVNP reached clinical use, therefore, it is imperative that long term safety data be collected to corroborate this favourable safety profile.

Unfortunately, the attempted perfusate analysis using a Luminex assay to assess both the perfusate and urine for markers of kidney injury and key cytokines was not successful, thus significantly limiting the conclusions that can be drawn from this work. A re-run of these experiments will be performed in due course. Due to the fact the purchase for the assay was made via a hospital endowment fund, the assay was delivered to a hospital ward without notification to the primary investigator. As a result, the assay was not immediately refrigerated, thus rendering the assay potentially unviable. Given the substantial expense, an attempt to use the assay was made, which, as stated, was unsuccessful.

The aforementioned studies, by Pool and Thompson, whilst demonstrating the feasibility of MSC delivery via normothermic perfusion of kidneys, failed to demonstrate beneficial benefits in outcomes. Indirect benefits were only demonstrated by Thompson et al., who reported a reduction in urinary NGAL, the commonly used biomarker for kidney injury, downregulation of IL-1β and upregulation of IL-10, with MSC treatment (Thompson et al., 2020). In addition, as highlighted in Brasile's work, the MSC treatment group demonstrated a reduction in inflammatory cytokines and increased ATP secretion. Although this study utilised a non-blood-based perfusate and therefore has significant scientific design differences, which limits direct comparisons (Brasile et al., 2019).

There are numerous limitations to this study that warrant discussion. Firstly, the small number of kidneys in the treatment group. This was partly due to time constraints, logistical challenges and a reduction in available kidneys for research during the COVID pandemic; generalisable conclusions are therefore limited. Secondly, although the method of harvesting the ADRC population and delivery (i.e., 'point-of-care' delivery) is not thought to require culture expansion, the number of cells produced ranged from 1.2 to 1.5 million cells. This is in contrast to comparable studies that used 50 million cells (Thompson et al., 2020), which may account for the lack of treatment effect evident. Thirdly, it is not known the impact of organ procurement and cold storage on the viability and function of ADRC. Although

satisfactory viability was evident in the cells harvested in this study (over 80%), more data on function is required to further understanding. Fourthly, as aforementioned, due to study design there was not an opportunity to label the ADRC cells to better understand cell function and treatment efficacy.

RNA sequencing was able to highlight differential gene expression between the control and ADRC groups. Genes such as HAS2 and DDIT4L were upregulated with ADRC treatment and have previously been demonstrated to play a role in renal tubule production and renal injury models, respectively (Fattahi et al., 2021; Soulié et al., 2014). Conversely, genes such as ESM1, were upregulated in the control group. Mechanistic information is not obtained via RNA sequencing, yet it infers potential avenues for further research by highlighting possible cellular pathways. Whilst gene ontology attempts to map the differential gene expression to biological pathways by way of clustering, the exact mechanisms remain elusive. It is important to note that due to small numbers, particularly in the treatment group, this study is underpowered to make strong conclusions with regards to gene variability. The RNA sequencing described in this work, in our opinion, highlights the potential impact of ADRC when administered via normothermic perfusion, yet does not conclusively demonstrate whether this impact is positive or negative. A strength of this work is the independent pathway analysis performed by experts in bioinformatics. Data was obtained, with subsequent gene mapping, differential gene expression and gene ontology analysis all performed by an independent third party (GeneWiz, Azenta). It is important to note, however, that due to the small sample size and the variability within the samples that the power of these findings is limited and should be interpreted with caution. The role of RNA sequencing data in this setting highlights potential avenues for further scientific study. Further work is critical, therefore, to investigate the highlighted biological pathways and differentially expressed genes when investigating the mechanistic actions of ADRC in this and similar kidney models.

The kidneys were perfused in this study for a period of four hours. It is reported that prolonged perfusion of grafts (over 5 hours) with whole blood-based products may have detrimental effects, including high vascular resistance and glomerular necrosis (D et al., 2017). Of note, no adverse effects were demonstrated with this duration of perfusion in this study. Concern with prolonged blood-based perfusion, along with the logistical challenges with obtaining blood products, has led to a focus on non-blood-based products such as EMS, Steen solution and artificial haemoglobin-based oxygen carriers (HBOC) in the pursuit of longer perfusion durations (i.e., beyond 6 hours). The duration of four hours was chosen for

two specific reasons: 1) this duration with blood-based perfusion has been shown to be safe and provides a sufficient period of assessment, and 2) from a clinical delivery perspective, we suggest that the four-hour duration provides time to shift implantation of grafts from the most challenging times between 02:00 and 06:00 hours to daylight hours. Longer perfusion durations may yet be proven to have additional reconditioning benefits; however, shifting a transplant from 04:00 to 16:00, for example, does not improve the clinical delivery of transplantation from the surgeon's perspective.

6.5 Conclusions

This study demonstrates that an ADRC population can be obtained from the donor kidneys' peri-renal adipose tissue and delivered via an EVNP circuit. No difference was found between EVNP and EVNP plus ADRC treatment with regard to urine output, perfusion parameters or histological grading. Although differential gene expression was demonstrated, it is not clear whether ADRC treatment had a positive impact on the grafts. More work is required to assess in more detail the ADRC population obtained in this manner to understand better the possible regenerative effects shown in other organ systems. Of importance, the delivery of ADRC, however, did not demonstrate any adverse effects, such as glomerular thrombus, in this study. The positive findings with MSC delivery via kidney perfusion, thus far, have been found in non-blood-based perfusates, which warrants further exploration.

CHAPTER 7: CONCLUSIONS

Marginal Kidneys and Marginal Recipients

This retrospective analysis of transplants over a two-year period reclassified both donors and recipients as per the latest national kidney allocation scheme. The work highlights the broad impact of high-risk donors combined with high-risk recipients. Time off dialysis, or graft survival, may be comparable, yet this comes at a cost with higher rates of delayed graft function, length of hospital stay and use of hospital resources in the postoperative period. These are important considerations for future amendments to the allocation scheme. With a high proportion of the highest-risk recipients (R4) in our current waiting list, there will be an ongoing high proportion of the high-risk combination of R4/D4 transplants. Techniques to better evaluate high-risk kidneys and reduce rates of delayed graft function, such as NRP or EVNP, will be critical in delivering this service.

The Introduction of a New Clinical Perfusion Service to Improve Organ utilisation in an acute Tertiary Hospital

Introducing a new technology such as EVNP to a busy tertiary hospital was logistically challenging. Specific obstacles of note included sourcing blood products, ordering consumables with protracted lead times and arterial cannulation in cases of variant anatomy. In a hospital facility without a dedicated transplant theatre, delivering this technology placed a strain on the already stretched theatre department, which is a consideration in providing post-procurement technologies on a broader scale.

Clinical Experience of Ex Vivo Normothermic Perfusion

EVNP was critical in the viability assessment of 7 clinical kidneys in both the deceased and living donor setting, which were transplanted successfully following a period of perfusion. This thesis outlines the novel use of this technology in cases of donors with rhabdomyolysis, renal artery stenosis and in a case of dual kidney transplantation. The technology also assessed a graft with prolonged cold ischaemic time in a circumstance where two intended recipients were found to be unfit for transplant, requiring a third recipient to be identified and prepared for theatre.

Therapeutic Efficacy of Mesenchymal Stromal Cell Population Delivered via Isolated Organ Perfusion - A Systematic Review

The systematic review included evaluates the current evidence for the delivery of regenerative cell therapies, such as mesenchymal stromal cells, via isolated solid organ perfusion in the kidney and liver. There is a body of evidence for the growing immune-modulatory role and anti-inflammatory properties of MSC; however, more work is required to demonstrate the benefit in human transplant models further to translate these therapies to clinical trials. In addition, work on both dosing and safety are crucial as we progress towards clinical use.

Delivery of ADRC Therapy During Ex-Vivo Normothermic Perfusion

This study demonstrated the delivery of ADRC during a 4-hour period of normothermic perfusion of human kidneys that had been declined for transplantation. Unique to this work was the harvest of this regenerative cell population from a point-of-care source, from the perirenal adipose tissue of the donor graft. Although no differences were seen in perfusion parameters and urine output, delivery of the therapy did not demonstrate any adverse events such as glomerular thrombus.

Closing statement

This thesis demonstrates the feasibility of a clinical EVNP service as a utilisation tool to facilitate decision making in renal transplantation. Specific recipient and donor combinations, i.e., high risk “R4/D4” transplants are hypothesised to be theoretical targets for the reconditioning benefits that EVNP may provide.

Furthermore, the work described adds to the current growing body of evidence for the use of EVNP as a vehicle to deliver targeted therapy with the aim of minimising the deleterious effects of ischaemic injury. Herein, we demonstrated the novel use of donor peri-renal adipose tissue as the source of regenerative cells delivered directly to the donor organ. The limitations of this source of ADRC, as outlined, are numerous. There are challenges with dosing and the unknown condition and number of cell populations within the adipose tissue until harvested (i.e., amount and quality of cell population limited to the tissue available).

Furthermore, ADRCs have the advantage of not requiring culture expansion prior to use, and therefore it is a point of discussion that the lack of therapeutic benefit demonstrated herein may be due to inadequate dosing delivered in this study. More work is required to assess the number and quality of ADRC within donor peri-renal tissue and to evaluate how the process of organ procurement and cold ischaemic time impacts the function of this cell group, and what cell number is required to achieve therapeutic benefit. Importantly, further work should consider the impact of dosing on the safety of this therapy. No adverse clotting events were demonstrated in this work following the treatment of ADRC via EVNP. To allay concerns on longer term safety, such as tumorigenesis, specifically designed autotransplant animal studies or longer-term clinical studies are likely to be required.

BIBLIOGRAPHY

Amador, A., Grande, L., Martí, J., Deulofeu, R., Miquel, R., Solá, A., Rodriguez-Laiz, G., Ferrer, J., Fondevila, C., Charco, R., Fuster, J., Hotter, G., & García-Valdecasas, J. C. (2007). Ischemic Pre-Conditioning in Deceased Donor Liver Transplantation: A Prospective Randomized Clinical Trial. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *7*(9), 2180–2189. https://doi.org/10.1111/j.1600-6143.2007.01914.x

Andre, M., Huang, E., Everly, M., & Bunnapradist, S. (2014). The UNOS Renal Transplant Registry: Review of the Last Decade. *Clinical Transplants*, 1–12.

Ankrum, J., & Karp, J. M. (2010). Mesenchymal Stem Cell Therapy: Two Steps Forward, One Step Back. *Trends Mol Med*, *16*(5), 203–209. https://doi.org/10.1016/j.molmed.2010.02.005

Annual Activity Report. (n.d.). In *ODT Clinical—NHS Blood and Transplant*. https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/

Archived Reference Costs | NHS Improvement. (n.d.). https://improvement.nhs.uk/resources/reference-costs/

Ardehali, A., Esmailian, F., Deng, M., Soltesz, E., Hsich, E., Naka, Y., Mancini, D., Camacho, M., Zucker, M., Leprince, P., Padera, R., Kobashigawa, J., & PROCEED II trial investigators. (2015). Ex-Vivo Perfusion of Donor Hearts for Human Heart Transplantation (PROCEED II): A Prospective, Open-Label, Multicentre, Randomised Non-Inferiority Trial. *Lancet (London, England)*, *385*(9987), 2577–2584. https://doi.org/10.1016/S0140-6736(15)60261-6

ASTRAL Investigators, Wheatley, K., Ives, N., Gray, R., Kalra, P. A., Moss, J. G., Baigent, C., Carr, S., Chalmers, N., Eadington, D., Hamilton, G., Lipkin, G., Nicholson, A., & Scoble, J. (2009). Revascularization versus medical therapy for

renal-artery stenosis. *The New England Journal of Medicine*, *361*(20), 1953–1962. https://doi.org/10.1056/NEJMoa0905368

Aubert, O., Reese, P. P., Audry, B., Bouatou, Y., Raynaud, M., Viglietti, D., Legendre, C., Glotz, D., Empana, J.-P., Jouven, X., Lefaucheur, C., Jacquelinet, C., & Loupy, A. (2019). Disparities in Acceptance of Deceased Donor Kidneys Between the United States and France and Estimated Effects of Increased US Acceptance. *JAMA Internal Medicine*. https://doi.org/10.1001/jamainternmed.2019.2322

Avlonitis, V. S., Wigfield, C. H., Kirby, J. A., & Dark, J. H. (2005). The hemodynamic mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *5*(4 Pt 1), 684–693. https://doi.org/10.1111/j.1600-6143.2005.00755.x

Ayala-Cuellar, A. P., Kang, J. H., Jeung, E. B., & Choi, K. C. (2018). Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation. *Biomol Ther (Seoul)*. https://doi.org/10.4062/biomolther.2017.260

Ayorinde, J. O., Summers, D. M., Pankhurst, L., Laing, E., Deary, A. J., Hemming, K., Wilson, E. C., Bardsley, V., Neil, D. A., & Pettigrew, G. J. (2019). PreImplantation Trial of Histopathology In renal Allografts (PITHIA): A stepped-wedge cluster randomised controlled trial protocol. *BMJ Open*, *9*(1), e026166. https://doi.org/10.1136/bmjopen-2018-026166

Bajema, I. M., & Rotmans, J. I. (2018). Histological Manifestations of Rhabdomyolysis in the Kidney. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *33*(12), 2113–2114. https://doi.org/10.1093/ndt/gfy162

Balfoussia, D., Yerrakalva, D., Hamaoui, K., & Papalois, V. (2012). Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *10*(2), 87–100. https://doi.org/10.6002/ect.2011.0167

Banfi, A., Muraglia, A., Dozin, B., Mastrogiacomo, M., Cancedda, R., & Quarto, R. (2000). Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. *Experimental Hematology*, *28*(6), 707–715. https://doi.org/10.1016/S0301-472X(00)00160-0

Baraniak, P. R., & McDevitt, T. C. (2010). Stem Cell Paracrine Actions and Tissue Regeneration. *Regenerative Medicine*, *5*(1), 121–143. https://doi.org/10.2217/rme.09.74

Barros, S. A., Tennant, R. W., & Cannon, R. E. (2003). Molecular structure and characterization of a novel murine ABC transporter, Abca13. *Gene*, *307*, 191–200. https://doi.org/10.1016/s0378-1119(03)00465-7

Baulier, E., Favreau, F., Le Corf, A., Jayle, C., Schneider, F., Goujon, J.-M., Feraud, O., Bennaceur-Griscelli, A., Hauet, T., & Turhan, A. G. (2014). Amniotic Fluid-Derived Mesenchymal Stem Cells Prevent Fibrosis and Preserve Renal Function in a Preclinical Porcine Model of Kidney Transplantation. *Stem Cells Translational Medicine*, *3*(7), 809–820. https://doi.org/10.5966/sctm.2013-0186

Baxter, M. A., Wynn, R. F., Jowitt, S. N., Wraith, J. E., Fairbairn, L. J., & Bellantuono, I. (2004). Study of Telomere Length Reveals Rapid Aging of Human Marrow Stromal Cells Following in Vitro Expansion. *Stem Cells (Dayton, Ohio)*, *22*(5), 675–682. https://doi.org/10.1634/stemcells.22-5-675

Belzer, F. O., Ashby, B. S., Gulyassy, P. F., & Powell, M. (1968). Successful seventeen-hour preservation and transplantation of human-cadaver kidney. *The New England Journal of Medicine*, *278*(11), 608–610. https://doi.org/10.1056/NEJM196803142781108

Bentas, W., Jones, J., Karaoguz, A., Tilp, U., Probst, M., Scheuermann, E., Hauser, I. A., Jonas, D., & Gossmann, J. (2008). Renal Transplantation in the Elderly: Surgical Complications and Outcome with Special Emphasis on the Eurotransplant Senior Programme. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *23*(6), 2043–2051. https://doi.org/10.1093/ndt/gfm912

Block, G. J., Ohkouchi, S., Fung, F., Frenkel, J., Gregory, C., Pochampally, R., DiMattia, G., Sullivan, D. E., & Prockop, D. J. (2009). Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. *Stem Cells (Dayton, Ohio)*, *27*(3), 670–681. https://doi.org/10.1002/stem.20080742

Boffa, C., van de Leemkolk, F., Curnow, E., Homan van der Heide, J., Gilbert, J., Sharples, E., & Ploeg, R. J. (2017). Transplantation of Kidneys From Donors With Acute Kidney Injury: Friend or Foe? *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *17*(2), 411–419. https://doi.org/10.1111/ajt.13966

Brasile, L., Henry, N., Orlando, G., & Stubenitsky, B. (2019). Potentiating Renal Regeneration Using Mesenchymal Stem Cells. *Transplantation*, *103*(2), 307–313. https://doi.org/10.1097/TP.0000000000002455

Brasile, L., & Stubenitsky, B. (2017). Bioengineering Approach to Immunomodulation. In *Kidney Transpl., Bioeng., and Regen.: Kidney Transpl. In the Regen. Med. Era* (pp. 861–872). Elsevier Inc. https://doi.org/10.1016/B978-0-12-801734-0.00061-8

Butler, C. R., Perkins, J. D., Johnson, C. K., Blosser, C. D., Bakthavatsalam, R., Leca, N., & Sibulesky, L. (2019). Burden of Excess Mortality after Implementation of the New Kidney Allocation System May Be Borne Disproportionately by Middle-Aged Recipients. *PloS One*, *14*(1), e0210589. https://doi.org/10.1371/journal.pone.0210589

Callaghan, C. J., Harper, S. J. F., Saeb-Parsy, K., Hudson, A., Gibbs, P., Watson, C. J. E., Praseedom, R. K., Butler, A. J., Pettigrew, G. J., & Bradley, J. A. (2014). The Discard of Deceased Donor Kidneys in the UK. *Clinical Transplantation*, *28*(3), 345–353. https://doi.org/10.1111/ctr.12319

Carden, D. L., & Granger, D. N. (2000). Pathophysiology of Ischaemia– Reperfusion Injury. *The Journal of Pathology*, *190*(3), 255–266. https://doi.org/doi:10.1002/(SICI)1096-9896(200002)190:3<255::AID-PATH526>3.0.CO;2-6

Carlis, R. D., Centonze, L., Migliorini, M., Pitoni, L., Cerchione, R., Lauterio, A., & Carlis, L. D. (2023). ABDOMINAL NORMOTHERMIC REGIONAL PERFUSION IN DONATION AFTER CIRCULATORY DEATH: ORGAN VIABILITY OR ORGAN PRESERVATION? *European Journal of Transplantation*, 113–120. https://doi.org/10.57603/EJT-013

Cervelli, V., Gentile, P., De Angelis, B., Calabrese, C., Di Stefani, A., Scioli, M. G., Curcio, B. C., Felici, M., & Orlandi, A. (2011). Application of Enhanced Stromal Vascular Fraction and Fat Grafting Mixed with PRP in Post-Traumatic Lower Extremity Ulcers. *Stem Cell Research*, *6*(2), 103–111. https://doi.org/10.1016/j.scr.2010.11.003

Chatterjee, P., Brown, P., Cuzzocrea, S., Zacharowski, K., Stewart, K., Mota-Filipe, H., Mcdonald, M., & Thiemermann, C. (2001). Calpain inhibitor-1 reduces renal

ischemia/reperfusion injury in the rat. *Kidney International*, *59*, 2073–2083. https://doi.org/10.1046/j.1523-1755.2001.00722.x

Chaudhry, D., Evison, F., & Sharif, A. (2024). Survival of patients with kidney failure awaiting transplantation stratified by age and ethnicity: Population-based cohort analysis. *The British Journal of Surgery*, *111*(1), znae001. https://doi.org/10.1093/bjs/znae001

Chen, C.-B., Zheng, Y.-T., Zhou, J., Han, M., Wang, X.-P., Yuan, X.-P., Wang, C.-X., & He, X.-S. (2017). Kidney Transplantation from Donors with Rhabdomyolysis and Acute Renal Failure. *Clinical Transplantation*, *31*(8). https://doi.org/10.1111/ctr.13021

Chen, F., Chen, N., Xia, C., Wang, H., Shao, L., Zhou, C., & Wang, J. (2023). Mesenchymal Stem Cell Therapy in Kidney Diseases: Potential and Challenges. *Cell Transplantation*, *32*, 09636897231164251. https://doi.org/10.1177/09636897231164251

Chen, L., Xiao, D., Tang, F., Gao, H., & Li, X. (2020). CAPN6 in disease: An emerging therapeutic target (Review). *International Journal of Molecular Medicine*, *46*(5), 1644–1652. https://doi.org/10.3892/ijmm.2020.4734

Chen, Z., Chen, L., & Chen, H. (2021). The Impact of COVID-19 on the Clinical Trial. *PLOS ONE*, *16*(5), e0251410. https://doi.org/10.1371/journal.pone.0251410

Chouchani, E. T., Pell, V. R., Gaude, E., Aksentijevic, D., Sundier, S. Y., Robb, E. L., Logan, A., Nadtochiy, S. M., Ord, E. N. J., Smith, A. C., Eyassu, F., Shirley, R., Hu, C. H., Dare, A. J., James, A. M., Rogatti, S., Hartley, R. C., Eaton, S., Costa, A. S. H., … Murphy, M. P. (2014). Ischaemic Accumulation of Succinate Controls Reperfusion Injury through Mitochondrial ROS. *Nature*, *515*(7527), 431–435. https://doi.org/10.1038/nature13909

Collins, G. M., Bravo-Shugarman, M., & Terasaki, P. I. (1969). KIDNEY PRESERVATION FOR TRANSPORTATION: Initial Perfusion and 30 Hours' Ice Storage. *The Lancet*, *294*(7632), 1219–1222. https://doi.org/10.1016/S0140-6736(69)90753-3

Cravedi, P., Mothi, S. S., Azzi, Y., Haverly, M., Farouk, S. S., Pérez-Sáez, M. J., Redondo-Pachón, M. D., Murphy, B., Florman, S., Cyrino, L. G., Grafals, M., Venkataraman, S., Cheng, X. S., Wang, A. X., Zaza, G., Ranghino, A., Furian, L., Manrique, J., Maggiore, U., … Riella, L. V. (2020). COVID-19 and Kidney Transplantation: Results from the TANGO International Transplant Consortium. *American Journal of Transplantation*. https://doi.org/10.1111/ajt.16185

Cypel, M., Yeung, J. C., Machuca, T., Chen, M., Singer, L. G., Yasufuku, K., de Perrot, M., Pierre, A., Waddell, T. K., & Keshavjee, S. (2012). Experience with the First 50 Ex Vivo Lung Perfusions in Clinical Transplantation. *The Journal of Thoracic and Cardiovascular Surgery*, *144*(5), 1200–1206. https://doi.org/10.1016/j.jtcvs.2012.08.009

D, U., Mf, B., Q, L., A, N., Ld, B., T, D. U., Ed, P., Wm, B. I., Da, G., & C, Q. (2017). Development of a prolonged warm ex vivo perfusion model for kidneys donated after cardiac death. *The International Journal of Artificial Organs*, *40*(6). https://doi.org/10.5301/ijao.5000586

Dahmane, D., Audard, V., Hiesse, C., Pessione, F., Bentaarit, B., Barrou, B., Rondeau, E., Cohen, S., Lang, P., & Grimbert, P. (2006). Retrospective follow-up of transplantation of kidneys from 'marginal' donors. *Kidney International*, *69*(3), 546–552. https://doi.org/10.1038/sj.ki.5000102

Dahmen, M., Becker, F., Pavenstädt, H., Suwelack, B., Schütte-Nütgen, K., & Reuter, S. (2019). Validation of the Kidney Donor Profile Index (KDPI) to Assess a Deceased

Donor's Kidneys' Outcome in a European Cohort. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-47772-7

De Ugarte, D. A., Morizono, K., Elbarbary, A., Alfonso, Z., Zuk, P. A., Zhu, M., Dragoo, J. L., Ashjian, P., Thomas, B., Benhaim, P., Chen, I., Fraser, J., & Hedrick, M. H. (2003). Comparison of Multi-Lineage Cells from Human Adipose Tissue and Bone Marrow. *Cells Tissues Organs*, *174*(3), 101–109. https://doi.org/10.1159/000071150

Demiselle, J., Augusto, J.-F., Videcoq, M., Legeard, E., Dubé, L., Templier, F., Renaudin, K., Sayegh, J., Karam, G., Blancho, G., & Dantal, J. (2016). 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: Official Journal of the European Society for Organ Transplantation*, *29*(4), 432–442. https://doi.org/10.1111/tri.12722

