Effect of remote ischaemic preconditioning of liver transplant recipients: a pilot randomized controlled feasibility study.

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Abstract:

Ischaemia Reperfusion (IR) injury is a major cause of post-operative morbidity, mortality and graft loss following Orthotopic Liver Trasnplantation (OLT). There is no current accepted treatment for IR injury. The recent drive to implant more marginal grafts, which are more susceptible to IR injury makes this clinical problem a key research goal. Remote Ischaemic Preconditioning (RIPC) has been shown to ameliorate hepatic IR injury in small animal models. Whether recipient RIPC can reduce IR injury in human liver transplant recipients has not previously been investigated.

Methods:

Forty patients with end stage liver disease undergoing liver transplantation were randomized to RIPC or a sham control group. RIPC was induced through three 5 minute cycles of alternate ischaemia and reperfusion using an orthopaedic tourniquet to the left lower limb under general anaesthesia prior to commencement of the abdominal procedure. The aim of the study was to determine the safety and feasibility of limb RIPC in patients with end stage cirrhosis. Data on clinical outcomes was collected prospectively (minimum 3 month follow up) and the function of the graft was also evaluated. Plasma cytokine levels were measured at baseline, 2 hours post reperfusion and at 24 hours post-operatively.

Results:

45 of 51 patients approached (88%) were willing to enroll in the study. 5 patients were then excluded and 40 patients were randomized of which 20 underwent RIPC. RIPC was able to be performed in all patients randomized to the RIPC group. There was no evidence of localized complications following RIPC. One patient died in the control group and 1 further graft was lost in the control group due to a non-IR related

issue. Median AST levels on the third post-operative day showed a slightly higher trend in the RIPC group than in the control group (221iU vs 149iU, p=1.00) but this was not statistically significant.

Conclusions:

RIPC is acceptable and can be carried out safely in patients with advanced liver disease. This pilot feasibility study has not demonstrated evidence of a reduction in IR injury or clinical benefit. A longer follow up, a larger study or an altered preconditioning protocol may be required.

Liver transplantation is the treatment of choice for both acute and chronic end stage liver disease. As outcomes following transplantation have improved, the indications for liver transplantation have been widened and a shortage of suitable organ donors has developed. This has resulted in an increased use of grafts from marginal donors such as the elderly and those with a fatty liver from obesity (extended criteria donors) and especially the use of grafts from donors following cardiac death (DCD). The use of liver DCD grafts in the UK has increased from 6.9% in 2005[1] to 19.1% of grafts implanted in 2013[2].

Ischaemia Reperfusion (IR) injury is the damage that happens to an organ when its blood supply is interrupted and reconstituted. It is a major cause of morbidity and mortality following liver transplantation and is believed to account for up to 10% of early graft loss[3].

Grafts from extended criteria donors are particularly prone to IR injury and in UK centres, the implantation of DCD grafts is associated with a 2 fold increase in risk of graft loss and recipient mortality which is maintained for 3 years post transplantation[1]. Due to this increased susceptibility to IR injury and the poor clinical outcome, grafts from extended criteria donors are often discarded. The ability to ameliorate IR injury would therefore improve outcome following liver transplantation, reduce early graft loss and the need for re-transplantation and would allow the safe implantation of more marginal grafts increasing the potential donor organ pool. There is no current accepted treatment for IR injury and as such the development of strategies to treat IR injury remains a key clinical concern in the field of solid organ transplantation.

Ischaemic Preconditioning (IPC), first described in a canine cardiac model[4], is the process by which short periods of ischaemia to the target organ protect that organ

during further more substantial periods of ischaemia. Despite robust evidence of the protective benefit of direct IPC in small animal models[5,6], direct IPC has been shown in small animal models to impair liver regeneration[7, 8]. Although this effect has not been shown on donor grafts in human liver transplantation, a multivariate analysis has shown that direct IPC is an independent predictor for post-operative morbidity[9] following hepatic resection surgery in humans.

Ten small studies have investigated the effect of direct IPC of donor livers in the setting of deceased donor liver transplantation and a recent meta-analysis has shown that donor IPC of donor grafts lead to a large reduction in recipient mortality and incidence of primary graft non-function although this difference was not statistically significant[10].

Remote Ischaemic Preconditioning (RIPC), again first described in a canine cardiac model[11], is the process by which preconditioning of one organ or vascular bed provides protection to distant organs or vascular beds during a sustained period of ischaemia. RIPC has been shown by our own group and others to ameliorate hepatic IR injury in small animal models[12, 13, 14, 15]. It has also been translated into clinical benefit in patients undergoing both cardiac surgery[16] and major vascular surgery[17]. In major liver surgery, RIPC was shown in a pilot RCT to reduce liver IR injury as indicated by a reduction in post operative transaminases and increased ICG clearance. Successful use of RIPC in liver transplant recipients would avoid the complexity of organizing and the ethics of preconditioning at the multiple donor sites and the potential risk of impairing graft regeneration following implantation.

