Mannitol and renal graft injury in patients undergoing deceased donor renal transplantation – A randomized controlled clinical trial

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BMC Nephrology  □ BMC Series

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DOI: 10.21203/rs.2.24370/v2

SUBJECT AREAS
Urology & Nephrology

KEYWORDS
mannitol, renal transplantation, I/R injury, renoprotection, biology-derived biomarker
Abstract

Background

Ischaemia/reperfusion (I/R) injury is associated with renal tissue damage during deceased donor renal transplantation. Evidence is weak regarding the effect of mannitol attenuating I/R injury during graft reperfusion in renal transplant recipients. We tested the effect of mannitol to reduce renal graft injury represented by 16 serum biomarkers, which are involved in different relevant pathophysiological pathways. Our primary outcome were differences in biomarker concentrations between the mannitol and the placebo group 24 hours after graft reperfusion. Additionally, to account for biomarker concentrations before transplantation, a linear mixed model was applied.

Methods

34 patients undergoing deceased donor renal transplantation were randomly assigned at the Medical University of Vienna to receive either 20% mannitol or 0.9% NaCl placebo solution before and after graft reperfusion. 16 serum biomarkers (MMP1, CHI3L1, CCL2, MMP8, HGF, GH, FGF23, Tie2, VCAM1, TNFR1, IGFBP7, IL18, NGAL, Endostatin, CystC, KIM1) were measured preoperatively and 24 hours after graft reperfusion using Luminex assays and ELISA.

Results

16 patients in each group were analysed. Tie2 differed 24 hours after graft reperfusion between both groups (p = 0.011). Change of log2 transformed concentration levels over time differed significantly in four biomarkers (VCAM1,Endostatin, KIM1, GH; p=0.007; p = 0.013; p = 0.004; p = 0.033; respectively) out of 16 between both groups.

Conclusion

Our study showed insufficient effects of mannitol on I/R injury in patients undergoing deceased renal transplantation. Thus, we do not support the routinely use of mannitol to attenuate I/R injury.

Background

Renal transplantation is the treatment of choice for patients with end-stage-renal disease. Renal ischaemia and reperfusion (I/R) injury is one of the leading causes mediating acute kidney injury (AKI) in the native as well as in the transplanted kidney. Re-oxygenation after ischaemia results in tissue...
injury due to apoptosis and necrosis, and activation of inflammatory pathways triggering innate immune responses.\textsuperscript{4}

Mannitol, an osmotic diuretic, is routinely used to improve renal function after deceased donor renal transplantation.\textsuperscript{5} It increases renal blood flow, supposedly attenuates I/R injury during graft reperfusion and acts as a free radical scavenger.\textsuperscript{6} Additionally, it promotes the release of prostaglandins in the kidney leading to vasodilatation and consequently to an increased urine flow in the renal tubulars.\textsuperscript{7} However, these beneficial effects are only based on weak evidence and the renoprotective potency of mannitol during renal transplantation is still lacking.

Estimated glomerular filtration rate (eGFR) after transplantation based on serum creatinine (sCr) is mainly used for the clinical assessment of renal function. Even though, measurement of sCr is still the state of the art to estimate renal function, the sensitivity is low for detecting AKI of biopsy proven tubular injury.\textsuperscript{8,9} Therefore, in the last decade a major focus of research was to detect new molecular biomarkers (BM) involved in disease relevant pathophysiological pathways.

In the chronic disease, BM were identified following a systems biology approach, which is based on a silico-derived molecular model of diabetic kidney disease. A parsimonious set of BM covering multiple relevant pathways was validated in a large group of patients with type II diabetes and was able to improve the predictive power for eGFR loss on top of clinical covariates.\textsuperscript{10,11} Similarly, promising BM for AKI, delayed graft function and renal transplant outcome were identified.\textsuperscript{12–17} Consequently, we tested the intraoperative use of mannitol on a set of 16 BM representing different molecular processes involved in ischaemic kidney injury, inflammation and tubular damage.

Methods

This prospective double-blinded, randomized trial was performed at the Department of Anaesthesia, Intensive Care Medicine and Pain Medicine and the Department of Nephrology and Dialysis, Medical University of Vienna, Vienna, Austria. The trial was approved by the local ethics committee of the Medical University of Vienna in 2014 (Chairman: J. Zezula, MD) (EK 2021/2014) and was registered at
ClinicalTrials.gov (NCT02705573) and EudraCT (2014-005391-29) and conducted according to the Declaration of Helsinki and Good Clinical Practice.

Written informed consent was obtained from all patients. Patients with end-stage renal disease between 18-80 years of age undergoing deceased donor renal transplantation were included. Exclusion criterion was a known allergy to mannitol. All patients were hemodialysed shortly before renal transplantation.

Non-heart-beating donors were not included. Hypothermic machine perfusion of deceased donor kidneys was not performed.

Randomization

Patient allocated to the mannitol group received a 20% mannitol solution in a dose of 5 mL/kg bodyweight (BW) (Concentration: 5 mL = 1 gram) The placebo group received 0.9% NaCl solution in a dose of 5mL/kg BW. The maximum dose of the study medication was restricted to 500 mL. A bolus of 100 mL of the study solution was administered shortly before graft reperfusion. The remaining study solution was infused till the end of surgery. Randomization and blinding of the study drug were performed by the pharmacy. The patient, the attending anesthesiologist, and the research team were unaware of the group allocation.

