Transplant International

ORIGINAL ARTICLE

Early experience with hypothermic machine perfusion of living donor kidneys – a retrospective study

Michael A. J. Moser[1,2], Nathan Ginther[1], Yigang Luo[1,2], Gavin Beck[1,2], Ronn Ginther[2], Marla Ewen[2], Rhianna Matsche-Neufeld[2], Ahmed Shoker[2,3] & Grzegorz Sawicki[4,5]

1 Department of Surgery, University of Saskatchewan, Saskatoon, SK, Canada
2 Saskatchewan Renal Transplant Program, Saskatoon, SK, Canada
3 Department of Medicine, University of Saskatchewan, Saskatoon, SK, Canada
4 Department of Pharmacology, University of Saskatchewan, Saskatoon, SK, Canada
5 Department of Clinical Chemistry, Medical University of Wroclaw, Wroclaw, Poland

Correspondence
Dr. Michael A. J. Moser, Department of Surgery, University of Saskatchewan, 103 Hospital Drive, Saskatoon, SK S7N 0W8, Canada.
Tel.: 306-655-5319;
fax: 306-844-1522;
e-mail: mike.moser@usask.ca

Summary

Although hypothermic machine perfusion (HMP) has been shown to be beneficial to deceased donor kidneys, the effect of HMP on living donor kidneys (LDK) is unknown. LDK are subjected to minutes of normothermic ischemia at the time of recovery. Comparison of 16 LDK preserved by HMP with 16 LDK preserved by static cold storage (SCS). Outcomes of interest are resistive indices (RI), both while on HMP and postoperatively, and creatinine clearance (CrCl). Injury markers NGAL and LDH were seen in the perfusate of LDK in amounts similar to what is found for donation after neurological determination of death kidneys. Compared to SCS kidneys, CrCl was significantly higher in the HMP group from days 2 through 7 post-transplant [ie: day 7 (78.8 ± 5.4 vs. 54.0 ± 4.6 ml/min, $P = 0.005$)]. CrCl at 1 year was higher in the HMP group (81.2 ± 5.8 vs. 70.0 ± 5.3 ml/min, $P = 0.03$). Early post-transplant RI was significantly lower in the HMP group (0.61 ± 0.02 vs. 0.71 ± 0.02, $P < 0.0001$). Our data support the assertion that injury does occur during LDK procurement and suggest that some of this injury may be reversed with HMP, resulting in more favorable early RI and graft function compared to SCS kidneys.

Transplant International 2017; 30: 706–712

Key words
ischemia reperfusion injury, kidney, living donor, organ preservation and procurement

Received: 8 November 2016; Revision requested: 31 December 2016; Accepted: 24 March 2017; Published online: 21 May 2017

Introduction

The living donor kidney (LDK) is considered to be the ideal kidney for transplant purposes in terms of organ function and immunologic benefit. Yet there is an inevitable period of a few minutes where the kidney is subjected to ischemia at 37 °C (normothermic ischemia) from the time the artery is stapled to the arrival of the kidney on the back table and the initiation of cold flush. In cases of donation after neurological determination of death (DNDD), the amount of time that the kidney is subjected to normothermic ischemia is minimal at the time of procurement. Yet it is the normothermic ischemia that occurs during donation after circulatory determination of death (DCDD) procurement that is thought to be responsible for the injury that occurs to DCDD kidneys.

Randomized controlled studies [1] and systematic reviews [2] have confirmed the benefit of hypothermic machine perfusion (HMP) in kidney transplantation. Initial studies had suggested that the benefits were greatest for expanded criteria donor kidneys [3], older

doi:10.1111/tri.12964

kidneys [4], and DCDD kidneys [5]. However, a more recent analysis confirms a benefit for all deceased donor transplant kidneys [6]. However, the use of HMP to improve the preservation of living donor kidneys has not yet been reported in the literature.

Hypothermic machine perfusion provides the opportunity to analyze the perfusion fluid for proteins and other molecules that are released during preservation. Work in our laboratory and other laboratories has identified several biomarkers that provide information about preservation injury [7–10]. LDH has in the past been the most studied marker of renal injury. More recently, NGAL (neutrophil gelatinase-associated lipocalin) has been shown to be a sensitive and specific marker of kidney injury in serum, urine, and perfusates [8,11].