Although the mechanism by which RIPC and IPC protect organs from IR injury remain unknown, the process of performing RIPC in the recipient more closely resembles successful animal models in which preconditioning is performed in the

individual in which the reperfusion injury occurs and therefore may be more efficacious than IPC of donor livers[18]. RIPC of renal transplant recipients has been investigated in 2 recent trials including both living[19] and deceased[20] donors with 1 trial demonstrating an improvement in early graft function[20]. No trial as yet has investigated RIPC in liver transplant recipients and there are therefore fundamental issues which have to be addressed. These include the willingness of patients, their clinicians and the transplant anaesthetists to support such a trial. Patients undergoing liver transplantation mainly have end stage cirrhosis and the risk of limb conditioning in patients with jaundice and a coagulopathy are unknown. Finally the conditioning protocol which has been used in other clinical applications may not be optimal with the altered metabolism and haemodynamics of end stage liver disease?cirrhosis.

The aims of this study was to perform a prospective randomized controlled feasibility study to address these issues and to obtain preliminary data on which to design a further prospective trial to determine efficacy and cost effectiveness.

Methods:

A single centre double blinded open prospective randomised controlled trial was performed at the Royal Free Hospital following approval by the NHS National Research Ethics Service (11/H0720/4) and the Royal Free Hospital/University College London medical school ethical committee (8191). The trial involved randomization of adult recipients undergoing deceased donor liver transplantation and was registered with ClinicalTrials.gov: Number NCT00796588. The protocol has been previously published[21].

Patients above the age of 18 undergoing first elective deceased donor liver transplantation were enrolled with informed consent in the study for randomization. All graft types were included. Exclusion criteria are contained in Table 1.

51 patients were approached for recruitment to the study of which 6 patients were unwilling to recruit to the trial and 5 patients were excluded. 40 patients were randomized to a sham control group and a RIPC group. Randomisation was performed, following induction of anaesthesia, using a sealed envelope method by the study fellow who was not involved with the transplant surgery or post operative care (CONSORT flowchart, Figure 1).

Both patients and clinical teams, including the transplant surgeon, were blinded as to which group the patient was randomized to.

Endpoints:

The primary endpoints were:

1: Feasibility to recruit patients with end stage cirrhosis to undergo limb preconditioning prior to commencement of liver transplantation.

2: Investigate the safety of limb pre-conditioning in patients with end stage cirrhosis. This included the assessment of any clinical evidence of a deep vein thrombosis or pulmonary embolus, the development of localized cellulitis and pain or paresthesia of the left lower limb following RIPC and subsequent surgery. Secondary endpoints are listed in Table 2 and include mortliaty, morbidity as measured using Clavien Dindo classification, graft function assessed using day 3 aspartate transferase (AST) levels[22] and early allograft dysfunction (EAD) as defined by Olfthoff et al[23].

Donor organ and patient demographics were recorded as …..?

The preconditioning stimulus:

Following induction of anaesthesia but before skin incision, the left lower limb was covered with 2 layers of stockinette and a wide pneumatic tourniquet was applied to the left middle thigh in accordance with safe and recommended practices by the Association of Peri-operative Registered Nurses (AORN)[24]. To induce RIPC through transient ischaemia, the tourniquet was inflated to 200mmHg for 5 minutes and then deflated for 5 minutes to reperfuse the leg. This was repeated 2 more times and completed prior to the abdominal incision for the transplant procedure. The control group had placement of the tourniquet, which was not inflated?

Liver transplantation:

Grafts were identified and retrieved through the dedicated UK National Organ Retrieval Service (NORS) according to national standards of organ retrieval from deceased donors[25] (NHSBT). All grafts were perfused in situ and transported in cold University of Wisconsin (UW) solution (Organ Recovery Systems, Chicago) at a

maximum pressure of 200mmHg following aortic cannulation. The liver graft was further flushed with ice cold UW solution on the back bench via the hepatic artery, portal vein and the bile duct once retrieved and transported in cold storage. Grafts which were stored and transported using normothermic perfusion using the OrganOx system as part of a contemporaneous trial were excluded from this study.

All patients were monitored intra-operatively via invasive central arterial and venous catheters Implantation of the liver graft was performed by standard piggy-back and caval replacement techniques. Veno-venous bypass was not employed in any patient randomsied in this trial. Grafts were flushed with 500-1000mls warm 4.5% human albumin solution (Bio Products Laboratory) via the portal vein following implantation of the graft and immediately prior to blood re-perfusion to remove residual UW solution and waste material accumulated during cold ischaemia. 1g of methylprednisolone (Pharmacia) was given intravenously during the anhepatic phase as part of standard anaesthetic protocol.

Post operative management:

Post-operatively all patients were managed in the intensive care unit.

Haemoglobin levels were maintained below 10g/L to reduce the risk of graft thrombosis.

Platelets and fresh frozen plasma were not administered unless there was active bleeding post operatively resulting in cardiovascular compromise or abdominal compartment syndrome. Patients were routinely started on subcutaneous thromboprophylaxis on the first post operative day.

All patients underwent a Doppler ultrasound scan of the liver vessels on the first, third and fifth post-operative day and daily bloods including coagulation profiles and serum transaminases.

Patients were extubated on the first post-operative day unless there was a clinical need for ongoing respiratory support and triple therapy immunosuppression was commenced on day 1 post-operatively. If there was evidence of early renal impairment, monoclonal antibody therapy was given in place of triple therapy immunosuppression.