Randomization sequence was created by an investigator with no clinical involvement in the trial using simple randomization procedures.

Protocol:

Anesthesia was induced with 2-3 μg kg⁻¹ BW fentanyl and 2-3 mg kg⁻¹ BW propofol. Muscle relaxation was performed at the discretion of the attending anesthesiologist. Narcotrend guided anesthesia was maintained with sevoflurane in 30% oxygen. Additional fentanyl was administered according to patient’s requirements. We kept end-tidal CO₂ at near 4.7 kPa. Non-invasive blood pressure was measured in 5-minute intervals. Normothermia was maintained with forced-air warming. According to clinical standards, all patients received a central venous line. Central venous blood gas samples were obtained hourly.
Fluid administration was esophageal Doppler guided (Cardio Q, Deltex Medical, Chichester, UK) according to a previous published algorithm.\textsuperscript{18} A balanced crystalloid solution (Elomel isoton; Fresenius Kabi, Austria) was used for intraoperative fluid replacement therapy. All patients received a baseline infusion rate of 2 mL kgBW\textsuperscript{-1} h\textsuperscript{-1}. We performed intraoperative goal-directed fluid management using esophageal Doppler monitoring (CardioQ; DeleteX Medical, Chichester, UK). Our fluid management was based on the algorithm published by the Anesthesia Working Group of the ‘Enhanced Recovery after Surgery (ERAS) Society\textsuperscript{18} and slightly modified.

We placed the esophageal Doppler probe after induction of anesthesia. After the characteristic Doppler signal was displayed, a fluid challenge of 250mL was administered to assess stroke volume (SV) response. If the SV increased > 10\% (i.e. fluid responder), a further fluid bolus was administered. This was repeated as often as no further increase of more than 10\% in SV was detected. In fluid non-responders we treated coexisting hypotensive episodes, which were defined by a mean arterial pressure (MAP) < 70 mmHg in normotensive and < 80 mmHg in hypertensive patients, with vasopressor titration at the discretion of the attending anesthesiologist.

Hemodynamic parameters were re-evaluated at least every 15 minutes (or more frequently in case of significant hemodynamic changes, e.g. blood loss). When SV dropped more than 10\%, we administered a further fluid bolus according to the above described algorithm.

Blood units were given as necessary. Transfusion trigger was a hemoglobin concentration of 7.0 mg dL\textsuperscript{-1}. However, if there was any clinical sign of organ hypoxemia (e.g. lactic acidosis) blood units were given earlier at the discretion of the attending anesthesiologist.

During the study period the use of diuretics was not allowed.

\textit{Blood Samples}

Blood samples for BM measurements were taken in Z Serum Separator Clot Activator vacutainers (Greiner Bio-One, Austria). Blood was drawn shortly before induction of anesthesia and 24 hours after graft reperfusion. Samples were allowed to clot for 30 min at room temperature before centrifuging at
1000g for 10 min at room temperature. Serum was removed and stored at -80°C until further processing.

**Measurements**

Demographic data, comorbidities, renal-replacement therapy, residual urinary output, long-term medication and preoperative laboratory values were obtained from patient’s medical records. Renal transplant specific data including arterial and venous vascular clamp times, intraoperative fluid requirements, blood loss, hemodynamic parameters and anesthesia specific management were recorded.

Donor and organ specific information including age, sex, laboratory values, diuresis, catecholamine support and cold and warm ischaemia time were provided by Eurotransplant.

Potassium, urinary output, creatinine, BUN and GFR were measured 24 hours after graft reperfusion.

**Luminex Serum Measurements**

Measurements of serum samples were carried out using three panels: Luminex 3-plex, Luminex 12-plex and ELISA. Quantikine ELISAs for human serum TIM-1/KIM-1/HAVCR (catalog no. DSKM100, R&D Systems, Minneapolis, MN) were used for measurement of KIM1 serum concentrations. Assays were processed according to the procedure guidelines provided. The optical density of each plate was measured within 30 min using a TriStar² LB 942 Modular Multimode Microplate Reader (Berthold Technologies, Bad Wildbad, Germany) set to 450 nm and wavelength correction set to 570 nm. The standard curve was generated using MikroWin2010 v5.21 software with a four-parameter logistic (4-PL) curve fit.

Three markers (Endostatin, CystC and NGAL) were measured using a Human Premixed Multi-Analyte Kit (catalog no.LXSAHM-03; R&D Systems). Samples were diluted 1:50 using Calibrator Diluent RD6-52 provided in the assay kit. The remaining twelve BM (MMP1, MMP8, HGF, GH, CHI3L1, TIE2, TNFR1, VCAM1, CCL2, FGF23, IL18 and IGFBP7) were measured using a Human Premixed Multi-Analyte Kit (catalog no.LXSAHM-12; R&D Systems) and a samples dilution of 1:2. Both assays were processed according to assay procedure provided by the manufacturer and measured on a Luminex 200
(Luminex Corporation, Austin, TX) with xPONENT software (version 3.1.971.0) set according to assay instructions.

Calibration and verification of the Luminex 200 was performed once a week using Luminex 200 Performance Verification Kit (catalog no. 40-276, Merck Millipore, Billerica, MA) and Calibration Kit (catalog no. 40-275, Merck Millipore).