Several potential mechanisms to explain the benefits of HMP have been proposed, from the reduction in tissue concentrations of free radicals [12] to its effect on vascular resistance [13]. Resistive indices can be measured while the kidney is on the HMP apparatus, and these have been shown to have prognostic significance regarding graft function once the kidney is implanted [14]. Resistive indices can also be measured indirectly via a Doppler ultrasound postoperatively. Resistive indices in the early postoperative period have been shown to reflect the status of the donor kidney itself [15,16], while in the longer term, resistive indices even in the implanted *donor* kidney have been shown to correlate with the status of the vessels in the *recipient* [17].

Our rationale for attempting HMP preservation of living donor kidneys was that the time from when the renal artery is stapled, until the vein can be stapled and divided, the kidney removed and brought to the back table to have the vascular staple lines cut off and flush initiated seems like a very long time to a transplant surgeon. Even worse is the fact that the temperature of the kidney during this ischemic period is 37 °C and from our experience, the time can vary from 2½ to 4 min of normothermic ischemia.

The purpose of our study is to document our initial experience with HMP of 16 living donor kidneys and to compare these to historical controls. The main outcomes of interest are early creatinine clearance, and then creatinine clearance at 6 months and 1 year, and secondary outcomes of interest are resistive indices on both HMP apparatus and in the postoperative Doppler ultrasounds.

Patients and methods

Our study is a retrospective review of 16 cases of living donor kidney transplants in which HMP was utilized (November 2012–October 2014). We estimated it would take a very large sample size to find a difference in creatinine clearance, graft survival, or delayed graft function, as in the work of Moers *et al.* [18]; therefore, sample size was calculated with vascular resistance in mind, based on the work by Dion *et al.* [14] comparing machine cold perfused kidneys to kidneys preserved using static cold storage. Our study was powered to detect a difference of 0.1 in Doppler vascular resistance with an SD of 0.105, an alpha of 0.05, and 1-beta of 0.8, and the sample size calculation yielded $n = 15$ per group. We requested support for 16 machine cold perfusion LKD kidneys for the purposes of this pilot study.

Recipient characteristics as well as daily serum creatinine and creatinine at 6 and 12 months were obtained from the medical records chart. All kidneys were retrieved laparoscopically with the use of a handport via a 6-cm midline incision in the epigastrium for the final steps in the dissection and ultimately for kidney extraction. Ultrasonic dissection rather than cautery was used, and vascular staplers were used to divide the renal artery and vein. There were no conversions to open procedure and no intraoperative complications nor need for blood transfusions in any of the donors. Kidneys were brought to the back table, then rapidly cooled, and flushed with 1 liter of Custodiol® (Essential Pharmaceuticals, Ewing, NJ, USA). Kidneys in the SCS group were stored in Custodiol, doubly wrapped, and then packed in ice in a cooler.

Kidneys in the HMP group were connected to the HMP apparatus using the atraumatic end adapter for Lifeport® Kidney Transporter (Organ Recovery Systems, Chicago, IL, USA) and then perfused at 2–4 °C using one liter of KPS-1 (Organ Recovery Systems). Cold ischemic time (CIT) was measured from the time the cold flush was initiated on the back table until the arterial clamp was removed, and the kidney was allowed to reperfuse at the time of implantation and included time on the HMP apparatus.

Once the kidney was removed from the apparatus and the solution was ready to be discarded, 80cc of perfusate was aspirated, centrifuged to remove the cells, and stored at −80 °C until the samples could be batch analyzed.

Neutrophil gelatinase-associated lipocalin level was measured using an ELISA kit (Abcam, Toronto, ON, Canada) and expressed as μg of protein per ml of perfusate. LDH activity was quantified using an LDH activity assay (Sigma-Aldrich, St. Louis, MO, USA). Enzyme activity is expressed as the amount of NADH generated from the conversion of lactate into pyruvate.

Ultrasound Doppler results on each patient were obtained from a digital radiology Picture Archiving and

Communication System (PACS); all segmental renal artery resistive indices recorded in the final report were averaged, as previously reported [14], to provide a value for use in the group comparisons. Reports of resistive indices from the Lifeport pump were downloaded directly from the Lifeport machines. Some of the resistive indices ($n = 4$ patients) were lost when, on one occasion, the apparatus was sent out for repairs.