DiRito, J. R., Hosgood, S. A., Tietjen, G. T., & Nicholson, M. L. (2018). The Future of Marginal Kidney Repair in the Context of Normothermic Machine Perfusion. *American Journal of Transplantation*, *18*(10), 2400–2408. https://doi.org/10.1111/ajt.14963

Domagala, P., Gorski, L., Wszola, M., Kieszek, R., Diuwe, P., Goralski, P., Drozdowski, J., Ostaszewska, A., Gozdowska, J., Ciszek, M., Trzebicki, J., Durlik, M., Paczek, L., Chmura, A., & Kwiatkowski, A. (2019). Successful Transplantation of Kidneys from Deceased Donors with Terminal Acute Kidney Injury. *Renal Failure*, *41*(1), 167–174. https://doi.org/10.1080/0886022X.2019.1590209

Domingues, C. C., Kundu, N., Kropotova, Y., Ahmadi, N., & Sen, S. (2019). Antioxidant-upregulated mesenchymal stem cells reduce inflammation and improve fatty liver

disease in diet-induced obesity. *Stem Cell Research & Therapy*, *10*(1), 280. https://doi.org/10.1186/s13287-019-1393-8

Dreyer, G. J., Hemke, A. C., Reinders, M. E. J., & de Fijter, J. W. (2015). Transplanting the Elderly: Balancing Aging with Histocompatibility. *Transplantation Reviews (Orlando, Fla.)*, *29*(4), 205–211. https://doi.org/10.1016/j.trre.2015.08.003

Echterdiek, F., Schwenger, V., Döhler, B., Latus, J., Kitterer, D., Heemann, U., & Süsal, C. (2019). Kidneys From Elderly Deceased Donors—Is 70 the New 60? *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02701

Eggenhofer, E., Luk, F., Dahlke, M. H., & Hoogduijn, M. J. (2014). The Life and Fate of Mesenchymal Stem Cells. *Frontiers in Immunology*, *5*, 148. https://doi.org/10.3389/fimmu.2014.00148

Eirin, A., Zhu, X.-Y., Ebrahimi, B., Krier, J. D., Riester, S. M., van Wijnen, A. J., Lerman, A., & Lerman, L. O. (2015). Intrarenal Delivery of Mesenchymal Stem Cells and Endothelial Progenitor Cells Attenuates Hypertensive Cardiomyopathy in Experimental Renovascular Hypertension. *Cell Transplantation*, *24*(10), 2041–2053. https://doi.org/10.3727/096368914X685582

Eltzschig, H. K., & Eckle, T. (2011). Ischemia and reperfusion—From mechanism to translation. *Nature Medicine*, *17*(11), 1391–1401. https://doi.org/10.1038/nm.2507

Erices, A., Conget, P., & Minguell, J. J. (2000). Mesenchymal progenitor cells in human umbilical cord blood. *British Journal of Haematology*, *109*(1), 235–242. https://doi.org/10.1046/j.1365-2141.2000.01986.x

Farney, A. C., Rogers, J., Orlando, G., al-Geizawi, S., Buckley, M., Farooq, U., al-Shraideh, Y., & Stratta, R. J. (2013). Evolving Experience Using Kidneys from Deceased Donors with Terminal Acute Kidney Injury. *Journal of the American College of Surgeons*, *216*(4), 645–655; discussion 655-656. https://doi.org/10.1016/j.jamcollsurg.2012.12.020

Fattahi, F., Saeednejad Zanjani, L., Habibi Shams, Z., Kiani, J., Mehrazma, M., Najafi, M., & Madjd, Z. (2021). High expression of DNA damage-inducible transcript 4 (DDIT4) is associated with advanced pathological features in the patients with colorectal cancer. *Scientific Reports*, *11*, 13626. https://doi.org/10.1038/s41598-021-92720-z

Feng, Z., Ting, J., Alfonso, Z., Strem, B. M., Fraser, J. K., Rutenberg, J., Kuo, H. C., & Pinkernell, K. (2010a). Fresh and Cryopreserved, Uncultured Adipose Tissue-Derived Stem and Regenerative Cells Ameliorate Ischemia– Reperfusion-Induced Acute Kidney Injury. *Nephrol Dial Transplant*, *25*(12), 3874–3884. https://doi.org/10.1093/ndt/gfq603

Feng, Z., Ting, J., Alfonso, Z., Strem, B. M., Fraser, J. K., Rutenberg, J., Kuo, H. C., & Pinkernell, K. (2010b). Fresh and Cryopreserved, Uncultured Adipose Tissue-Derived Stem and Regenerative Cells Ameliorate Ischemia-Reperfusion-Induced Acute Kidney Injury. *Nephrol Dial Transplant*, *25*(12), 3874–3884. https://doi.org/10.1093/ndt/gfq603

Ferreira, E. de S., Moreira, T. R., da Silva, R. G., da Costa, G. D., da Silva, L. S., Cavalier, S. B. de O., Silva, B. O., Dias, H. H., Borges, L. D., Machado, J. C., & Cotta, R. M. M. (2020). Survival and analysis of predictors of mortality in patients undergoing replacement renal therapy: A 20-year cohort. *BMC Nephrology*, *21*(1), 502. https://doi.org/10.1186/s12882-020-02135-7

First National Report of the Emergency Laparoscopic and Laparotomy Scottish Audit | Turas | Learn. (n.d.). Retrieved 29 November 2023, from https://learn.nes.nhs.scot/16164

Fischer, U. M., Harting, M. T., Jimenez, F., Monzon-Posadas, W. O., Xue, H., Savitz, S. I., Laine, G. A., & Cox, C. S. (2009). Pulmonary Passage Is a Major Obstacle for

Intravenous Stem Cell Delivery: The Pulmonary First-Pass Effect. *Stem Cells and Development*, *18*(5), 683–692. https://doi.org/10.1089/scd.2008.0253

Fraser, J. K., Hicok, K. C., Shanahan, R., Zhu, M., Miller, S., & Arm, D. M. (2014). The Celution((R)) System: Automated Processing of Adipose-Derived Regenerative Cells in a Functionally Closed System. *Adv Wound Care (New Rochelle)*, *3*(1), 38–45. https://doi.org/10.1089/wound.2012.0408

Fraser, J. K., Wulur, I., Alfonso, Z., & Hedrick, M. H. (2006a). Fat Tissue: An Underappreciated Source of Stem Cells for Biotechnology. *Trends Biotechnol*, *24*(4), 150–154. https://doi.org/10.1016/j.tibtech.2006.01.010

Fraser, J. K., Wulur, I., Alfonso, Z., & Hedrick, M. H. (2006b). Fat Tissue: An Underappreciated Source of Stem Cells for Biotechnology. *Trends in Biotechnology*, *24*(4), 150–154. https://doi.org/10.1016/j.tibtech.2006.01.010

Frei, U., Noeldeke, J., Machold-Fabrizii, V., Arbogast, H., Margreiter, R., Fricke, L., Voiculescu, A., Kliem, V., Ebel, H., Albert, U., Lopau, K., Schnuelle, P., Nonnast-Daniel, B., Pietruck, F., Offermann, R., Persijn, G., & Bernasconi, C. (2008). Prospective Age-Matching in Elderly Kidney Transplant Recipients–a 5-Year Analysis of the Eurotransplant Senior Program. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *8*(1), 50–57. https://doi.org/10.1111/j.1600-6143.2007.02014.x

Friedenstein, A. J., Chailakhyan, R. K., & Gerasimov, U. V. (1987). Bone marrow osteogenic stem cells: In vitro cultivation and transplantation in diffusion chambers. *Cell and Tissue Kinetics*, *20*(3), 263–272. https://doi.org/10.1111/j.1365-2184.1987.tb01309.x

Friedewald, J. J., Samana, C. J., Kasiske, B. L., Israni, A. K., Stewart, D., Cherikh, W., & Formica, R. N. (2013). The Kidney Allocation System. *Surgical Clinics of North America*, *93*(6), 1395–1406. https://doi.org/10.1016/j.suc.2013.08.007

Gallinat, A., Feldkamp, T., Schaffer, R., Radünz, S., Treckmann, J. W., Minor, T., Witzke, O., Paul, A., & Sotiropoulos, G. C. (2011). Single-Center Experience With Kidney Transplantation Using Deceased Donors Older Than 75 Years. *Transplantation*, *92*(1), 76–81. https://doi.org/10.1097/TP.0b013e31821d2687

Garcia-Olmo, D., Garcia-Arranz, M., & Herreros, D. (2008). Expanded Adipose-Derived Stem Cells for the Treatment of Complex Perianal Fistula Including Crohn's Disease. *Expert Opin Biol Ther*, *8*(9), 1417–1423. https://doi.org/10.1517/14712598.8.9.1417

García-Valdecasas, J. C., Tabet, J., Valero, R., Taurá, P., Rull, R., García, F., Montserrat, E., González, F. X., Ordi, J., Beltran, J., López-Boado, M. A., Deulofeu, R., Angás, J., Cifuentes, A., & Visa, J. (1998). Liver Conditioning after Cardiac Arrest: The Use of Normothermic Recirculation in an Experimental Animal Model. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *11*(6), 424–432.

Gentile, P., Orlandi, A., Scioli, M. G., Di Pasquali, C., Bocchini, I., & Cervelli, V. (2012). Concise Review: Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Implications for Tissue Engineering Therapies in Regenerative Surgery. *Stem Cells Translational Medicine*, *1*(3), 230–236. https://doi.org/10.5966/sctm.2011-0054

Gerbase-DeLima, M., de Marco, R., Monteiro, F., Tedesco-Silva, H., Medina-Pestana, J. O., & Mine, K. L. (2020). Impact of Combinations of Donor and Recipient Ages and Other Factors on Kidney Graft Outcomes. *Frontiers in Immunology*, *11*, 954. https://doi.org/10.3389/fimmu.2020.00954

Gharibi, B., Ghuman, M., & Hughes, F. J. (2016). DDIT4 regulates mesenchymal stem cell fate by mediating between HIF1α and mTOR signalling. *Scientific Reports*, *6*, 36889. https://doi.org/10.1038/srep36889

Gregorini, M., Corradetti, V., Pattonieri, E. F., Rocca, C., Milanesi, S., Peloso, A., Canevari, S., De Cecco, L., Dugo, M., Avanzini, M. A., Mantelli, M., Maestri, M., Esposito, P., Bruno, S., Libetta, C., Dal Canton, A., & Rampino, T. (2017). Perfusion of Isolated Rat Kidney with Mesenchymal Stromal Cells/Extracellular Vesicles Prevents Ischaemic Injury. *Journal of Cellular and Molecular Medicine*, *21*(12), 3381–3393. https://doi.org/10.1111/jcmm.13249

Gridelli, B., & Remuzzi, G. (2000). Strategies for Making More Organs Available for Transplantation. *The New England Journal of Medicine*, *343*(6), 404–410. https://doi.org/10.1056/NEJM200008103430606

Gu, D., Zou, X., Ju, G., Zhang, G., Bao, E., & Zhu, Y. (2016). Mesenchymal Stromal Cells Derived Extracellular Vesicles Ameliorate Acute Renal Ischemia Reperfusion Injury by Inhibition of Mitochondrial Fission through miR-30. *Stem Cells Int*, *2016*, 2093940. https://doi.org/10.1155/2016/2093940

Ham, S. W., Kumar, S. R., Wang, B. R., Rowe, V. L., & Weaver, F. A. (2010). Late outcomes of endovascular and open revascularization for nonatherosclerotic renal artery disease. *Archives of Surgery (Chicago, Ill.: 1960)*, *145*(9), 832–839. https://doi.org/10.1001/archsurg.2010.183

Hameed, A. M., Lu, D. B., Patrick, E., Xu, B., Hu, M., Chew, Y. V., Keung, K., P'ng, C. H., Gaspi, R., Zhang, C., Robertson, P., Alexander, S., Thomas, G., Laurence, J., De Roo, R., Wong, G., Miraziz, R., O'Grady, G., Yuen, L., … Pleass, H. C. (2019). Brief Normothermic Machine Perfusion Rejuvenates Discarded Human Kidneys. *Transplantation Direct*, *5*(11), e502. https://doi.org/10.1097/TXD.0000000000000944

Herrera, M. B., Bussolati, B., Bruno, S., Fonsato, V., Romanazzi, G. M., & Camussi, G. (2004). Mesenchymal Stem Cells Contribute to the Renal Repair of Acute Tubular Epithelial Injury. *International Journal of Molecular Medicine*, *14*(6), 1035–1041.

Hessheimer, A. J., Coll, E., Torres, F., Ruíz, P., Gastaca, M., Rivas, J. I., Gómez, M., Sánchez, B., Santoyo, J., Ramírez, P., Parrilla, P., Marín, L. M., Gómez-Bravo, M. Á., García-Valdecasas, J. C., López-Monclús, J., Boscá, A., López-Andújar, R., Fundora-Suárez, J., Villar, J., … Fondevila, C. (2018). Normothermic Regional Perfusion versus Super Rapid Recovery in Controlled Donation after Circulatory Death Liver Transplantation. *Journal of Hepatology*. https://doi.org/10.1016/j.jhep.2018.12.013

Hilbrands, L. B., Duivenvoorden, R., Vart, P., Franssen, C. F. M., Hemmelder, M. H., Jager, K. J., Kieneker, L. M., Noordzij, M., Pena, M. J., Vries, H. de, Arroyo, D., Covic, A., Crespo, M., Goffin, E., Islam, M., Massy, Z. A., Montero, N., Oliveira, J. P., Roca Muñoz, A., … ERACODA Collaborators. (2020). COVID-19-related mortality in kidney transplant and dialysis patients: Results of the ERACODA collaboration. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *35*(11), 1973–1983. https://doi.org/10.1093/ndt/gfaa261

Holthoff, J. H., Chandrashekar, K., & Juncos, L. A. (2022). The Role of Esm-1 in Diabetic Kidney Disease: More Than Just a Biomarker. *Kidney360*, *3*(12), 1998–2000. https://doi.org/10.34067/KID.0004952022

Horwitz, E. M., Gordon, P. L., Koo, W. K. K., Marx, J. C., Neel, M. D., McNall, R. Y., Muul, L., & Hofmann, T. (2002). Isolated Allogeneic Bone Marrow-Derived Mesenchymal Cells Engraft and Stimulate Growth in Children with Osteogenesis Imperfecta: Implications for Cell Therapy of Bone. *Proceedings of the National*

Academy of Sciences of the United States of America, *99*(13), 8932–8937. https://doi.org/10.1073/pnas.132252399

Horwitz, E. M., Le Blanc, K., Dominici, M., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Deans, R. J., Krause, D. S., Keating, A., & International Society for Cellular Therapy. (2005). Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*, *7*(5), 393–395. https://doi.org/10.1080/14653240500319234

Hosgood, S. A., Barlow, A. D., Dormer, J., & Nicholson, M. L. (2015). The Use of Ex-Vivo Normothermic Perfusion for the Resuscitation and Assessment of Human Kidneys Discarded Because of Inadequate in Situ Perfusion. *Journal of Translational Medicine*, *13*, 329. https://doi.org/10.1186/s12967-015-0691-x

Hosgood, S. A., Barlow, A. D., Hunter, J. P., & Nicholson, M. L. (2015). Ex Vivo Normothermic Perfusion for Quality Assessment of Marginal Donor Kidney Transplants. *The British Journal of Surgery*, *102*(11), 1433–1440. https://doi.org/10.1002/bjs.9894

Hosgood, S. A., & Nicholson, M. L. (2011). First in man renal transplantation after ex vivo normothermic perfusion. *Transplantation*, *92*(7), 735–738. https://doi.org/10.1097/TP.0b013e31822d4e04

Hosgood, S. A., & Nicholson, M. L. (2017). An Assessment of Urinary Biomarkers in a Series of Declined Human Kidneys Measured During Ex Vivo Normothermic Kidney Perfusion. *Transplantation*, *101*(9), 2120–2125. https://doi.org/10.1097/TP.0000000000001504

Hosgood, S. A., Patel, M., & Nicholson, M. L. (2013). The Conditioning Effect of Ex Vivo Normothermic Perfusion in an Experimental Kidney Model. *The Journal of Surgical Research*, *182*(1), 153–160. https://doi.org/10.1016/j.jss.2012.08.001

Hosgood, S. A., Saeb-Parsy, K., Hamed, M. O., & Nicholson, M. L. (2016). Successful Transplantation of Human Kidneys Deemed Untransplantable but Resuscitated by Ex Vivo Normothermic Machine Perfusion. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(11), 3282–3285. https://doi.org/10.1111/ajt.13906

Hosgood, S. A., Saeb-Parsy, K., Wilson, C., Callaghan, C., Collett, D., & Nicholson, M. L. (2017). 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*, *7*(1), e012237. https://doi.org/10.1136/bmjopen-2016-012237

Hosgood, S. A., Thompson, E., Moore, T., Wilson, C. H., & Nicholson, M. L. (2018). Normothermic Machine Perfusion for the Assessment and Transplantation of Declined Human Kidneys from Donation after Circulatory Death Donors. *The British Journal of Surgery*, *105*(4), 388–394. https://doi.org/10.1002/bjs.10733

Hou, B., Song, H., Cao, H., Yang, L., Sun, D., & Shen, Z. (2019). [Effects of bone marrow mesenchymal stem cells combined with normothermic mechanical perfusion on biliary epithelial cells donated after cardiac death in rats]. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*, *31*(9), 1137–1142. https://doi.org/10.3760/cma.j.issn.2095-4352.2019.09.016

Houtgraaf, J. H., den Dekker, W. K., van Dalen, B. M., Springeling, T., de Jong, R., van Geuns, R. J., Geleijnse, M. L., Fernandez-Aviles, F., Zijlsta, F., Serruys, P. W., & Duckers, H. J. (2012). First Experience in Humans Using Adipose Tissue-Derived Regenerative Cells in the Treatment of Patients with ST-segment Elevation Myocardial Infarction. *Journal of the American College of Cardiology*, *59*(5), 539–540. https://doi.org/10.1016/j.jacc.2011.09.065

Houtzager, J. H. E., Hemelrijk, S. D., Post, I. C. J. H., Idu, M. M., Bemelman, F. J., & van Gulik, T. M. (2021). The Use of the Oxygenated AirdriveTM Machine Perfusion System in Kidney Graft Preservation: A Clinical Pilot Study. *European Surgical Research*, *61*(6), 153–162. https://doi.org/10.1159/000513493

Hu, H., & Zou, C. (2017). Mesenchymal Stem Cells in Renal Ischemia-Reperfusion Injury: Biological and Therapeutic Perspectives. *Current Stem Cell Research & Therapy*, *12*(3), 183–187. https://doi.org/10.2174/1574888X11666161024143640

Huang, E., Segev, D. L., & Rabb, H. (2009). Kidney Transplantation in the Elderly. *Seminars in Nephrology*, *29*(6), 621–635. https://doi.org/10.1016/j.semnephrol.2009.07.011

Humar, A., Denny, R., Matas, A. J., & Najarian, J. S. (2003). Graft and Quality of Life Outcomes in Older Recipients of a Kidney Transplant. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *1*(2), 69–72.

Ibrahim, M., Greenhall, G. H. B., Summers, D. M., Mumford, L., Johnson, R., Baker, R. J., Forsythe, J., Pettigrew, G. J., Ahmad, N., & Callaghan, C. J. (2020). Utilization and Outcomes of Single and Dual Kidney Transplants from Older Deceased Donors in the United Kingdom. *Clinical Journal of the American Society of Nephrology: CJASN*, *15*(9), 1320–1329. https://doi.org/10.2215/CJN.02060220

Ittrich, H., Lange, C., Tögel, F., Zander, A. R., Dahnke, H., Westenfelder, C., Adam, G., & Nolte-Ernsting, C. (2007). In Vivo Magnetic Resonance Imaging of Iron Oxide-Labeled, Arterially-Injected Mesenchymal Stem Cells in Kidneys of Rats with Acute Ischemic Kidney Injury: Detection and Monitoring at 3T. *Journal of Magnetic Resonance Imaging: JMRI*, *25*(6), 1179–1191. https://doi.org/10.1002/jmri.20925

Iwagami, M., Tomlinson, L. A., Mansfield, K. E., Casula, A., Caskey, F. J., Aitken, G., Fraser, S. D. S., Roderick, P. J., & Nitsch, D. (2017). Validity of estimated prevalence of decreased kidney function and renal replacement therapy from primary care electronic health records compared with national survey and registry data in the United Kingdom. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *32*(suppl_2), ii142–ii150. https://doi.org/10.1093/ndt/gfw318

Jm, K., J, E., I, L., Jy, C., S, G., M, H., P, U., P, Y., R, J., D, B., I, M., A, G., D, G., La, R., & M, S. (2017). Normothermic Ex Vivo Kidney Perfusion Following Static Cold Storage-Brief, Intermediate, or Prolonged Perfusion for Optimal Renal Graft Reconditioning? *American Journal of Transplantation : Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *17*(10). https://doi.org/10.1111/ajt.14294

Johnson, R. J., Bradbury, L. L., Martin, K., Neuberger, J., & UK Transplant Registry. (2014). Organ Donation and Transplantation in the UK-the Last Decade: A Report from the UK National Transplant Registry. *Transplantation*, *97 Suppl 1*, S1–S27. https://doi.org/10.1097/01.TP.0000438215.16737.68

Kako, K., Kato, M., Matsuoka, T., & Mustapha, A. (1988). Depression of membrane-bound Na+-K+-ATPase activity induced by free radicals and by ischemia of kidney. *The American Journal of Physiology*, *254*(2 Pt 1), C330-337. https://doi.org/10.1152/ajpcell.1988.254.2.C330

Kanazawa, H., Fujimoto, Y., Teratani, T., Iwasaki, J., Kasahara, N., Negishi, K., Tsuruyama, T., Uemoto, S., & Kobayashi, E. (2011). Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. *PloS One*, *6*(4), e19195. https://doi.org/10.1371/journal.pone.0019195

Kang, J.-W., Kim, S.-J., Cho, H.-I., & Lee, S.-M. (2015). DAMPs activating innate immune responses in sepsis. *Ageing Research Reviews*, *24*(Pt A), 54–65. https://doi.org/10.1016/j.arr.2015.03.003

Kaths, J. M., Echeverri, J., Chun, Y. M., Cen, J. Y., Goldaracena, N., Linares, I., Dingwell, L. S., Yip, P. M., John, R., Bagli, D., Mucsi, I., Ghanekar, A., Grant, D. R., Robinson, L. A., & Selzner, M. (2017). Continuous Normothermic Ex Vivo Kidney Perfusion Improves Graft Function in Donation After Circulatory Death Pig Kidney Transplantation. *Transplantation*, *101*(4), 754–763. https://doi.org/10.1097/TP.0000000000001343

Kaths, J. M., Spetzler, V. N., Goldaracena, N., Echeverri, J., Louis, K. S., Foltys, D. B., Strempel, M., Yip, P., John, R., Mucsi, I., Ghanekar, A., Bagli, D., Robinson, L., & Selzner, M. (2015). Normothermic Ex Vivo Kidney Perfusion for the Preservation of Kidney Grafts prior to Transplantation. *Journal of Visualized Experiments: JoVE*, *101*, e52909. https://doi.org/10.3791/52909

Kayler, L. K., Garzon, P., Magliocca, J., Fujita, S., Kim, R. D., Hemming, A. W., Howard, R., & Schold, J. D. (2009). Outcomes and Utilization of Kidneys from Deceased Donors with Acute Kidney Injury. *American Journal of Transplantation*, *9*(2), 367–373. https://doi.org/10.1111/j.1600-6143.2008.02505.x

Khalil, M. A. M., Tan, J., Khan, T. F. T., Khalil, M. A. U., & Azmat, R. (2017). Dual Kidney Transplantation: A Review of Past and Prospect for Future. *International Scholarly Research Notices*, *2017*. https://doi.org/10.1155/2017/2693681

Khoubnasabjafari, M., Ansarin, K., & Jouyban, A. (2015). Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *BioImpacts : BI*, *5*(3), 123–127. https://doi.org/10.15171/bi.2015.20

Kidney Allocation System—Professional Education—OPTN. (n.d.). https://optn.transplant.hrsa.gov/learn/professional-education/kidney-allocation-system/

Kidney Donor Profile Index (KDPI) Guide for Clinicians—OPTN. (n.d.). Retrieved 5 November 2023, from https://optn.transplant.hrsa.gov/professionals/by-topic/guidance/kidney-donor-profile-index-kdpi-guide-for-clinicians/

Kidney Offering Scheme. (n.d.). ODT Clinical - NHS Blood and Transplant. Retrieved 5 November 2023, from https://www.odt.nhs.uk/odt-structures-and-standards/odt-hub-programme/kidney-offering-scheme/

Kim, H.-S., Na Choi, S. J., & Lee, H. K. (2019). Successful Kidney Transplantation Using a Deceased Donor Graft With Fibromuscular Dysplasia. *Transplantation Proceedings*, *51*(8), 2842–2844. https://doi.org/10.1016/j.transproceed.2019.02.057

King, N. and P., J. (2014). Ethical Issues in Stem Cell Research and Therapy. *Stem Cell Res Ther*, *5*(85).

