Early remote ischaemic preconditioning leads to sustained improvement in allograft function following live donor kidney transplantation; long term outcomes in the REPAIR randomised trial

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Note at the time of the original clinical trial Raymond MacAllister and Kristin Veighey were based at UCL, however this paper has been prepared since both have moved to the above institutions.

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ABSTRACT

Background: The REnal Protection Against Ischaemia Reperfusion in transplantation (REPAIR) study examined the utility of remote ischaemic preconditioning (RIPC) prior toliving donor kidney transplantation. The primary endpoint, GFR at 12 months, although statistically not significant, suggested the potential for RIPC to improve kidney function. A key secondary endpoint, CKD-EPI eGFR collected from site reported creatinine values, demonstrated significant potential to improve eGFR at 3 months and 1 year. Here we present eGFR collected yearly, up to 5 years following transplantation.

Methods: In this double blind randomised controlled trial we enrolled 406 adult live donor kidney transplant donor-recipient pairs at kidney transplant centres in the UK and Europe. Pairs were randomised using a factorial design to: sham RIPC, early RIPC only (immediately pre-surgery), late RIPC only (24 hours pre-surgery) or dual RIPC (early and late RIPC). RIPC consisted of 4x5-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg above systolic blood pressure separated by 5-minute periods of cuff deflation. For sham RIPC low pressure inflations of 40 mmHg were used. Importantly, the intervention was performed prior to anaesthetic induction.

Results: There was a sustained improvement in eGFR with early RIPC compared to control from 3 months to 5 years (adjusted mean difference 4.71 ml/min/1.73m2; 95% CI 1.54 to 7.89; p=0.004). Observed mortality or graft loss was lower with RIPC, confirming safety.

Conclusions: RIPC is safe (as demonstrated in this study), and when administered prior to anaesthetic induction improves long term kidney function following living donor transplantation.

Trial Registration: ISRCTN30083294.

Full protocol available at: www.lshtm.ac.uk/repair

Keywords: Ischaemia-reperfusion, kidney, transplant, preconditioning

INTRODUCTION

Kidney transplantation is the optimal treatment for suitable patients with end-stage renal disease (ESRD)[1]. However, a shortage of donors and eventual failure of the allograft means that many patients require dialysis for long-term therapy. During surgery, the kidney sustains ischaemic damage between interruption of its blood supply in the donor and reperfusion in the recipient. Reperfusion causes a second injury, and this composite ischaemia-reperfusion injury (IR injury) determines the function of the transplanted kidney in the immediate post-operative period and may increase the risk of acute and chronic rejection.[2] Reducing IR injury to the kidney should result in a healthier kidney at implantation, and ultimately one with a longer lifespan.

One method of rendering organs resistant to IR injury is ischaemic preconditioning (IPC), which utilises sub-lethal ischaemia (preconditioning stimulus) to induce a state of protection against subsequent prolonged ischaemia.[3] This protection is biphasic; an early phase occurs within minutes of the preconditioning stimulus (lasting for up to four hours) and a late phase occurs 24 hours after the preconditioning stimulus (lasting for up to 72 hours).[4,5,6] The difficulties of directly applying IPC stimuli to vital organs in humans has precluded its assessment in adequately powered clinical trials. However preconditioning has a systemic phenotype (remote ischaemic preconditioning; RIPC), and this facet of preconditioning could protect against IR injury to the kidney.[7] RIPC is activated by brief periods of ischaemia to a limb, and a number of small-scale clinical studies have demonstrated potentially protective effects in humans.[8].

The REnal Protection Against Ischaemia-Reperfusion in transplantation (REPAIR) trial suggested there is potential for early RIPC to improve kidney function one year after transplant.[9] REPAIR found little evidence of an effect of late RIPC but there was a suggestion that early RIPC improved iohexol glomerular filtration rate (GFR) (difference in mean GFR 3.08 ml/min/1.73m^2; 95% CI -0.89 to 7.04; p=0.13) and better evidence of a benefit on estimated (e)GFR using centrally measured creatinine at the time of the Iohexol test (difference in means 4.98 ml/min/1.73m^2; 95% CI 1.13 to 8.83; p=0.011). There were no major safety concerns around RIPC, although as expected RIPC caused transient pain/paraesthesia and minor petechiae related to cuff inflation. It is important to note that the mechanism of RIPC has not been fully elucidated, and anaesthetic agents have been implicated in both potentiating[10] and abrogating[11] RIPC. Therefore in this study, we elected to deliver RIPC prior to the induction of anaesthetic agents, hence allowing activation of this innate protective reflex prior to administration of potentially confounding agents.