As no commercial quality controls (QC) were available for multiplex assays, Pooled Normal Human Plasma K3 EDTA (catalog no. IPLA-N-100ml-K3 EDTA, Innovative Research, Novi, MI) was spiked with recombinant proteins (R&D Systems) and diluted to different concentrations to act as low, medium and high QC samples for the Luminex panel and the KIM1 ELISA. All samples were measured as two technical replicates. If replicate measurements display a coefficient of variation over 12%, the sample was remeasured. In addition, at least 10% of all samples were remeasured on a different plate to follow FDA and EMA guidelines which recommends incurred sample reanalysis with percentage difference between these samples below 20%. More than 66% of incurred sample reanalysis fulfilled this requirement and are therefore in concordance with FDA and EMA guidelines. Values out of quantifiable range were set to 0.5 and 1.5 times lower and upper quantification limits, respectively.

**Statistical Analysis**

Statistical analysis was performed using IBM SPSS Statistics (Version 25) and SAS 9.4 (Cary, NC). Normal distribution of the data was assessed with Kolmogorov-Smirnov test. Normally distributed data were presented as mean (standard deviation); not normally distributed data were given as median and percentile. Chi-square test was accomplished for comparing categorical variables.

Interval variables between groups prior to transplantation were compared using a Mann-Whitney-U-test or a Student’s t-test. Post transplantation a mixed linear model adjusted for donor kidney pairs was used to assess our primary outcome: differences in serum BM concentrations between both study groups 24 hours after graft reperfusion. Additionally, in order to take preoperative BM concentrations into account another mixed linear model for concentration changes over time for each of the sixteen biomarkers was performed. Concentrations were standardised for each group and biomarker (mean = 0, standard deviation = 1) to be able to compare the different models. In the
model the dependent variable was the standardised concentration. Independent variables were treatment group and time point of measurement and their interaction. Additionally, we used the intercept and slope as random effects. (online Supplement)

A p-value < 0.05 was considered as statistically significant.

Sample size consideration

Based on previous BM concentration measurements the standard deviation of the log2 transformed values in median (1\textsuperscript{st}, 3\textsuperscript{rd} quartile) is 0.86 (0.5 – 1.06). Therefore, in order to detect an effect size of 0.86 with a power of 80% using a two-sample t-test 17 patients in each study group were estimated to be sufficient to detect an significant difference in at least 50% of all BM.

Results

In total 34 patients with end-stage renal disease undergoing deceased donor renal transplantation were enrolled between January 2018 and July 2018. 17 patients received mannitol and 17 patients received placebo (Figure 1). One patient in the mannitol group deceased within 24 hours after transplantation; therefore, no follow-up measurement was available. Another patient from the placebo group was lost to follow up due to missing samples. Both patients were excluded from the analysis.

Demographic and morphometric data are summarised in table 1. Donor specific characteristics are available in the online supplement are shown in table 2.

Intraoperative characteristics including duration of anesthesia and surgery, fluid and hemodynamic data, anesthetic variables and central venous blood gas variables are shown in table 3.

Routine parameters measured 24 hours after graft reperfusion including potassium, urinary output, creatinine, BUN and GFR were compared between the two groups; none of those parameters displayed a significant difference (Table 4).

Concentration levels for all 16 BM before transplantation and 24 hours after graft reperfusion grouped by treatment are illustrated in Figure 2. Out of the 16 BM only Tie2 showed significant differences in concentration between the placebo group and the mannitol group 24 hours after graft reperfusion ($p = 0.011$) (Table 5)

In order to take BM concentrations before transplantation into account, linear mixed models were
applied for changes in BM concentrations pre transplantation and 24 hours after graft reperfusion.

Change of log2 transformed concentration levels over time differed significantly between the groups in four out of 16 biomarkers only (online Supplement, eAppendix 1, Table S1). In the mannitol group there was a higher increase of VCAM1 than the placebo group ($p = 0.007$). On the contrary, KIM1 increased significantly less in patients receiving mannitol as compared to those receiving placebo ($p = 0.004$). Endostatin and GH concentrations decreased significantly less in the mannitol group as compared to the placebo groups ($p = 0.013$ and $p =0.033$, respectively).

Discussion
This study tested the efficiency of mannitol to reduce I/R injury during kidney transplantations. eGFR is the standard clinical parameter to evaluate kidney function but lacks sensitivity for small changes in the filtration rate and is not able to reflect pathophysiological processes. Therefore, we used a set of 16 serum BM representing different pathways involved in ischaemic injury and inflammation in patients with end-stage renal disease undergoing deceased donor renal transplantation.

Although mannitol has been used for over 30 years in clinical practice, there is only weak evidence of its beneficial effects. Renoprotection e.g. superoxide radical scavenging, increased renal blood flow and/or less tubular necrosis were mostly found based on preclinical animal studies. Specifically, data for the renoprotective potency during renal transplantation are still lacking. Data from clinical studies, mostly performed in 1980’s revealed that mannitol reduced the incidence of acute tubular necrosis and decreased the number of dialysis required after transplantation. However no beneficial long-term effect could be observed in those studies.