Kol, A., Walker, N. J., Galuppo, L. D., Clark, K. C., Buerchler, S., Bernanke, A., & Borjesson, D. L. (2013). Autologous point-of-care cellular therapies variably induce equine mesenchymal stem cell migration, proliferation and cytokine expression. *Equine Veterinary Journal*, *45*(2), 193–198. https://doi.org/10.1111/j.2042-3306.2012.00600.x

Kramann, R., Couson, S. K., Neuss, S., Kunter, U., Bovi, M., Bornemann, J., Knüchel, R., Jahnen-Dechent, W., Floege, J., & Schneider, R. K. (2011). Exposure to Uremic Serum Induces a Procalcific Phenotype in Human Mesenchymal Stem Cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(9), e45-54. https://doi.org/10.1161/ATVBAHA.111.228601

Laing, R. W., Stubblefield, S., Wallace, L., Roobrouck, V. D., Bhogal, R. H., Schlegel, A., Boteon, Y. L., Reynolds, G. M., Ting, A. E., Mirza, D. F., Newsome, P. N.,

Mergental, H., & Afford, S. C. (2020). The Delivery of Multipotent Adult Progenitor Cells to Extended Criteria Human Donor Livers Using Normothermic Machine Perfusion. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.01226

Lathan, R., Ghita, R., & Clancy, M. J. (2019). Stem Cells to Modulate IR: A Regenerative Medicine-Based Approach to Organ Preservation. *Current Transplantation Reports*, *6*(2), 146–154. https://doi.org/10.1007/s40472-019-00240-7

Lee, C. M., Scandling, J. D., Shen, G. K., Salvatierra, O., Dafoe, D. C., & Alfrey, E. J. (1996). THE KIDNEYS THAT NOBODY WANTED: Support for the Utilization of Expanded Criteria Donors: 1. *Transplantation*, *62*(12), 1832–1841.

Lee, K. W., Park, J. B., Cha, S. R., Lee, S. H., Chung, Y. J., Yoo, H., Kim, K., & Kim, S. J. (2020). Dual Kidney Transplantation Offers a Safe and Effective Way to Use Kidneys from Deceased Donors Older than 70 Years. *BMC Nephrology*, *21*(1), 3. https://doi.org/10.1186/s12882-019-1664-8

Levitt, M. (2015). Could the Organ Shortage Ever Be Met? *Life Sciences, Society and Policy*, *11*, 6. https://doi.org/10.1186/s40504-015-0023-1

Li, J., Peng, Q., Yang, R., Li, K., Zhu, P., Zhu, Y., Zhou, P., Szabó, G., & Zheng, S. (2021). Application of Mesenchymal Stem Cells During Machine Perfusion: An Emerging Novel Strategy for Organ Preservation. *Frontiers in Immunology*, *12*, 713920. https://doi.org/10.3389/fimmu.2021.713920

Lin, K., Matsubara, Y., Masuda, Y., Togashi, K., Ohno, T., Tamura, T., Toyoshima, Y., Sugimachi, K., Toyoda, M., Marc, H., & Douglas, A. (2008). Characterization of Adipose Tissue-Derived Cells Isolated with the Celution System. *Cytotherapy*, *10*(4), 417–426. https://doi.org/10.1080/14653240801982979

Lipowsky, H. H., Bowers, D. T., Banik, B. L., & Brown, J. L. (2018). Mesenchymal Stem Cell Deformability and Implications for Microvascular Sequestration. *Annals of*

Biomedical Engineering, *46*(4), 640–654. https://doi.org/10.1007/s10439-018-1985-y

Liu, J., Litt, L., Segal, M. R., Kelly, M. J. S., Pelton, J. G., & Kim, M. (2011). Metabolomics of Oxidative Stress in Recent Studies of Endogenous and Exogenously Administered Intermediate Metabolites. *International Journal of Molecular Sciences*, *12*(10), 6469–6501. https://doi.org/10.3390/ijms12106469

Lohmann, S., Pool, M. B. F., Rozenberg, K. M., Keller, A. K., Moers, C., Møldrup, U., Møller, B. K., Lignell, S. J. M., Krag, S., Sierra-Parraga, J. M., Faro, M. L. L., Hunter, J., Hoogduijn, M. J., Baan, C. C., Leuvenink, H. G. D., Ploeg, R. J., Eijken, M., & Jespersen, B. (2021). Mesenchymal stromal cell treatment of donor kidneys during ex vivo normothermic machine perfusion: A porcine renal autotransplantation study. *American Journal of Transplantation*, *21*(7), 2348–2359. https://doi.org/10.1111/ajt.16473

Lopes, J. A., & Jorge, S. (2013). The RIFLE and AKIN Classifications for Acute Kidney Injury: A Critical and Comprehensive Review. *Clinical Kidney Journal*, *6*(1), 8–14. https://doi.org/10.1093/ckj/sfs160

Lum, E. L., Homkrailas, P., Abdalla, B., Danovitch, G. M., & Bunnapradist, S. (2022). Cold Ischemia Time, Kidney Donor Profile Index, and Kidney Transplant Outcomes: A Cohort Study. *Kidney Medicine*, *5*(1), 100570. https://doi.org/10.1016/j.xkme.2022.100570

Mahmood, A., Lu, D., Lu, M., & Chopp, M. (2003). Treatment of Traumatic Brain Injury in Adult Rats with Intravenous Administration of Human Bone Marrow Stromal Cells. *Neurosurgery*, *53*(3), 697–702; discussion 702-703. https://doi.org/10.1227/01.neu.0000079333.61863.aa

Malek, M., & Nematbakhsh, M. (2015). Renal Ischemia/Reperfusion Injury; from Pathophysiology to Treatment. *Journal of Renal Injury Prevention*, *4*(2), 20–207. https://doi.org/10.12861/jrip.2015.06

Malinin, T. I. (1996). Remembering Alexis Carrel and Charles A. Lindbergh. *Texas Heart Institute Journal*, *23*(1), 28–35.

Mallon, D. H., Riddiough, G. E., Summers, D. M., Butler, A. J., Callaghan, C. J., Bradbury, L. L., Bardsley, V., Broecker, V., Saeb-Parsy, K., Torpey, N., Bradley, J. A., & Pettigrew, G. J. (2015). Successful Transplantation of Kidneys From Elderly Circulatory Death Donors by Using Microscopic and Macroscopic Characteristics to Guide Single or Dual Implantation. *American Journal of Transplantation*, *15*(11), 2931–2939. https://doi.org/10.1111/ajt.13349

Manara, A. R., Mumford, L., Callaghan, C. J., Ravanan, R., & Gardiner, D. (2020). Donation and Transplantation Activity in the UK during the COVID-19 Lockdown. *The Lancet*, *396*(10249), 465–466. https://doi.org/10.1016/S0140-6736(20)31692-5

Martin, J. L., Gruszczyk, A. V., Beach, T. E., Murphy, M. P., & Saeb-Parsy, K. (2019). Mitochondrial mechanisms and therapeutics in ischaemia reperfusion injury. *Pediatric Nephrology (Berlin, Germany)*, *34*(7), 1167–1174. https://doi.org/10.1007/s00467-018-3984-5

Matsushita, Y., Motoyama, D., Ito, T., Sugiyama, T., Otsuka, A., Sano, M., Inuzuka, K., & Miyake, H. (2020). Kidney transplantation from a living donor with renal artery fibromuscular dysplasia: A case report on arterial grafting of the donor renal artery. *IJU Case Reports*, *3*(5), 201–203. https://doi.org/10.1002/iju5.12188

Mazilescu, L. I., Goto, T., John, R., Rosales, R., Ganesh, S., Yu, F., Noguchi, Y., Kawamura, M., Dezard, V., Gao, F., Urbanellis, P., Parmentier, C., Konvalinka, A., Bagli, D. J., Reichman, T. W., Robinson, L. A., & Selzner, M. (2024). Combining Oxygenated Cold Perfusion With Normothermic Ex Vivo Perfusion Improves the

Outcome of Donation After Circulatory Death Porcine Kidney Transplantation. *Transplantation*, *108*(1), 184–191. https://doi.org/10.1097/TP.0000000000004734

Mazilescu, L. I., Urbanellis, P., Kaths, M. J., Ganesh, S., Goto, T., Noguchi, Y., John, R., Konvalinka, A., Mucsi, I., Ghanekar, A., Bagli, D. J., Turgeon, J., Karakeusian Rimbaud, A., Hébert, M.-J., Dieudé, M., Alleys, I., Dore, E., Boilard, E., Overkleeft, H. S., … Selzner, M. (2021). Prolonged Normothermic Ex Vivo Kidney Perfusion Is Superior to Cold Nonoxygenated and Oxygenated Machine Perfusion for the Preservation of DCD Porcine Kidney Grafts. *Transplantation Direct*, *7*(10), e751. https://doi.org/10.1097/TXD.0000000000001218

Mendel, L., Albano, L., Bentellis, I., Yandza, T., Bernardi, C., Quintens, H., Tibi, B., Jourdan, J., Durand, M., Amiel, J., & Chevallier, D. (2018). Safety of Dual Kidney Transplantation Compared to Single Kidney Transplantation from Expanded Criteria Donors: A Single Center Cohort Study of 39 Recipients. *Transplant International*, *31*(10), 1110–1124. https://doi.org/10.1111/tri.13280

Merrick, D., Sakers, A., Irgebay, Z., Okada, C., Calvert, C., Morley, M. P., Percec, I., & Seale, P. (2019). Identification of a Mesenchymal Progenitor Cell Hierarchy in Adipose Tissue. *Science*, *364*(6438). https://doi.org/10.1126/science.aav2501

Mhaske, S. M., Patil, B., Patwardhan, S. K., Gopalakrishnan, G., Shelke, U. R., & Pamecha, Y. G. (2019). Outcome following renal autotransplantation in renal artery stenosis. *Urology Annals*, *11*(1), 46–52. https://doi.org/10.4103/UA.UA_39_18

Mildmay-White, A., & Khan, W. (2017). Cell Surface Markers on Adipose-Derived Stem Cells: A Systematic Review. *Current Stem Cell Research & Therapy*, *12*(6), 484–492. https://doi.org/10.2174/1574888X11666160429122133

Miñambres, E., Suberviola, B., Dominguez-Gil, B., Rodrigo, E., Ruiz-San Millan, J. C., Rodríguez-San Juan, J. C., & Ballesteros, M. A. (2017). Improving the Outcomes of Organs Obtained From Controlled Donation After Circulatory Death Donors

Using Abdominal Normothermic Regional Perfusion. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *17*(8), 2165–2172. https://doi.org/10.1111/ajt.14214

Miyazaki T, K. Y., Toriyama K, Kobori M, Torii S. (2005). Isolation of Two Human Fibroblastic Cell Populations with Multiple but Distinct Potential of Mesenchymal Differentiation by Ceiling Culture of Mature Fat Cells from Subcutaneous Adipose Tissue. *Differentiation*, *73*, 69–78.

Moeckel, G. W. (2018). Pathologic Perspectives on Acute Tubular Injury Assessment in the Kidney Biopsy. *Seminars in Nephrology*, *38*(1), 21–30. https://doi.org/10.1016/j.semnephrol.2017.09.003

Moghadamyeghaneh, Z., Hanna, M. H., Fazlalizadeh, R., Obi, Y., Foster, C. E., Stamos, M. J., & Ichii, H. (2017). A Nationwide Analysis of Kidney Autotransplantation. *The American Surgeon*, *83*(2), 162–169.

Montero, N., Redondo-Pachón, D., Pérez-Sáez, M. J., Crespo, M., Cruzado, J. M., & Pascual, J. (2018). Dual Kidney Transplantation as a Strategy to Use Expanded Criteria Donors: A Systematic Review. *Transplant International*, *31*(8), 838–860. https://doi.org/10.1111/tri.13157

Mordant, P., Nakajima, D., Kalaf, R., Iskender, I., Maahs, L., Behrens, P., Coutinho, R., Iyer, R. K., Davies, J. E., Cypel, M., Liu, M., Waddell, T. K., & Keshavjee, S. (2016). Mesenchymal Stem Cell Treatment Is Associated with Decreased Perfusate Concentration of Interleukin-8 during Ex Vivo Perfusion of Donor Lungs after 18-Hour Preservation. *Journal of Heart and Lung Transplantation*, *35*(10), 1245–1254. https://doi.org/10.1016/j.healun.2016.04.017

Morigi, M., Benigni, A., Remuzzi, G., & Imberti, B. (2006). The Regenerative Potential of Stem Cells in Acute Renal Failure. *Cell Transplantation*, *15 Suppl 1*, S111-117. https://doi.org/10.3727/000000006783982449

Morigi, M., Imberti, B., Zoja, C., Corna, D., Tomasoni, S., Abbate, M., Rottoli, D., Angioletti, S., Benigni, A., Perico, N., Alison, M., & Remuzzi, G. (2004). Mesenchymal Stem Cells Are Renotropic, Helping to Repair the Kidney and Improve Function in Acute Renal Failure. *Journal of the American Society of Nephrology: JASN*, *15*(7), 1794–1804. https://doi.org/10.1097/01.asn.0000128974.07460.34

Munk, A., Duvald, C. S., Pedersen, M., Lohmann, S., Keller, A. K., Møller, B. K., Ringgaard, S., Buus, N. H., Jespersen, B., & Eijken, M. (2022). Dosing Limitation for Intra-Renal Arterial Infusion of Mesenchymal Stromal Cells. *International Journal of Molecular Sciences*, *23*(15), 8268. https://doi.org/10.3390/ijms23158268

Nakano, H., Nakajima, A., Sakon-Komazawa, S., Piao, J.-H., Xue, X., & Okumura, K. (2006). Reactive Oxygen Species Mediate Crosstalk between NF-kappaB and JNK. *Cell Death and Differentiation*, *13*(5), 730–737. https://doi.org/10.1038/sj.cdd.4401830

Nasralla, D., Coussios, C. C., Mergental, H., Akhtar, M. Z., Butler, A. J., Ceresa, C. D. L., Chiocchia, V., Dutton, S. J., García-Valdecasas, J. C., Heaton, N., Imber, C., Jassem, W., Jochmans, I., Karani, J., Knight, S. R., Kocabayoglu, P., Malagò, M., Mirza, D., Morris, P. J., … Consortium for Organ Preservation in Europe. (2018). A Randomized Trial of Normothermic Preservation in Liver Transplantation. *Nature*, *557*(7703), 50–56. https://doi.org/10.1038/s41586-018-0047-9

Nasralla, D., Lembach, H., Mergental, H., Mirza, D., Friend, P., Muiesan, P., & Perera, M. (2020). Ex Situ Arterial Reconstruction During Normothermic Perfusion of the

Liver. *Transplantation Direct*, *6*(9). https://doi.org/10.1097/TXD.0000000000001040

Navarro, A. P., Sohrabi, S., Reddy, M., Carter, N., Ahmed, A., & Talbot, D. (2008). Dual Transplantation of Marginal Kidneys from Nonheart Beating Donors Selected Using Machine Perfusion Viability Criteria. *The Journal of Urology*, *179*(6), 2305–2309; discussion 2309. https://doi.org/10.1016/j.juro.2008.01.113

Net, M., Valero, R., Almenara, R., Barros, P., Capdevila, L., López-Boado, M. A., Ruiz, A., Sánchez-Crivaro, F., Miquel, R., Deulofeu, R., Taurá, P., Manyalich, M., & García-Valdecasas, J. C. (2005). The Effect of Normothermic Recirculation Is Mediated by Ischemic Preconditioning in NHBD Liver Transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *5*(10), 2385–2392. https://doi.org/10.1111/j.1600-6143.2005.01052.x

Nguefouet Momo, R. E., Donato, P., Ugolini, G., Nacchia, F., Mezzetto, L., Veraldi, G. F., Marletta, S., Cavallo, E., Eccher, A., Giambanco, A., Cenzi, D., & Boschiero, L. (2020). Kidney transplantation from living donor with monolateral renal artery fibromuscular dysplasia using a cryopreserved iliac graft for arterial reconstruction: A case report and review of the literature. *BMC Nephrology*, *21*, 451. https://doi.org/10.1186/s12882-020-02097-w

Nicholson, M. L., & Hosgood, S. A. (2013). Renal Transplantation after Ex Vivo Normothermic Perfusion: The First Clinical Study. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *13*(5), 1246–1252. https://doi.org/10.1111/ajt.12179

Nooshabadi, V. T., Mardpour, S., Yousefi-Ahmadipour, A., Allahverdi, A., Izadpanah, M., Daneshimehr, F., Ai, J., Banafshe, H. R., & Ebrahimi-Barough, S. (2018). The

Extracellular Vesicles-Derived from Mesenchymal Stromal Cells: A New Therapeutic Option in Regenerative Medicine. *Journal of Cellular Biochemistry*, *119*(10), 8048–8073. https://doi.org/10.1002/jcb.26726

Oedayrajsingh-Varma, M., van Ham, S., Knippenberg, M., Helder, M., Klein-Nulend, J., Schouten, T., Ritt, M., & van Milligen, F. (2006). Adipose Tissue-Derived Mesenchymal Stem Cell Yield and Growth Characteristics Are Affected by the Tissue-Harvesting Procedure. *Cytotherapy*, *8*(2), 166–177. https://doi.org/10.1080/14653240600621125

Oniscu, G. C., Randle, L. V., Muiesan, P., Butler, A. J., Currie, I. S., Perera, M. T. P. R., Forsythe, J. L., & Watson, C. J. E. (2014). In Situ Normothermic Regional Perfusion for Controlled Donation after Circulatory Death–the United Kingdom Experience. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *14*(12), 2846–2854. https://doi.org/10.1111/ajt.12927

Opelz, G., & Terasaki, P. I. (1982). Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. *Transplantation*, *33*(1), 64–68. https://doi.org/10.1097/00007890-198201000-00013

Pacienza, N., Santa-Cruz, D., Malvicini, R., Robledo, O., Lemus-Larralde, G., Bertolotti, A., Marcos, M., & Yannarelli, G. (2019). Mesenchymal Stem Cell Therapy Facilitates Donor Lung Preservation by Reducing Oxidative Damage during Ischemia. *Stem Cells International*, *2019*. https://doi.org/10.1155/2019/8089215

Padilla, M., Coll, E., Fernández-Pérez, C., Pont, T., Ruiz, Á., Pérez-Redondo, M., Oliver, E., Atutxa, L., Manciño, J. M., Daga, D., Miñambres, E., Moya, J., Vidal, B., Dueñas-Jurado, J. M., Mosteiro, F., Rodríguez-Salgado, A., Fernández-García, E., Lara, R., Hernández-Marrero, D., … Domínguez-Gil, B. (2021). Improved short-term outcomes of kidney transplants in controlled donation after the circulatory

determination of death with the use of normothermic regional perfusion. *American Journal of Transplantation*, *21*(11), 3618–3628. https://doi.org/10.1111/ajt.16622

Pak, J. (2012). Autologous Adipose Tissue-Derived Stem Cells Induce Persistent Bone-like Tissue in Osteonecrotic Femoral Heads. *Pain Physician*, *15*(1), 75–85.

Parasuraman, R., Attallah, N., Venkat, K. K., Yoshida, A., Abouljoud, M., Khanal, S., & Greenbaum, A. (2004). Rapid progression of native renal artery fibromuscular dysplasia following kidney donation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *4*(11), 1910–1914. https://doi.org/10.1111/j.1600-6143.2004.00581.x

Pattonieri, E. F. (2014). MP591PRE-TRANSPLANT GRAFT RECONDITIONING WITH MESENCHYMAL STROMAL CELLS IN NON HEART BEATING DONOR EXPERIMENTAL MODEL. *Nephrology Dialysis Transplantation*, *29*(suppl_3), iii528–iii538. https://doi.org/10.1093/ndt/gfu179

Pearson, R., Asher, J., Jackson, A., Mark, P. B., Shumeyko, V., & Clancy, M. J. (2021). Viability assessment and utilization of declined donor kidneys with rhabdomyolysis using ex vivo normothermic perfusion without preimplantation biopsy. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *21*(3), 1317–1321. https://doi.org/10.1111/ajt.16329

Pearson, R., Geddes, C., Mark, P., Clancy, M., & Asher, J. (2021). Transplantation of kidneys after normothermic perfusion: A single center experience. *Clinical Transplantation*, *35*(10), e14431. https://doi.org/10.1111/ctr.14431

Pearson, R., Murray, E., Thomson, P. C., Mark, P. B., Clancy, M. J., & Asher, J. (2021). The New UK National Kidney Allocation Scheme With Maximized ‘R4-D4’ Kidney Transplants: Better Patient-to-Graft Longevity Matching May Be at the

Cost of More Resources. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *19*(11), 1133–1141. https://doi.org/10.6002/ect.2021.0129

Pearson, R., Wubetu, J., Jackson, A., & Kingsmore, D. (2021). Living donor kidney transplant following nephrectomy for renal artery stenosis with arterial reconstruction and viability assessment using ex vivo normothermic perfusion. *BMJ Case Reports*, *14*(11), e245273. https://doi.org/10.1136/bcr-2021-245273

Peired, A. J., Sisti, A., & Romagnani, P. (2016). Mesenchymal Stem Cell-Based Therapy for Kidney Disease: A Review of Clinical Evidence. *Stem Cells International*, *2016*, 4798639. https://doi.org/10.1155/2016/4798639

Peris, A., Lazzeri, C., Bonizzoli, M., Guetti, C., Tadini Buoninsegni, L., Fulceri, G., Ticali, P. F., Chiostri, M., Li Marzi, V., Serni, S., & Migliaccio, M. L. (2018). A Metabolic Approach during Normothermic Regional Perfusion in Uncontrolled Donors after Circulatory Death-A Pilot Study. *Clinical Transplantation*, *32*(10), e13387. https://doi.org/10.1111/ctr.13387

Pfeiffer, T., Böhner, H., Voiculescu, A., Sarbia, M., Grabensee, B., & Sandmann, W. (2002). Even severe renal artery fibromuscular dysplasia is no contraindication for living donor renal transplantation: Report of two successful cases with venous grafting of the donor renal artery. *Transplantation Proceedings*, *34*(8), 3113–3116. https://doi.org/10.1016/s0041-1345(02)03559-5

Phinney, D. G., Hwa Lee, R., & Boregowda, S. V. (2023). Revisiting the Mesenchymal 'Stem vs. Stromal' Cell Dichotomy and Its Implications for Development of Improved Potency Metrics. *Stem Cells (Dayton, Ohio)*, *41*(5), 444–452. https://doi.org/10.1093/stmcls/sxad019

Ponticelli, C. (2014). Ischaemia-Reperfusion Injury: A Major Protagonist in Kidney Transplantation. *Nephrol Dial Transplant*, *29*(6), 1134–1140. https://doi.org/10.1093/ndt/gft488

Pool, M. B. F., Vos, J., Eijken, M., van Pel, M., Reinders, M. E. J., Ploeg, R. J., Hoogduijn, M. J., Jespersen, B., Leuvenink, H. G. D., & Moers, C. (2020). Treating Ischemically Damaged Porcine Kidneys with Human Bone Marrow- and Adipose Tissue-Derived Mesenchymal Stromal Cells During Ex Vivo Normothermic Machine Perfusion. *Stem Cells and Development*, *29*(20), 1320–1330. https://doi.org/10.1089/scd.2020.0024

Pool, M., Eertman, T., Sierra Parraga, J., 't Hart, N., Roemeling-van Rhijn, M., Eijken, M., Jespersen, B., Reinders, M., Hoogduijn, M., Ploeg, R., Leuvenink, H., & Moers, C. (2019). Infusing Mesenchymal Stromal Cells into Porcine Kidneys during Normothermic Machine Perfusion: Intact MSCs Can Be Traced and Localised to Glomeruli. *International Journal of Molecular Sciences*, *20*(14), 3607. https://doi.org/10.3390/ijms20143607

Pradhay, G., Gopidas, G. S., Karumathil Pullara, S., Mathew, G., Mathew, A. J., Sukumaran, T. T., Pavikuttan, N., & Sudhakaran, R. (n.d.). Prevalence and Relevance of Multiple Renal Arteries: A Radioanatomical Perspective. *Cureus*, *13*(10), e18957. https://doi.org/10.7759/cureus.18957

Querard, A.-H., Foucher, Y., Combescure, C., Dantan, E., Larmet, D., Lorent, M., Pouteau, L.-M., Giral, M., & Gillaizeau, F. (2016). Comparison of Survival Outcomes between Expanded Criteria Donor and Standard Criteria Donor Kidney Transplant Recipients: A Systematic Review and Meta-Analysis. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *29*(4), 403–415. https://doi.org/10.1111/tri.12736

Raman, G., Adam, G. P., Halladay, C. W., Langberg, V. N., Azodo, I. A., & Balk, E. M. (2016). Comparative Effectiveness of Management Strategies for Renal Artery Stenosis: An Updated Systematic Review. *Annals of Internal Medicine*, *165*(9), 635–649. https://doi.org/10.7326/M16-1053

Rao, P. S., Schaubel, D. E., Guidinger, M. K., Andreoni, K. A., Wolfe, R. A., Merion, R. M., Port, F. K., & Sung, R. S. (2009). A Comprehensive Risk Quantification Score for Deceased Donor Kidneys: The Kidney Donor Risk Index. *Transplantation*, *88*(2), 231–236. https://doi.org/10.1097/TP.0b013e3181ac620b

Ravaioli, M., Baldassare, M., Vasuri, F., Pasquinelli, G., Laggetta, M., Valente, S., De Pace, V., Neri, F., Siniscalchi, A., Zanfi, C., Bertuzzo, V. R., Caraceni, P., Trerè, D., Longobardi, P., & Pinna, A. D. (2018). Strategies to Restore Adenosine Triphosphate (ATP) Level After More than 20 Hours of Cold Ischemia Time in Human Marginal Kidney Grafts. *Annals of Transplantation*, *23*, 34–44.