Measurement of cytokines:

In both groups, 10mls of peripheral arterial blood was collected at the following time intervals:

- baseline (following induction of anaesthesia), before knife to skin?
- immediately before the recipient's portal vein and cava were cross clamped,
- 2 hours post reperfusion of the portal vein,
- 24 hours post-operatively.

Blood was collected in BD vacutainer plasma tubes (BD, UK). Samples were immediately centrifuged at 1000g for ten minutes and the plasma was stored at -80^{0}C until analysis. IL2, IL6, IL10, TNFα and IFNγ were measured by legendPLEX (Biolegend, UK)- Human Th Cytokine Mix and Match Subpanel. IL8 (Biolegend, UK) and IL17A (Biolegend, UK) were measured by ELISA via commercial kits.

Oxygenation levels during preconditioning:

Two 2mls bloods samples were collected simultaneously. A venous blood sample was collected from the recipients left foot in lithium heparin gas syringes (BD Preset,

UK), after 4.5 minutes of lower limb vascular occlusion while the tourniquet was still inflated or at the same time point in the sham group. A simultaneous peripheral arterial blood sample was collected from the arterial line and identically managed. Oxygen levels, lactate levels and acid base status were measured instantly from both samples on a RAPIDPoint 500 blood gas analyser (Siemens, Surrey, UK).

Statistical analysis and power calculation:

A power calculation is not required for a pilot feasibility study[26] however guidance would suggest that 40 patients would be suitable for a feasibility study[27] with 20 patients randomized to RIPC and 20 patients randomized to a sham.

Continuous variables were expressed as median (+ inter-quartile range) or mean (± standard deviation) as appropriate and comparisons between the groups were analysed by Mann Whitney-U test or Students' T-Test as appropriate. Binary outcomes were expressed as frequency counts and percentages and comparisons between the groups were analysed by Chi-squared tests on Statistical Package for Social Sciences (SPSS) (IBM, Chicago, IL, USA) and Prism 5 (Graphpad, USA).

Results:

Feasibility and recruitment:

Fifty–one patients were approached of which 45 (88%) were willing to enroll in the trial. Five patients were subsequently excluded due to concern regarding possible risk from intermittent limb ischaemia from the tourniquet. Four of the five patients had a prior history of thromboembolic disease and 1 patient had varicose veins of the left lower limb. The remaining 40 patients were randomized with 20 randomized to RIPC and 20 to a sham control. The patients were well matched at baseline. The characteristics of the recipients and donors are shown in Tables 3 and 4.

All patients randomized to undergo preconditioning were successfully preconditioned prior to abdominal incision. There was no evidence of haemodynamic instability or vagal response either during the cuff inflation or after reperfusion of the limb. Visual inspection of the limb following preconditioning showed no evidence of bruising/haematoma formation.

No patient complained of pain or parasthesia post-operatively. There was no clinical evidence of DVT or PE formation in any patient.

Secondary end-points:

1: 90 day graft and patient survival

One patient in the control group died peri-operatively as a result of significant intra-operative haemorrhage and primary graft non-function (PGNF). There was no 90 day mortality in the RIPC group. One patient in the control group required retransplantation on the 4th post-operative day following the discovery of an incidental adeno-carcinoma in the donor's gallbladder.

2: Complications:

There were no significant differences in incidences of post-operative complications between the groups (Table 5) including infective complications and incidence of AKI.

3: Graft function:

Aspartate transferase levels on the 3rd post operative day were trending to be higher in the preconditioning group but this did not reach significance (221iU (82-434) vs 149iU (103-370), p=1.00). There was a also a trend to higher incidence of EAD in the preconditioned group but this was not significant (10 vs 7, p=0.523). Although median transaminase levels, in the first week post transplantation, were higher in recipients that underwent RIPC, median bilirubin (27μmol/L (19-37) vs 41μmol/L (23-74), p=0.087) and alkaline phosphatase levels (215iU/L (168-293) vs 275iU/L (218-351), p=0.126), at day 7 post transplantation, were trending to be lower in recipients who were preconditioned although this was not statistically significant. By 3 months post transplant both groups were similar in all measured indices.

4: Acute cellular rejection:

Incidence of acute cellular rejection was low with only one episode proven by biopsy in the control group and no episodes in the preconditioning group.

5: ITU and total hospital stay:

Patients in the preconditioning group spent longer in ITU post operatively (4 days vs 3 days, p=0.372) and in hospital post transplantation (20 days vs 16 days, p=0.409), although this was not statistically significant.

Limb oxygenation during RIPC:

Arterial oxygen levels measured from the radial artery during the preconditioning stimulus were similar between the preconditioned and control groups (28.87(±9.73)kPa vs 30.43(±12.63)kPa, p=0.757). Venous oxygen levels measured at the same time in the lower limb during the preconditioning stimulus were significantly lower in the preconditioning group than the control group (7.53(4.94-9.28)kPa vs 15.06(8.67-19.00)kPa, p=0.004) however the venous pO2 levels do not support the creation of localized ischaemia during RIPC (figure 2).

Plasma cytokine levels:

Plasma levels of IL6, IL8, IL10 and IL17a were significantly raised from baseline at 2 hours post reperfusion and had returned to near baseline levels within 24 hours (Figure 3). Plasma levels of IL2, IFN-γ and TNF-α did not change during the peri-transplant period (Table 6). The median IL-6 levels in the preconditioned group were significantly lower than in the control group (487.99 (221.65-1232.37)pg/ml vs1062.3 (221.5-25903.85)pg/ml, p=0.013) (figure 4). Median levels of all other cytokines measured were similar between both groups and are shown in figure 4.