Our standard immunosuppression consisting of tacrolimus/MMF/prednisone was initiated in all patients except for one patient in each group, who received cyclosporine A/MMF/prednisone. Creatinine clearance for postoperative days 1 through 7 was calculated using the Jeliffe equation for calculation of creatinine clearance for a creatinine that is not yet at steady state. The Cockcroft–Gault equation was used to calculate creatinine clearance in the donor preoperatively and in the recipient at 6 and 12 months. Our program does not do protocol biopsies. Instead, a biopsy is generally performed if rejection is suspected based on a rise in serum creatinine by more than 10%, and no other causes (dehydration, calcineurin inhibitor toxicity) are identified.

Continuous variables were expressed as mean ± standard deviation unless otherwise stated. Analyses were carried out using SPSS 23 (Armonk, NY, USA). Student's *t*-test and the chi-squared test were used as appropriate to compare groups.

Our study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio # 14-299). There is no conflict of interest to declare, and no support, financial or otherwise, was provided by Organ Recovery Systems.

Results

Analysis of perfusates for NGAL and LDH revealed significant release of these markers by LDK when compared to DNDD and DCDD kidneys, suggesting that significant injury occurs in the LDK during normothermic ischemia. The NGAL levels are similar for DNDD and LDK kidneys despite the shorter preservation time (and hence "accumulation time") for LDK (876 ± 375 min for DNDD vs. 213 ± 36 min for LDK, $P < 0.0001$, Figure S1). Obviously, because these levels are obtained from perfusates, there is no way to compare the levels for SCS kidneys.

The demographics of the 16 kidney transplants in each of the HMP and SCS groups are shown in Table 1. Living donors were similar in terms of age, gender, and calculated creatinine clearance. Cold ischemic time (CIT) and Warm Ischemic Time (WIT) were significantly longer in the HMP group compared to the SCS group. Delayed graft function, primary nonfunction, or acute rejection within 1 year of transplant did not occur in any of the 32 transplants.

Creatinine clearance was significantly higher in the HMP group starting as early as postoperative day 2 (47.3 ± 19.5 vs. 35.6 ± 13.2 ml/min, $P = 0.04$) and persisting at 1 year post-transplant (81.2 ± 23.2 vs. 70.0 ± 20.4 ml/min, $P = 0.04$, Fig. 1).

Resistance to flow during HMP decreased with time, particularly in the first hour of perfusion and was markedly reduced at the end of perfusion compared to the beginning of perfusion (0.22 ± 0.07 vs. 0.38 ± 0.14, $P < 0.001$). This reduction of perfusion resistance was similar to the typical pattern seen with perfusion of deceased donor kidneys (Figure S2). The improvements in vascular resistance persisted in the early postoperative period; the resistive indices determined by Doppler ultrasound were significantly less for the HMP group compared to the SCS group (0.61 ± 0.072 vs. 0.71 ± 0.064, $P = 0.0001$, Fig. 2).

Discussion

Significant levels of injury markers noted in the perfusate of the LDK in our study even after a short preservation time suggest that injury does occur to LDK, possibly due to the normothermic ischemia that is inevitable during procurement. As the use of HMP has been shown to be beneficial for all types of deceased donor kidneys retrieved for transplant [5], it seems reasonable that HMP could also protect the LDK from injury. Our pilot study suggests that early function and function at 6 months and 1 year may be improved with HMP compared to SCS. Within minutes after HMP is initiated, an improvement in resistance is noted, similar to the effect seen in the case of deceased donor kidneys, and this improvement in resistance appears to persist into the early postoperative period based on Doppler ultrasound.

The term "warm ischemic time" (WIT) is somewhat ill-defined and can denote both the time from asystole to cold flush in DCDD donors (we call this "procurement WIT") as well as the time during which the kidney gradually warms up during the time of vascular anastomoses ("implantation WIT"); yet in the latter the kidney probably does not get above 10 °C. Instead of "warm" ischemia, the ischemia that occurs during procurement of LDK and at the time of the 5 min no-touch time in DCDD kidney recovery represents "normothermic ischemia." DCDD recovery also subjects kidneys to up to 90 minutes of hypoperfusion during

Transplant International 2017; 30: 706–712

Table 1. Donor and recipient characteristics.