A retrospective analysis based on an e-survey in 2011 showed that the use of diuretics during renal transplantation does not improve renal graft survival. Although, of the lack of of high quality studies mannitol is still routinely used in some high-volume transplantation centres throughout renal transplantation.

It seems unlikely that mannitol affects long-term graft function; we thus focused on the immediate postoperative period. eGFR is still considered to be the best clinical marker to predict renal graft
function. Direct measurements, however, are mostly infeasible; rather equitation based on demographic parameters, which’s accuracy to reflect real renal function in kidney transplant patients are limited\textsuperscript{26}, are used to estimate eGFR. Therefore, we measured a panel of promising BM to assess the renoprotective effect of mannitol.

We expected to see effects of mannitol reflected in the BM profile of the study patients. However, out of 16 BM only Tie2 was significantly different between the mannitol and the placebo group 24 hours after transplantation. If BM concentrations before transplantation are taken into account only four BM (KIM1, Endostatin, GH and VCAM1) showed significant different changes in concentration over time. The Angiopoetin(Ang)/Tie2 system is an important regulator for vessel stabilization and destabilization, and further to cope with injuries caused by ischemia and inflammation through binding of Ang1 to Tie2 as well as interactions of Ang2 as a context-dependent Tie2 antagonist. Shedding of Tie2 due to proteolytic cleavage occurs upon various stimuli such as vascular endothelial growth factor (VEGF) and result in blocking of downstream signaling as soluble Tie2 acts as a competitive ligand for Ang1 and Ang2.\textsuperscript{27} A higher sTie serum level has been seen in serveral clinical conditions such as inflammation and cardiovascular disease.\textsuperscript{28 29} Additionally, sTie to has been shown to be correlated to VEGF which is dramatically upregulated under hypoxic circumstances.\textsuperscript{30} Though, the exact mechanism of sTie2 in ischemia is not well understood it plays an important role in the regulation of angiogenesis and vascular inflammation. In our study Tie2 concentrations 24 hours after transplantation were significantly higher in the placebo group. But, after taking pre transplantation concentrations into account by comparing the changes of concentrationen over time Tie2 was no longer significantly different between study groups.

KIM1, NGAL and IGFBP7 are specific biomarkers to detect renal tubular injury.\textsuperscript{31 32} KIM1 increased significantly less in the mannitol group over 24 hours after graft reperfusion. However, there were no differences in NGAL and IGFBP7 in both groups over time. As concentrations of these three BM measured 24 hours after reperfusion did not differ significantly between both groups, a protective effect of mannitol on tubular damages remains still questionable.
I/R injury is associated with inflammation and plays a crucial role in tissue damage and immune cell recruitment. One important protein is VCAM1, which is triggered by cytokines leading to endothelial cell-leukocytes adhesion and trans-endothelial migration into inflamed tissue. In vitro studies confirmed VCAM1 as a major contributor for leukocyte recruitment and consequently tissue damage during I/R injury.\textsuperscript{33} Upregulation of VCAM1 correlates with structural tubular damage and fibrotic changes.\textsuperscript{34} In our study population VCAM1 significantly increased over time in the mannitol group but no difference could be detected in VCAM1 concentrations 24 hours after graft reperfusion. Endostatin is the c-terminal fragment of collagen XVII, which is expressed in the membranes of glomerular, tubular epithelium, and vascular endothelium cells.\textsuperscript{35, 36} Endostatin increases significantly in patients with AKI.\textsuperscript{37} In our study population there was a decrease of Endostatin 24 hours after transplantation, however there was no difference between both study groups. IL18 is a proinflammatory cytokine and is a mediator and biomarker of ischaemic tissue damage.\textsuperscript{38} Tubular epithelial cells express NGAL in response to tubulointerstitial cell injury which is mainly released by leucocytes, the loop of Henle and the collecting ducts.\textsuperscript{39} NGAL is significantly higher in patients with delayed graft dysfunction after transplantation. Cystatin C is a promising serum BM for all-cause AKI with a pooled sensitivity, specificity and AUROC of 0.82, 0.82 and 0.89, respectively.\textsuperscript{40} FGF23 is an early predictor and fast responding biomarker for AKI\textsuperscript{41} and is significantly higher in patients developing AKI after cardiac surgery and ICU patients.\textsuperscript{42} There are few BM which display statistically significant differences in concentration changes over time or in concentrations 24 hours post transplantation but the BM panel as a whole provides little evidence for differences in the study groups. Additionally, we measured routine clinical parameters such as creatinine, urinary output and potassium. There was no difference in those parameters, which further underlines the lack of efficacy of mannitol in patients undergoing deceased renal transplantation. Adequate intraoperative hydration during renal transplantation is associated with a lower incidence of
delayed graft function.\textsuperscript{43,44} Thus, the strength of our study is esophageal Doppler guided fluid management and stroke volume optimization in all of our patients. Therefore, we can exclude differences in fluid volume status as a potential confounder. Additionally, protein measurements were carried out strictly according to protocol – samples were stored at -80°C and never thawn prior to measurements. Rigorous QC control according to FDA and EMA guidelines ensure accurate and reproducible results.

Since the effect of mannitol begins within minutes after administration it seems unlikely that a single dose of mannitol might improve renal function in the long-term. Therefore, we focused on the effect within the 24 hours after graft reperfusion evaluated with our set of 16 BM. Because of our small sample size we were not able to draw any conclusions regarding postoperativ long-term renal graft function e.g. requirement of dialysis. Furthermore, we did not assess our biomarkers in healthy patients without any renal dysfunction which might have provided valuable addintional physiological renal background information.