There was no significant difference in post reperfusion levels of Il-6, IL8, IL10 and IL17a in patients that went on to develop early allograft dysfunction, an infective complication post-operatively or the need for prolonged organ support.

Discussion:

This is the first trial to prospectively investigate RIPC of liver transplant recipients as far as we are aware . It has demonstrated that RIPC is feasible, acceptable to patients and safe to perform in liver transplant recipients.

Recruitment to the trial was satisfactory with a consent rate of 88% and a post randomization drop out of 0%. RIPC was successfully completed in all patients. No patient suffered a complication secondary to RIPC and in no patient was surgery delayed as result of undergoing RIPC. This study satisfied the primary objectives of the feasibility study[21].

Ninety day mortality and graft loss was low in this cohort of patients with one peri-operative death and only one other graft loss within the study period. It is therefore unsurprising that RIPC was unable to demonstrate any benefit by a reduction in 90 day morbidity and mortality in this small population of patients.

In UK centres, current 90 day graft loss and patient mortality following elective liver transplantation is 3.5% and 6.9% respectively[28] and as such designing a trial based on these end-points would be difficult due to the required recruitment rate necessary to demonstrate a significant improvement by one intervention. Further secondary end-

points were therefore chosen as they may identify more subtle differences in outcomes post transplantation and aid the design of a future cost-effectiveness study. Common post operative complications were documented including infective complications and post operative organ dysfunction as these would indicate a poorly functioning graft. Patients who underwent preconditioning had a higher incidence of post operative acute kidney injury (9 vs 7) and need for renal replacement therapy (5 vs 3). The mean days ventilated post operatively was similar between the 2 groups (2 vs 2). Severe IR injury results in a systemic inflammatory response and end organ damage. AKI is a particular problem post AKI and documented rates in the literature range from 14% to 94%[29–34]. A recent audit of incidence of AKI at the Royal Free Hospital found that AKI occurs in around 50% of patients undergoing liver transplantation. Sixteen patients (40%) of patients developed an AKI so this is representative of our general patient cohort. These data would suggest that RIPC did not reduce the incidence of end organ dysfunction following IR injury.

In such a small pilot study that was not powered to detect a significant difference in mortality and graft loss, surrogate markers were assessed. Graft function including AST levels on the 3rd post-operative day (which have been shown to correlate strongly with both graft survival and recipient outcomes[22]) and EAD (which has also been shown to be associated with an increased risk of graft loss and recipient mortality[23]) were both measured. Median AST levels on day 3 were non-significantly higher than in the control group again suggesting that in its current form RIPC has not reduced IR injury in the liver graft and is not associated with an improvement in graft or recipient outcome. This is further reflected by a non-statistically significant higher incidence of EAD in the RIPC group (50% vs 37%, p=0.523). It should however be noted that patients who underwent RIPC had a non significant reduction in bilirubin

levels (27μmol/L vs 41μmol/L, p=0.087) at day 7 contrarily suggesting evidence of better early graft function.

Taken together this data shows that although RIPC is safe and feasible to be performed in patients undergoing liver transplantation, in its current form it does not provide evidence of clinical benefit to liver transplant recipients in this small patient group.

Results from previous studies of RIPC have been conflicting. Despite initial success in children undergoing cardiac surgery[16] and promising results from a phase II trial[35], a recent large randomized trial of RIPC in cardiac surgery has failed to shown any significant benefit gained from RIPC[36]. In the setting of clinical transplantation 2 trials of RIPC have been performed. The results however are conflicting with 1 trial demonstrating evidence of improved early graft function[20] whist 1 trial failed to demonstrate any improvement in graft function or a reduction in early biomarkers[19].

It is unclear from the published manuscripts whether or not supplementary oxygen was administered to the patients in these trials during the pre- or intraoperative period. In this study we investigated the degree of hypoxia which was achieved using the pneumatic cuff. One important finding from our study was that although the venous pO2 in the limb was significantly lower in patients undergoing RIPC compared with controls, true hypoxia was not achieved in the limb during the preconditioning stimulus as assessed by ……. There could be several reasons for this, including errors in cuff inflation. However the standard deviation of the venous pO2 levels is small (6.19kPa) suggesting that this is not the case. It may be that the high FiO2 delivered to the patients during the transplant procedure, including during the period of preconditioning stimulus, prevented significant tissue hypoxia in the conditioned

limb. This would suggest that 5 minutes of tourniquet inflation was insufficient to create localized ischaemia in the limb in these patients. This mirrors the results from direct preconditioning of liver grafts in donors prior to organ retrieval in which 5 minutes of ischaemia was found not to be of any benefit[37] but 10 minutes of ischaemia was shown to provide a degree of protection demonstrated by a reduction in markers of liver injury[38]. The optimal protocol for RIPC in humans remains to be established. A pilot study of RIPC in patients undergoing liver resection using 10 minute cycles to perform the preconditioning stimulus showed evidence of a reduction in liver injury as demonstrated by a reduction in post operative transaminases and improve ICG clearance and as such a further trial with 10 minute cycles is warranted.