Remuzzi, G., Cravedi, P., Perna, A., Dimitrov, B. D., Turturro, M., Locatelli, G., Rigotti, P., Baldan, N., Beatini, M., Valente, U., Scalamogna, M., Ruggenenti, P., & Dual Kidney Transplant Group. (2006). Long-Term Outcome of Renal Transplantation from Older Donors. *The New England Journal of Medicine*, *354*(4), 343–352. https://doi.org/10.1056/NEJMoa052891

Renal Artery Stenosis | NIDDK. (n.d.). National Institute of Diabetes and Digestive and Kidney Diseases. Retrieved 29 January 2021, from https://www.niddk.nih.gov/health-information/kidney-disease/renal-artery-stenosis

Renner, P., Eggenhofer, E., Popp, F., Geissler, E., Schlitt, H., & Dahlke, M. (2012). FEASIBILITY OF CONTINUOUS EX VIVO KIDNEY PERFUSION WITH MESENCHYMAL STEM CELLS TO PREVENT ISCHEMIA-REPERFUSION INJURY IN MICE. *Transplant International*, *25*, 11–11.

Rigo, F., De Stefano, N., Navarro-Tableros, V., David, E., Rizza, G., Catalano, G., Gilbo, N., Maione, F., Gonella, F., Roggio, D., Martini, S., Patrono, D., Salizzoni, M., Camussi, G., & Romagnoli, R. (2018). Extracellular Vesicles from Human Liver Stem Cells Reduce Injury in an Ex Vivo Normothermic Hypoxic Rat Liver Perfusion Model. *Transplantation*, *102*(5), e205. https://doi.org/10.1097/TP.0000000000002123

Rigotti, P., Capovilla, G., Bella, C. D., Silvestre, C., Donato, P., Baldan, N., & Furian, L. (2014). A Single-Center Experience with 200 Dual Kidney Transplantations. *Clinical Transplantation*, *28*(12), 1433–1440. https://doi.org/10.1111/ctr.12475

Riordan, N. H., Ichim, T. E., Min, W.-P., Wang, H., Solano, F., Lara, F., Alfaro, M., Rodriguez, J. P., Harman, R. J., Patel, A. N., Murphy, M. P., Lee, R. R., & Minev, B. (2009). Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *Journal of Translational Medicine*, *7*, 29. https://doi.org/10.1186/1479-5876-7-29

Roberts, G., Holmes, J., Williams, G., Chess, J., Hartfiel, N., Charles, J. M., McLauglin, L., Noyes, J., & Edwards, R. T. (2022). Current costs of dialysis modalities: A comprehensive analysis within the United Kingdom. *Peritoneal Dialysis International: Journal of the International Society for Peritoneal Dialysis*, *42*(6), 578–584. https://doi.org/10.1177/08968608211061126

Robinson, L., Gedroyc, W., Reidy, J., & Saxton, H. M. (1991). Renal Artery Stenosis in Children. *Clinical Radiology*, *44*(6), 376–382. https://doi.org/10.1016/s0009-9260(05)80653-1

Rocca, C., Milanesi, S., Corradetti, V., Pattonieri, E. F., Valsania, T., Gregorini, M., Dugo, M., De Cecco, L., Benzoni, I., Bianco, C., Avanzini, M. A., Mantelli, M., Ingo, D., Maestri, M., Cannone, M., Esposito, P., Rampino, T., & Dal Canton, A. (2015). SP770PRE-CONDITIONING OF RENAL GRAFT WITH MESENCHYMAL

STROMAL CELLS PREVENTS ISCHEMIA DAMAGE UPREGULATING GENE INVOLVED IN ENERGY SAVING. *Nephrology Dialysis Transplantation*, *30*(suppl_3), iii631–iii632. https://doi.org/10.1093/ndt/gfv201.03

Rodger, D., & Venter, B. (2023). A fair exchange: Why living kidney donors in England should be financially compensated. *Medicine, Health Care, and Philosophy*, *26*(4), 625–634. https://doi.org/10.1007/s11019-023-10171-x

Rosen, E. D., & Spiegelman, B. M. (2014). What We Talk about When We Talk about Fat. *Cell*, *156*(1–2), 20–44. https://doi.org/10.1016/j.cell.2013.12.012

Rozenberg, K., & Al, E. (2020). What Happens When You Administer Mesenchymal Stromal Cells During Normalthermic Machine Perfusion of Porcine Kidneys: Possible and Helpful, or Not? : *Journal of the Nuffield Department of Surgical Sciences*, *1*(2). https://doi.org/10.37707/jnds.v1i2.89

Sagban, T. A., Baur, B., Rump, L. C., Schelzig, H., Grabitz, K., & Balzer, K. M. (2014). Long-term graft outcome after renal arterial reconstruction during living related kidney transplantation. *Langenbeck's Archives of Surgery*, *399*(4), 441–447. https://doi.org/10.1007/s00423-014-1167-9

Saidi RF, H. K. S. (2014). Challenges of Organ Shortage for Transplantation: Solutions and Opportunities. *Int J Org Transplant Med*, *5*(3), 87–96.

Salahudeen, A. K., Clark, E. C., & Nath, K. A. (1991). Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo. *The Journal of Clinical Investigation*, *88*(6), 1886–1893. https://doi.org/10.1172/JCI115511

Salvadori, M., Rosso, G., & Bertoni, E. (2015a). Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World Journal of Transplantation*, *5*(2), 52–67. https://doi.org/10.5500/wjt.v5.i2.52

Salvadori, M., Rosso, G., & Bertoni, E. (2015b). Update on Ischemia-Reperfusion Injury in Kidney Transplantation: Pathogenesis and Treatment. *World J Transplant*, *5*(2), 52–67. https://doi.org/10.5500/wjt.v5.i2.52

Santos, G. P. A., de Andrade, L. G. M., Valiatti, M. F., Contti, M. M., Nga, H. S., & Takase, H. M. (2019). Kidney transplantation with donors in severe acute kidney injury. Should we use these organs? Retrospective Case Series. *Jornal Brasileiro De Nefrologia: 'Orgao Oficial De Sociedades Brasileira E Latino-Americana De Nefrologia*, *41*(4), 534–538. https://doi.org/10.1590/2175-8239-JBN-2018-0264

Sathian, B., Asim, M., Banerjee, I., Pizarro, A. B., Roy, B., van Teijlingen, E. R., do Nascimento, I. J. B., & Alhamad, H. K. (2020). Impact of COVID-19 on Clinical Trials and Clinical Research: A Systematic Review. *Nepal Journal of Epidemiology*, *10*(3), 878–887. https://doi.org/10.3126/nje.v10i3.31622

Sato, Y., Bando, H., Di Piazza, M., Gowing, G., Herberts, C., Jackman, S., Leoni, G., Libertini, S., MacLachlan, T., McBlane, J. W., Pereira Mouriès, L., Sharpe, M., Shingleton, W., Surmacz-Cordle, B., Yamamoto, K., & van der Laan, J. W. (2019). Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider. *Cytotherapy*, *21*(11), 1095–1111. https://doi.org/10.1016/j.jcyt.2019.10.001

Schamberger, B., Lohmann, D., Sollinger, D., Stein, R., & Lutz, J. (2018). Association of Kidney Donor Risk Index with the Outcome after Kidney Transplantation in the Eurotransplant Senior Program. *Annals of Transplantation*, *23*, 775–781. https://doi.org/10.12659/AOT.909622

Shah, T., Bunnapradist, S., Hutchinson, I., Pravica, V., Cho, Y. W., Mendez, R., Mendez, R., & Takemoto, S. K. (2008). The Evolving Notion of 'Senior' Kidney Transplant Recipients. *Clinical Transplantation*, *22*(6), 794–802. https://doi.org/10.1111/j.1399-0012.2008.00881.x

Shapey, I. M., & Muiesan, P. (2013). Regional Perfusion by Extracorporeal Membrane Oxygenation of Abdominal Organs from Donors after Circulatory Death: A Systematic Review. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *19*(12), 1292–1303. https://doi.org/10.1002/lt.23771

Sharma, V., Shaw, A., Lowe, M., Summers, A., van Dellen, D., & Augustine, T. (2020). The Impact of the COVID-19 Pandemic on Renal Transplantation in the UK. *Clinical Medicine*, *20*(4), e82–e86. https://doi.org/10.7861/clinmed.2020-0183

Shiely, F., Foley, J., Stone, A., Cobbe, E., Browne, S., Murphy, E., Kelsey, M., Walsh-Crowley, J., & Eustace, J. A. (2021). Managing Clinical Trials during COVID-19: Experience from a Clinical Research Facility. *Trials*, *22*(1), 62. https://doi.org/10.1186/s13063-020-05004-8

Sierra Parraga, J. M., Rozenberg, K., Eijken, M., Leuvenink, H. G., Hunter, J., Merino, A., Moers, C., Møller, B. K., Ploeg, R. J., Baan, C. C., Jespersen, B., & Hoogduijn, M. J. (2019). Effects of Normothermic Machine Perfusion Conditions on Mesenchymal Stromal Cells. *Frontiers in Immunology*, *10*, 765. https://doi.org/10.3389/fimmu.2019.00765

Sierra-Parraga, J. M., Eijken, M., Hunter, J., Moers, C., Leuvenink, H., Møller, B., Ploeg, R. J., Baan, C. C., Jespersen, B., & Hoogduijn, M. J. (2017). Mesenchymal Stromal Cells as Anti-Inflammatory and Regenerative Mediators for Donor Kidneys During Normothermic Machine Perfusion. *Stem Cells and Development*, *26*(16), 1162–1170. https://doi.org/10.1089/scd.2017.0030

Sies, H. (2015). Oxidative stress: A concept in redox biology and medicine. *Redox Biology*, *4*, 180–183. https://doi.org/10.1016/j.redox.2015.01.002

Smith, T. B., Nicholson, M. L., & Hosgood, S. A. (2021). Advances in Hypothermic and Normothermic Perfusion in Kidney Transplantation. *Transplantology*, *2*(4), Article 4. https://doi.org/10.3390/transplantology2040044

Soliman, H., Theret, M., Scott, W., Hill, L., Underhill, T. M., Hinz, B., & Rossi, F. M. V. (2021). Multipotent stromal cells: One name, multiple identities. *Cell Stem Cell*, *28*(10), 1690–1707. https://doi.org/10.1016/j.stem.2021.09.001

Soulié, P., Chassot, A., Ernandez, T., Montesano, R., & Féraille, E. (2014). Spatially restricted hyaluronan production by Has2 drives epithelial tubulogenesis in vitro. *American Journal of Physiology. Cell Physiology*, *307*(8), C745-759. https://doi.org/10.1152/ajpcell.00047.2014

Statistics about Organ Donation—NHS Organ Donation Register | Organ Donation—English. (n.d.). https://www.organdonation.nhs.uk/supporting-my-decision/statistics-about-organ-donation/

Stavely, R., & Nurgali, K. (2020). The emerging antioxidant paradigm of mesenchymal stem cell therapy. *Stem Cells Translational Medicine*, *9*(9), 985–1006. https://doi.org/10.1002/sctm.19-0446

Stephenson, T. P., O'Donoghue, E. P., Hendry, W. F., & Wickham, J. E. (1973). Preservation of human kidneys for transplantation: Preliminary results with a Gambro perfusion machine. *British Medical Journal*, *1*(5850), 379–381. https://doi.org/10.1136/bmj.1.5850.379

Stratta, R. J., Farney, A. C., Orlando, G., Farooq, U., Al-Shraideh, Y., Palanisamy, A., Reeves-Daniel, A., Doares, W., Kaczmorski, S., Gautreaux, M. D., Iskandar, S. S., Hairston, G., Brim, E., Mangus, M., El-Hennawy, H., Khan, M., & Rogers, J. (2016). Dual Kidney Transplants from Adult Marginal Donors Successfully Expand the Limited Deceased Donor Organ Pool. *Clinical Transplantation*, *30*(4), 380–392. https://doi.org/10.1111/ctr.12697

Strubbe-Rivera, J. O., Schrad, J. R., Pavlov, E. V., Conway, J. F., Parent, K. N., & Bazil, J. N. (2021). The mitochondrial permeability transition phenomenon elucidated by cryo-EM reveals the genuine impact of calcium overload on mitochondrial structure and function. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-020-80398-8

Summers, D. M., Johnson, R. J., Hudson, A., Collett, D., Watson, C. J., & Bradley, J. A. (2013). Effect of Donor Age and Cold Storage Time on Outcome in Recipients of Kidneys Donated after Circulatory Death in the UK: A Cohort Study. *Lancet (London, England)*, *381*(9868), 727–734. https://doi.org/10.1016/S0140-6736(12)61685-7

Summers, D. M., Watson, C. J. E., Pettigrew, G. J., Johnson, R. J., Collett, D., Neuberger, J. M., & Bradley, J. A. (2015). Kidney Donation after Circulatory Death (DCD): State of the Art. *Kidney International*, *88*, 241–249. https://doi.org/10.1038/ki.2015.88

Sundström, J., Bodegard, J., Bollmann, A., Vervloet, M. G., Mark, P. B., Karasik, A., Taveira-Gomes, T., Botana, M., Birkeland, K. I., Thuresson, M., Jäger, L., Sood, M. M., VanPottelbergh, G., & Tangri, N. (2022). Prevalence, outcomes, and cost of chronic kidney disease in a contemporary population of 2·4 million patients from 11 countries: The CaReMe CKD study. *The Lancet Regional Health – Europe*, *20*. https://doi.org/10.1016/j.lanepe.2022.100438

Suzuki, S., Toledo-Pereyra, L. H., Rodriguez, F. J., & Cejalvo, D. (1993). Neutrophil Infiltration as an Important Factor in Liver Ischemia and Reperfusion Injury. Modulating Effects of FK506 and Cyclosporine. *Transplantation*, *55*(6), 1265–1272. https://doi.org/10.1097/00007890-199306000-00011

Ta, S., B, B., H, S., K, G., & M, D. (2014). Vascular challenges in renal transplantation. *Annals of Transplantation*, *19*. https://doi.org/10.12659/AOT.890893

Takada, M., Nadeau, K. C., Hancock, W. W., Mackenzie, H. S., Shaw, G. D., Waaga, A. M., Chandraker, A., Sayegh, M. H., & Tilney, N. L. (1998). Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation*, *65*(12), 1533–1542. https://doi.org/10.1097/00007890-199806270-00001

Tan, J. W., W; Xu, A; Liao, L; Zheng, F; Messinger, S; Sun, X; Chen, J; Yang, S; Cai, J; Gao, X; Pileggi, A; Ricordi, C. (2012). Induction Therapy With Autologous Mesenchymal Stem Cells in Living-Related Kidney Transplants: A Randomized Controlled Trial. *JAMA*, *307*, 1169–1177.

Tanriover, B., Mohan, S., Cohen, D. J., Radhakrishnan, J., Nickolas, T. L., Stone, P. W., Tsapepas, D. S., Crew, R. J., Dube, G. K., Sandoval, P. R., Samstein, B., Dogan, E., Gaston, R. S., Tanriover, J. N., Ratner, L. E., & Hardy, M. A. (2014). Kidneys at Higher Risk of Discard: Expanding the Role of Dual Kidney Transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *14*(2), 404–415. https://doi.org/10.1111/ajt.12553

Tarng, D. C., Tseng, W. C., Lee, P. Y., Chiou, S. H., & Hsieh, S. L. (2016). Induced Pluripotent Stem Cell-Derived Conditioned Medium Attenuates Acute Kidney Injury by Downregulating the Oxidative Stress-Related Pathway in Ischemia-Reperfusion Rats. *Cell Transplant*, *25*(3), 517–530. https://doi.org/10.3727/096368915X688542

Therneau, T. M., until 2009), T. L. (original S.->R port and R. maintainer, Elizabeth, A., & Cynthia, C. (2023). *survival: Survival Analysis* (3.5-7) [Computer software]. https://cran.r-project.org/web/packages/survival/index.html

Thompson, E. R., Bates, L., Ibrahim, I. K., Sewpaul, A., Stenberg, B., McNeill, A., Figueiredo, R., Girdlestone, T., Wilkins, G. C., Wang, L., Tingle, S. J., Scott, W. E., III, de Paula Lemos, H., Mellor, A. L., Roobrouck, V. D., Ting, A. E., Hosgood,

S. A., Nicholson, M. L., Fisher, A. J., … Wilson, C. H. (2020). Novel Delivery of Cellular Therapy to Reduce Ischemia Reperfusion Injury in Kidney Transplantation. *American Journal of Transplantation*. https://doi.org/10.1111/ajt.16100

Thompson, E. R., Ibrahim, I. K., Bates, L., Sewpaul, A., Stenberg, B., McNeill, A., Scott, W. E., Roobrouck, V., Ting, A., Ali, S., Sheerin, N., Fisher, A. J., & Wilson, C. H. (2018). Feasibility of Delivering Cell Therapy during Kidney Ex Vivo Normothermic Machine Perfusion. *Cytotherapy*, *20*(5), S117–S118. https://doi.org/10.1016/j.jcyt.2018.02.350

Tingle, S. J., Figueiredo, R. S., Moir, J. A. G., Goodfellow, M., Thompson, E. R., Ibrahim, I. K., Bates, L., Talbot, D., & Wilson, C. H. (2020). Hypothermic Machine Perfusion Is Superior to Static Cold Storage in Deceased Donor Kidney Transplantation: A Meta-Analysis. *Clinical Transplantation*, *34*(4). https://doi.org/10.1111/ctr.13814

Togel, F., Hu, Z., Weiss, K., Isaac, J., Lange, C., & Westenfelder, C. (2005). Administered Mesenchymal Stem Cells Protect against Ischemic Acute Renal Failure through Differentiation-Independent Mechanisms. *Am J Physiol Renal Physiol*, *289*(1), F31-42. https://doi.org/10.1152/ajprenal.00007.2005

Tolar, J., Le Blanc, K., Keating, A., & Blazar, B. R. (2010). Concise Review: Hitting the Right Spot with Mesenchymal Stromal Cells. *Stem Cells (Dayton, Ohio)*, *28*(8), 1446–1455. https://doi.org/10.1002/stem.459

Torres Crigna, A., Daniele, C., Gamez, C., Medina Balbuena, S., Pastene, D. O., Nardozi, D., Brenna, C., Yard, B., Gretz, N., & Bieback, K. (2018). Stem/Stromal Cells for Treatment of Kidney Injuries With Focus on Preclinical Models. *Front Med (Lausanne)*, *5*. https://doi.org/10.3389/fmed.2018.00179

Toyserkani, N. M., Jørgensen, M. G., Tabatabaeifar, S., Jensen, C. H., Sheikh, S. P., & Sørensen, J. A. (2017). Concise Review: A Safety Assessment of Adipose-Derived Cell Therapy in Clinical Trials: A Systematic Review of Reported Adverse Events. *Stem Cells Translational Medicine*, *6*(9), 1786–1794. https://doi.org/10.1002/sctm.17-0031

Tran, G., Ramaswamy, K., Chi, T., Meng, M., Freise, C., & Stoller, M. L. (2015). Laparoscopic Nephrectomy with Autotransplantation: Safety, Efficacy and Long-Term Durability. *The Journal of Urology*, *194*(3), 738–743. https://doi.org/10.1016/j.juro.2015.03.089

Transplant Activity Report. (n.d.). In *NHS Organ Donation Register | Organ Donation—English*.