Conclusion

Few of the measured BM showed statistically differences in concentrations 24 hours after transplantation between the mannitol and the placebo group as well as differences in concentration changes over time. Nevertheless the whole BM panel displayed limited evidence for the supposedly renoprotective effect of mannitol in patients exposed to reperfusion injury after deceased renal transplantation. We thus do not support the routine use of mannitol during graft reperfusion. This study awaits confirmation in an outcome trial.

List Of Abbreviations

| Abbreviation | Description                   |
|--------------|-------------------------------|
| CCL2         | Chemokine (C-C motif) ligand-2 |
| CHI3L1       | Chitinase-3-like protein-1    |
| CystC        | Cystatin C                    |
| Endostatin   | Endostatin                    |
| FGF23        | Fibroblast growth factor-23   |
| GH           | Growth hormone                |
Declarations

**Disclosure:** The authors declare no competing interests.

**Funding:** This work was supported by the Medical University of Vienna.

**Authors’ Contributions**

C.R.: data acquisition, writing and preparation of the manuscript

K.H.: proteomics measurements, contributed to data quality control and contributed to writing of the manuscript.

S.S.: study protocol, data acquisition

M.F.: data acquisition

E.F.: study protocol, writing and preparation of the manuscript

A.K.: data processing and analysis and contributed to writing of the manuscript.

B.K.: study protocol, writing and preparation of the manuscript

**References**

1. Kaballo MA, Canney M, O’Kelly P, Williams Y, O’Seaghdha CM, Conlon PJ. A comparative analysis of survival of patients on dialysis and after kidney transplantation. *Clin Kidney J* 2018; **11**: 389-93

2. Rabbat CG, Thorpe KE, Russell JD, Churchill DN. Comparison of mortality risk for
dialysis patients and cadaveric first renal transplant recipients in Ontario, Canada. *J Am Soc Nephrol* 2000; **11**: 917–22

3. Ponticelli C. Ischaemia-reperfusion injury: A major protagonist in kidney transplantation. *Nephrol Dial Transplant* 2014; **29**: 1134–40

4. Kezić A, Stajic N, Thaiss F. Innate Immune Response in Kidney Ischemia/Reperfusion Injury: Potential Target for Therapy. *J Immunol Res* 2017;

5. Cosentino M, Breda A, Sanguedolce F, et al. The use of mannitol in partial and live donor nephrectomy: An international survey. *World J Urol* 2013; **31**: 977–82

6. Lugo-Baruqui JA, Ayyathurai R, Sriram A, Pragatheeshwar KD. Use of Mannitol for Ischemia Reperfusion Injury in Kidney Transplant and Partial Nephrectomies—Review of Literature. *Curr Urol Rep* 2019; **20**

7. Bragadottir G, Redfors B, Ricksten SE. Mannitol increases renal blood flow and maintains filtration fraction and oxygenation in postoperative acute kidney injury: a prospective interventional study. *Crit Care* 2012;

8. Thomas ME, Blaine C, Dawnay A, et al. The definition of acute kidney injury and its use in practice. *Kidney Int* Elsevier Masson SAS; 2015; **87**: 62–73

9. Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *Am J Transplant* 2006; **6**: 1639–45

10. Mayer P, Mayer B, Mayer G. Systems biology building a useful model from multiple markers and profiles. *Nephrol Dial Transplant* 2012; **27**: 3995–4002

11. Mayer G, Heerspink HJL, Aschauer C, et al. Systems biology-derived biomarkers to predict progression of renal function decline in type 2 diabetes. *Diabetes Care* 2017; **40**: 391–7

12. Van Timmeren MM, Vaidya VS, Van Ree RM, et al. High urinary excretion of kidney
injury molecule-1 is an independent predictor of graft loss in renal transplant recipients. *Transplantation* 2007; **84**: 1625-30

13. Einecke G, Sis B, Reeve J, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520-31

14. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre J V. Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury. *Kidney Int* 2002; **62**: 237-44

15. Mishra J, Barasch J, Edelstein CL, et al. Urine NGAL and IL-18 are Predictive Biomarkers for Delayed Graft Function Following Kidney Transplantation. *Am J Transplant* 2006; **6**: 1639-45

16. Lohkamp LN, Öllinger R, Chatzigeorgiou A, Illigens BMW, Siepmann T. Intraoperative biomarkers in renal transplantation. Nephrology. 2016.