	Hypothermic machine perfusion (n = 16)	Static cold storage (n = 16)	P-value
Donor age (years)	44.3 ± 14.1	40.4 ± 12.4	0.42*
Donor gender (M:F)	7:9	7:9	–
Donor kidney side (L:R)	15:1	15:1	–
Donor baseline serum creatinine (μmol/l)	71.0 ± 10.9	74.6 ± 16.0	0.27*
Donor calculated creatinine clearance (ml/min)	115.6 ± 30.9	117.3 ± 30.4	0.87*
Cold ischemic time (min)	213 ± 36	159 ± 16	**<0.001***
Time on machine cold perfusion (min) (n = 12)	196 ± 16	N/A	
Warm ischemic time at the time of implantation (min)	46 ± 18	26 ± 5	**<0.01***
Recipient age (years)	45.3 ± 13.7	40.8 ± 12.4	0.48*
Recipient preoperative creatinine (μmol/l)	849 ± 477	639 ± 232	0.12*
Recipient with diabetes	3	2	0.63†
Recipient retransplant	3	2	0.63†
Immunosuppression (Tac/MMF/Pred): (CsA/MMF/Pred)	15:1	15:1	–
Acute rejection within 1 year of transplant	0	0	–

Numbers are mean ± standard deviation.

Tac/MMF/Pred = tacrolimus/mycophenolate mofetil/prednisone.

CsA/MMF/Pred = cyclosporine A/mycophenolate mofetil/prednisone.

*Calculated using two sided t-test.

†Calculated using chi-squared test.

Bold text indicates significant p-values ($p < 0.05$).

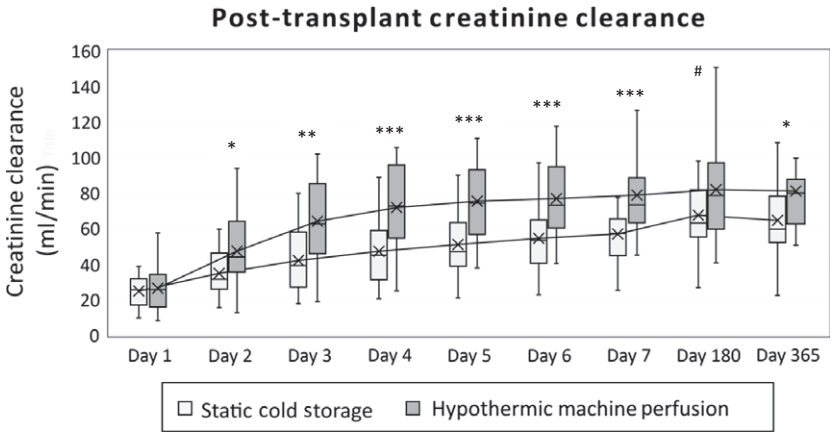

Figure 1 Post-transplant creatinine clearance (n = 16 per point). HMP, hypothermic machine perfusion; SCS, static cold storage. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, $^{\#}P = 0.07$. Error bars represent standard deviation.

withdrawal of life-supporting treatments, during which time there is relative normothermic ischemia. In the case of DNDD, high concentrations of inflammatory cytokines resulting from severe brain injury are thought to contribute to significant damage during preservation and then at the time of reperfusion [19,20], yet the normothermic ischemia time between cessation of perfusion and the initiation of cold flush is negligible in most cases. For LDK, there should not be any hypotension, nor should there be high levels of inflammatory cytokines, yet the period of a few minutes of normothermic ischemia may be responsible for significant injury and the presence of significant levels of markers of injury in the perfusion fluid.

The findings of improved early function and reduced resistive indices are similar to those shown in studies of expanded criteria and DCDD kidneys when HMP was compared to SCS. The average serum creatinine at 1 year in our SCS group (135 ± 48.7 μmol/l) and our HMP group (111 ± 29 μmol/l, $P = 0.03$ vs. SCS group)

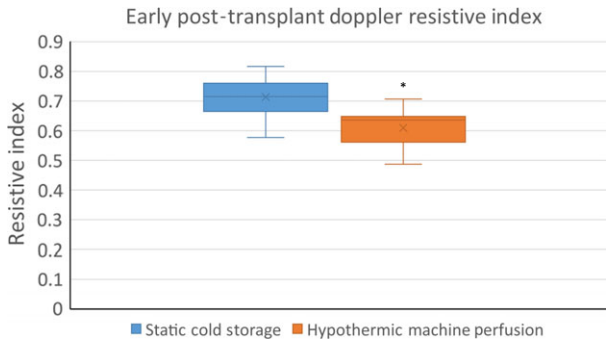

Figure 2 Early post-transplant Doppler resistive index. HMP, hypothermic machine perfusion; SCS, Static cold storage. *$P < 0.001$. Error bars represent standard deviation.