Tt, P., Ll, F., Tq, N., Mc, V., S, F., Fj, B., J, K., T, V., D, K., R, G., & Mb, R. (2019). Histological characteristics of Acute Tubular Injury during Delayed Graft Function predict renal function after renal transplantation. *Physiological Reports*, *7*(5). https://doi.org/10.14814/phy2.14000

Tuttle, K. R. (2020). Impact of the COVID-19 Pandemic on Clinical Research. *Nature Reviews Nephrology*, *16*(10), 562–564. https://doi.org/10.1038/s41581-020-00336-9

Vallant, N. (2018). *Use of Mesenchymal Stem Cells for Preconditioning of Kidney Grafts in an Ex-Vivo Kidney Perfusion Model* [PhD Thesis, Imperial College London]. https://doi.org/10.25560/71363

Van Der Wijk, J., Slooff, M. J. H., Rijkmans, B. G., & Kootstra, G. (1980). Successful 96- and 144-hour experimental kidney preservation: A combination of standard machine preservation and newly developed normothermic *ex vivo* perfusion. *Cryobiology*, *17*(5), 473–477. https://doi.org/10.1016/0011-2240(80)90057-7

van Dijk, A., Naaijkens, B. A., Jurgens, W. J. F. M., Nalliah, K., Sairras, S., van der Pijl, R. J., Vo, K., Vonk, A. B. A., van Rossum, A. C., Paulus, W. J., van Milligen, F. J., & Niessen, H. W. M. (2011). Reduction of infarct size by intravenous injection of uncultured adipose derived stromal cells in a rat model is dependent on the time point of application. *Stem Cell Research*, *7*(3), 219–229. https://doi.org/10.1016/j.scr.2011.06.003

van Dorn, A. (2020). COVID-19 and Readjusting Clinical Trials. *The Lancet*, *396*(10250), 523–524. https://doi.org/10.1016/S0140-6736(20)31787-6

Verstegen, M. M. A., Mezzanotte, L., Ridwan, R. Y., Wang, K., de Haan, J., Schurink, I. J., Sierra Parraga, J. M., Hoogduijn, M., Kessler, B. M., Huang, H., Hall, S. R. R., Ijzermans, J. N. M., Löwik, C. W. G. M., van der Laan, L. J. W., & de Jonge, J. (2020). First Report on Ex Vivo Delivery of Paracrine Active Human Mesenchymal Stromal Cells to Liver Grafts During Machine Perfusion. *Transplantation*, *104*(1), e5–e7. https://doi.org/10.1097/TP.0000000000002986

Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., Nolta, J., Phinney, D. G., & Sensebe, L. (2019). Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy*, *21*(10), 1019–1024. https://doi.org/10.1016/j.jcyt.2019.08.002

Wadei, H. M., Heckman, M. G., Rawal, B., Taner, C. B., Farahat, W., Nur, L., Mai, M. L., Prendergast, M., & Gonwa, T. A. (2013). Comparison of kidney function between donation after cardiac death and donation after brain death kidney transplantation. *Transplantation*, *96*(3), 274–281. https://doi.org/10.1097/TP.0b013e31829807d1

Walker, P. A., Shah, S. K., Jimenez, F., Gerber, M. H., Xue, H., Cutrone, R., Hamilton, J. A., Mays, R. W., Deans, R., Pati, S., Dash, P. K., & Cox, C. S. (2010). Intravenous Multipotent Adult Progenitor Cell Therapy for Traumatic Brain Injury: Preserving

the Blood Brain Barrier via an Interaction with Splenocytes. *Experimental Neurology*, *225*(2), 341–352. https://doi.org/10.1016/j.expneurol.2010.07.005

Walter, D. H., & Dimmeler, S. (2002). Endothelial Progenitor Cells: Regulation and Contribution to Adult Neovascularization. *Herz*, *27*(7), 579–588. https://doi.org/10.1007/s00059-002-2427-y

Wang, Y., He, J., Pei, X., & Zhao, W. (2013). Systematic review and meta-analysis of mesenchymal stem/stromal cells therapy for impaired renal function in small animal models. *Nephrology (Carlton, Vic.)*, *18*(3), 201–208. https://doi.org/10.1111/nep.12018

Wang, Y., Hemmelder, M. H., Bos, W. J. W., Snoep, J. D., de Vries, A. P. J., Dekker, F. W., & Meuleman, Y. (2021). Mapping health-related quality of life after kidney transplantation by group comparisons: A systematic review. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *36*(12), 2327–2339. https://doi.org/10.1093/ndt/gfab232

Watson, C., Johnson, R., & Mumford, L. (2020). *Overview of the evolution of the UK kidney allocation schemes*. https://www.repository.cam.ac.uk/handle/1810/303038

Weber, B. R., & Dieter, R. S. (2014). Renal Artery Stenosis: Epidemiology and Treatment. *International Journal of Nephrology and Renovascular Disease*, *7*, 169–181. https://doi.org/10.2147/IJNRD.S40175

Wolfe, R. A., Ashby, V. B., Milford, E. L., Ojo, A. O., Ettenger, R. E., Agodoa, L. Y., Held, P. J., & Port, F. K. (1999). Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *The New England Journal of Medicine*, *341*(23), 1725–1730. https://doi.org/10.1056/NEJM199912023412303

Wu, D. A., Robb, M. L., Forsythe, J. L. R., Bradley, C., Cairns, J., Draper, H., Dudley, C., Johnson, R. J., Metcalfe, W., Ravanan, R., Roderick, P., Tomson, C. R. V., Watson, C. J. E., Bradley, J. A., & Oniscu, G. C. (2019). Recipient Comorbidity and Survival Outcomes after Kidney Transplantation: A UK-wide Prospective Cohort Study. *Transplantation*. https://doi.org/10.1097/TP.0000000000002931

Wu, D. A., Watson, C. J., Bradley, J. A., Johnson, R. J., Forsythe, J. L., & Oniscu, G. C. (2017). Global Trends and Challenges in Deceased Donor Kidney Allocation. *Kidney International*, *91*(6), 1287–1299. https://doi.org/10.1016/j.kint.2016.09.054

Wu, L., Cao, H., Tian, X., Zheng, W., Yuan, M., Li, X., Tian, X., Wang, Y., & Song, H. (2022). Bone marrow mesenchymal stem cells modified with heme oxygenase-1 alleviate rejection of donation after circulatory death liver transplantation by inhibiting dendritic cell maturation in rats. *International Immunopharmacology*, *107*, 108643. https://doi.org/10.1016/j.intimp.2022.108643

Wu, M.-Y., Yiang, G.-T., Liao, W.-T., Tsai, A. P.-Y., Cheng, Y.-L., Cheng, P.-W., Li, C.-Y., & Li, C.-J. (2018). Current Mechanistic Concepts in Ischemia and Reperfusion Injury. *Cellular Physiology and Biochemistry*, *46*(4), 1650–1667. https://doi.org/10.1159/000489241

Xi, J., Zheng, W., Chen, M., Zou, Q., Tang, C., & Zhou, X. (2023). Genetically engineered pigs for xenotransplantation: Hopes and challenges. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/fcell.2022.1093534

Xie, X., Yang, X., Wu, J., Tang, S., Yang, L., Fei, X., & Wang, M. (2022). Exosome from indoleamine 2,3-dioxygenase-overexpressing bone marrow mesenchymal stem cells accelerates repair process of ischemia/reperfusion-induced acute kidney injury by regulating macrophages polarization. *Stem Cell Research & Therapy*, *13*(1), 367. https://doi.org/10.1186/s13287-022-03075-9

Yang, L., Cao, H., Sun, D., Hou, B., Lin, L., Shen, Z.-Y., & Song, H.-L. (2020). Bone Marrow Mesenchymal Stem Cells Combine with Normothermic Machine Perfusion to Improve Rat Donor Liver Quality-the Important Role of Hepatic Microcirculation in Donation after Circulatory Death. *Cell and Tissue Research*, *381*(2), 239–254. https://doi.org/10.1007/s00441-020-03202-z

Yang, L., Cao, H., Sun, D., Lin, L., Zheng, W.-P., Shen, Z.-Y., & Song, H.-L. (2020). Normothermic Machine Perfusion Combined with Bone Marrow Mesenchymal Stem Cells Improves the Oxidative Stress Response and Mitochondrial Function in Rat Donation After Circulatory Death Livers. *Stem Cells and Development*, *29*(13), 835–852. https://doi.org/10.1089/scd.2019.0301

Zhao, H., Alam, A., Soo, A. P., George, A. J. T., & Ma, D. (2018). Ischemia-Reperfusion Injury Reduces Long Term Renal Graft Survival: Mechanism and Beyond. *EBioMedicine*, *28*, 31–42. https://doi.org/10.1016/j.ebiom.2018.01.025

Zhao, Q., Ren, H., & Han, Z. (2016). Mesenchymal stem cells: Immunomodulatory capability and clinical potential in immune diseases. *Journal of Cellular Immunotherapy*, *2*(1), 3–20. https://doi.org/10.1016/j.jocit.2014.12.001

Zheng, Y.-T., Chen, C.-B., Yuan, X.-P., & Wang, C.-X. (2018). Impact of Acute Kidney Injury in Donors on Renal Graft Survival: A Systematic Review and Meta-Analysis. *Renal Failure*, *40*(1), 649–656. https://doi.org/10.1080/0886022X.2018.1535982

Zhou, L., Song, Q., Shen, J., Xu, L., Xu, Z., Wu, R., Ge, Y., Zhu, J., Wu, J., Dou, Q., & Jia, R. (2017). Comparison of Human Adipose Stromal Vascular Fraction and Adipose-Derived Mesenchymal Stem Cells for the Attenuation of Acute Renal Ischemia/Reperfusion Injury. *Sci Rep*, *7*. https://doi.org/10.1038/srep44058

Zhou, Y., Xu, H., Xu, W., Wang, B., Wu, H., Tao, Y., Zhang, B., Wang, M., Mao, F., Yan, Y., Gao, S., Gu, H., Zhu, W., & Qian, H. (2013). Exosomes released by human

umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. *Stem Cell Research & Therapy*, *4*(2), 34. https://doi.org/10.1186/scrt194

Zuk, P. A., Zhu, M., Ashjian, P., De Ugarte, D. A., Huang, J. I., Mizuno, H., Alfonso, Z. C., Fraser, J. K., Benhaim, P., & Hedrick, M. H. (2002). Human Adipose Tissue Is a Source of Multipotent Stem Cells. *Molecular Biology of the Cell*, *13*(12), 4279–4295. https://doi.org/10.1091/mbc.e02-02-0105

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P., Lorenz, H. P., & Hedrick, M. H. (2001). Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. *Tissue Engineering*, *7*(2), 211–228. https://doi.org/10.1089/107632701300062859

APPENDIX A – Business Case for EVNP

Ex vivo normothermic kidney perfusion

[redacted] *Consultant Transplant Surgeon, Queen Elizabeth University Hospital, Glasgow*

Specific Aims

The renal transplant unit at the Queen Elizabeth University Hospital in Glasgow are proposing to develop a service for viability assessment and reconditioning to facilitate the safe transplantation of marginal kidneys using ex vivonormothermic perfusion (EVNP).
Ex vivo normothermic perfusion of kidneys is a technique developed by Professor Mike Nicholson, originallyat the University of Leicester and now at the University of Cambridge. It perfuses deceased donor kidneys with warmed, oxygenated blood, allowing some recovery of the kidney in the same way that the kidney starts to recover after transplantation, and provides an opportunity to assess that recovery to determine whether a kidneywould be likely to function if transplanted without exposing the patient to the risks of a failed transplant.

The benefits to the West of Scotland renal transplant programme and its patients are:

Aim 1: Viability assessment for safe transplantation of kidneys currently not used

1.1 Assessment of recovery of the most marginal kidneys, not currently used

1.2. Assessment of poorly perfused kidneys, which are at risk of thrombosis after reperfusion and therefore notcurrently used.

1.3 Assessment of the integrity of ureteric blood supply for kidneys received with stripped ureters

Aim 2: Reconditioning of kidneys

2.1. Acceptance of kidneys with long cold ischaemic times

2.2. Local use of kidneys for back-up recipients, e.g. after positive crossmatch for the index recipient

Aim 3: Future therapeutic possibilities

3.1. Direct intra-renal administration of agents to ameliorate ischaemia-reperfusion injury

3.2. Direct intra-renal administration of anti-virals, e.g. to allow safer use of organs from hepatitis C positive donors

Significance

A.1. Background

The West of Scotland Renal Transplant Unit has a focus on maximising access to renal transplantation, as wefeel it is clearly the optimal mode of renal replacement therapy for both duration and quality of patients' lives, as well as being an extraordinarily cost effective intervention compared with hospital haemodialysis. This philosophy is widely supported, including by the Scottish Government, who have signed up to the NHSBT Taking Organ Transplantation to 2020 strategy, which aims to achieve a 50% increase in transplants by 2020.

A remaining barrier to achieving the increased numbers of transplants, and the transformation in patients' lives that will produce, is the number of offers of kidneys felt to be unsuitable for transplantation. The status quo is illustrated in figure 1 which shows the outcomes of deceased donor kidney offers to the Glasgow transplant unit in 2016-17. Although 86 of the offers led to one or more kidney transplants, 35 were declined due to anticipated poor function and a further 23 were declined as inappropriate for the named recipient.

If the 50% increase in transplants is to be delivered, it will only be by using higher risk kidneys, but this has tobe implemented safely which will require transformations in working practices at both the national (NHSBT) andlocal transplant centre levels.

Increasing the transplantable kidney pool is particularly important for highly sensitised recipients. These arepatients with pre-formed antibodies against other human tissue types, which are formed after previous exposureto other tissue types, most commonly following pregnancy, blood transfusions or previous transplants. Although with modern immunosuppression a patient can typically receive a successful transplant from a donor with different tissue types, when there is pre-formed antibody to a particular mismatched tissue type, transplant is precluded. Patients with high levels of antibodies thus have reduced opporunities for transplants, as even a near perfectly-matched donor cannot be used if there is a antibody to the only mismatched antigen.

NHSBT measures levels of sensitisation using the calculated reaction frequency (cRF), the percentage ofa pool of 10,000 previous organ donors to whom a recipient has antibodies. In its online chance of transplant calculator, NHSBT divides these levels of sensitisation into five bands: 0-10%, 10-40%, 40-85%, 85-95% and 95-100%. We have examined the effect of these levels of sensitisation on the waiting time for transplant by comparing the cRF band and waiting times of patients on our active transplant list on the 19th July 2017 and theresults are presented in figure 2.

While on the national level there is likely to be more intelligent offering of higher risk organs matched to appropriate recipients, locally we need to optimise our use of the kidneys offered, and we believe that viability assessement and reconditioning with EVNP may allow some of the kidneys declined for expected poor functionto be used.

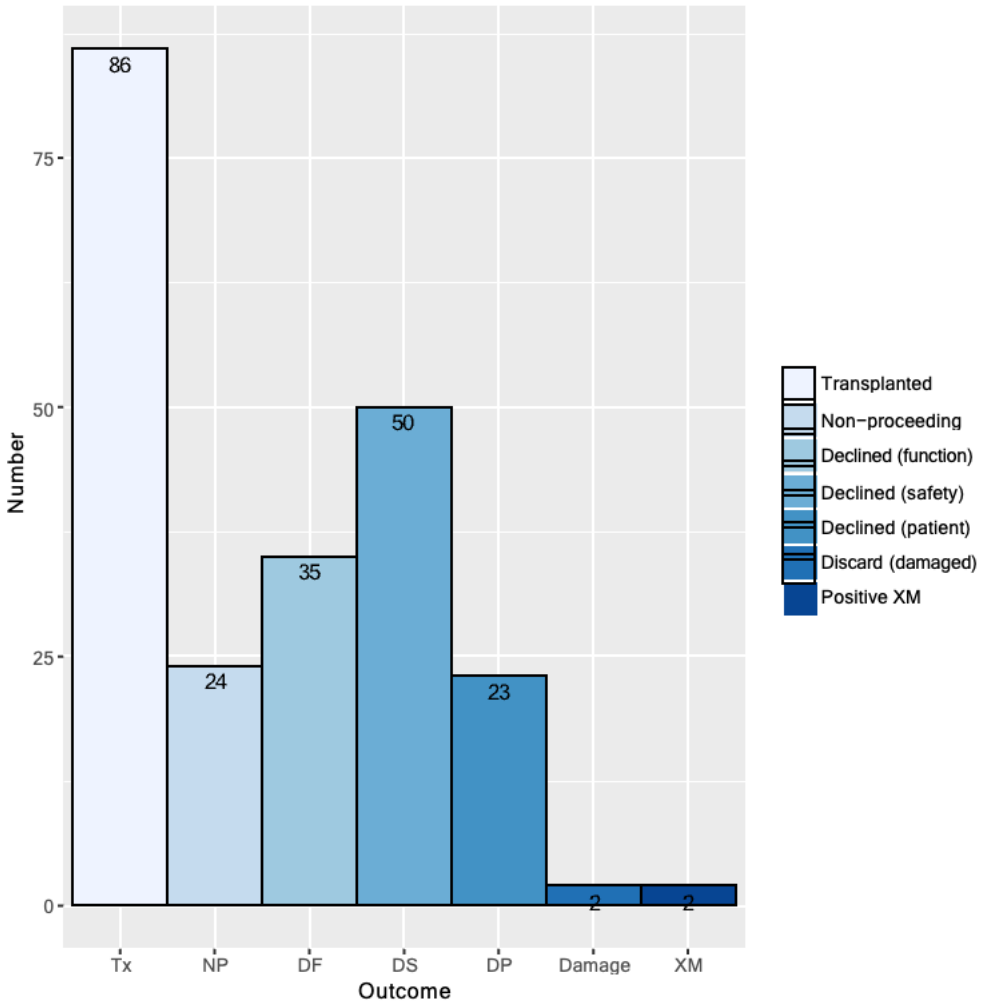


Figure 1: Outcomes of deceased donor kidney offers to Glasgow in 2016-17. The numbers counted are of the donors, and in some cases we were offered both kidneys from the same donor.

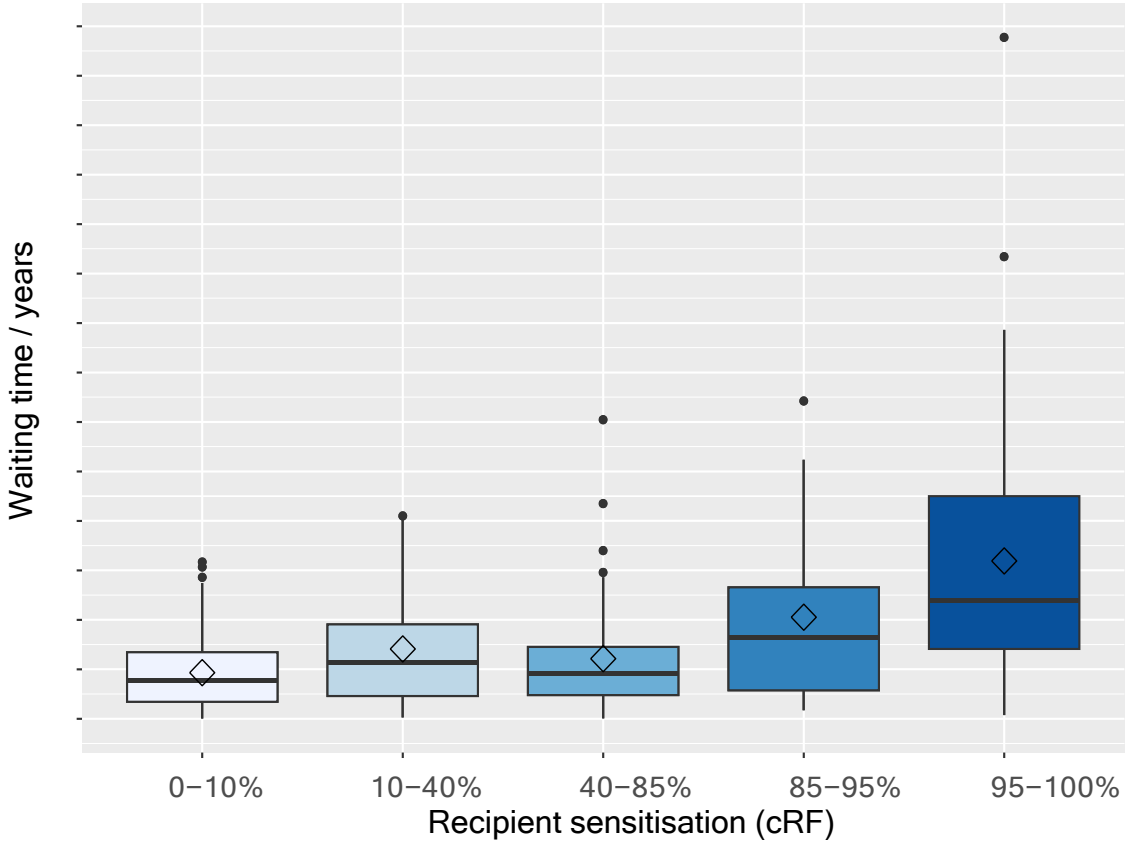


Figure 2: Waiting times of patients on the active transplant list by cRF band. The boxes indicate the interquartile range, the lines the full range, the dots indicate out-liers, the central bars indicate the medians and the diamonds indicate the means. The differences were statistically significant (Kruskal-Wallis test, $p<0.001$), with the 85-95% and 95-100% group found to have significantly longer waiting times onpost-hoc testing using the Dunn's non-parametric comparison test.

B. EVNP

Ex vivo normothermic perfusion is a technique to provide warmed, oxygenated red blood cell solution to the kidneyto reperfuse it and restore normal physiological levels of ATP, used as an energy source for essential processesin human cells. It is a technique developed by Professor Mike Nicholson and Dr Sarah Hosgood at the Universityof Leicester, using standard cardiopulmonary bypass equipment which can be used at the transplant centre afterreceiving the kidney. The isolated perfusion system includes a centrifugal pump, membrane oxygenator and heatexchanger.

B.1. Viability assessment with EVNP

EVNP allows the kidney to be assessed for its viability for transplant based on the quality of perfusion and calculation of intrarenal vascular resistance based on measurement of flow rate and pressure.
In cases of kidneys where delayed graft function is particularly undesirable, such as for transplanting kidneysfrom very elderly donors into very frail recipients, EVNP can predict a high chance of immediate function if the kidney is able to make urine while on the circuit – additionally creatinine or inulin can be added to the fluids to allow an estimation of glomerular filtration rate.

B.2. Reconditioning with EVNP

The initial experiments in a porcine model in Leicester showed that the combination of haemoglobin, oxygenand a normothermic environment allow the kidney to replenish depleted ATP stores[1], and this was followed upwith a clinical trial which demonstrated an incidence of delayed graft function of 11% in DCD kidneys[2], compared with rates of 75-90% in published large DCD series.

The potential benefits of EVNP compared with static cold storage include:[3]

- Full ATP regeneration and metabolism prior to transplantation
- Potential to extend acceptable cold ischaemic time
- Protective gene upgregulation prior to exposure to complement, leukocytes and platelets
- Separation of the biochemical and immunological components of ischaemia-reperfusion injury
- Potential for drug delivery in a metabolically active ex vivo environment

The benefits of normothermic perfusion technologies have already been seen in Glasgow from the kidney transplants from DCD donors managed with normothermic regional perfusion by the Scottish Organ Retrieval Team in 2013. These all had immediate function apart from kidneys from two donors with pre-mortem acute kidney injury. The function at one and three years of the DCD transplants following NRP was also much better than standard DCD with eGFR comparable to living donor kidneys transplanted in the same year (figures 3 and4) and follow-up data for up to four years indicates these transplants resulted in excellent graft survival (figure 5)and patient survival similar to recipients of DBD kidneys (figure 6).

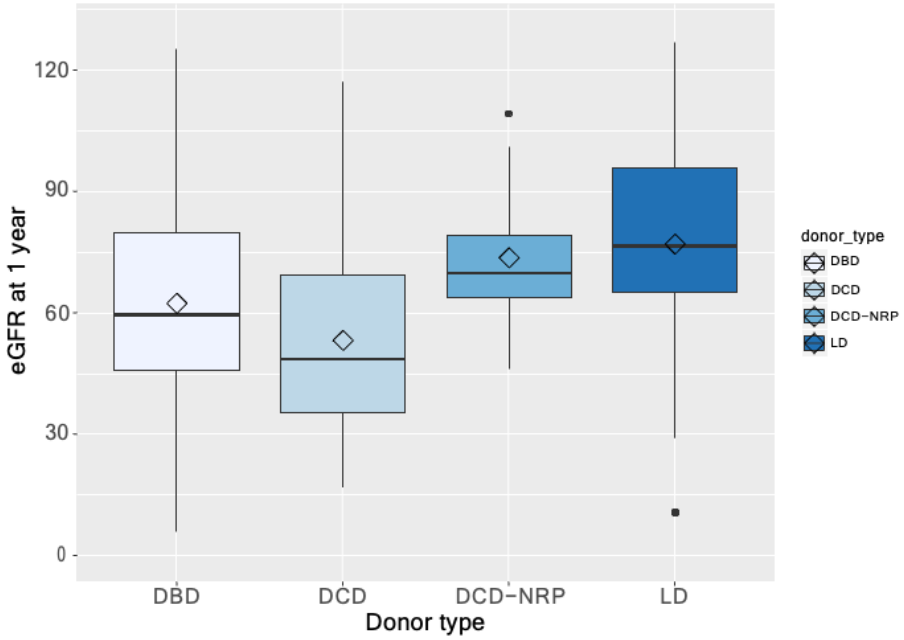


Figure 3: Transplant function at one year. The box plots represent data in the same way as for figure 2. ANOVA with Bonferroni post-hoc testing demonstrated that the NRP and living donor eGFR were both significantly higher than standard DCD (p=0.001).

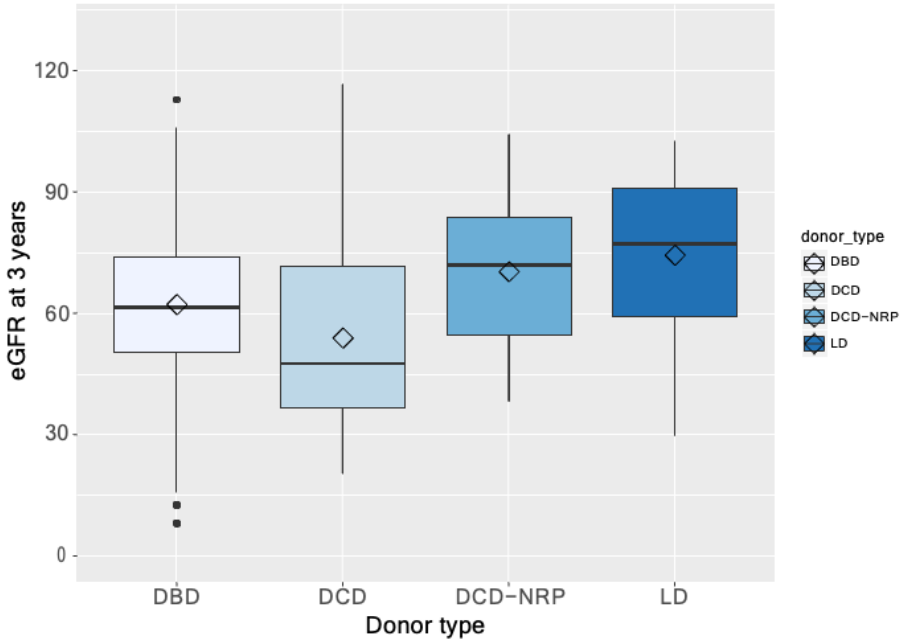


Figure 4: Transplant function at three years. The box plots represent data in the same way as for figure 2. ANOVA with Bonferroni post-hoc testing demonstrated that living donors were had statistically significantly higher function than standard DCD (p=0.009), but the other differences between groups did not reach statistical significance.