In this paper, we report clinical outcomes up to 5 years of recipients enrolled in REPAIR – eGFR (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)[12]), graft loss and mortality.

METHODS

Study design and participants

REPAIR was a multi-centre factorial double-blind randomised controlled trial assessing the impact of RIPC on kidney function following live donor kidney transplantation. Full details of the study design have previously been reported.[9] In brief, 406 pairs of transplant recipients and donors were recruited from kidney transplant centres in the UK, the Netherlands, Belgium and France. Patients were excluded who were aged <18 years, on medicines that modulate preconditioning pathways (ATP-sensitive potassium channel opening or blocking drugs, or ciclosporin), had iodine sensitivity (contraindicating iohexol), or required antibody removal (ABO or HLA incompatible transplants).

Ethical approval for the study in the UK was given by the Joint University College London/University College London Hospitals Committees on the Ethics of Human Research (Reference number: 09/H0715/48) and by local Research Ethics Committees for sites outside the UK. REPAIR was registered with the International Standard Randomised Controlled Trial Register (ISRCTN; reference number 300832940).

Randomisation and blinding

Recipients and donors were randomised equally to control (sham RIPC), early RIPC alone (immediately pre-surgery), late RIPC alone (24 hours pre-surgery) and dual RIPC (RIPC 24 hours and immediately pre-surgery) groups. Donor and recipient were randomised to the same intervention group. Randomisation was by a web-based service with random permuted blocks stratified by centre. Unblinded research staff not involved in sample collection or data analysis performed enrolment and preconditioning procedures. All other research personnel at each centre, including those responsible for assessing outcomes, remained blinded to treatment allocation.

Procedures

The active RIPC procedure consisted of four, five-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg above systolic blood pressure, separated by five-minute periods of cuff deflation. The sham RIPC procedure consisted of four five-minute inflations on the upper arm to 40 mmHg separated by five-minute periods of cuff deflation. Active or sham RIPC sequences were completed before administration of anaesthetic agents. Donors were followed up to day three post-transplant and recipients up to 5 years post-transplant.

Outcomes

The primary outcome (iohexol GFR at 12 months) has already been reported.[9] In this paper we focus on the pre-specified secondary outcomes of eGFR at three months, 12 months, and then annually up to 5 years after transplantation, graft loss and mortality. eGFR data at all follow-up points were derived from

site reported serum creatinine measures using the CKD-EPI formula.[10] We also report graft and patient outcomes up to 5 years, another pre-specified secondary outcome in this study.

Statistical analysis

The effect of RIPC on eGFR over all follow-up visits (at three, 12 months, and 2-5 years after transplantation) was examined using a linear mixed effects model for repeated measures with indicator variables for early RIPC, late RIPC and time of visit. This analysis includes all four of the randomised groups, with those received dual RIPC included in both the early RIPC vs sham and the late RIPC vs sham comparisons. An unstructured residual variance–covariance matrix allowed for the anticipated correlation between repeated measures of eGFR on the same patient. A second mixed effects model evaluated the treatment effects at each follow-up time point by including an interaction between treatment and visit. This model was used to predict the average eGFR by treatment group at each visit.

A large interaction between early and late RIPC on mean GFR was not expected and the trial was under-powered to detect small interactions. However, this was formally tested by including an interaction term between early and late RIPC. Irrespective of the result of the interaction tests, a linear mixed effects model was used to compare eGFR between the combined RIPC arms (early, late and dual) and the arm receiving no RIPC. All analyses adjusted for the donor's baseline eGFR.

For patients with a missing eGFR due to death or graft loss, a value of 0 was imputed, and the impact of this was tested in a secondary sensitivity analysis. Data were included from all patients who had an eGFR measure at one or more of the visits (or had 0 imputed for death or graft loss), providing an unbiased estimate of the treatment effect under the assumption that data were missing at random given the pattern of eGFR at the other time points, treatment group and donor eGFR. The distribution of eGFR was examined to assess whether any transformations were necessary in order to adhere to the assumptions of the analysis models.

Graft survival and mortality up to 5 years post transplant were compared between treatment groups using Kaplan-Meier plots and a Cox proportional hazards regression model with indicator variables for early RIPC and late RIPC. In view of the small numbers of events observed, graft loss and mortality were combined into a single endpoint as an additional analysis to that stated in the Statistical Analysis Plan. All primary analyses were intention to treat, and a secondary per protocol analysis was also undertaken. Stata version 15.1 was used for all analyses.