is in keeping with and seems slightly lower, respectively, compared to values reported in other studies of LDK [21–23]. In one study [21], comparing laparoscopic to open donor nephrectomy, the mean creatinine at 1 year in the laparoscopic group was 130 ± 46 μmol/l. In another study [22], comparing different approaches to living donor nephrectomy, the one-year creatinine was 132 ± 56 μmol/l.

This is the only report of the use of HMP for living donor kidneys that we are aware of. The sample size of most pilot studies is necessarily small, nonetheless, in this study, a statistically significant difference was shown for RI, upon which the sample size was calculated. A difference in CrCl was unexpected and will need to be confirmed with larger and/or randomized prospective studies. Our study is also limited by biases that are inherent in a retrospective historical design. However, the potential biases that are present in our study might tend to, instead, favor the SCS group, including significantly shorter cold ischemic time, shorter implantation WIT [24], a trend toward lower initial creatinine, and fewer second transplants and recipients with diabetes. The CIT was also, on average, 48 min longer for the patients in the HMP group. The differences in WIT at the time of implantation are due to the fact that one very experienced and very efficient senior surgeon did many of the SCS transplants. This translated to a slightly shorter CIT as well, due to the rapidity with which he could close the donor and make the incision, and skeletonize the vessels in the recipient. He had performed several hundred transplants over the previous 30 years and was very efficient with no wasted moves. After his retirement, none of the new surgeons on the team had his level of experience, and furthermore, residents were becoming involved in transplants, often participating in half of the venous anastomosis. Because minimal backtable work is needed on LDK, the time taken to put the kidney on HMP ranged from 15 to 20 min and did not add significantly to the CIT compared to the steps required to put a kidney into SCS. The use of an end-to-end cannula (Organ Recovery Systems) did not traumatize the vessels, nor did it result in a loss of length except in one case where 2 mm was trimmed back because of an irregular edge. In another single case, there were two arteries on a left kidney; in this case, both arteries were flushed on the back table, while only the dominant, larger artery was connected to the HMP cannula. A recent publication has suggested that retrograde venous perfusion is a safe option for patients with multiple vessels [25]. In the future, when we encounter living donor kidneys with multiple arteries, we may consider retrograde perfusion via the vein.

It has been suggested and often repeated that for there to be a benefit from HMP that cold perfusion needs to be used for over 4 h. Yet data from a porcine model that vascular benefits are seen after as little as 1 or 4 h of HMP [13]. The vascular benefits seem to be related to the increased release of nitric oxide and decreased release of endothelin-1 [26]. It has further been suggested that a two hour "preconditioning" period of HMP including oxygenation after static cold storage improves early renal function [27], and that this may be equivalent to HMP continuously during the whole preservation period [28]. These studies and our observations that resistive indices decrease over the first hour or less support that benefits occur well before the 4-h suggested minimum and that a short period of HMP can be helpful. In our study, the HMP was performed without oxygenation, but it would be interesting to do another pilot with an oxygenated HMP setup as this may provide further benefits. Some programs do LDK transplants in two adjacent operating rooms, such that CIT is reduced to minutes; in these cases, it would be impractical to use HMP. Nonetheless, it is possible that a few hours of HMP may have benefits that outweigh a very brief CIT. The costs of the consumables used in HMP are probably much less than the costs of running two operating rooms simultaneously.

The cost of perfusion fluid and consumables that are used each time HMP is performed has been estimated at $1480 per case at our institution. However, several cost analyses of HMP in deceased donor transplantation suggest that HMP is cost-effective even if there is, but, a modest increase in graft survival [29,30]. An immunologic benefit has also been suggested from HMP [31], possibly a byproduct of reduced injury to the kidney during preservation. This benefit becomes even more

important to consider in cases of LDK being transplanted into highly sensitized patients. Given that the rate of DGF is very low in living donor kidney transplants, estimated at 3.6% in a UNOS dataset of over 64 000 patients [32], it is unlikely that an improvement in the rate of DGF by HMP will be shown except in extremely large series. As living donor kidneys are increasingly shipped to the recipient center and CIT is increased, the rate of DGF could increase, and in this case, it may be possible to demonstrate an improvement in DGF. If graft survival was prolonged by even just a few weeks because a greater number of nephrons are preserved in going from donor to recipient, then the cost of the perfusion fluid and consumables is easily justified when one considers the costs of dialysis for those few weeks.