It is widely accepted that IR injury results in systemic cytokine release and activation of the systemic response syndrome with resulting end-organ damage[39, 40]. TNFα, is a key cytokine shown to be upregulated early following IR injury and to promote recruitment of lymphocytes to the ischaemic injury[41]. Other cytokines that have been implicated in IR injury in small animal models include IL-2[42], IL-6[43], IL8[44], IL-17[45] and IFNγ[43] and these were measured in this study. A previous observational study in humans undergoing liver transplantation[46] measured circulating serum cytokine levels at 24 hours post transplantation and found that the in the majority of patients, circulating levels were below the detectable level especially IFNγ (99%) and TNFα (77%). These results are similar to our results which show that systemic cytokine levels were below the detectable levels in the majority of patients at 24 hours post reperfusion. Although 17 patients developed an AKI as measured by the AKIN criteria – suggesting a systemic inflammatory response and end organ damage,

plasma levels of IL2, IFNγ and TNFα were not significantly raised from base line in our patients even 2 hours post reperfusion suggesting that these cytokines may not be involved in the systemic inflammatory response post transplant. This is in keeping with results from canine lung IR injury which showed no elevation in serum IL-2, IFNγ and TNFα although they were significantly elevated in bronchial alveolar lavage samples[47] showing they may be involved in the local inflammatory response.

In the current study, circulating levels of IL-6, IL-8, IL-17A and IL-10 were elevated at 2 hours post reperfusion and were similar to baseline levels by 24 hours. Similar peaks of circulating levels of IL-6 and Il-8 were seen in patients at 2 hours post liver resection[48] however circulating levels remained elevated at 24 hours post operatively in comparison to this study when levels returned to near baseline. This may reflect the fact that post liver transplant patients are immunosuppressed whilst they are not following liver resection surgery. Similarly the peak of plasma Il-10 levels at 2 hours in our patients likely represents the anti inflammatory effect of the intravenous dose of methylprednisolone given during the anhepatic phase of the transplant. In this study there were significantly lower levels of circulating IL-6 in patients who underwent RIPC. However the significance of this result is unclear. Plasma IL-6 levels post reperfusion showed a positive correlation with the calculated donor risk index[49] suggesting that IL-6 levels vary with the quality of donor organ. Higher IL-6 levels post liver resection have been shown to be associated with increased risk of post-operative complications and bile leaks[48]. However plasma IL-6 levels were not higher in patients who developed either EAD or AKI which are associated with poor quality grafts. Furthermore although patients undergoing RIPC had lower levels of IL-6 post reperfusion, there was no evidence that this was associated with a reduction in graft injury or an improvement in clinical outcomes.

In conclusion, to our knowledge, this is the first trial to investigate RIPC of liver transplant recipients and has shown that RIPC is feasible and safe in liver transplant recipients. In its current form it does not appear to provide any clinical benefit detectable within the first 3 months post transplantation. Venous blood gas measurements taking from the limb during the preconditioning period suggest that 5 minute cycles are insufficient to create localized ischaemia in the limb.

We would suggest that a pilot RCT of RIPC vs sham with an altered preconditioning protocol for example of three 10 minute cycles is evaluated prior to considering a larger scale study aimed a determining efficacy and cost effectiveness.

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Table 1: Exclusion Criteria

Exclusion criteria:	Re-transplantation Patients under 16 years of age Super-urgent transplantation Lack of informed consent Combined liver and kidney transplantation Peripheral vascular disease Varicose veins Localized limb infection Prior history of thrombo-embolic disease Inclusion in another interventional trial

Table 2: Primary and secondary trial endpoints.

Primary endpoints:	Ability to recruit patients to the trial Feasibility of performing RIPC in liver transplant recipients Safety of RIPC in liver transplant recipients
Secondary endpoints:	90 day recipient mortality 90 day graft loss AST levels on the third post-operative day[22] Incidence of Acute Kidney injury and need for Renal Replacement therapy Length of stay in Intensive Care and total hospital stay Incidence of vascular thrombotic events Incidence of biliary complications Incidence of post-operative infections Incidence of acute rejection in the first months post

	transplantation Circulating cytokine levels 2 hours post reperfusion of the liver graft

Table 3: Base-line recipient characteristics (mean ± SD).

Recipient characteristics	RIPC	Control	P vaue
Gender (M:F)	18:2	16:4	0.661
Age	55 (±10)	54 (±9)	0.758
MELD	15 (±5)	13 (±5)	0.190
UKELD	55 (±5)	52 (±5)	0.085

Table 4: Baseline donor and Transplant characteristics (mean ± SD)

Donor and Transplant characteristics	RIPC	Control	P value
Gender (M:F)	16:4	13:7	0.731
Age	43 (±14)	47 (±16)	0.376
Type of graft			0.723
DBD	15	17	
DCD	3	2	
Domino	1	0	
Split	1	1	
Cold ischaemic time (mins)	470 (±140)	455 (±157)	0.750
Warm ischaemic time (mins)	44 (±14)	42 (±11)	0.546