RESULTS

Between January 2010 and April 2013, 406 donor recipient pairs were randomised: 99 to sham RIPC; 102 to early RIPC; 103 to late RIPC; and 102 to dual RIPC. The baseline characteristics were reasonably well-balanced across the four treatment arms at baseline (Table 1). By end of May 2018 all patients had reached 5 years of follow-up. At 1 year, information was available for 96% of patients. The corresponding percentages for 2, 3, 4 and 5 years were 95%, 91%, 88% and 93% respectively, with follow-up completion similar across the trial arms (eFigure 1). Data from 2-5 years follow-up are reported here for the first time.

Effect of RIPC on GFR

There was strong evidence that mean eGFR up to 5 years post-transplant was higher in the early RIPC group compared to control (adjusted difference in mean eGFR=4.71 ml/min/1.73m^2; 95% confidence interval (CI) 1.54 to 7.89; p=0.004) and little evidence of a benefit of late RIPC (adjusted difference in mean eGFR=1.35; 95% CI: -1.83 to 4.53; p=0.41) (Table 2). There was no evidence of an interaction between early and late RIPC (p=0.91). However, the results indicated a clinically important benefit of combined RIPC arms (early, late and dual) compared to no RIPC (eTable 1).

There was no evidence that the effect of early RIPC differed by visit (p=0.78 interaction test) or that the effect of late RIPC differed by visit (p=0.36). Further, when the treatment effect was estimated for each visit from 3 months up to five years after transplantation, eGFR was consistently higher in the early RIPC group than in the control group (Figure 1A, Table 2). There was a lag of at least 2 years for the mean eGFR in the early RIPC arm to reduce to that in the control arm. At each visit the mean eGFR was higher in the late RIPC group than in the control group although the evidence for a treatment effect was weak (Figure 1B, Table 2).

Results were similar from a sensitivity analysis without imputation of 0 eGFR for patients with graft loss or death (eTable 2). The adjusted mean difference between early RIPC and control was 4.50 (95% CI 1.57 to 7.43; p=0.003) and the adjusted mean difference between late RIPC and control was 1.49; (95% CI -1.44 to 4.42; p=0.32).

Results from the per-protocol analysis of 362 donor recipient pairs (89% of 406 randomised pairs), after excluding those pairs where the intervention was not undertaken or incomplete, were very similar (eTable 3). There was evidence for higher mean eGFR with early RIPC compared to control, which was sustained to 5 years post-transplant, and little evidence for an effect of late RIPC.

Effect of RIPC on graft loss and mortality

Up to 5 years, 23 patients had experienced graft loss and 21 patients had died, figures consistent with transplant registry data in the UK.[13] The proportion with these outcomes was numerically lower amongst those who had received early RIPC or late RIPC compared to the control group, but the study was underpowered to detect differences in these endpoints (Table 2; Figure 2; eTable 4; eFigure 2, eFigure 3). However, the data on mortality do not raise any concerns about the safety of RIPC and the only apparent adverse events were the previously reported transient pain/paraesthesia at the time of preconditioning, and minor petechiae related to cuff inflation (eTable 5).

DISCUSSION

REPAIR is the largest phase 3 clinical trial to estimate the effect of RIPC on clinically relevant end-points in kidney transplantation and has over 90% completeness for long term (5 year) follow-up. Although there was some evidence for an effect of RIPC on iohexol-measured GFR at 1 year (primary outcome of the trial),there was good evidence for an effect on eGFR at both 3 months and 1 year. This paper reports the sustained benefit over 5 years of follow-up. This benefit was observed in those recipients who had undergone preconditioning immediately pre-transplant i.e. 'early' RIPC, either alone or in combination with delayed ('late') preconditioning. There was no evidence of an effect of delayed ('late') preconditioning on kidney function, although results do not rule out a small benefit over the longer term. RIPC had minimal morbidity, no serious or sustained adverse effects, and was low cost and of little inconvenience to patients.

The discovery that limb ischaemia activates a whole-body systemic reflex that may limit tissue injury to vital organs in animals and humans[7] has stimulated a large number of clinical trials to detect clinically meaningful protective effects. There have been several previous studies in kidney transplantation, which have shown heterogeneity in the way in which ischaemic conditioning has been applied.[14] Most were small studies that were not powered to detect differences on clinical endpoints, which is likely to have contributed to the heterogeneity in their findings. The largest previous study to date, of 225 recipients of deceased donor kidney transplantation, did not find a benefit of preconditioning.[15] However, there were important differences in the preconditioning protocol; preconditioning was given to recipients only and performed after initiation of anaesthetic while we preconditioned both donor and recipient prior to initiation of anaesthetic. Additionally the heterogeneity in the clinical phenotypes of deceased donors would suggest that a much greater sample size would be necessary to infer any conclusions in the clinical trial setting, and that clinical trial outcomes of cadaveric and live donors cannot be directly compared.