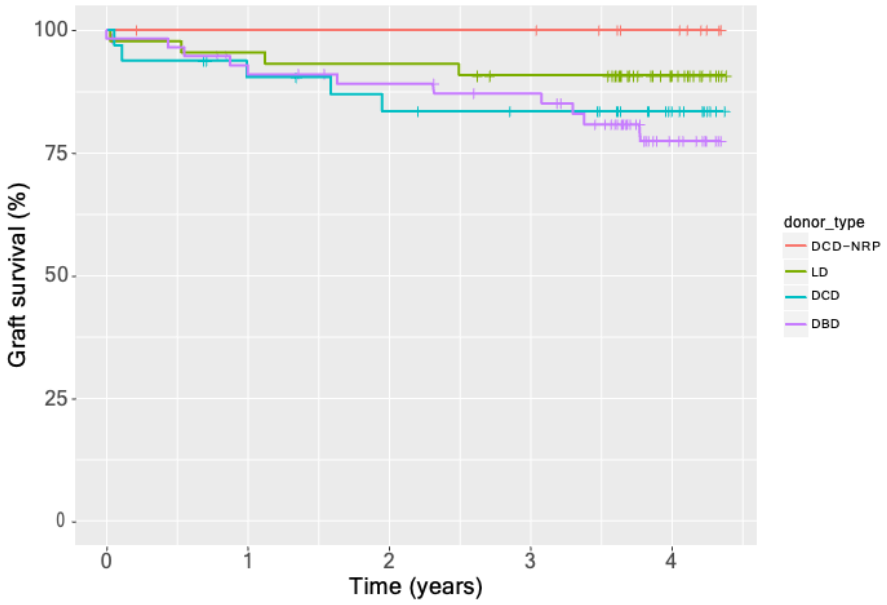


Figure 5: Transplant survival at up to four years represented by a Kaplan-Meier actuarial survival curve for transplants in 2013. The analysis is censored for death with functioning graft. The differences in transplant survival were not statistically significant (Logrank test, p=0.255).

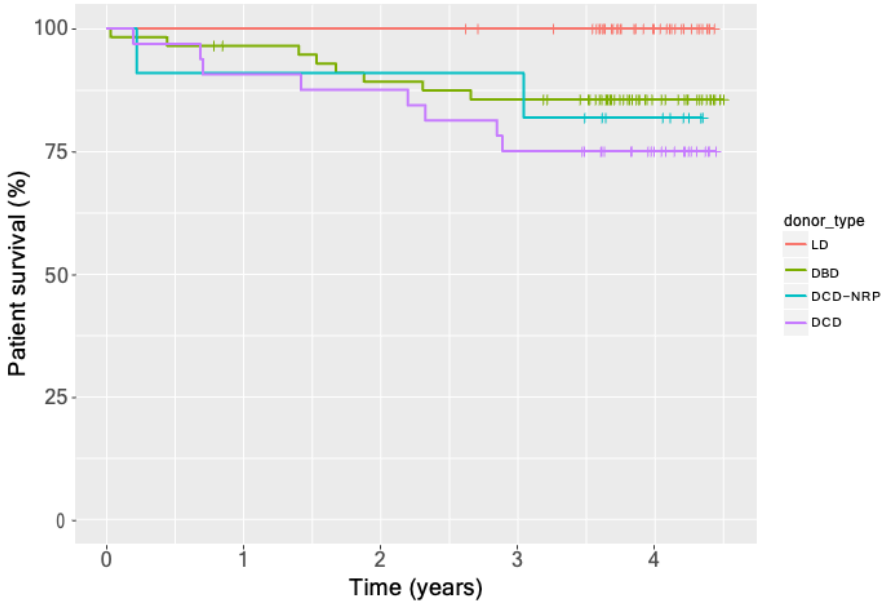


Figure 6: Patient survival at up to four years represented by a Kaplan-Meier actuarial survival curve for patients transplanted in 2013. Recipients of living donor kidneys had the best survival, which was statistically significant (Logrank test, p=0.014). Both of the patients with DCD-NRP kidneys died with functioning transplants. The colour scheme is different to figure 5 above.

C: Potential for EVNP in Glasgow

We examined the outcomes of deceased donor kidney offers in figure 1. On further examination of the data recorded, there were 20 declined offers which might have led to a successful transplant if EVNP for viability assessment were available, and a further two declined at the screening call stage which might have been considered with EVNP for reconditioning.
The twenty possible cases were then categorised into a high potential group, where the kidney would definitely have been transplanted if it passed viability assessment on the score developed by Nicholson, a medium potential group where there were other issues but the kidney may have been used after EVNP, and a low potential group where the kidney probably would not have been used even with good EVNP assessment although might have been suitable for an urgent recipient, such as a patient with failing vascular access. There were 6 in the high potential group, 10 in the medium potential group and 4 in the low potential group (see figure 7).

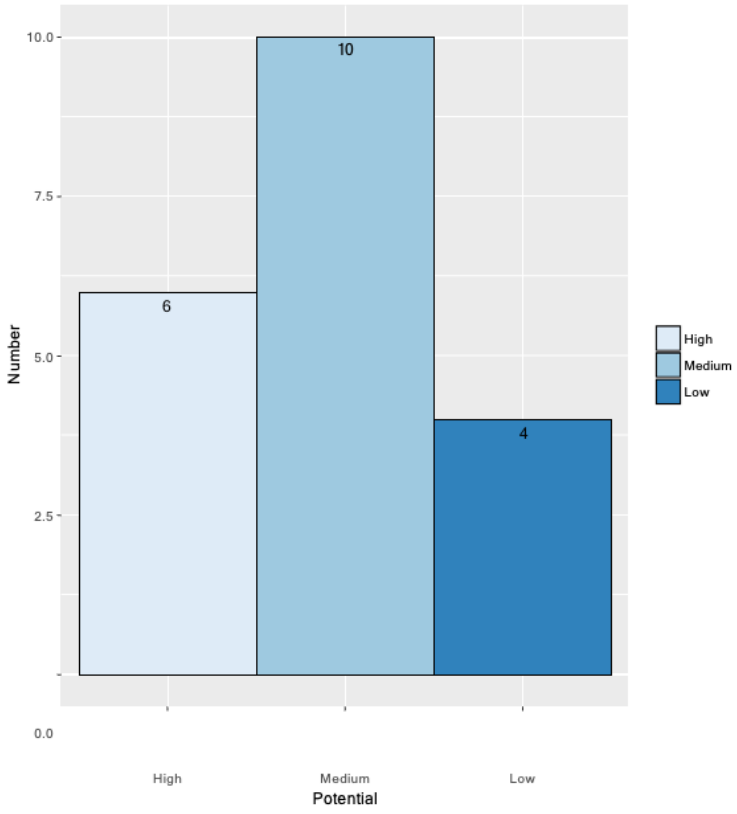


Figure 7: Potential for EVNP. The bar chart shows the numbers of declined kidney offers where the availability of EVNP would have provided a high, medium or low chance of the kidney being transplanted.

D: Indications for EVNP

The main anticipated indications for EVNP within the West of Scotland programme in 2017 would be:

- Viability assessment of extremely marginal DCD kidneys, such as those with prolonged cold ischaemictimes
- Assessment of potential for recovery in poorly perfused kidneys
- Assessment of the adequacy of ureteric blood supply in kidneys with stripped ureters
- Prediction of immediate function in kidneys from very elderly donors intended for elderly recipients

By adopting this approach, we would intend to increase access to transplantation for those highly sensitisedpatients who have very few options and cannot afford to miss any donor offers, but at the same time cannot riskdeveloping further sensitisation in the wake of a failed transplant.
Using EVNP to restore ATP levels will also facilitate the use of kidneys with prolonged cold ischaemic times,such as occurs when a kidney has been to another centre first and found to be unsuitable for their recipient, or where our recipient has a positive cytotoxic crossmatch and we are informed by NHSBT only at a late stage thatwe may reallocate the kidney locally to a backup recipient – and therefore need time to call in and prepare that recipient.
The likely reduction in incidence and duration of delayed graft function which would be expected from the results of the programme in Leicester[2] will also bring significant cost savings[4] and may improve long-term transplant function[5].

E: Future possibilities

One of the more intriguing aspects of EVNP is the potential for drug delivery in a metabolically active ex vivo environment, where potential toxicity to non-renal organs is not a concern, and so permits administration of veryeffective high doses without the risk of systemic side-effects. The potential drugs fall into two broad groups:

- Drugs to attenuate the effects of ischaemia-reperfusion injury
- Drugs to reduce the risk of donor-transmitted disease, such as antivirals for hepatitis C positive donors

These however are future possibilities, not plans to be adopted in 2017. As such interventions are researchedand developed, having the facility for EVNP here would ensure our patients do not lose access to these further opportunities for a successful transplant.

F: Costs

It is anticipated that the capital costs (table 1) will be funded by a capital bid to NSD and that the recurrent cost ofconsumables (table 2) will be covered by NHS Greater Glasgow & Clyde. If EVNP allows additional transplants to be performed, we believe this will be cost effective compared to dial-ysis: in 2008-9 the average cost of dialysis in the UK was £30,800 per patient per year; against this a functioningrenal transplant leads to a cost saving of £25,800 pa after the first year[6].

Table 1: Capital costs

Description	Supplier	Unit cost (ex-VAT)	Unit cost (+VAT)
Bio Medicus 560 Console	Medtronic	17250.00	20700.00
Affinity Pump External Drive Motor	Medtronic	2650.00	3180.00
TX50 Flow Transducer (Adult)	Medtronic	1750.00	2100.00
Holder for Affinity Oxygenator	Medtronic	500.00	600.00
Hico Variotherm 555 Warming Unit	Hirtz & Co.	4410.83	5293.00
Hose extension with Hansen couplings	Hirtz & Co.	320.83	385.00
Tilt-resistant safety stand	Hirtz & Co.	355.83	427.00
2-channel infusion pumps	Imed/Carefusion	4000.00	4800.00
Kidney stage for perfusion (x4)	Precision Surgical	3980.00	4776.00
Steel perfusion trolley	Medtronic	6000.00	7200.00
Total capital cost		41217.50	49461.00

Table 2: Consumables used for each kidney

Description	Supplier	Unit price (ex-VAT)	Unit price (+VAT)
Circuit consumables			
Paediatric tubing set with oxygenator	Medtronic	350.00	420.00
Cannulae (14F and 16F)	Edward Lifesciences	25.56	30.67
Drip "giving" set	Carefusion	6.00	7.20
Priming fluids			
Sodium bicarbonate 8.4%	Fresenius	3.00	3.60
Mannitol 10%	Sigma Aldrich	3.50	4.20
Eporprostenol/Prostacyclin 1.5mg vial	GlaxoSmithKline	45.00	54.00
Augmentin 1.2g vial	GlaxoSmithKline	1.06	1.27
Synthamen 9 / Ringer's / Cerenvit / 5% dextrose	Baxter	31.50	37.80
Insulin / heparin	NovoNordisk / PoM	15.00	18.00
Dexamethasone	Organon	5.00	6.00
Total cost per kidney		485.62	582.74

References

[1] A Bagul, SA Hosgood, and ML Nicholson. “Experimental renal preservation by normothermic resuscitationperfusion with autologous blood”. In: *British Journal of Surgery* 95 (2008), page 111.

[2] AD Barlow, SA Hosgood, J Hunter, et al. “Quality assessment of human kidneys using ex vivo normothermicperfusion [M4]”. In: *17th British Transplantation Society Annual Congress, Glasgow*. 2014.

[3] SA Hosgood, E van Heurn, and ML Nicholson. “Normothermic machine perfusion of the kidney: Betterconditioning and repair?” In: *Transplant International* (2014).

[4] C Wilson, S Stamp, HL Wyrley-Birch, et al. “The early economic costs of DGF in DCD kidney transplantation[P147]”. In: *17th Annual Congress of the British Transplantation Society, Glasgow*. 2014.

[5] AJ MacLaren, W Jassem, DW Gray, et al. “Delayed graft function in renal transplantation: aetiology, man-agement and long term significance”. In: *Clincal Transplantation* 13 (1999), page 266.

[6] National Kidney Federation. *Transplantation Cost Effectiveness*. 2010. URL: http://www.kidney.org.uk/archives/news-archive-2/campaigns-transplantation-trans-cost-effect/

APPENDIX B – Material Clinical Perfusion Checklist

Materials checklist: EVNP

Checklists		Notes
Kidney offer received:		
Arrange transfer through Amvale Courier		Quote 'Glasgow research organs'
Contact theatres staff – assess theatre space for perfusion		
Contact Ward 4 nursing staff and advise graft en route		
Haematology:		
Call haematology and state 'EVNP' kidney requiring one unit of O Neg		
If clinical EVNP – provide CHI of recipient		
Ensure blood collection slip is sent to blood bank		
Print blood transfusion return form and ensure completion		
Equipment:		
☐ Benching set + mallet for ice		
☐ 4-0 Vicryl ties (x3)		
☐ Kidney steel tray		
☐ Temperature probe		
☐ Soltran 1L		
☐ Saline for ice x2		
☐ Small swabs x1		
☐ Large packs x1		
☐ Alaris infusion pumps x4		
☐ Tubing clamps		
☐ Patch clamps		
☐ 95%0_2/CO_2 gas		
☐ Medtronic perfusion set		
Consumables:		
☐ Ringer's solution		As over
☐ Prostacyclin 0.5mg (Flolan)		16ml in 100ml of Saline 0.9%
☐ 5% Dextrose 100ml		At 4mls per hour
☐ Synthamin 17 500ml		As over
☐ Cernevit multivitamin 1 vial		As over
☐ Insulin (Actrapid 100iu)		Direct to circuit (DTC)
☐ Sodium bicarbonate 8.4% 20ml		DTC - Initial administration: 27ml
☐ Mannitol 10% 20ml		DTC
☐ Dexamethasone 3.75mg		DTC
☐ Heparin 1000iu/ml x 3ml		DTC – 3ml
☐ Soltran 1L at 4°C		For post-EVNP flush
☐ 1 unit of O-neg PRCs		DTC

Set up: EVNP

- ☐ Turn on Medtronic Bio-Console
- ☐ Ensure heater/cooler pre-filled
- ☐ Prepare supplements as described – record all serial numbers

- ☐ **Set up IV infusions** and connect 4 x Alaris infusion pumps

	Infusion 1	**Infusion 2**	**Infusion 3**	**Infusion 4**
Drug	Flolan – reconstitute with fluid provided in pack – 16ml	None	Cernevit – reconstitute in 10ml saline	None
Infusion bag	100ml Saline	100ml 5% dextrose	500ml Synthamin 17	500ml Ringer's
Rate	4ml/hr	4ml/hr	20ml/hr	4ml/hr – *match urine output*

- ☐ Scrub up (you will need a second pair of hands from now on)
- ☐ **Assemble the perfusion set** (aseptic)

- ☐ Connect the IV infusion lines
- ☐ Connect the pressure transducer
- ☐ Assemble the sterile perfusion chamber on a draped trolley and connect ¼ inch tubing to the base, and opposing end to the venous reservoir.
- ☐ Ensure all taps are closed
- ☐ Attach 95%0_2/CO_2 via tubing
- ☐ Attach water heater – *ensure filled with sterile water*

Priming the system:

- Add 300mls Ringer's through the IV line
- Add heparin – *do this first*
- Add PRCs
- Add mannitol, insulin, dexamethasone, 27mls bicarbonate to the reservoir

- **Remove air from the pump head**
- Set to 1000rpm and allow the perfusate to circulate
- Turn on the oxygen, set the flow rate to 0.1L/min
- Turn on the water heater and set to 37.4°C
- Turn on the alaris pumps with flow rate as described

- **Zero the pressure line –** *prime by withdrawing 10mls of perfusate and injecting it into the line, then attach to the pressure transducer and open the line – it should read zero.*

- **Take a blood gas** – add more sodium bicarbonate if require to maintain a pH of 7.3-7.5

Attaching the kidney:

- Cannulate ureter with nelation catheter
- Renal artery and vein pre-prepared – *connect to ¼ tubing with Water's clamps*
- Flush the kidney with 200mls of Ringer's to remove the perseveration fluid
- Weigh the kidney
- Prime the cannula with Ringer's to remove the air

- Under aseptic conditions, clamp the tubing one 5cm beyond flow probe and the second 10cm further downstream.
- Cut the tubing between the clamps
- Prime the arterial cannula (with Ringer's) and try to remove all air from the tubing – *partly release clamps/venting via straight connector*
- Connect the tubing

Perfusing the kidney:

- Turn up pump speed until pressure reaches 75mmHg (approx. 1400-1500rpm)
- Monitor renal blood flow and urine output
- Adjust rate of Ringer's solution to match urine output
- Take arterial and venous sample at 30 and 60 minutes – *add sodium bicarbonate as necessary*

To complete the perfusion:

- Document macroscopic appearance
- Total urine output
- Clamp the arterial tubing and attach the IV line containing 1L of Soltran and place the kidney on ice
- **Continue until effluent is clear**
- **Weigh the kidney**
- **+/- biopsy**
- **Complete full data record (see data record sheet)**

Disposal of consumables:

- Drain perfusate into a receptacle and dispose as per hospital policy
- All consumables to be incinerated as clinical waste

APPENDIX C – Study Protocol

DELIVERY OF ADIPOSE-DERIVED REGENERATIVE CELL THERAPY DURING EX-VIVO NORMOTHERMIC PERFUSION OF KIDNEYS

Aim: To assess whether adipose-derived regenerative cells can be administered during ex-vivo normothermic perfusion of the human kidney and to evaluate the effect this has on the immunogenic environment.

PROTOCOL VERSION NUMBER AND DATE

Sept 2019 – Version 0.3

RESEARCH REFERENCE NUMBERS

IRAS Number:	269613
SPONSORS Number:	GN19RE465
FUNDERS Number:	Not applicable.

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:
...../...../.....

.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Date:
13/11/2019

.....

Name: (please print): Mr Robert Pearson

.....

LIST of CONTENTS

GENERAL INFORMATION	**Page No.**
TITLE PAGE	1
RESEARCH REFERENCE NUMBERS	1
SIGNATURE PAGE	2
LIST OF CONTENTS	3
KEY STUDY CONTACTS	4
STUDY SUMMARY	4
FUNDING	5
ROLE OF SPONSOR AND FUNDER	5
ROLES & RESPONSIBILITIES OF STUDY STEERING GROUPS AND INDIVIDUALS	5
STUDY FLOW CHART	6
SECTION 2	
1. BACKGROUND	1
2. RATIONALE	3
3. THEORETICAL FRAMEWORK	4
4. RESEARCH QUESTION/AIM(S)	4
5. STUDY DESIGN/METHODS	4
6. STUDY SETTING	7
7. SAMPLE AND RECRUITMENT	8
8. ETHICAL AND REGULATORY COMPLIANCE	9
9. DISSEMINATION POLICY	12
10. REFERENCES	13
11. APPENDICES	

KEY STUDY CONTACTS

Chief Investigator	Mr Robert Pearson MBBS MRes MRCS Robert.pearson5@nhs.net 07787391159
Study Co-ordinator	Mr Marc Clancy MBBS FRCS PhD Consultant Transplant and General Surgeon Marc.clancy@ggc.scot.nhs.uk
Sponsor	NHS Greater Glasgow & Clyde Sponsor Representative : Mrs Mary McAuley Research Performance Manager Clinical Research and Development Unit Dykebar Hospital Grahamston Road Paisley PA2 7DE
Joint-sponsor(s)/co-sponsor(s)	Not Applicable
Funder(s)	NHS Greater Glasgow & Clyde Renal owment Fund – Mr Marc Clancy
Key Protocol Contributors	Rashida Lathan PhD Rashida.lathan@glasgow.ac.uk

STUDY SUMMARY

Study Title	**DELIVERY OF ADIPOSE-DERIVED REGENERATIVE CELL THERAPY DURING EX-VIVO NORMOTHERMIC PERFUSION OF KIDNEYS**
Internal ref. no. (or short title)	ADRC Delivery During EVNP
Study Design	Randomised; Interventional; Design type: Treatment
Study Participants	Research kidneys as per NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group)
Planned Size of Sample (if applicable)	12 (6:6) – a maximum of 15 to allow for logistical issues
Follow up duration (if applicable)	Not applicable. Kidneys not transplanted.
Planned Study Period	18 months

Research Question/Aim(s)	To assess whether adipose-derived regenerative cells can be administered during ex-vivo normothermic perfusion of the human kidney and to evaluate the effect this has on the immunogenic environment.

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**NHS Greater Glasgow & Clyde Transplant and Renal Failure Endowment Fund**
Transplant and Renal Failure Endowment	Supported by the Transplant and Renal Failure Endowment Fund

ROLE OF STUDY SPONSOR AND FUNDER

The study will be sponsored by NHS Greater Glasgow & Clyde – Sponsor Representative is Mrs Mary McAuley, Research Performance Manager, Clinical Research and Development Unit, Dykebar Hospital. The sponsor will register the study on the clinical trials website, oversee costing analysis, provide insurance and indemnity for the study and assist with IRAS application and ethical approval.

The study design, conduct, data analysis and interpretation will be completed by the principal investigator, as will be with manuscript writing and dissemination of results. The sponsor controls the final decision regarding this study.

ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

This study does not contain study management groups or committees to oversee this laboratory study.

PROTOCOL CONTRIBUTORS

Mr Marc Clancy – Advice on study design, clinical support with EVNP service

Dr Rashida Lathan – Support with scientific study design

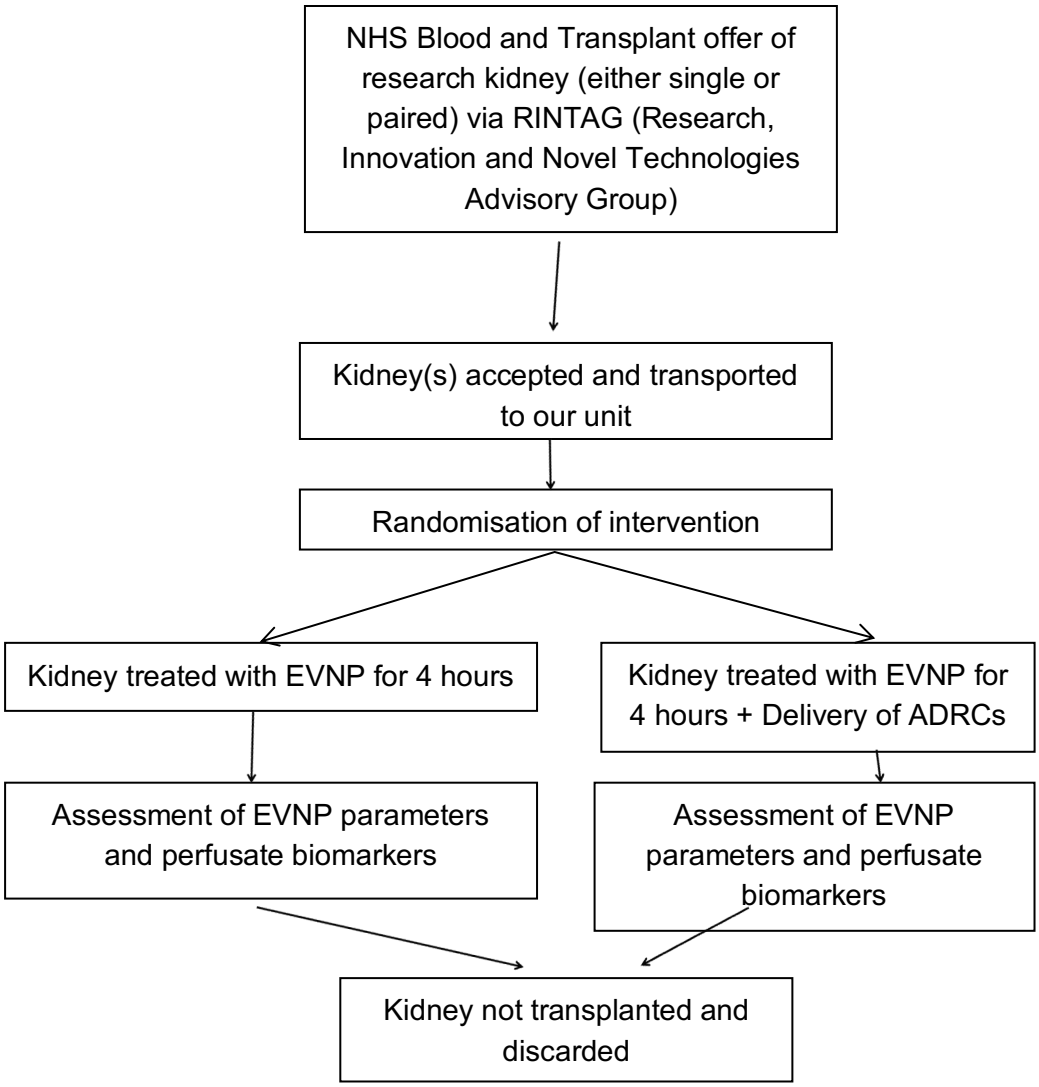
Dr John Stone – Support with scientific study design

Dr James Fildes – Support with scientific study design

Professor Patrick Mark – Supervision of study design and clinical support

KEY WORDS: EVNP; Regenerative Cells; Ischaemia-Reperfusion; Organ Preservation; Normothermic Perfusion

STUDY FLOW CHART:



STUDY PROTOCOL

DELIVERY OF ADIPOSE-DERIVED REGENERATIVE CELL THERAPY DURING EX-VIVO NORMOTHERMIC PERFUSION OF KIDNEYS

1 BACKGROUND

The greatest challenge for kidney transplantation remains how best to bridge the discrepancy between the increasing demand of those in need of a kidney transplant and the utilisable supply of grafts. In the UK and across Europe we lead the way in the use of donors from circulatory death (DCD), however, there remains a substantial and clinically significant shortfall. There are currently over 6000 patients on the waiting list for a kidney in the UK alone(1). The use of extended criteria donors (ECD), i.e. those from donors over 65 years of age, has increased the potential pool from which to obtain donation but this carries inherent challenges with graft survival and rates of delayed graft function (DGF). The proportion of ECD donations here in the UK now represents an increasing share, with 35% deceased donations being provided by donors over 60 years of age(2).