Table 5: Clinical outcomes in RIPC and control groups

	RIPC	Control	P value
3 month mortality	0	1	1.00
3 month graft loss	0	2	0.487
Day 3 AST	221 (82-434)	149 (103-370)	1.00
EAD	10	7	0.523
Mean ITU stay (days)	4 (±2)	3 (±3)	0.372
Mean days ventilated	2 (±1)	2 (±1)	0.758
Need for RRT	5	3	0.695
Portal vein thrombosis	0	0	1.00
Hepatic artery thrombosis	0	0	1.00
Biliary stenosis	2	1	0.501
Bile leak	1	2	0.459
Bacteraemia	3	2	0.549
Chest infection	0	1	0.349
Abdominal infection	3	2	0.549
Wound infection	4	7	0.303
Urine infection	2	3	0.517
Mean hospital stay (days)	31 (±46)	21 (±14)	0.409

Table 6: Plasma cytokine levels during the transplant period (Median + IQR) * denotes significance identified with Kruskal-Wallis.

Cytokine	Baseline (pg/ml)	Pre implantation (pg/ml)	Post reperfusion (pg/ml)	24 hours post-op (pg/ml)
IL-2	9.34 (4.08-35.11)	7.50 (4.08-40.40)	6.19 (4.08-17.13)	12.16 (4.08-42.29)
IL-6*	13.98 (8.27-44.80)	245 (150.97-375.12)	644.98 (338.31-1132.01)	21.58 (10.11-43.29)
IL-8*	0.88 (0-3.26)	8.23 (1.28-15.84)	30.59 (15.37-52.42)	0.88 (0-3.11)
IL-10*	4.22 (3.72-7.69)	9.83 (4.38-16.96)	540.74 (344.21-815.48)	7.37 94.56-35.26)
IL-17A*	2.14 (1.74-2.96)	2.40 (1.68-3.2)	2.94 (1.85-8.78)	1.86 (0.81-2.33)
IFNγ	57.13 (18.05-176.09)	32.66 (17.15-73.29)	31.41 (10.92-107.48)	17.35 (6.50-44.20)
TNFα	7.97 (3.5-53.16)	7.17 (3.5-33.79)	7.15 (5.46-8.58)	6.46 (3.5-8.58)

Figure 1: CONSORT flow diagram

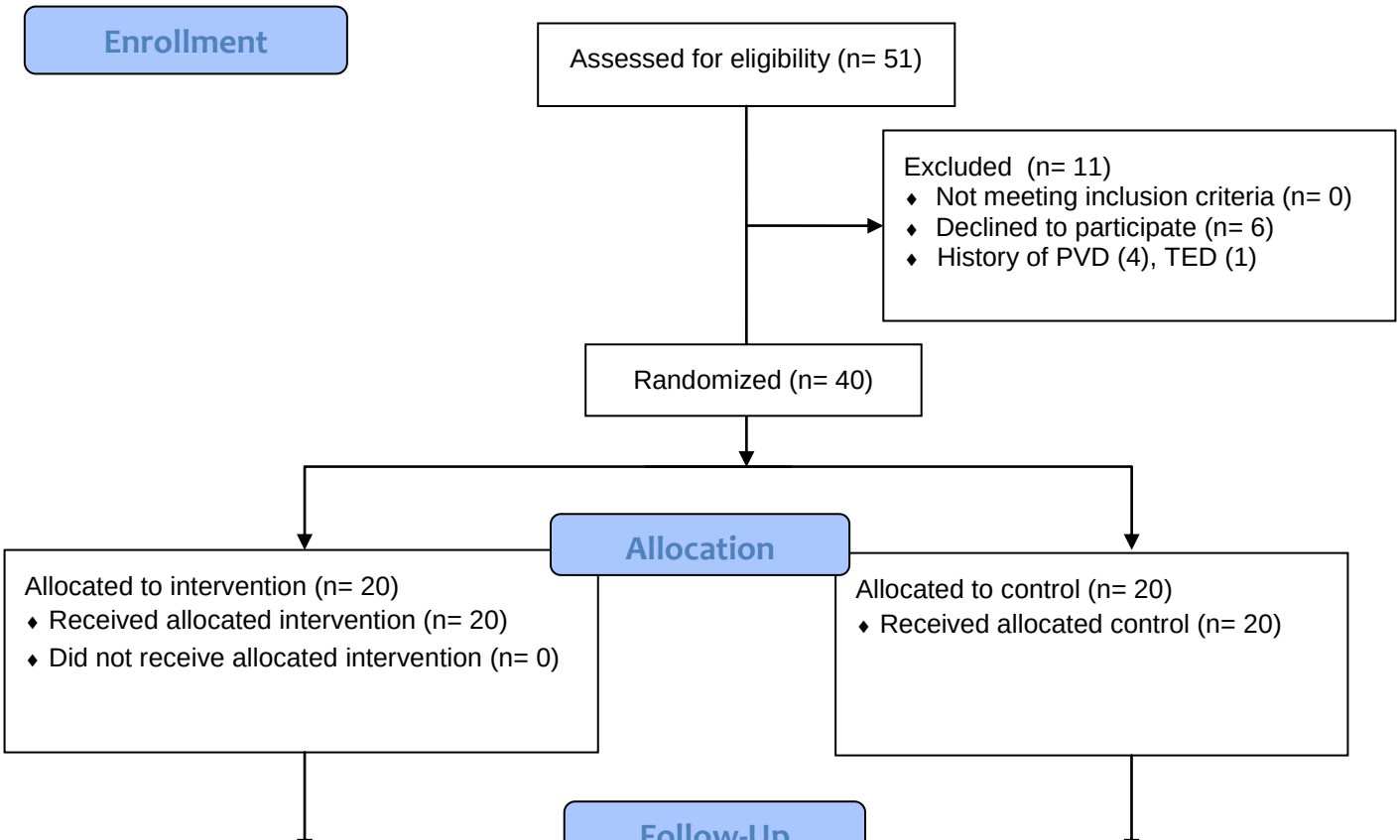


Figure 2: Venous oxygen levels in the limb during the preconditioning cycle.