17. Malhotra R, Siew ED. Biomarkers for the early detection and prognosis of acute kidney injury. *Clin J Am Soc Nephrol* 2017; **149-173**

18. Feldheiser A, Conroy P, Bonomo T, Cox B, Ruiz Garces T, Spies C. Development and feasibility study of an algorithm for intraoperative goal-directed haemodynamic management in noncardiac surgery. *J Int Med Res* 2012; **40**: 1227-41

19. Moledina DG, Hall IE, Thiessen-Philbrook H, et al. Performance of Serum Creatinine and Kidney Injury Biomarkers for Diagnosing Histologic Acute Tubular Injury. *Am J Kidney Dis* 2017; **70**

20. Lugo-Baruqui JA, Ayyathurai R, Sriram A, Pragatheeshwar KD. Use of Mannitol for Ischemia Reperfusion Injury in Kidney Transplant and Partial Nephrectomies—Review of Literature. *Curr Urol Rep* Current Urology Reports; 2019; **26**

21. Green RD, Boyer DA, Halasz N, Collins GM. Pharmacological protection of rabbit kidneys from normothermic ischemia. *Transplantation* 1979; **28**: 131-134
22. Van Valenberg PLJ, Hoitsma AJ, Tiggeler RGWL, Berden JHM, Van Lier HJJ, Koene RAP. Mannitol as an indispensable constituent of an intraoperative hydration protocol for the prevention of acute renal failure after renal cadaveric transplantation. Transplantation. 1987. p. 784-8

23. Weimar W, Geerlings W, Bijnen A, et al. A controlled study on the effect of mannitol on immediate renal function after cadaver donor kidney transplantation. Transplantation 1983; 35: 99-101

24. Tiggeler RGWL, Berden JHM, Hoitsma AJ, Koene RAP. Prevention of acute tubular necrosis in cadaveric kidney transplantation by the combined use of mannitol and moderate hydration. Ann Surg 1985; 201: 246-51

25. Hanif F, Macrae AN, Littlejohn MG, Clancy MJ, Murio E. Outcome of renal transplantation with and without intra-operative diuretics. Int J Surg 2011;

26. Raju DL, Grover VK, Shoker A. Limitations of glomerular filtration rate equations in the renal transplant patient. Clin Transplant 2005; 19: 259-68

27. Singh H, Hansen TM, Patel N, Brindle NPJ. The molecular balance between receptor tyrosine kinases Tie1 and Tie2 is dynamically controlled by VEGF and TNFα and regulates angiopoietin signalling. PLoS One 2012; 7

28. Chung NAY, Makin AJ, Lip GYH. Measurement of the soluble angiopoietin receptor tie-2 in patients with coronary artery disease: Development and application of an immunoassay. Eur J Clin Invest 2003; 33; 529-535

29. Koutroubakis IE, Xidakis C, Karmiris K, Sfiridaki A, Kandidaki E, Kouroumalis EA. Potential role of soluble angiopoietin-2 and Tie-2 in patients with inflammatory bowel disease. Eur J Clin Invest 2006; 36: 127-132

30. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - In control of vascular function. Nat. Rev. Mol. Cell Biol. 2006; 7: 359-371d
31. Bolignano D, Donato V, Coppolino G, et al. Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a Marker of Kidney Damage. *Am J Kidney Dis* 2008; **52**: 595-605

32. Fan W, Ankawi G, Zhang J, et al. Current understanding and future directions in the application of TIMP-2 and IGFBP7 in AKI clinical practice. *Clin Chem Lab Med* 2018; **57**: 567-576

33. Cassie S, Masterson MF, Polukoshko A, Viskovic MM, Tibbles LA. Ischemia/reperfusion induces the recruitment of leukocytes from whole blood under flow conditions. *Free Radic Biol Med* 2004; **36**: 1102-1111

34. Mrowka C, Sieberth HG. Circulating adhesion molecules ICAM-1, VCAM-1 and E-selectin in systemic vasculitis: marked differences between Wegener’s granulomatosis and systemic lupus erythematosus. *Clin Investig* 1994; **72**: 762–768

35. Tomono Y, Naito I, Ando K, et al. Epitope-defined Monoclonal Antibodies against Multiplexin Collagens Demonstrate that Type XV and XVIII Collagens are Expressed in Specialized Basement Membranes. *Cell Struct Funct* 2002; **27**: 9–20

36. Marneros AG, Olsen BR. Physiological role of collagen XVIII and endostatin. *FASEB J* 2005;

37. Jia HM, Zheng Y, Huang LF, et al. Derivation and validation of plasma endostatin for predicting renal recovery from acute kidney injury: a prospective validation study. *Crit care* Critical Care; 2018; **22**: 305

38. Malyszko J, Lukaszyk E, Glowinska I, Durlik M. Biomarkers of delayed graft function as a form of acute kidney injury in kidney transplantation. *Sci Rep* 2015; **15**

39. Wasung ME, Chawla LS, Madero M. Biomarkers of renal function, which and when? *Clin Chim Acta* Elsevier B.V.; 2015; **438**: 350–357

40. Yong Z, Pei X, Zhu B, Yuan H, Zhao W. Predictive value of serum cystatin C for acute kidney injury in adults: A meta-analysis of prospective cohort trials. *Sci Rep* 2017; **7**: 
Table 1: Patient characteristics and preoperative data

|                  | Mannitol (n=16) | Placebo (n=16) | p-Value |
|------------------|-----------------|----------------|---------|
| **Age, yrs**     | 62 [57, 71]     | 53 [45, 68]    | 0.10    |
| **Height, cm**   | 167 ± 10        | 170 ± 15       | 0.58    |
| **Weight, kg**   | 74 ± 17         | 78 ± 17        | 0.67    |
| **Gender, no. (%)** |                 |                | 0.64    |
| Men              | 9 (56)          | 9 (56)         |         |
| Women            | 7 (44)          | 7 (44)         |         |
| **Comorbidities, no. (%)** |             |                |         |
| Hypertension     | 15 (94)         | 15 (94)        | 1.00    |
| Diabetes         | 5 (31)          | 1 (6)          | 0.13    |
| Insulin use      | 4 (25)          | 1 (6)          |         |
| Non-insulin use                      | 1 (6) | 0 (0) |
|-------------------------------------|-------|-------|
| Pulmonary                           | 2 (13)| 5 (31)| 0.39 |