It is well documented in the literature that the volatile anaesthetic agents can pharmacologically mimic the effects of RIPC, so-called 'anaesthetic preconditioning'.[9] It was with this in mind that we elected to perform RIPC in advance of any anaesthetic being administered. Patients who undergo kidney transplantation typically receive induction with propofol and maintenance with volatile anaesthetic agents, and a survey of a random subset of 4 centres in REPAIR confirmed this practice. Since the initial results of REPAIR were published, it is now understood that propofol can inhibit RIPC.[10] This has been postulated as the reason why large studies in cardiac surgery utilising propofol anaesthesia, most notably the ERICCA study[16] of over 1600 patients, demonstrated no effect of RIPC, whilst studies such as that by Zarbock et al.[17], who avoided propofol, demonstrated that RIPC was effective, both in reducing cardiac injury but also in reducing the incidence of acute kidney injury (AKI). It is important to note that RIPC can cause some discomfort, and therefore clinical studies investigating the effects of anaesthetic agents on preconditioning are urgently

needed, in order to determine if RIPC could be delivered during anaesthetic to minimise this discomfort. It should be noted that in the setting of kidney transplantation we chose not to extend the relatively short anaesthetic time by 30 minutes to facilitate RIPC; clearly in areas such as cardiac surgery it would be pertinent that this approach could be considered whilst the patient is being set up for surgery. Additionally, performing RIPC during anaesthetic would maintain true 'blindedness' in any clinical study.

In many ways, live donor kidney transplantation is an ideal model for investigating RIPC. Surgery is carefully scheduled, and this facilitates the application of a preconditioning stimulus before surgery and prior to the administration of a general anaesthetic. This enables the early and late effects of preconditioning to be tested in the presence of intact neuro-hormonal reflexes, conditions that are likely necessary to optimally activate protection.[18] Preconditioning both donors and recipients ensures that the donor kidney undergoes preconditioning in advance of its ischaemic insult, as well as potentially modulating the reperfusion injury in the recipient. In addition, live donor kidney ischaemia times are relatively consistent, which reduces the variability of kidney injury and kidney function after surgery and improves the ability to detect an effect of RIPC.

REPAIR used plasma clearance of iohexol (a direct measure of glomerular filtration) to measure GFR at 12 months, which constituted the primary endpoint of the trial. However, the observed variation of iohexol clearance was greater than expected, due to the greater complexity of the Iohexol test and the potential for human error in administering the full dose and in accurately documenting the timing of the subsequent blood samples. Variability in the Iohexol results may have been one of the reasons that we were unable to demonstrate evidence of an effect of early RIPC on GFR despite the consistency of the estimated impact shown by the different methods and over follow-up.

In light of the lack of a robust demonstration of the effects of early RIPC on GFR at 12 months, it is the data beyond 12 months that give confidence in the protective effect of early RIPC in renal transplantation. For pragmatic reasons, eGFR (CKD-EPI formula) was used as the measure of graft function during the 5 year follow up. This simpler measure, which is routinely performed at all clinic visits, could be collected easily and without expense, thus ensuring a more complete dataset – data were obtained from up to 95% of available patients at each time point. Between 12 months and five years, eGFR remained approximately 10% higher in the group randomised to early RIPC. The effect on eGFR when using site reported creatinine was similar to the effect we previously reported for centralised analyses of serum creatinine at 12 months,[9] which further supports the reliability of the long-term findings. In fact, when centrally measured creatinine was used at 12 months the treatment effect was larger and more precise (4.98; 1.13 to 8.83; p=0.011) which would suggest our long term results underestimate the clinical effect of the intervention.

There are around 28,000 patients on dialysis (hemo- or peritoneal dialysis) in the UK,[19] with a median waiting time of approximately 2.3 years for the deceased donor kidneys that become available for transplantation every year[11]. There is significant morbidity and annual mortality among prevalent haemodialysis patients[19]. Dialysis also imposes substantial and permanent restrictions on lifestyle and has substantial economic costs[11,19]. Therefore, approaches that maximise the lifespan of each transplanted kidney will benefit patients directly, contribute to a reduction in the transplant list and moderate the costs of renal replacement therapy. Given the current annual rate of decline of eGFR after kidney transplantation in the UK of 0.7 ml/min/1.73m^2,[19] a patient starting out after transplantation with a 5ml/min/1.73m^2 advantage might expect several years of extension to the lifespan of the transplant. The cost of this intervention amounts to no more than a 40-minute procedure that causes a transient unpleasant sensation. A single, phase 3 trial does not usually change medical practice, and therefore there is an urgent need for further well designed clinical studies in this area.

