META-ANALYSIS

A systematic review and meta-analysis of donor ischaemic preconditioning in liver transplantation

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SUMMARY

Ischaemic preconditioning (IPC) is a strategy to reduce ischaemia–reperfusion (IR) injury. Its benefit in human liver transplantation is unclear. The aim of this study was to analyse the current evidence for donor IPC in liver transplantation. Systematic review and meta-analysis of studies involving IPC of liver transplant donors. Ovid Medline, Embase and Cochrane CENTRAL were searched up until January 2015. Data retrieved included the primary outcomes of 1-year mortality, incidence of primary graft non-function (PGNF) and retransplantation. Secondary outcomes included aspartate aminotransferase (AST) levels on day 3 post-op. Pooled odds ratios (ORs) were calculated for dichotomous data and mean weighted ratios for continuous data. Ten studies included 593 patients (286 IPC; 307 control). IPC was associated with a reduction in mortality at 1 year (6% vs. 11%) although this was not statistically significant (OR 0.54, 95% C.I. 0.28–1.04, $P = 0.06$). The IPC group had a significantly lower day 3 AST level (WMD −66.41iU, $P = 0.04$). This meta-analysis demonstrates that IPC reduces liver injury following transplantation and produces a large reduction in 1-year mortality which was not statistically significant. Confirmation of clinical benefit from IPC requires an adequately powered prospective RCT.

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Key words
ischaemic preconditioning, ischaemic reperfusion injury, liver transplantation, morbidity, mortality

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Introduction

Liver transplantation is the only effective treatment for both acute and chronic liver failure. A total of 780 deceased donor liver transplants were performed in the UK in 2014–2015 [1]. Due to a recent widening of the eligibility criteria, demand for this scarce resource far outstrips supply of suitable organs. The use of grafts from extended criteria donors in the UK has expanded to meet demand with the use of grafts from donors following cardiac death (DCD) increasing from 6.9% in 2005 [2] to 19.1% of grafts inserted in 2013 [1]. Ischaemia–reperfusion (IR) injury is the injury that happens to an organ when its blood supply is interrupted and reconstituted and is a major cause of morbidity, mortality and graft loss following liver transplantation – accounting for up to 10% of early graft loss from brain dead donors (DBD) [3]. DCD grafts are associated with a twofold increase in risk of graft loss and recipient mortality in UK centres [2]. A key factor to the reduced outcomes seen with the use of DCD grafts is their susceptibility to IR injury and the associated complications. There are no current accepted treatments for IR injury and as such the development of

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strategies to prevent or reduce IR injury is a key research goal.

Ischaemic preconditioning (IPC) was first described in 1986 [4]. This process of inducing short periods of nonlethal ischaemia to a target organ has been shown to provide protection to the same or other organs during a subsequent sustained ischaemic insult. The reduction in liver damage with IPC has been demonstrated in different small animal models of hepatic ischaemia [5, 6], but its role in clinical transplantation remains to be proven. Several small trials have investigated IPC in the donor prior to organ recovery.

The aim of this study was to evaluate the current evidence in the medical literature regarding the use of IPC in human liver transplantation.

Methods

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [7]. The study protocol was registered with the University of York Centre for Reviews and Dissemination international prospective register of systematic reviews (2015: CRD42015016055).

The medical literature was searched for RCT's examining the effect of IPC in the clinical setting of deceased donor liver transplantation.

All trials including patients undergoing deceased donor liver transplantation were included in the study. Patients undergoing living donor liver transplantation were excluded.

Data was retrieved from the published studies. The primary outcomes chosen for the analysis were early graft failure and retransplantation within 3 months and mortality within 1 year. The secondary outcomes were episodes of acute rejection, length of time spent in the intensive therapy unit (ITU) and in hospital, number of days ventilated, incidence of postoperative transient renal support, infective complications and aspartate transferase (AST) levels on the 3rd postoperative day [8]. Papers were included irrespective of language. Both RCTs and matched cohort studies were included. Studies based on overlapping cohorts of patients were excluded.