- **Chronic Intermittent Dialysis, no. (%)**
  - 11 (69) | 13 (82) | 0.70 |
- **Peritoneal Dialysis, no. (%)**
  - 5 (31)  | 3 (19)  | 0.70 |
- **GFR, mL min\(^{-1}\).1.73m\(^{-2}\)**
  - 9.34 (2.7) | 10.4 (3.4) | 0.36 |
- **Residual Urine Output (mL 24hrs\(^{-1}\))**
  - 500 [0, 1000] | 250 [0, 750] | 0.54 |
- **Patients with > 500mL residual Urine volume, no. (%)**
  - 8 (50)  | 9 (56)  | 0.73 |

**Long-Term Medication, no. (%)**

- **Beta Blocker**
  - 13 (82) | 12 (75) | 0.67 |
- **ACE Inhibitors/AT1 Blocker**
  - 8 (50)  | 9 (56)  | 0.72 |
- **Diuretics**
  - 4 (25)  | 5 (31)  | 0.69 |

**Preoperative Laboratory**

- **Hemoglobin, g/dL**
  - 11.8 [10.7, 12.5] | 11.1 [10.4, 12.2] | 0.70 |
- **Haematocrit, %**
  - 35.7 [32.5, 38.3] | 33.2 [31.9, 37.4] | 0.67 |
- **Creatinine, mg/dL**
  - 7.5 [5.5, 8.7] | 8.3 [5.5, 9.8] | 0.47 |
- **CRP, mg/dL**
  - 0.2 [0.1, 0.5] | 0.2 [0.1, 0.7] | 0.64 |

Table 1: Summary statistics are presented as counts, percentages of patients, means ±SD, and median [25\(^{th}\) percentile, 75\(^{th}\) percentile]. All P-values are for unpaired Student’s t-tests, Mann-Whitney-U test or chi-square tests as appropriate. ASA, American Society of Anesthesiologists physical status; ACE, angiotensin converting enzyme; AT1, angiotensin; CRP, C-reactive protein
|                         | Mannitol (n=16) | Placebo (n=16) |
|-------------------------|----------------|----------------|
| **Age, yrs**            | 55 (range 40, 75) | 60 (range 41, 69) |
| **Sex**                 |                |                |
| Men, (%)                | 8 (50) | 11 (69) |
| Women, (%)              | 8 (50) | 5 (31) |
| **Laboratory**          |                |                |
| Creatinine mg/dL        | 0.7 [0.6, 1.0] | 0.9 [0.7, 1.1] |
| BUN, mg/dL              | 25 [14, 42]  | 19 [17, 46]   |
| **Diuresis, mL 24h⁻¹**  | 2730 [1860, 4560] | 2800 [2155, 4540] |
| **Noradrenaline, µg kg⁻¹min⁻¹** | 0.15 [0.06, 0.19] | 0.16 [0.04, 0.22] |
| **Duration of Ischaemia** |                |                |
| Cold, min               | 774 [480, 1071]   | 768 [543, 1150] |
| Warm, min               | 45 [35, 53]      | 44 [30, 58]   |
Table 2: Summary characteristics of donor specific data are presented as medians [25th percentile, 75th percentile]. All P-values are for unpaired Student’s t-tests or Mann-Whitney-U tests as appropriate. BUN, blood urea nitrogen

Table 3: Intraoperative parameters

|                      | Mannitol (n=16) | Placebo (n=16) | P       |
|----------------------|----------------|----------------|---------|
| **Duration**         |                |                |         |
| Anesthesia, hrs      | 3.6 [3.1, 4.1] | 3.8 [3.4, 4.3] | 0.35    |
| Surgery, hrs         | 2.6 [2.2, 3.2] | 2.9 [2.4, 3.5] | 0.55    |
| Arterial Clamp, min  | 16 [10, 24]    | 24 [12, 29]    | 0.25    |
| Venous Clamp, min    | 22 [16, 25]    | 20 [15, 25]    | 0.47    |
| **Fluid & Hemodynamics** |            |                |         |
| Total Fluid, mL      | 1.870 ±817     | 1.823 ±558     | 0.85    |
| Blood Loss, mL       | 200 [100, 588] | 175 [0, 375]   | 0.49    |
| MAP, mmHg            | 77 [74, 91]    | 79 [74, 82]    | 0.74    |
| SV, mL               | 51 [43, 82]    | 64 [52, 72]    | 0.74    |
| CO, L                | 3.1 [2.5, 5.0] | 4.3 [2.9, 4.7] | 0.97    |
| CVP, mmHg            | 13 ±4          | 12 ±4          | 0.47    |
### Anesthesia Variables

| Variable             | Lower Limit | Upper Limit | P Value |
|----------------------|-------------|-------------|---------|
| Propofol, mg         | 123,        | 200         | 0.92    |
| Fentanyl, µg         | 500,        | 750         | 0.92    |
| Sevoflurane TWA, %   | 1.5,        | 3.0         | 0.36    |
| SpO₂, %              | 98,         | 99          | 0.15    |
| Core T°C             | 36.3,       | 36.4        | 0.32    |
| Phenylephrine        |             |             |         |
| No. of Patients, (%) | 8, (50)     | 11, (69)    | 0.78    |
| Cumulative Dose, mg  | 0.02,       | 0.4         | 0.99    |
| Noradrenaline        |             |             |         |
| No. of Patients, (%) | 5, (32)     | 5, (32)     | 1.00    |