CONCLUSION

REPAIR demonstrates that RIPC causes a clinically meaningful enhancement of kidney allograft function following live donor transplantation over five years of follow-up, with the likelihood that RIPC extends the life of the transplanted kidney by several years.

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Trial Steering Committee

Tom Meade (Chair). Externals – Adam McLean, John Forsythe and Lisa Silas. Investigators – Raymond MacAllister, Mark Harber, Kristin Veighey and Chris Watson. Consumers – Neil Woodnick and Francesca Crozier. CTU - Rosemary Knight and Tim Clayton.

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Rajesh Kharbanda (Chair), Alan Jardine and Joan Morris. Cono Ariti provided the statistical support to the DMC in the early stages of the trial and drafted the initial statistical analysis plan.

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DECLARATION OF INTERESTS

KV, JN, TC, RK, SR, MH, RMcA, JWDeF – nothing to declare

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Raymond MacAllister (Clinical Pharmacologist and Consultant Physician) was the Chief Investigator on REPAIR and was involved in the design and conduct of the trial, was a member of the TSC and PMG and provided input into the manuscript.

Tim Clayton (Medical statistician, Clinical Trials Unit, LSHTM) was a co-applicant on REPAIR and involved in the design and conduct of the trial (including the statistical analysis plan), was a member of the TSC and PMG and provided input into the manuscript.

Rosemary Knight (Senior Trial Manager, Clinical Trials Unit, LSHTM) had overall responsibility for the REPAIR trial ensuring that all relevant approvals were in place, set up and training of sites, delivering the project on time and to budget.

Jennifer Nicholas (Medical Statistician, Clinical Trials Unit, LSHTM) was the unblinded statistician supporting the DMC, and provided support in preparation of the statistical analysis plan. She conducted the statistical analysis and provided input into the manuscript.

Steven Robertson (Senior Data Manager, Clinical Trials Unit, LSHTM) had overall responsibility for all aspects of data management including involvement in design and development of the CRF and eCRF. He was also responsible for data cleaning and preparation for final analyses and was a member of the PMG.

Kristin Veighey (Consultant Nephrologist, Wessex Kidney Centre, formally Research Fellow at UCL) was a member of the PMG and TSC. Kristin carried out site induction and training and co-ordinated sample

collection and transfer for storage and analysis at UCL. She also organised and performed analysis of samples collected during the trial.

Mark Harber (Consultant Nephrologist, Royal Free Hospital) was involved in the design and conduct of the trial, was a member of the TSC and provided input into the manuscript.

Chris Watson (Professor of Renal Transplantation at Cambridge University) was involved in the design and conduct of the trial, was a member of the TSC and provided input into the manuscript.

Johan W De Fijter (Professor of Nephrology at Leiden University) was involved in the design and conduct of the trial and provided input into the manuscript.

Stavros Loukogeorgakis (Paediatric Surgical Registrar) was involved in the design and conduct of the trial.

Neil Dalton performed the primary endpoint analysis, contributed to the design of the trial and inputted into this manuscript.

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Table 1: Baseline characteristics of recipients and donors, by treatment group