MEDLINE, Embase and Cochrane Central Register of Controlled Trials (CENTRAL) databases were searched up to and including the second week of January 2015 using the following search algorithm: ((((hepatic OR liver) adj3 (transplant$ or graft$)).ti,ab.) or (exp Liver Transplantation/)) and (((isch?emic adj(preconditioning or pre conditioning or pre-conditioning)).ti,ab.) or (exp Ischaemic Preconditioning/)).

Two authors independently reviewed the titles. Appropriate studies were identified, and those appropriate following review of abstracts were sourced in full. Two authors independently extracted data from the studies selected for full paper review. Disagreements were resolved by consensus or where necessary by the senior author.

Statistical analysis

Data on the selected primary and secondary outcomes were entered into a meta-analysis. Dichotomous outcomes were analysed based on event rates using pooled odds ratio (OR) whilst continuous outcomes were analysed using a weighted means difference (WMD). The analysis was performed on REVMAN 5® software (The Nordic Cochrane Centre, Copenhagen, Denmark) using a random-effects DerSimonian-Laird model, and results were reported with 95% confidence intervals. A P value of less than 0.05 or an odds ration not crossing 1 was considered to be significant. Heterogeneity was assessed using τ^2, χ^2 and I^2 measures and was deemed significant if $P < 0.10$ or I^2 was greater than 30%. In keeping with REVMAN's default setting, trials with zero events in each arm of a variable were excluded from the analysis of that variable. If a variable had zero events in one arm, 0.5 events were added to that arm to allow an odds ratio and confidence intervals to be calculated.

Results

Following the initial search (Fig. 1), 458 studies were identified of which 305 remained following removal of duplicates. The 305 titles and abstracts were reviewed, and 19 studies were selected for full review. Reasons for study exclusion were animal model (125), review, editorial or reply (73), irrelevant topic (64), no full text or abstract available (20) and duplicated patient dataset (4).

Of the 19 studies reviewed in detail 9 were excluded – reasons for exclusion were conference abstracts (7), duplicated patient dataset (1) and living donor study (1).

Data from ten studies were further analysed [9–18] which included 593 patients (286 IPC; 307 control). The characteristics and results of each study are included in Tables 1 and 2. Only DBD grafts were included in these studies. No grafts underwent

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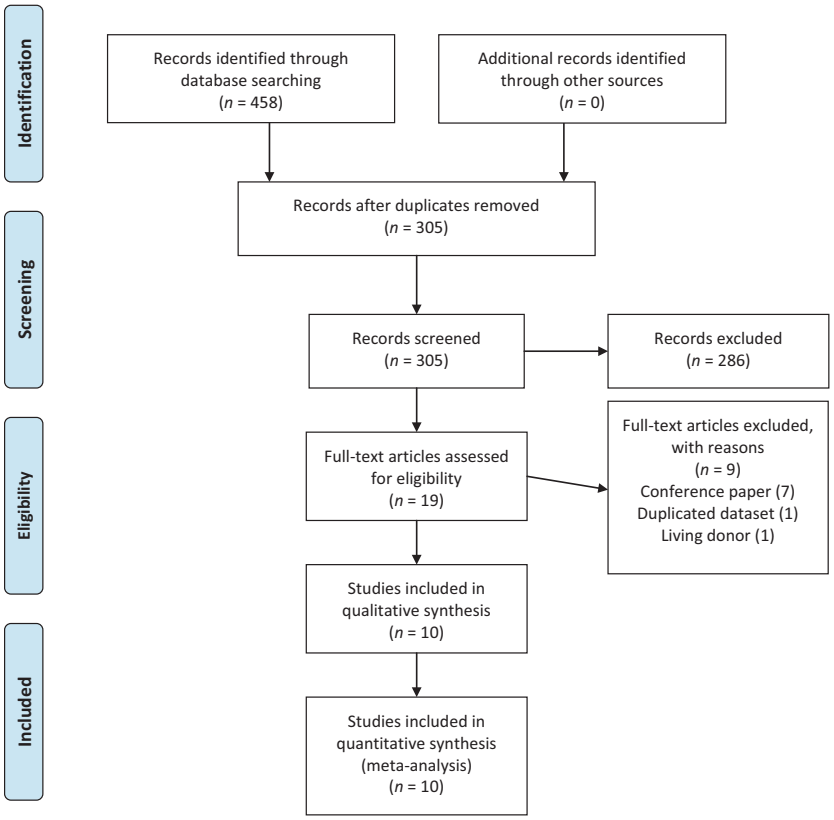