National kidney paired donation programs have led to an increase in the number of living donor kidney transplants by up to 20% per year and have also increased the chances that highly sensitized patients can receive a kidney transplant. In the current model, donors are required to travel to the transplant center where their matched recipient will receive his or her transplant, and this includes an added cost and inconvenience. The possibility of transporting living donor kidneys from one center to another is being explored to reduce the inconvenience to the donor [33]. This may help eliminate one of the perceived obstacles to being a donor in a paired donation program and perhaps even lead to an increase in donor numbers as a result. If the transportation of living donor kidneys occurs more frequently in the future, then HMP will become an important consideration, particularly, if transport needs to occur over long distances resulting in markedly increased CIT.

Our early experience suggests that significant levels of markers of injury are seen in LDK procurement, and that HMP may be beneficial to LDK in terms of early changes to vascular resistance and early function. A larger series or a randomized controlled trial should be pursued.

Authorship

MM, NG and GS: contributed to the study design, data acquisition, analysis, drafting of the manuscript and manuscript revisions. YL, GB, RG and ME: contributed to the study design, data acquisition and manuscript revisions. RMN: contributed to the data acquisition, analysis, drafting of the manuscript and manuscript revisions. AS: contributed to the study design, analysis, drafting of the manuscript and manuscript revisions.

Funding

University of Saskatchewan Department of Surgery Research Chair Funds. Saskatchewan Renal Transplant Program Research Startup Funds.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgements

The authors acknowledge the transplant teams from the Saskatchewan Transplant Program and the London Health Sciences Centre Transplant Program for their support and hard work.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. LDH and NGAL activity in perfusates of transplant kidneys from living donors (LDK), donors after neurological determination of death (DNDD), and donors after circulatory determination death (DCDD).

Figure S2. Typical change in resistance with time for living donor kidneys (LDK) and donation after neurological determination of death kidneys (DNDD).

REFERENCES

1. Moers C, Smits JM, Maathuis M-HJ, *et al.* Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**: 7.
2. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. *Br J Surg* 2013; **100**: 991.
3. Treckmann J, Moers C, Smits JM, *et al.* Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. *Transpl Int* 2011; **24**: 548.
4. Gallinat A, Moers C, Treckmann J, *et al.* Machine perfusion versus cold storage for the preservation of kidneys from donors ≥65 years allocated in the Eurotransplant senior Programme. *Nephrol Dial Transpl* 2012; **27**: 4458.
5. Jochmans I, Moers C, Smits JM, *et al.* Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death. *Ann Surg* 2010; **252**: 756.
6. Gill J, Dong J, Eng M, Landsberg D, Gill J. Pulsatile perfusion reduces the risk of delayed graft function in deceased donor kidney transplants, irrespective of donor type and cold ischemic time. *Transplantation* 2014; **97**: 668.