	Control (N=99)	Early RIPC (N=102)	Late RIPC (N=103)	Dual RIPC (N=102)
N with baseline data	95	98	99	99
Recipient male, N (%)	58 (61·1)	71 (72·4)	65 (65·7)	73 (73·7)
Donor male, N (%)	41 (43·2)	47 (48·0)	47 (47·5)	41 (41·4)
Recipient ethnicity, N (%)				
White	76 (80·0)	81 (82·7)	86 (86·9)	78 (78·8)
Asian	5 (5·3)	8 (8·2)	4 (4·0)	6 (6·1)
Black	5 (5·3)	5 (5·1)	5 (5·1)	11 (11·1)
Other	6 (6·3)	3 (3·1)	4 (4·0)	2 (2·0)
Not stated	3 (3·2)	1 (1·0)	0 (0)	2 (2·0)
Donor ethnicity, N (%)				
White	78 (82·1)	75 (76·5)	85 (85·9)	81 (81·8)
Asian	3 (3·2)	10 (10·2)	4 (4·0)	4 (4·0)
Black	5 (5·3)	5 (5·1)	4 (4·0)	10 (10·1)
Other	5 (5·3)	4 (4·1)	4 (4·0)	3 (3·0)
Not stated	4 (4·2)	4 (4·1)	2 (2·0)	1 (1·0)
Recipient age, mean (range), years	46·8 (18,74)	47·6 (18,77)	45·9 (18,76)	45·3 (19,72)
Donor age, mean (range), years	50·2 (20,77)	50·8 (22,77)	49·1 (22,77)	49·2 (22,75)
Recipient BMI, mean (SD), kg/m^2*	26·3 (4·9)	26·2 (4·7)	25·5 (4·2)	24·8 (4·4)
Donor BMI, mean (SD), kg/m^2*	25·9 (3·2)	26·8 (4·0)	25·2 (4·0)	26·5 (3·9)
Recipient systolic BP, mean (SD), mmHg*	132 (19·0)	138 (19·0)	133 (19·7)	133 (15·3)
Donor systolic BP, mean (SD), mmHg*	122 (13·3)	126 (15·6)	126 (15·5)	124 (15·3)
Recipient creatinine, mean (SD), µmol/L	635 (292·6)	607 (256·1)	622 (296·7)	643 (261·4)
Donor creatinine, mean (SD), µmol/L	71 (13·2)	73 (15·9)	73 (16·9)	75 (16·5)
Donor GFR (mL/min per 1·73 m^2)	96·3 (13·0)	95·1 (16·4)	95·5 (15·5)	93·3 (16·8)
Donor medical history, N (%)				
Previous kidney transplant**	8 (8·1)	5 (4·9)	6 (5·8)	10 (9·8)
Dialysis prior to transplant	51 (53·7)	51 (52·0)	46 (46·5)	57 (57·6)
Glomerulonephritis	19 (20·0)	18 (18·4)	13 (13·1)	17 (17·2)
-biopsy proven	13 (13·7)	13 (13·3)	10 (10·1)	16 (16·2)
Pyelonephritis	3 (3·2)	2 (2·0)	4 (4·0)	2 (2·0)
Diabetes	11 (11·6)	13 (13·3)	6 (6·1)	7 (7·1)
Polycystic Kidney	13 (13·7)	12 (12·2)	18 (18·2)	13 (13·1)
Hypertension	31 (32·6)	41 (41·8)	38 (38·4)	48 (48·5)
Renal vascular disease	1 (1·1)	2 (2·0)	4 (4·0)	2 (2·0)
Aetiology uncertain	7 (7·4)	6 (6·1)	7 (7·1)	7 (7·1)
Other diagnoses	47 (49·5)	49 (50·0)	54 (54·5)	53 (53·5)

Abbreviations: N, number; SD, standard deviation; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate.

*These characteristics were recorded for fewer than the 391 participants who had baseline data: recipient BMI n=389, donor BMI n=356, recipient systolic BP n=389, donor systolic BP n=388.

**This characteristic was recorded for all participants (N=406)

Table 2: Effect of RIPC on eGFR (ml/min/1.73m^2), graft loss and death up to 5 years post-transplant, intention to treat analysis

Outcome	Control (N=202)		Early RIPC (N=204)		Difference in means	P-value
	N	Mean (SD)	N	Mean (SD)	(95% CI)*	
eGFR						
All visits					4.71 (1.54 to 7.89)	0.004
3 months	191	53.7 (17.7)	193	57.7 (18.3)	5.03 (1.67 to 8.39)	0.003
12 months	191	56.6 (19.6)	197	59.7 (20.4)	3.76 (0.02 to 7.51)	0.049
24 months	188	53.7 (20.8)	194	57.4 (21.0)	4.27 (0.35 to 8.19)	0.033
36 months	185	52.0 (21.8)	185	57.1 (22.4)	5.34 (1.17 to 9.51)	0.012
48 months	179	50.2 (24.2)	177	55.3 (26.0)	5.38 (0.58 to 10.18)	0.028
60 months	185	48.2 (25.0)	192	52.3 (25.5)	4.93 (0.06 to 9.80)	0.047
		N (%) with outcome		**N (%) with outcome**	**Hazard ratio (95% CI)****	
Graft loss	202	13 (6.4)	204	10 (4.9)	0.73 (0.32 to 1.67)	0.462
Death	202	12 (5.9)	204	9 (4.4)	0.72 (0.30 to 1.70)	0.450
Graft loss or death	202	25 (12.4)	204	19 (9.3)	0.72 (0.40 to 1.31)	0.278
	Control (N=201)		**Late RIPC (N=205)**		**Difference in means**	**P-value**
	N	**Mean (SD)**	**N**	**Mean (SD)**	**(95% CI)***	
eGFR						
All visits					1.35 (-1.83 to 4.53)	0.405
3 months	189	55.3 (17.8)	195	56.2 (18.4)	1.36 (-1.99 to 4.72)	0.426
12 months	193	58.0 (20.7)	195	58.3 (19.5)	1.02 (-2.73 to 4.77)	0.594
24 months	189	55.0 (20.7)	193	56.2 (21.2)	1.74 (-2.18 to 5.65)	0.385
36 months	184	54.1 (21.5)	186	55.0 (23.1)	2.13 (-2.04 to 6.29)	0.317
48 months	176	51.4 (26.1)	180	54.1 (24.3)	4.65 (-0.15 to 9.45)	0.058
60 months	185	49.1 (26.4)	192	51.4 (24.3)	2.97 (-1.90 to 7.84)	0.232
		N (%) with outcome		**N (%) with outcome**	**Hazard ratio (95% CI)****	
Graft loss	201	11 (5.5)	205	12 (5.9)	1.04 (0.46 to 2.36)	0.923
Death	201	13 (6.5)	205	8 (3.9)	0.58 (0.24 to 1.39)	0.219
Graft loss or death	201	24 (11.9)	205	20 (9.8)	0.79 (0.43 to 1.42)	0.428