### Central Venous Blood Gas Analysis

| Parameter       | Lower Limit | Upper Limit | P Value |
|-----------------|-------------|-------------|---------|
| pH              | 7.36 ±0.1   | 7.37 ±0.1   | 0.40    |
| pCO₂, mmHg      | 44 ±7       | 46 ±7       | 0.34    |
| pO₂, mmHg       | 49;         | 52          | 0.38    |
| Hb, g/dL        | 9.7 ±1.4    | 9.6 ±1.3    | 0.62    |
Table 3: Summary characteristics of intraoperative measurements presented as means ± SD or medians [25th percentile, 75th percentile]. All P-values are for unpaired Student’s t-tests or Mann-Whitney-U tests as appropriate. MAP, mean arterial pressure; TWA, time weighted average; SV, stroke volume; FTc, corrected flow time; CO, cardiac output, CVP, central venous pressure, pCO₂, partial pressure of carbon dioxide; pO₂, partial pressure of oxygen; BE, base excess; Hb, hemoglobin

|                     | Mannitol (n=16) | Placebo (n=16) | P       |
|---------------------|----------------|----------------|---------|
| Na⁺ \( \text{mmol/L} \) | 136 ± 3        | 138 ± 2        | 0.04    |
| K⁺ \( \text{mmol/L} \) | 4.7 ± 0.7      | 4.7 ± 0.4      | 0.93    |
| Lactate \( \text{mmol/L} \) | 0.8 ± 0.2      | 0.8 ± 0.3      | 0.49    |

Table 4: Postoperative parameters within 24 hours

Table 4: Summary characteristics of postoperative measurements 24 hours after graft reperfusion.

Data are presented as means ± SD or medians [25th percentile, 75th percentile]. All P-values are for a
mixed linear model adjusted for the correlated nature of paired donor kidneys. BUN, blood urea nitrogen; GFR, glomerular filtration rate

Table 5: Biology-derived Biomarkers
|          | Mannitol                | Placebo                | p-value |
|----------|-------------------------|------------------------|---------|
| MMP1     | 3492 [3040, 4910]       | 3828 [3299, 6556]      | 0.360   |
| CHI3L1   | 277789 ±119127          | 290668 ±43544          | 0.882   |
| CCL2     | 244 [208, 314]          | 240 [196, 303]         | 0.711   |
| MMP8     | 17414 ±11898            | 13001 ±7340            | 0.126   |
| HGF      | 1488 ±847               | 1736 ±1124             | 0.800   |
| GH       | 286 [189, 598]          | 511 [292, 981]         | 0.387   |
| FGF23    | 170 [67, 1673]          | 215 [116, 722]         | 0.183   |
| Tie2     | 12563 [9594, 18103]     | 16357 [14596, 20411]   | 0.011   |
| VCAM1    | 2057315 [1483384, 3106136] | 1741057 [1422526, 2331264] | 0.354   |
| TNFR1    | 36465 ±14344            | 32838 ±10274           | 0.272   |
| IGFBP7   | 19960 ±4097             | 19385 ±2886            | 0.556   |
| IL18     | 158 [130, 208]          | 221 [171, 304]         | 0.202   |
| NGAL     | 252684 [202155, 340047] | 206354 [182217, 273962] | 0.091   |
| Endostatin | 314261 ±123046      | 307918 ±74018          | 0.981   |
| CystC    | 2405546 [1716719, 2976273] | 2023359 [1794789, 2449441] | 0.994   |
| KIM1     | 249 ±197                | 227 ±108               | 0.624   |

Table 5: Testing for differences in BM concentrations 24 hours after transplantation. Data are presented as means ±SD or medians [25th percentile, 75th percentile]. All concentrations are given in pg/mL A mixed linear model adjusted for paired donor kidneys was applied.
Figures
Figure 1: Patient flow chart

- Assessed for eligibility (n=42)
  - Excluded (n=8)
    - Surgery postponed (n=4)
    - Not consented (n=4)
  - Randomized (n=34)
    - Allocation
      - Allocated to Mannitol (n=17)
        - Received Intervention (n=17)
      - Allocated to Placebo (n=17)
        - Received Intervention (n=17)
    - Follow-Up
      - Lost to follow-up (n=1)
        - Deceased (n=1)
      - Lost to follow-up (n=1)
        - Measurement not done (n=1)
    - Analysis
      - Analysed (n=16)
      - Analysed (n=16)

Figure 1

CONSORT 2010 patients flow chart
Concentration levels (log2 transformed) for all 16 biomarkers before transplantation and 24 hours after graft reperfusion grouped by treatment. All concentrations are given in pg/mL.

The quantifiable range of assays is indicated by areas shaded in blue.

Supplementary Files
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