7. Moser MJ, Arcand S, Lin H-B, *et al.* Protection of the transplant kidney from preservation injury by inhibition of Matrix Metalloproteinases. *PLoS One* 2016; **11**: e0157508.
8. Bhangoo RS, Hall IE, Reese PP, Parikh CR. Deceased-donor kidney perfusate and urine biomarkers for kidney allograft outcomes: a systematic review. *Nephrol Dial Transpl* 2012; **27**: 3305.
9. Moers C, Varnav OC, van Heurn E, *et al.* The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. *Transplantation* 2010; **90**: 966.
10. Parikh CR, Hall IE, Bhangoo RS, *et al.* Associations of perfusate biomarkers and pump parameters with delayed graft function and deceased donor kidney allograft function. *Am J Transpl* 2016; **16**: 1526.
11. Mishra J, Ma Q, Kelly C, *et al.* Kidney NGAL is a novel early marker of acute injury following transplantation. *Pediatr Nephrol* 2006; **21**: 856.
12. Vaziri N, Thuillier R, Favreau FD, *et al.* Analysis of machine perfusion benefits in kidney grafts: a preclinical study. *J Transl Med* 2011; **9**: 15.
13. Gallinat A, Efferz P, Paul A, Minor T. One or 4 h of "in house" reconditioning by machine perfusion after cold storage improves reperfusion parameters in porcine kidneys. *Transpl Int* 2014; **27**: 1214.
14. Dion M, McGregor T, McAlister V, Luke P, Sener A. Hypothermic machine perfusion improves Doppler ultrasonography resistive indices and long-term allograft function after renal transplantation: a single-centre analysis. *BJU Int* 2014; **116**: 932.
15. Kolonko A, Chudek J, Zejda JE, Wiecek A. Impact of early kidney resistance index on kidney graft and patient survival during a 5-year follow-up. *Nephrol Dial Transpl* 2011; **27**: 1225.
16. Saracino A, Santarsia G, Latorraca A. Early assessment of renal resistance index after kidney transplant can help predict long-term renal function. *Nephrol Dial Transpl* 2006; **21**: 2916.
17. Rein P, Woss E, Lhotta K. Renal resistance index–think of more than just the kidney. *Clin Kidney J* 2010; **3**: 333.
18. Moers C, Pirenne J, Paul A, Ploeg RJ. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2012; **366**: 770.
19. Stroo I, Stokman G, Teske GJD, *et al.* Chemokine expression in renal ischemia/reperfusion injury is most profound during the reparative phase. *Int Immun* 2010; **22**: 433.
20. Cavaillé-Coll M, Bala S, Velidedeoglu E, *et al.* Summary of FDA workshop on Ischemia Reperfusion injury in kidney transplantation. *Am J Transpl* 2013; **13**: 1134.
21. Derweesh IH, Goldfarb DA, Abreu SC, *et al.* Laparoscopic live donor nephrectomy has equivalent early and late renal function outcomes compared with open donor nephrectomy. *Urology* 2005; **65**: 862.
22. Hoda MR, Hamza A, Greco F, Wagner S, Fischer K, Fornara P. Early and late graft function after laparoscopic hand-assisted donor nephrectomy for living kidney transplantation: comparison with open donor nephrectomy. *Urol Int* 2010; **84**: 61.
23. Serrano OK, Kirchner V, Bangdiwala A, *et al.* Evolution of living donor nephrectomy at a single center. *Transplantation* 2016; **100**: 1299.
24. Tennankore KK, Kim SJ, Alwayn IPJ, Kiberd BA. Prolonged warm ischemia time is associated with graft failure and mortality after kidney transplantation. *Kidney Int* 2016; **89**: 648.
25. Hobeika MJ, Dar WA, Hall DR, Bynon JS. Retrograde flushing of living donor renal allografts via the renal vein: a simple, effective technique. *Transplantation* forthcoming 2017. doi:10.1097/TP0000000000001525.
26. Gallinat A, Fox M, Luer B, Efferz P, Paul A, Minor T. Role of pulsatility in hypothermic reconditioning of porcine kidney grafts by machine perfusion after cold storage. *Transplantation* 2013; **96**: 538.
27. Koetting M, Frotscher C, Minor T. Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. *Transpl Int* 2010; **23**: 38.
28. Gallinat A, Paul A, Efferz P, *et al.* Hypothermic reconditioning of porcine kidney grafts by short-term preimplantation machine perfusion. *Transplantation* 2010; **93**: 787.
29. Wszola M. Long-term medical and economical benefit of machine perfusion kidney storage in comparison to cold storage. *Ann Transpl* 2009; **14**: 24.
30. Groen H, Moers C, Smits JM, *et al.* Cost-effectiveness of Hypothermic machine preservation versus static cold storage in renal transplantation. *Am J Transpl* 2012; **12**: 1824.
31. Ciancio G, Gaynor JJ, Sageshima J, *et al.* Favorable outcomes with machine perfusion and longer pump times in kidney transplantation: a single-center, observational study. *Transplantation* 2010; **90**: 882.
32. Redfield RR, Scalea JR, Zens TJ, *et al.* Predictors and outcomes of delayed graft function after living-donor kidney transplantation. *Transpl Int* 2016; **29**: 81.
33. Allen R, Pleass H, Clayton PA, Woodroff C, Ferrari P. Outcomes of kidney paired donation transplants in relation to shipping and cold ischemia time. *Transpl Int* 2016; **29**: 425.