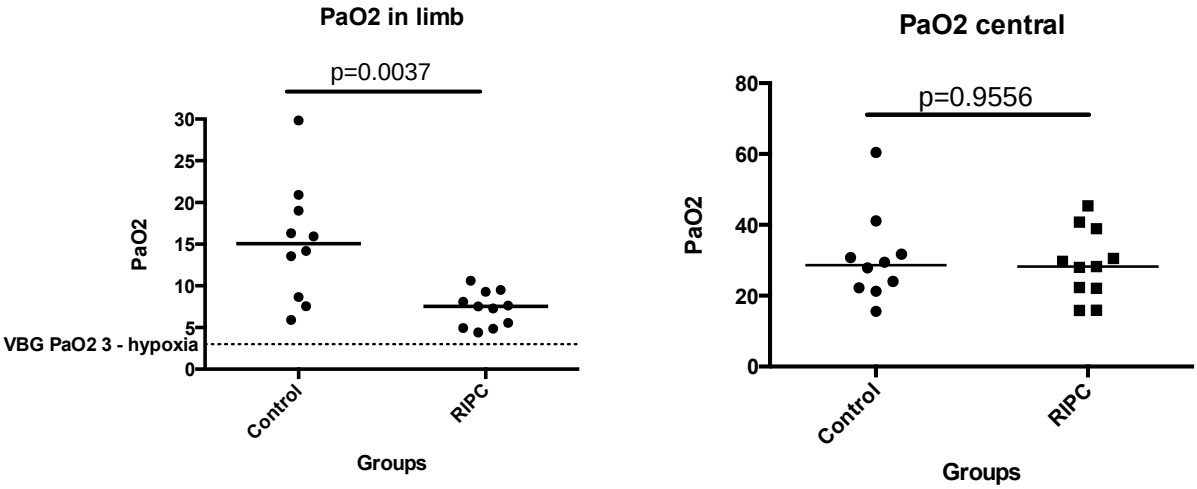
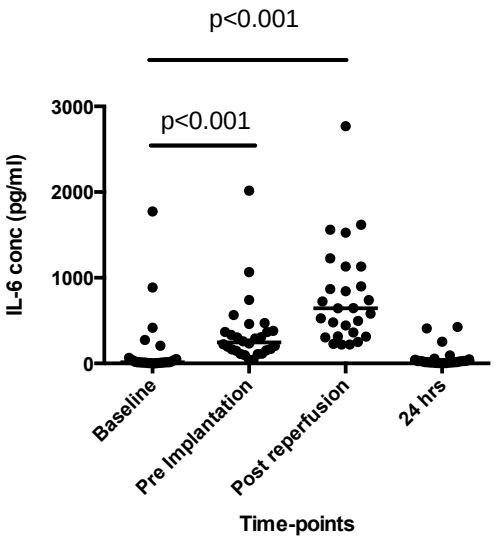
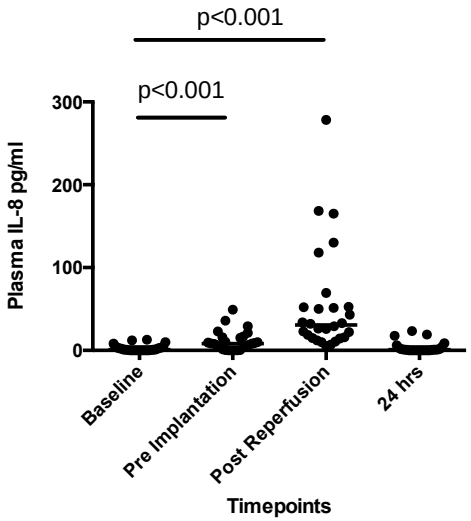


Figure 3: Circulating cytokine levels that showed a significant increase during liver transplant and at 24 hours post op.