* Early RIPC vs. Control / late RIPC vs control adjusted for baseline donor eGFR by CKD-EPI and factorial design

** Early RIPC vs. Control / late RIPC vs control adjusted for factorial design

Abbreviations: N, number of observations; SD, standard deviation; CI, confidence interval

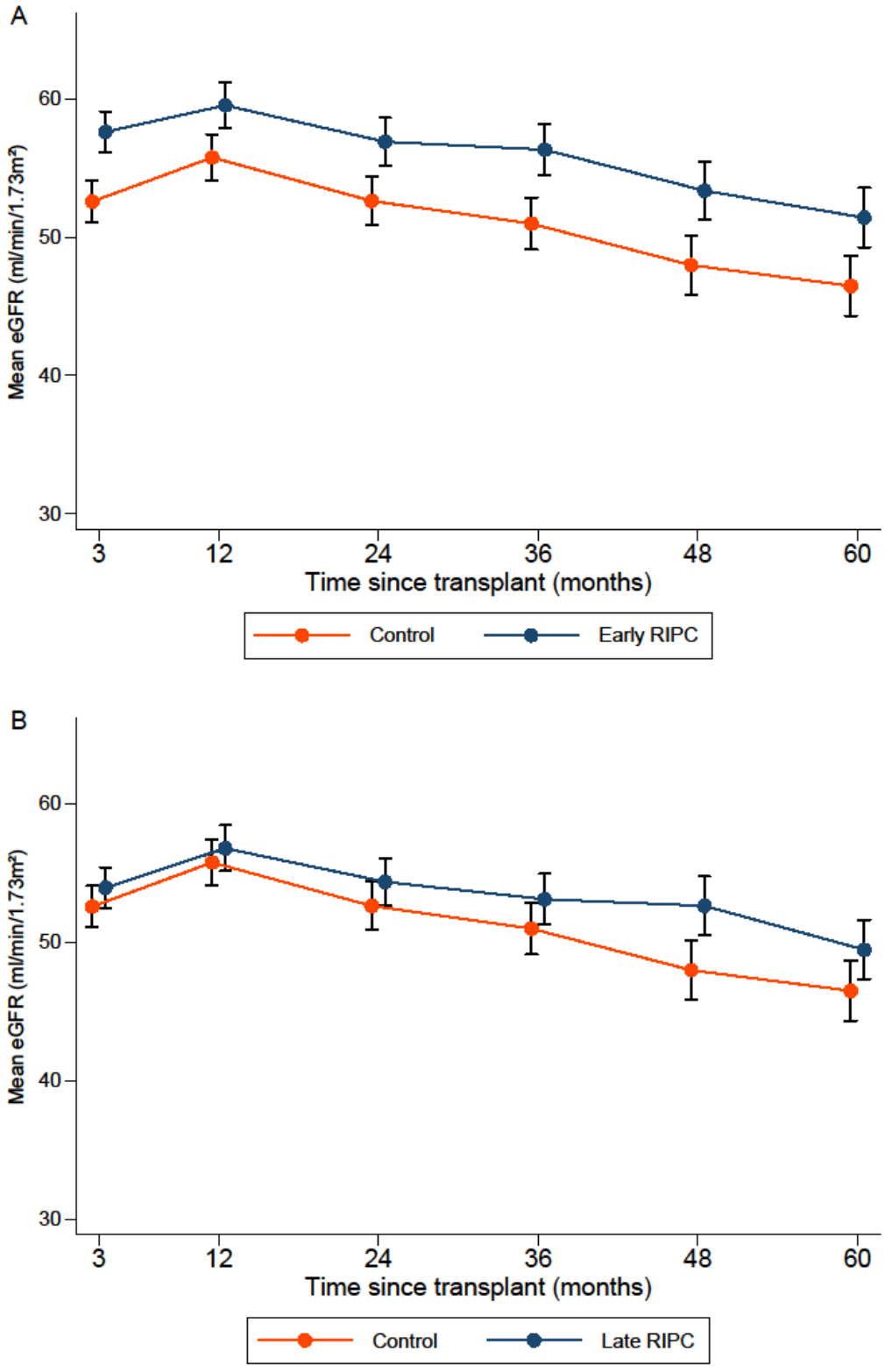

Figure 1: Effect of A. Early RIPC and B. Late RIPC on eGFR over 60 months follow-up, intention to treat analysis