The nature of DCD kidney transplantation, with inherent warm ischaemia time and unpredictable perfusion during the hypotensive agonal phase, creates the environment for ischaemia-reperfusion injury(3-5). Organs recovered in this setting are usually obtained through rapid laparotomy following circulatory arrest and in situ cold perfusion fluid is circulated through the grafts at time of procurement before being transported to their recipient transplantation unit via static cold storage (SCS) i.e. on ice. The cold perfusion fluid followed by SCS cools the organ temperature to approximately 4 degrees, dampening the metabolic activity and tissue demand. The period of warm ischaemia followed by cooling and cold storage, is thought to be key contributory factor underpinning ischaemia-reperfusion injury (IRI) and the resulting increased rates of primary non-function (PNF), delayed graft function (DGF) and long-term graft survival rates are well described (6). To quantify the rate of DGF, two large European multi-centre studies reported rates as high as 50.2% and 63.4% for controlled DCD donors (7,8).

In order to mitigate this pathophysiology there is increasing interest in kidney perfusion techniques, both at the time of organ procurement, and following explantation i.e. ex-vivo perfusion. The optimal timing, duration, temperature and contents of the perfusate are all independent areas of ever-emerging research.

Normothermic perfusion of organs at the recipient centre (so called Ex-vivo normothermic perfusion, or EVNP) has been demonstrated in a clinical setting thus far in heart(9), lung(10), liver(11) and more recently kidney solid organ transplantation(12). The perfusion circuit allows warmed, oxygenated blood to be circulated through the kidney, re-establishing a pseudo-physiological state, and crucially facilitates the following opportunities: A period of reconditioning by re-establishing oxygenation and altering the mechanism of IRI; viability assessment to aid the transplant decision making process in marginal grafts; and a potential mode for therapeutic intervention.

The detrimental effects of cold ischaemia time (CIT) on transplant outcomes are well documented, with the duration of this hypothermic period being linked with renal allograft complications such as DGF, primary non-function (PNF), and long-term graft function(8,13–16). Furthermore, the occurrence of such complications is known to negatively impact patient survival as well as graft survival(17,18). Demonstrating the re-conditioning effects of EVNP, Hosgood et al. (2013) used an animal model where porcine kidneys were exposed to either 24 hours of CIT, or 23 hours followed by a one-hour period of EVNP. EVNP-treated porcine kidneys demonstrated lower intra-renal resistance and had improved metabolic function with evidence of less tubular injury(19). Kaths et al. also demonstrated in a porcine model that a longer period of EVNP (8 hours)

vs. SCS reduced intrarenal resistance, lowered lactate levels and cellular injury markers (lactate dehydrogenase and aspartate aminotrasferase) when these grafts were auto-transplanted(20). In an attempt to further elucidate the ideal length of perfusion time on the EVNP circuit, the same group compared 0, 1, 8 and 16 hour/s, respectively. The 16 hour group were treated with continuous EVNP (i.e. no SCS, as the others were all subjected to SCS prior to EVNP making a total of 16 hours post-procurement.) Their results demonstrated a reduction in peak serum creatinine and a higher 24-hour creatinine clearance at day 3 post-transplant in the continuous 16 hour group(21).

The first clinical pilot utilising EVNP in extended criteria donor kidneys demonstrated a reduction in the rate of DGF from 36% in standard ECD (i.e. static cold storage only) to 6% in EVNP-treated ECD renal allografts, with no detrimental effects on graft or patient survival at one year(22). Following on from this the group presented the successful transplantation of a declined kidney (deemed unsuitable by other transplantation units) which was reconditioned and assessed using EVNP(23). To further this exciting work, there is currently an ongoing randomised control trial assessing EVNP vs static cold storage in DCD donors to validate these promising results(24).

During the organ procurement process, if the kidney is seen to inadequately perfuse when flushed with in situ cold flush (typically Wisconsin solution) the term ‘inadequate in situ perfusion’ or ‘poor perfusion’ is often used. As many as 18% of kidneys from DCD donors are deemed unsuitable for transplant, with poor perfusion one of the most common reasons cited(25). The rationale for declining such organs is that the poor perfusion appearance indicates incomplete cooling (i.e. warm ischaemia) and raises concern of the potential for poor function post-transplantation. The aetiology of poor perfusion is multifactorial and potential causes include donor atherosclerosis, technical difficulties at procurement, complex anatomy, intravascular thrombosis, and vasospasm(23).

The perfusion of kidneys with EVNP which have been discarded for poor perfusion has been demonstrated by Hosgood et al. Initially as an experimental model, 22 kidneys were observed during a period of EVNP for appearance, creatinine clearance, renal blood flow, and urine output. This study highlighted that a significant proportion of these discarded kidneys may be suitable for transplantation(26). Following this encouraging data the first successful transplant of a human kidney which had been deemed untransplantable was performed in 2016(23). This demonstrates two key strengths of this technology, the resuscitative capacity and the opportunity to assess the viability of a graft to aid decision making, and therefore crucially having the potential to expand the pool of usable grafts.

In a porcine model, Kaths et al. (2018) further demonstrated the utility of EVNP in the assessment of grafts(27). Porcine kidneys were subjected to varying degrees of warm ischaemia time (0, 30 or 60 minutes) and then autotransplanted. Low intra-renal resistance and markers of acid-base balance, lactate and aspartate aminotransferase all correlated with the degree of warm ischaemia and with post-transplant function. This demonstrated that the assessment of perfusate biomarkers and composition provided a prediction of post-transplant function.

Hosgood eloquently described how EVNP can provide a viability assessment, by stating the technology *“not only allows a measure of injury but also provides an evaluation of recovery”*(28). Through a large body of work in kidneys declined by recipient centres their team have defined a quality assessment score, and graded kidneys from 1 (least injured) to 5 (the most severe injury) based on three parameters: 1) macroscopic appearance 2) renal blood flow and 3) urine output. Importantly, a score of 1-4 was deemed potentially transplantable. Kidneys scoring 1-3 were all transplanted without

complication with the largest DGF rate at 38% for those scoring 3(29). This work highlighted the potential underutilisation of our current offering system.

One of the key benefits of an EVNP circuit is the ability to deliver therapeutics to the solid organ whilst being perfused, thus allowing localised treatment to the organ whilst circumventing the systemic delivery of the agent to the transplant recipient. Numerous studies have investigated the potential benefit of agents added to the perfusion circuit in animal models, including stem cells, gene therapies and nanoparticles.

Stem cells have the unique property of pluripotency (i.e. the ability to differentiate into various cell types), whilst demonstrating regenerative properties in numerous in vitro models (30–33). More specifically, mesenchymal stromal cells (MSC), which can be derived from bone marrow, adipose tissue, placental tissue or amniotic fluid, have been shown to secrete anti-inflammatory cytokines, growth factors, and immunoregulatory mediators. In renal models MSCs have been shown to improve medullary inflammation and fibrosis in atherosclerotic renal artery stenosis(34,35)and preserve renal function and prevent fibrosis in a porcine transplant model (36). Furthermore, MSCs have shown the tendency to localise in sites of inflammation (37–39) and migrate to renal tissue which has been subject to injury (40,41).

The difficulty, however, is the clinical delivery of such a therapy. Studies have demonstrated that when delivered intravenously the cells are short-lived and invariably fail to migrate beyond the pulmonary circulation (42,43). Circumventing this, recent work by Sierra-Parraga et al. demonstrated that the administration of MSCs directly into the renal artery following a porcine model of ischaemia reperfusion facilitates the localisation and accumulation of the regenerative cells, predominantly within the renal cortex, adjacent to the glomeruli, capillary networks and tubules(44). Furthermore, Pool et al. (2019) demonstrated the feasibility of delivering bone-marrow derived mesenchymal stem cells in a normothermic perfusion circuit and reported the apparent clustering of the regenerative cells in the glomeruli but found a large attrition rate to only 10% during the period of perfusion(45).

Work by our own laboratory at the Institute of Cardiovascular Medicine, affiliated with Glasgow University, has attempted to elucidate the mechanistic effects of adipose-derived regenerative cells (ADRC) in a rodent model of ischaemia reperfusion injury. We have demonstrated that following ischaemia, ADRCs appear to improve renal function in the short- and medium-term, which was associated with an upregulation of key immuno-modulatory cytokines.

Adipose-derived regenerative cells (ADRC) can be delivered during EVNP without negotiating the pulmonary circuit, with direct access to the target organ during a period of perfusion within a closed circuit. To our knowledge, there are no published studies to date demonstrating the efficacy of adipose-derived regenerative cells within an ex vivo normothermic perfusion circuit to assess their role in amelioration of ischaemia-reperfusion injury within the human kidney following a period of static cold storage.

2 RATIONALE

Further understanding into the mechanism and role of ADRCs modulation of the immunogenic environment and evidence of this treatment's safety could advance this work to a clinical trial in which kidneys are perfused with EVNP, with or without ADRC administration, and then subsequently transplanted. This would allow an assessment of this therapy as a clinical benefit, potentially reducing the clinical impact of ischaemic damage and improve early transplant function.

3 THEORETICAL FRAMEWORK

The study would involve the administration of ADRC directly to the kidney via an ex-vivo perfusion circuit with warmed-oxygenated blood. The ulitilisation of this circuit provides us with a unique mode of delivering this therapeutic agent. ADRC have been shown by our own group, and numerous other centres, to have regenerative and reno-protective properties. During the period of perfusion, perfusate samples can be taken at various time points, and further analysed within our laboratory facilities for recognised markers of renal injury, including TGF-β, IL1- β, NGAL etc. (see Methods of Data Collection).

Urine production will be measured and analysed for creatinine clearance to assess the function of the kidney during perfusion. Furthermore, as the kidney is not subsequently transplanted, histology of the graft can be performed to assess levels of fibrosis, tubular damage and assess the migration and location of administered ADRCs.

4 RESEARCH QUESTION/AIM(S)

To assess whether adipose-derived regenerative cells can be administered during ex-vivo normothermic perfusion of the human kidney and to evaluate the effect this therapy has on the immunogenic environment.

4.1 Objectives

The objective of this study is to elucidate:

- Whether ADRC can be safely delivered to a kidney during ex-vivo normothermic perfusion
- To assess the viability of ADRC during perfusion
- To assess how ADRC treatment effects the immunogenic environment e.g. cytokine release, inflammatory mediate.
- To analyse how the ADRCs migrate and locate within the kidney graft during perfusion and once perfusion is completed.
- To further understand the mechanism of effect that the ADRC population has on the kidney at the molecular level.

4.2 Outcome

This study aims to improve understanding of both ADRCs as a therapy for renal injury, and the way for this cell type to be safely administered to an organ. Ultimately, progress on this subject could lead to translation of this therapy into the clinical environment by way of clinical trial if proven to be safe and efficacious in this ex-vivo model.

5 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYIS

Research kidneys offered as per NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group) to our unit. Transport subsequently arranged for graft to be couriered to Greater Glasgow and Clyde NHS Trust, Queen Elizabeth University Hospital, Glasgow. At this point, all identifiable information travelling

with the organ is destroyed. The organ is then perfused with warmed-oxygenated blood (37 degrees) for 4 hours, utilising a perfusion protocol produced and refined by Hosgood et al. in Cambridge, now used in multiple sites across the UK in Edinburgh, Newcastle, and Cambridge. Once established on the circuit, the graft will be administered with adipose-derived regenerative cells harvested from healthy human surplus adipose tissue, obtained through Glasgow Biorepository Lab.

Components of the perfusion system:

a) Hardware
    i. Bio-Console 560, pump head, flow probe, pressure transducer, temperature probe, bracket for Pixie oxygenator.
    ii. Hico-Variotherm heater/cooler.
    iii. Alaris infusion pumps x4, pump head, flow probe, pressure transducer.
    iv. Temperature probe and monitor
    v. Tubing clamps
    vi. 95 % 0_2/5 % CO_2 cylinder F size clinical grade + regulator (Carnet)

b) Disposables
    i. Disposable perfusion set: Medtronic (M449802A)
    ii. Stainless Steel Perfusion Chamber
    iii. Infusion sets x 4
    iv. Pressure transducer x 1
    v. IV blood set x 2
    vi. Arterial and venous cannula, nelation catheter for the ureter
    vii. ¼ inch tubing
    viii. ¼ inch straight connector
    ix. Y-connector

c) Consumables
    i. Ringer's solution
    ii. Prostacyclin 0.5 mg x 1 (IV infusion 16 ml in 100 ml 0.9% sodium chloride) (Flolan)
    iii. 5 % glucose 100 ml (4 ml per hour)
    iv. Synthamin 17 500 ml
    v. Cernevit multivitamin 1 vial
    vi. Insulin (Actrapid 100 iu)
    vii. Sodium bicarbonate 8.4 % 20 ml
    viii. Mannitol 10 % 20 ml

ix. Dexamethasone 3.75 mg

x. Sodium bicarbonate 8.4 % approximately 27 ml*

xi. Heparin 1000 iu/ml x 3ml

xii. Soltran 1L at 4°C

xiii. 1 unit of cross-matched red blood cells

5.1 Schedule of events

Area of Activity	Specific Activity	Duration (Minutes)	Undertaken by
Phone call	Receive offer of research kidney	10	PI (RP)
Phone call	Arrange courier of graft from donor unit	30	PI (RP)
QEUH	Graft arrives at QEUH	-	
QEUH	Organisation of where EVNP can take place at the given time	30	PI (RP)
QEUH	Liaise with theatre staff (Staff nurse band 6) re: space in theatres	10	PI (RP)
QEUH Theatres	Proceed with EVNP only if suitable space available and no disruption to theatre utility		
QEUH Theatres	Set up EVNP equipment	30	PI (RP)
QEUH Theatres	Run EVNP (time dependent on quality of graft)	120-240	PI (RP)
QEUH Theatres	Take samples of perfusate during EVNP	-	PI (RP)
QEUH Theatres	Administer regenerative cell population through EVNP circuit	-	PI (RP)
QEUH Theatres	Stop EVNP	-	PI (RP)
QEUH Theatres	Take required tissue biopsies from tissue for laboratory analysis	10	PI (RP)
QEUH Theatres	Discard kidney graft as per standard theatre protocol	15	Theatre nursing staff – standard procedure
ICAMS	Transfer samples to ICAMS facility at Glasgow University via Taxi	20	PI (RP)
ICAMS	Samples stored for further scientific analysis as per tissue transfer agreement	10	PI (RP)

Key points:

- Duration of perfusion: 4 hours
- No identifiable information stored with the kidney
- ADRC Harvest: Obtained through Glasgow Biorepository Laboratory (Application number 521) in which healthy adipose tissue is obtained from consenting individuals in which surplus tissue can be used for research purposes. This tissue

is then used to harvest and isolate the regenerative cell population, and stored for use in this scientific model.
- $50x10^6$ ADRCs administered at 60 minutes.
- ADRCs labelled with lipophilic dye (red fluorescent dye Cell Tracker CMPTx CT 24552)
- Perfusate samples taken at the following time points (minutes): 30, 55, 90, 120, 180, and 240.
- Measure of renal blood flow (measured by the Medtronic Bio-Console 560 device)
- Samples taken are transported via taxi and stored in our laboratory facility within Glasgow University.
- Kidney preserved for histological analysis. Imaged to assess location of ADRC following perfusion of the graft.
- Chemokine and cytokine analysis of perfusate samples, broad Luminex panel for cytokine and chemokine analysis to assess immunity, inflammation, oxidation and remodelling/repair, including the following:
    - TGF-β
    - KIM-1
    - MIP-1
    - IL-1β
    - NGAL
    - Indolamine-2,3-dioxygenase
    - Caspase-3
    - AKT
    - Nitric Oxide
    - 8-isoprostane
    - IL-10
- Urine collected, measured and tested for creatinine clearance.
    - Fractional excretion of sodium
- Overall assessment score of kidney (as per Hosgood et al. 2016) based on macroscopic appearance, urine output and renal blood flow.
- Tissue samples are stored for the duration of this study
- Kidney discarded as per standard theatre protocol

Data analysis will be performed by myself and Rashida Lathan, post-doctorate at the Institute of Cardiovascular and Molecular Science (ICAMS), affiliated with Glasgow University.

Statistical analysis will be performed using the software R Studio.

6 STUDY SETTING

In order to perform the ex-vivo perfusion in a suitable environment, this will take place at the theatre facility in Queen Elizabeth University Hospital (QEUH), Greater Glasgow and Clyde NHS Trust. Once allocated, the research kidney(s) will be transported to the hospital and prepared for attachment to the perfusion circuit.

Harvested ADRCs are pre-prepared and stored frozen at the ICAMS facility laboratory and when required transferred to the hospital for the sole purpose of perfusion.

This is a single centre study utilising the facilities of the Renal Transplant Surgical Department theatre suite, QEUH, and the laboratory facilities to which the hospital is affiliated, at the ICAMS facility, Glasgow.

All perfusate, urine and tissue sample analysis will be performed at the ICAMS laboratory facility.

7 SAMPLE AND RECRUITMENT

7.1 Eligibility Criteria

All research kidneys offered through the NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group) to the principal investigator will be considered eligible for this study. All kidneys will be from deceased donors who have research consent and have been refused by transplant units for clinical transplantation and are therefore offered to research units for use.

7.1.1 Inclusion criteria

- Deceased donor kidney(s) offered to the principal investigator through the national advisory group NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group)

7.1.2 Exclusion criteria

- Renal anatomy that precludes successful cannulation and attachment to the ex-vivo perfusion circuit i.e. multiple arteries without common stem (unable to perfuse entire graft)
- Significant ureteric injury that makes urinalysis and measurement of urine output not possible
- Evidence of extensive malignancy in graft that would have a structural detrimental effect of perfusion and/or renal blood flow

7.2 Sampling

Perfusate samples will be taken from the arterial limb of the perfusion circuit with two functions: 1) For real time adjustment of the circuit parameters i.e. to guide need for bicarbonate to adjust pH of perfusate 2) To sample perfusate for later analysis. Perfusate samples taken at the following time points (minutes): 30, 55, 90, 120, 180, and 240. Volume taken is 10ml.

Urine is collected separately and a single 10ml sample taken for scientific analysis.

7.2.1 Size of sample

The aim of the study is to perfuse six grafts with EVNP with administration of ADRCs and perfuse six grafts with EVNP without ADRCs. 15 total is estimated to allow for logistical issues with the study circuit, however, study will be completed when there are 6 in both groups.

7.2.2 Sampling technique

The national advisory group NHS Blood and Transplant RINTAG (Research, Innovation and Novel Technologies Advisory Group) offer deceased donor kidneys to research units for use in studies for the purpose of research and innovation. These grafts are offered once refused

by clinical transplantation units for reasons of poor perfusion, suspicion of malignancy, complex anatomy etc. All kidneys offered will be considered eligible and are then inspected for suitability as per exclusion criteria (see above).

7.3 Recruitment

No patient participants are required for this study.

7.3.1 Sample identification

Samples are pre-identified as described above by the independent national advisory group, RINTAG.

7.3.2 Consent

The consent for donation of organs is obtained pre-donation, and part of that is the potential use of such organs for use in scientific research, if deemed untranslatable. Kidneys used in this study will not be subsequently transplanted and therefore no consent is required for the use of the graft.

ADRCs are obtained from surplus healthy adipose tissue which is taken at time of operation whereby the patient has consented to the storage and use of this surplus tissue in scientific research. This tissue is fully anonymised at source (Glasgow Biorepository Laboratory – Application number 336) and no patient identifiable data is attached to the sample.

8 ETHICAL AND REGULATORY CONSIDERATIONS

The NHS Blood and Transplant national advisory group (RINTAG) is an independent body that oversees the use of organs that are deemed non-transplantable by clinical units within the UK. There is then a hierarchy of allocation to research units, in which grafts that could ultimately be transplanted (i.e. after investigation and/or treatment in a research setting) are provided priority. As a result, kidneys that would be offered to our unit are refused by both clinical transplantation units, but also research units that could potentially transplant the graft following treatment, thus maximising the utilisation of the donor pool. This study, therefore, is not reducing the utilisable pool of suitable grafts for transplantation. This work would ultimately aim to increase the understanding around both perfusion techniques and the therapeutic potential of regenerative cells administered in this context.

Numerous studies have used stem cells in wide-ranging clinical contexts demonstrating both safety and potential regenerative efficacy. In this study, ADRCs are harvested from patients who have consented to the use of surplus tissue (in this case adipose tissue) which is taken as part of an elective operation for a separate purpose (e.g. liposuction). The anonymised tissue is then stored by an independent facility (Glasgow Biorepository Laboratory – Application number 336) and processed in our laboratory facilities to extract the ADRC population.

8.1 Assessment and management of risk

Risks in this study include:

- Exposure to human blood and tissue
  - Deceased donor organs are donated if safe infectious disease status is confirmed prior to death. There is, however, a recent change in policy to allow donors with hepatitis C to donate in certain circumstances. Therefore, for the duration of this study, it is possible that a research kidney allocated via RINTAG could have a positive hepatitis C virology. This would be identified and be handled using standard precautions e.g. gloves, PPE (personal protective equipment) and disposed of as hazardous biological waste as per local protocol.

8.2 Research Ethics Committee (REC) and other Regulatory review & reports

Prior to commencement of this study a favourable opinion will be sought from both local research and development team and REC. All correspondence with REC regarding ethical approval will be retained.

- Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
- All correspondence with the REC will be retained.
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the REC of the end of the study.
- An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
- If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.
- Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

Regulatory Review & Compliance

Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance. Different arrangements for NHS and non NHS sites are described as relevant.

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

Amendments

If a substantial amendment is made to the REC application or the supporting documents, a valid notice of amendment will be submitted to the REC for consideration. If applicable, other specialist review bodies (e.g. RINTAG) need to be notified about substantial amendments in case the amendment affects their opinion of the study.

In the event of an amendment the local R&D office will be informed, including 'non-substantial' amendments. The process would include written correspondence with our local R&D sponsor.

The chief investigator/principal investigator is responsible for the decision to amend the protocol if deemed necessary and to decide whether an amendment is substantial or non-substantial.