A: IL-6



B: IL-8



C: IL-10

D: IL-17A

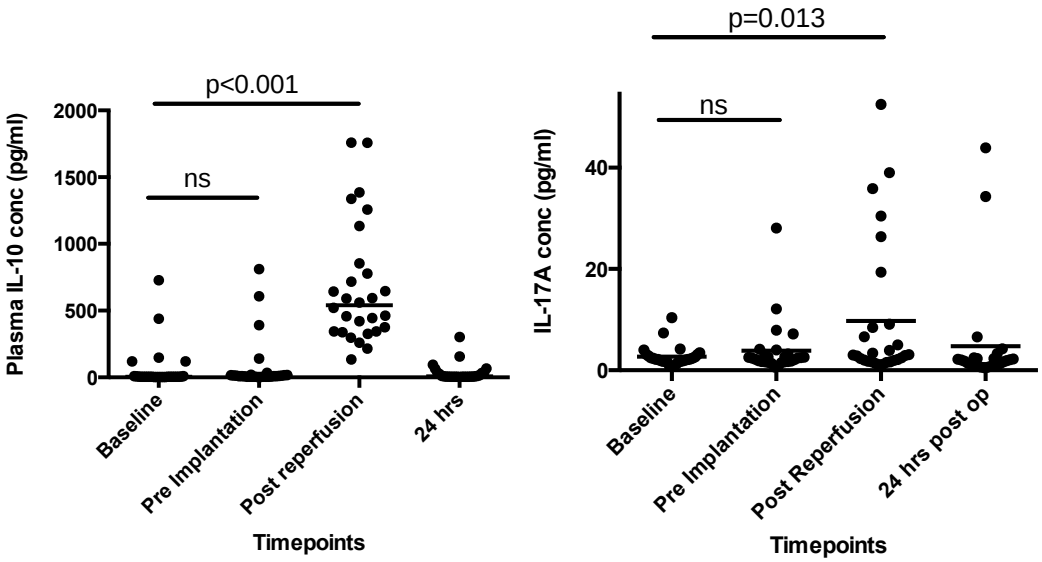
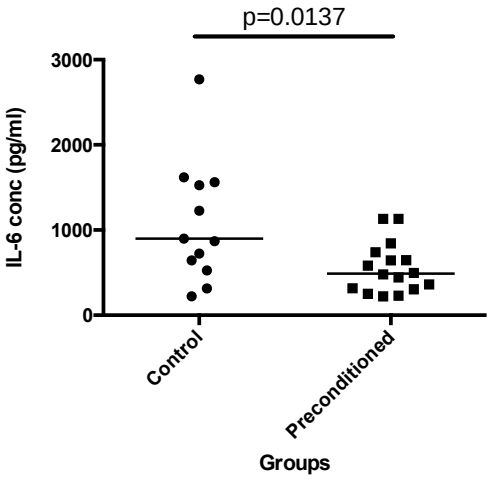


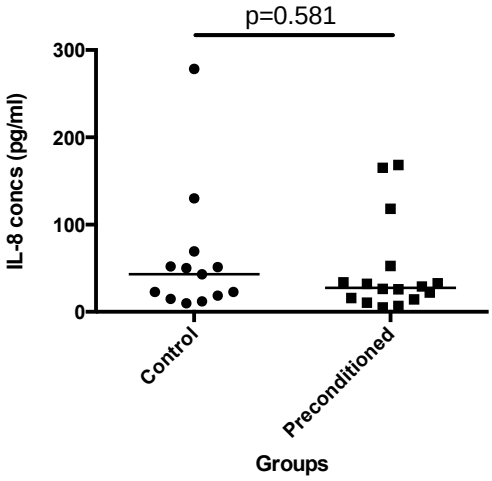
Figure A (IL-6) and Figure B (IL-8) demonstrate a significant increase in circulating cytokine levels following mobilization of the recipient liver but prior to hepatectomy and a further significant increase in cytokine levels at 2 hours post reperfusion. Levels returned to near baseline levels at 24 hours post operatively.
Figure C (IL-10) and Figure D (IL-17A) demonstrate significantly elevated cytokine levels at 2 hours post reperfusion only.

Figure 4: Circulating cytokine levels between the groups.

A: IL-6

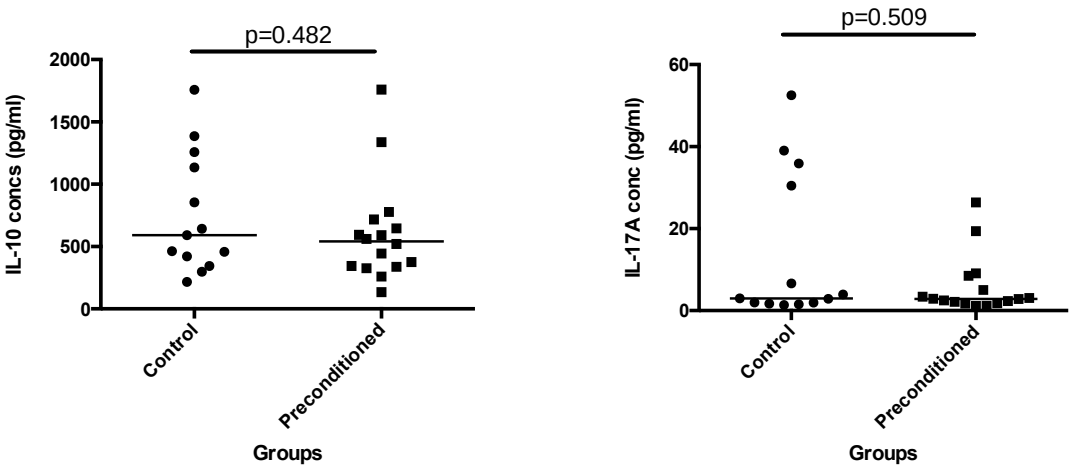


B: IL-8



C: IL-10

D: IL-17A



Only IL-6 levels (Figure A) were significantly reduced in patients undergoing RIPC.