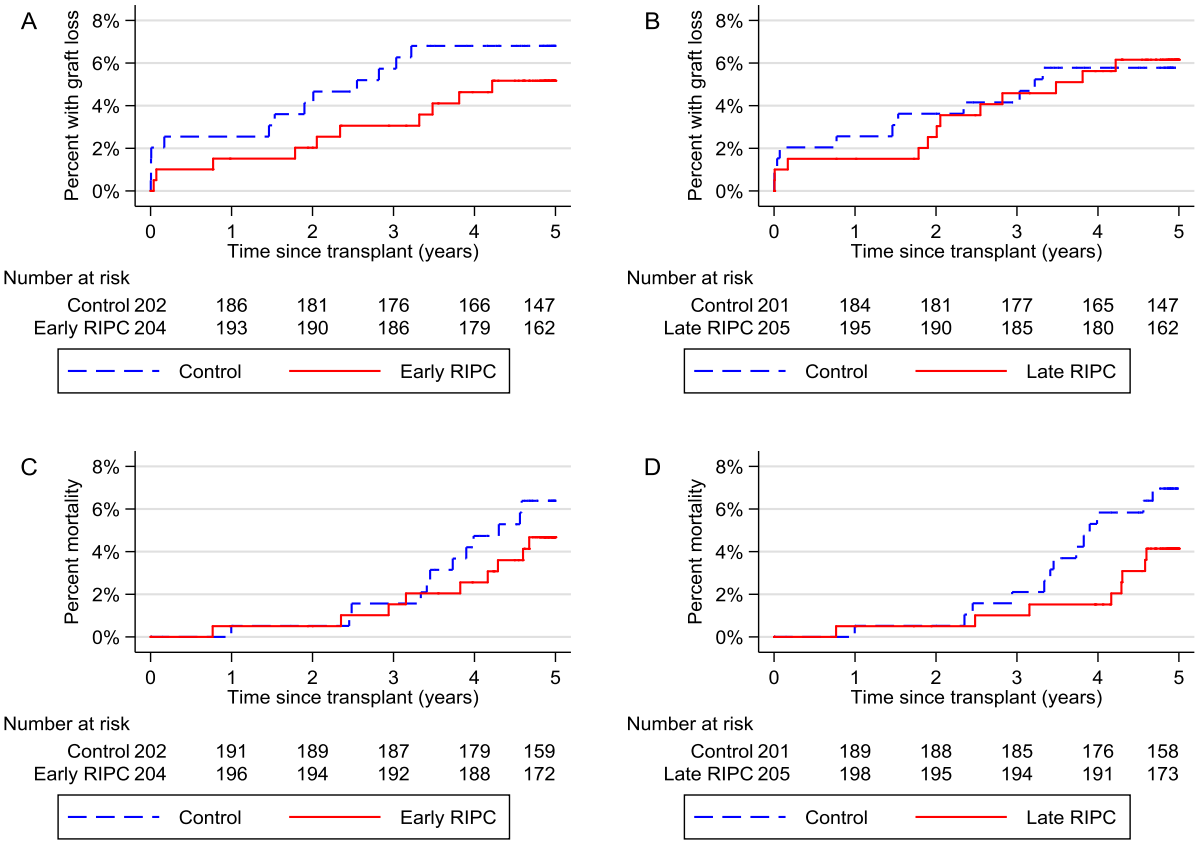

Figure 2 – Kaplan-Meier estimate of incidence of graft loss and mortality up to 5 years post-transplant, intention to treat analysis

Panels show: A. graft loss early RIPC vs control; B. graft loss late RIPC vs control; C. mortality early RIPC vs control; and D. mortality late RIPC vs control.