All amendment history will be tracked and retained.

8.3 Peer review

This study protocol has been reviewed by two independent individual experts in the field of clinical transplantation, adipose-derived regenerative cells and organ perfusion techniques.

8.4 Patient & Public Involvement

There has been no public involvement in the design or planned communication of final results.

8.5 Protocol compliance

All protocol deviations, non-compliances, or breaches from the approved protocol will be documented. The chief investigator (Robert Pearson), is the only member of the team sufficiently trained in the perfusion technique (external certification) and therefore adherence to the study protocol is enhanced by less intra-operator variability.

Significant and recurrent deviations from the protocol which are found to frequently recur are not acceptable, and will require immediate action. If considered a serious breach this may conclude this study.

8.6 Data protection and patient confidentiality

Regarding the Data Protection Act 1998, when the research kidney is offered to the chief investigator, patient identifiable data is provided and is transported with the organ. If accepted, upon arrival to the clinical unit all identifiable data will be destroyed using appropriate confidential waste disposal. The only information that will be stored with the research organ is the age of the graft, and not the date of birth.

Therefore, once the organ is used, and subsequent samples are taken for analysis they will be provided with a study number which has no relation to patient detail.

Further information:

- Personal information that is carried with the research organ will be destroyed once arrived at the clinical unit and all subsequent samples will be given a unique depersonalised study number
- All data will be stored using password protected folders and storage media.
- All data will be stored for 12 months following the conclusion of the study

- The chief investigator will be the only member of the team to accept the organ and be responsible for the disposal of patient identifiable information. From this point on, the graft is anonymised.

All investigators and contributors to this study must comply with the requirements of the Data Protection Act 1998 and will uphold the Act's core principles. The chief investigator is the data custodian.

8.7 Indemnity

All equipment used for this study is owned by either the Greater Glasgow and Clyde NHS Trust, Queen Elizabeth Hospital or the ICAMs laboratory facility, affiliated to Glasgow University. Damage to equipment therefore, will be covered at personal expense as part of the study budget.

8.8 Access to the final study dataset

The following members of the team will have access to the final dataset:

- Principal Investigator/Chief Investigator, Robert Pearson
- Sub-PI, Rashida Lathan
- Sub-PI, Rachanchai Chawangwongsanukun
- Sub-PI, Amir Fard

The chief investigator will be the data custodian.

9 DISSEMINIATION POLICY

9.1 Dissemination policy

The data that arises from this study will be owned by the ICAMS (Institute of Cardiovascular and Molecular Sciences), Glasgow University as part of my post-doctoral studies. The data will be analysed and tabulated and a final study report will be submitted. The investigators, Rashida Lathan, Amir Fard, and Rachanchai Chawangwongsanukun will be able to publish aspects of this data.

There are no plans to communicate results with families of those who donated, either by provision of the publication, or via a specifically designed newsletter, presentation etc.

The study protocol will be made available at request.

9.2 Authorship eligibility guidelines and any intended use of professional writers

Authorship of this data will be granted to significant contributors:

- Principal Investigator, Robert Pearson
- Sub-PI, Rashida Lathan
- Sub-PI, Amir Fard
- Sub-PI, Rachanchai Chawangwongsanukun
- Sub-PI, Mr John Asher
- PhD Supervisor, Mr Marc Clancy

- PhD Supervisor, Professor Patrick Mark
- Protocol contributor, John Stone
- Protocol contributor, James Fildes

10 REFERENCES

1. Statistics about organ donation - NHS Organ Donation Register | Organ Donation - English [Internet]. [cited 2019 Jan 23] Available from: https://www.organdonation.nhs.uk/supporting-my-decision/statistics-about-organ-donation/

2. Transplant activity report [Internet]. *NHS Organ Donation Register | Organ Donation - English* [cited 2019 Feb 5] Available from: /supporting-my-decision/statistics-about-organ-donation/transplant-activity-report/

3. Oniscu GC, Randle LV, Muiesan P et al. In situ normothermic regional perfusion for controlled donation after circulatory death–the United Kingdom experience. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2014; 14: 2846.

4. Peris A, Lazzeri C, Bonizzoli M et al. A metabolic approach during normothermic regional perfusion in uncontrolled donors after circulatory death-A pilot study. Clinical Transplantation 2018; 32.

5. Shapey IM, Muiesan P. Regional perfusion by extracorporeal membrane oxygenation of abdominal organs from donors after circulatory death: A systematic review. Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society 2013; 19: 1292.

6. Demiselle J, Augusto J-F, Videcoq M 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: Official Journal of the European Society for Organ Transplantation 2016; 29: 432.

7. Peters-Sengers H, Homan van der Heide JJ, Heemskerk MBA et al. Similar 5-Year Estimated Glomerular Filtration Rate Between Kidney Transplants From Uncontrolled and Controlled Donors After Circulatory Death-A Dutch Cohort Study. Transplantation 2017; 101: 1144.

8. Summers DM, Johnson RJ, Hudson A, Collett D, Watson CJ, Bradley JA. Effect of donor age and cold storage time on outcome in recipients of kidneys donated after circulatory death in the UK: A cohort study. Lancet (London, England) 2013; 381: 727.

9. Ardehali A, Esmailian F, Deng M et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): A prospective, open-label, multicentre, randomised non-inferiority trial. Lancet (London, England) 2015; 385: 2577.

10. Cypel M, Yeung JC, Machuca T et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. The Journal of Thoracic and Cardiovascular Surgery 2012; 144: 1200.

11. Nasralla D, Coussios CC, Mergental H et al. A randomized trial of normothermic preservation in liver transplantation. Nature 2018; 557: 50.

12. Hosgood SA, Nicholson ML. First in man renal transplantation after ex vivo normothermic perfusion. Transplantation. 2011 Oct 15;92(7):735-8.

13. Bilgin N, Karakayali H, Moray G et al. Outcome of renal transplantation from elderly donors. Transplantation Proceedings 1998; 30: 744.

14. Debout A, Foucher Y, Trébern-Launay K et al. Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation. Kidney International 2015; 87: 343.

15. 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. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2011; 11: 2647.

16. Ponticelli CE. The impact of cold ischemia time on renal transplant outcome. Kidney International 2015; 87: 272.

17. 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 International 2016; 89: 1331.

18. Wu WK, Famure O, Li Y, Kim SJ. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. Kidney International 2015; 88: 851.

19. Hosgood SA, Patel M, Nicholson ML. The conditioning effect of ex vivo normothermic perfusion in an experimental kidney model. The Journal of Surgical Research 2013; 182: 153.

20. Kaths JM, Echeverri J, Chun YM et al. Continuous Normothermic Ex Vivo Kidney Perfusion Improves Graft Function in Donation After Circulatory Death Pig Kidney Transplantation. Transplantation 2017; 101: 754.

21. 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. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2017; 17: 957.

22. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: The first clinical study. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2013; 13: 1246.

23. Hosgood SA, Saeb-Parsy K, Hamed MO, Nicholson ML. Successful Transplantation of Human Kidneys Deemed Untransplantable but Resuscitated by Ex Vivo Normothermic Machine Perfusion. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2016; 16: 3282.

24. 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.

25. Callaghan CJ, Harper SJF, Saeb-Parsy K et al. The discard of deceased donor kidneys in the UK. Clinical Transplantation 2014; 28: 345.

26. Hosgood SA, Barlow AD, Dormer J, Nicholson ML. The use of ex-vivo normothermic perfusion for the resuscitation and assessment of human kidneys discarded because of inadequate in situ perfusion. Journal of Translational Medicine 2015; 13: 329.

27. Kaths JM, Hamar M, Echeverri J et al. Normothermic ex vivo kidney perfusion for graft quality assessment prior to transplantation. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2018; 18: 580.

28. 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.

29. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. The British Journal of Surgery 2015; 102: 1433.

30. Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. Regenerative Medicine 2010; 5: 121.

31. Morigi M, Imberti B, Zoja C et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. Journal of the American Society of Nephrology: JASN 2004; 15: 1794.

32. Morigi M, Benigni A, Remuzzi G, Imberti B. The regenerative potential of stem cells in acute renal failure. Cell Transplantation 2006; 15 Suppl 1: S111.

33. Peired AJ, Sisti A, Romagnani P. Mesenchymal Stem Cell-Based Therapy for Kidney Disease: A Review of Clinical Evidence. Stem Cells International 2016; 2016: 4798639.

34. Eirin A, Zhu X-Y, Ebrahimi B et al. Intrarenal Delivery of Mesenchymal Stem Cells and Endothelial Progenitor Cells Attenuates Hypertensive Cardiomyopathy in Experimental Renovascular Hypertension. Cell Transplantation 2015; 24: 2041.

35. Nooshabadi VT, Mardpour S, Yousefi-Ahmadipour A et al. The extracellular vesicles-derived from mesenchymal stromal cells: A new therapeutic option in regenerative medicine. Journal of Cellular Biochemistry 2018; 119: 8048.

36. Baulier E, Favreau F, Le Corf A et al. Amniotic fluid-derived mesenchymal stem cells prevent fibrosis and preserve renal function in a preclinical porcine model of kidney transplantation. Stem Cells Translational Medicine 2014; 3: 809.

37. Horwitz EM, Gordon PL, Koo WKK et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proceedings of the National Academy of Sciences of the United States of America 2002; 99: 8932.

38. Mahmood A, Lu D, Lu M, Chopp M. Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. Neurosurgery 2003; 53: 697.

39. Tolar J, Le Blanc K, Keating A, Blazar BR. Concise review: Hitting the right spot with mesenchymal stromal cells. Stem Cells (Dayton, Ohio)2010; 28: 1446.

40. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. International Journal of Molecular Medicine 2004; 14: 1035.

41. Ittrich H, Lange C, Tögel F et al. In vivo magnetic resonance imaging of iron oxide-labeled, arterially-injected mesenchymal stem cells in kidneys of rats with acute ischemic kidney injury: Detection and monitoring at 3T. Journal of magnetic resonance imaging: JMRI 2007; 25: 1179.

42. Eggenhofer E, Luk F, Dahlke MH, Hoogduijn MJ. The life and fate of mesenchymal stem cells. Frontiers in Immunology 2014; 5: 148.

43. Fischer UM, Harting MT, Jimenez F et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. Stem Cells and Development 2009; 18: 683.

44. Sierra-Parraga JM, Eijken M, Hunter J et al. Mesenchymal Stromal Cells as Anti-Inflammatory and Regenerative Mediators for Donor Kidneys During Normothermic Machine Perfusion. Stem Cells and Development 2017; 26: 1162.

45. Pool M, Eertman T, Sierra Parraga J et al. Infusing Mesenchymal Stromal Cells into Porcine Kidneys during Normothermic Machine Perfusion: Intact MSCs Can Be Traced and Localised to Glomeruli. International Journal of Molecular Sciences *[*Internet*]* 2019; [cited 2019 Jul 30] 20: 3607.

11. APPENDICIES

11.1 Appendix 1- Required documentation

- CVs of all investigators

- Certification of sufficient training if performing perfusion technique without Chief Investigator present.

11.2 Appendix 3 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	0.1	July 2019	Rob Pearson	First draft
2	0.2	August 2019	J Stone / J Fildes	Alterations to study design
3	0.3	Sept 2019	Rob Pearson	Schedule of Events Added

APPENDIX D – Ethical Approval

London - Westminster Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

14 April 2020

Mr Robert Pearson
Clinical research fellow renal
transplant
Greater Glasgow and Clyde
1354
Govan
RoadG51
4TF

Dear Mr Pearson

Study title:	**Delivery of adipose-derived regenerative cell therapy during ex-vivo normothermic perfusion of kidneys.**
REC reference:	**20/LO/0256**
Protocol number:	**GN19RE465**
IRAS project ID:	**269613**

Thank you for your letter of 12th March 2020, responding to the Proportionate ReviewSub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved on behalf of the PRsub-committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentationas revised.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start ofthe study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisationmust confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission forresearch is available in the Integrated Research Application System.

For non-NHS sites, site management permission should be obtained in accordance with theprocedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from hostorganisations.

Registration of Clinical Trials

It is a condition of the REC favourable opinion that **all clinical trials are registered** on a publicly accessible database. For this purpose, 'clinical trials' are defined as the first four projectcategories in IRAS project filter question 2. Registration is a legal requirement for clinical trials of investigational medicinal products (CTIMPs), except for phase I trials in healthy volunteers (these must still register as a condition of the REC favourable opinion).

Registration should take place as early as possible and within six weeks of recruiting the first research participant at the latest. Failure to register is a breach of these approval conditions, unless a deferral has been agreed by or on behalf of the Research Ethics Committee (see herefor more information on requesting a deferral: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registratio n-research-project-identifiers/

As set out in the UK Policy Framework, research sponsors are responsible for making information about research publicly available before it starts e.g. by registering the research project on a publicly accessible register. Further guidance on registration is available at: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/transparency-respo nsibilities/

You should notify the REC of the registration details. We routinely audit applications forcompliance with these conditions.

Publication of Your Research Summary

We will publish your research summary for the above study on the research summaries sectionof our website, together with your contact details, no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, make a request to defer, or require further information, please visit:
https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

It is the responsibility of the sponsor to ensure that all the conditions are complied withbefore the start of the study or its initiation at a particular site (as applicable).

After ethical review: Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailedguidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators

- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study, including early termination of the study
- Final report

The latest guidance on these topics can be found at https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/.

Ethical review of research sites

The favourable opinion applies to all NHS/HSC sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of thestudy (see "Conditions of the favourable opinion" above).

Approved documents

The documents reviewed and approved by the Committee are:

Document	*Version*	*Date*
Confirmation of any other Regulatory Approvals (e.g. CAG) and all correspondence [NHS Biorepository Approval confirmation]	1.0	07 October 2019
Confirmation of any other Regulatory Approvals (e.g. CAG) and all correspondence [NHS Biorepository Application Document]	1.0	07 October 2019
Covering letter on headed paper [Covering Letter]	1	13 November 2019
IRAS Application Form [IRAS_Form_12032020]		12 March 2020
IRAS Application Form XML file [IRAS_Form_12032020]		12 March 2020
IRAS Checklist XML [Checklist_12032020]		12 March 2020
Other [Provisional Opinion Response]	1.0	12 March 2020
Research protocol or project proposal [Study Protocol]	1.0	12 March 2020
Summary CV for Chief Investigator (CI) [Research CV]	1	09 December 2018
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Study flow chart]	1	13 November 2019

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for ResearchEthics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received andthe application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events andonline learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

IRAS project ID: 269613	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this

Email: westminster.rec@hra.nhs.uk

Enclosures: "After ethical review – guidance for researchers" [SL-AR2]

APPENDIX E – Funding

R&D Finance Department
Ward 16, Dykebar Hospital
Grahamston Road
Paisley, PA2 7DE

Tel: 0141 314 0244
Fax: 0141 314 0276
tracie.coote@ggc.scot.nhs.uk

4 March 2020

Dear Mr Pearson

NHSGGC Endowment Fellowship Funding Award

Project ref: **GN19RE694**
Award value: **£14,484**
Cost centre: **G47395**

Further to your award of NHSGGC Endowments Fellowship funding, a new cost centre – as noted above - has been set up to administer the award. Please ensure that this cost centre is quoted on any orders or invoices related to the award. Expenditure should only be charged for items requested in your application.

Please note that due to the nature of the funding these awards must be administered by NHSGGC. To assist with managing project finances a detailed statement of expenditure will be sent to you on a biannual basis.

In line with the conditions of funding, please advise Tracie Coote in R&D Finance of the start date of the project. The start date must occur within 6 months of the award date. If you have any queries, please contact Tracie by email: tracie.coote@ggc.scot.nhs.uk.

If you intend to purchase goods through the University of Glasgow, they will have to set up a budget centre for you. Please give them a copy of this letter as confirmation of funds awarded. They should invoice quarterly in arrears based on actual expenditure incurred quoting our cost centre and your surname. All invoices should be sent to the above address marked for the attention of R&D Finance.

Please do not pay for any goods or services yourself, as NHSGGC is unable to make any personal payments to reimburse you.

Best wishes for the success of your project.

Yours sincerely

R&D Finance

APPENDIX F – RINTAG APPROVAL

Tooting Blood Donor Centre
75 Cranmer Terrace
London
SW17 0RB

Tel: 0203 123 8582
clare.denison@nhsbt.nhs.uk
Clare Denison
Innovation & Research – Lead Specialist ODT

Mr Robert Pearson
Department of Renal
Transplantation Queen Elizabeth
University Hospital 1354 Govan
Road
Glasgow G51 4TF

Sent via e-mail to:
Robert.pearson5@nhs.net

20 November 2020
Dear Mr Pearson,

RE: Delivery Of Adipose-Derived Regenerative Cell Therapy During Ex-Vivo Normothermic Perfusion Of Kidneys (ODT Study no. 107)

I am writing to thank you for contacting the Organ Donation and Transplantation Directorate of NHS Blood and Transplant regarding the above research proposal, wherein you request allocation of 12 untransplantable kidneys, until 1st May 2022.

The Research, Innovation and Novel Technologies Advisory Group (RINTAG) have thoroughly reviewed your proposal and I am pleased to confirm that your request has been approved.

Your study has gained an initial ranking of no. 2 in the research organ allocation scheme.

Thank you for sending us confirmation of your ethical approval (REC reference: 20/LO/0256). We will send you an agreement for you and your Sponsor to sign and return. Upon our receipt of the signed agreement and confirmation from your Sponsor that you are ready to begin, your study can commence.

Please note that your study should go live within 6 months of receiving this letter, or otherwise you will be asked to re-submit your application. If you have any queries then please feel free to contact me or the ODT Research team.

Yours sincerely,

Innovation & Research - Lead Specialist ODT

Copy to:

(ODT Research Project Manager)
(Specialist Nurse for Research)

APPENDIX G – EVNP Record Sheet

Ex-vivo Normothermic Perfusion Record Sheet

Date: _____ ID: _____

Weight (g) (Pre): _____
Weight (g) (Post): _____

Time (min)	Renal Blood Flow (ml)	Pump Speed (rpm)	Mean Arterial Pressure (mmHg)	Temp (□C)	pH on blood gas	Urine output
0						
5						
10						
15						
20						
25						
30						
35						
40						
45						
50						
55						
60						
Mean						

Record of Consumables

Consumable	Batch No.	Expiry Date	Quantity	Comments
Perfusion set				
Ringer's solution				
5% Glucose				
Synthamin 17				
Cernevit				
Insulin				
Sodium Bicarbonate 8.4%				
Mannitol 10%				
Dexamethasone 3.75mg				
Heparin 1000iu/ml				
Soltran				
Packed Red Cells				

Table 1 Ex-vivo normothermic perfusion (EVNP) assessment score

EVNP assessment	Point
Macroscopic assessment	
Grade I: Excellent perfusion (global pink appearance)	1
Grade II: Moderate perfusion (patchy appearance)	2
Grade III: Poor perfusion (global mottled and purple/black appearance)	3
Renal Blood flow	
Threshold ≥50 ml/min/100 g	0
Threshold <50 ml/min/100 g	1
Total urine output	
Threshold ≥43 ml	0
Threshold <43 ml	1

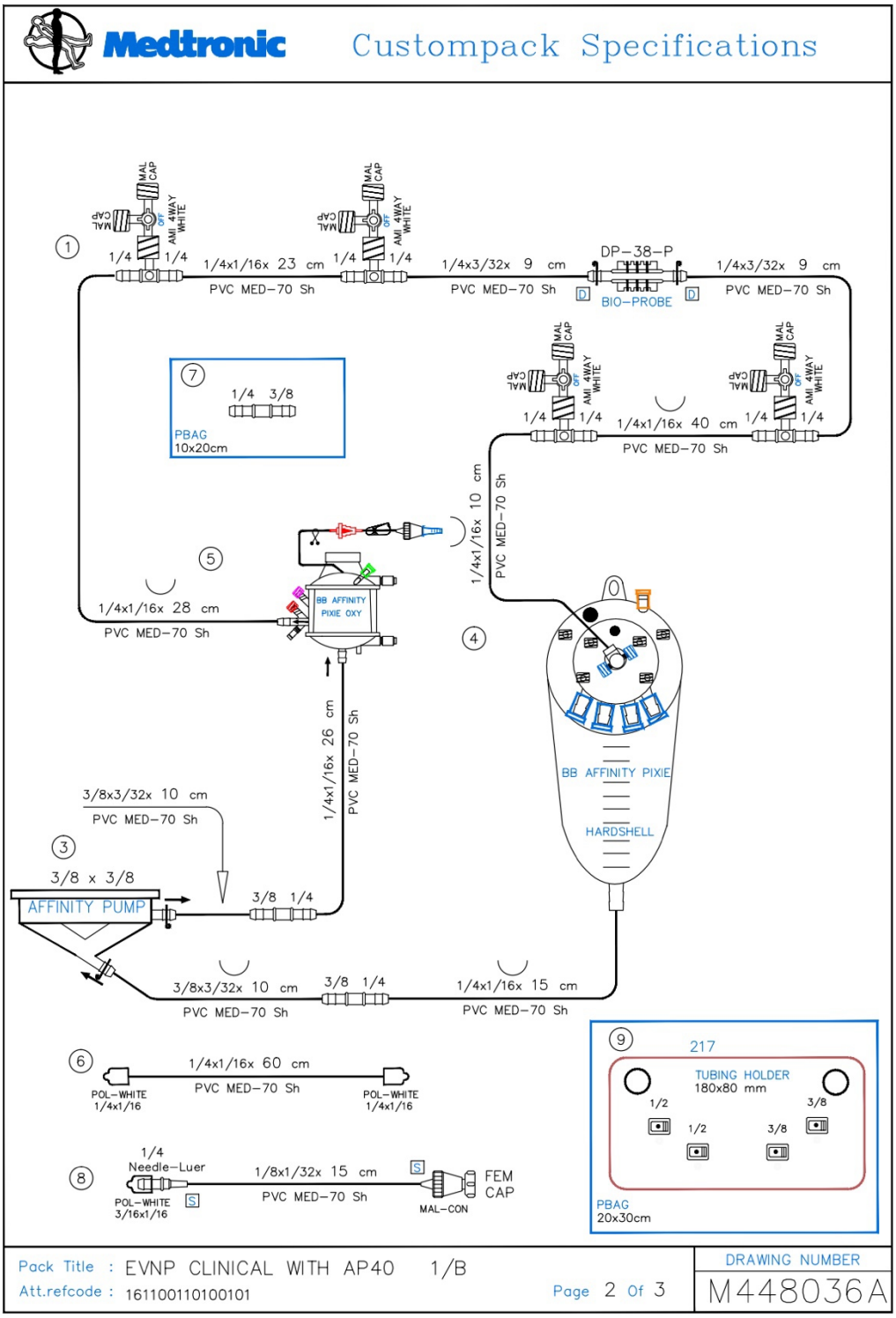
Macroscopic assessment, thresholds of renal blood flow and urine output. Scores ranges from 1 to 5, 1 indicating the least injury to 5 the most severe

APPENDIX H – Cost analysis for kidney transplantation

Costing as per The National Schedule of Reference Costs, 2016-17 - NHS trusts and NHS foundation trusts. (Archived Reference Costs | NHS Improvement, n.d.)

Healthcare Item	Unit Cost (£)	Currency / Service Code
Kidney Transplant, donor from brainstem death (DBD)	12888.13	LA02A
Kidney Transplant, donor from circulatory death (DCD)	12167.18	LA01A
Excess bed day beyond index admission, DBD	549	LA02A
Excess bed day beyond index admission, DCD	510	LA01A
Examination for Post-Transplantation of Kidney of Recipient (Inpatient)	239.19	LA13A
Examination for Post-Transplantation of Kidney of Recipient (Day case)	68	LA13A
Percutaneous Needle Biopsy of Lesion of Kidney, 19 years and over	934.93	YL02A
Transplant Failure and Rejection, without Interventions, with CC Score 0-1	1568.64	WH01D
Readmission with sepsis with no intervention (positive blood culture)	1429.47	WJ06J
Readmission with urinary tract infection	848.28	LA04M
Hospital Haemodialysis or Filtration, with Access via Haemodialysis Catheter	149.81	LD01A

APPENDIX I – Medtronic Custom Tubing Pack for use with Ex-Vivo Normothermic Perfusion



Medtronic Custom Tubing Pack specification. The single-use custom pack required for each clinical and research perfusion was uniquely designed for our unit.