Figure 1 PRISMA flow chart.

normothermic machine perfusion or reperfusion. There was no significant difference in mean length of cold ischaemic time between the treatment and control arms of the included studies.

Primary endpoints

Seven studies [9–15] (475 patients: 232 IPC, 243 control) included data on 1-year mortality (Fig. 2). There was significant variability in the time point at which patient mortality was calculated from 3 months up to 1 year. There was no significant heterogeneity between the studies (I^2 = 0%, P = 0.85). IPC was associated with a 45% reduction in postoperative mortality (6% vs. 11%), but this was not statistically significant (OR 0.54, 95% C.I. 0.28–1.04, P = 0.06).

Five studies [9–12,15] (322 patients: 152 IPC, 170 control) provided data on the incidence of primary graft nonfunction (PGNF) (Fig. 3). There was no significant heterogeneity between the studies (I^2 = 0%, P = 0.91). The IPC group had a lower incidence of PGNF (0.7% vs. 4%), but this was not statistically significant (OR 0.35, 95% C.I. 0.009–1.31, P = 0.12).

Six studies [10–12,14–16] (274 patients: 182 IPC, 192 control) commented on the need for retransplantation (Fig. 4). There was no significant heterogeneity between the studies (I^2 = 0%, P = 0.99). IPC was associated with a reduction in the incidence of retransplantation (3% vs. 4%), but this was not significant (OR 0.83, 95% C.I. 0.28–2.41, P = 0.73).

Secondary endpoints

Three studies [11,12,18] (149 patients: 68 IPC, 81 control) included data on day 3 AST levels (Fig. 5). There was no significant heterogeneity between the studies (I^2 = 0%, P = 0.46). AST levels on the 3rd postoperative day were significantly lower in patients who had been transplanted with grafts from IPC treated donors compared with controls [WMD −66.41 (−129.92 to −2.89) iU, P = 0.04].

Four studies [9,10,13,16] (240 patients: 121 IPC, 119 control) included data on length of ITU stay (Fig. 6). There was no significant heterogeneity between the studies (I^2 = 0%, P = 0.74). IPC was associated with an increase in length of ITU stay, but this was not

Table 1. Summary of included trials.

Author (year)	Amador *et al.* [9]		Cescon *et al.* [10]		Cescon *et al.* [11]		Franchello *et al.* [12]		Azoulay *et al.* [13]	
Duration of IPC	10 min		10 min		10 min		10 min		10 min	
Group	IPC	Ctl	IPC	Ctl	IPC	Ctl	IPC	Ctl	IPC	Ctl
No in group	30	30	23	24	19	20	30	45	46	45
1-year mortality	0	2	0	2	3	2	1	3	2	2
Incidence of PGNF	0	3	0	1	0	1	0	1	0	0
Incidence of retransplantation	–	–	2	2	1	1	1	2	0	0
Day 3 AST levels (iU/l)	644	819	–	–	–	–	270.12 (193.74)	281.46 (292.94)	–	–
Length of ITU stay (days)	6.8 (8)	6.7 (7)	–	–	–	–	–	–	15 (14)	12 (6)
Length of hospital stay (days)	24 (14)	24 (14)	–	–	–	–	13.4 (6.6)	18.12 (12.92)	38 (25)	31 (12)
Incidence of episodes of acute rejection	4	4	–	–	–	–	9	7	9	12

Table 2. Summary of included trials ctd.

Author (year)	Koneru *et al.* [14]		Koneru *et al.* [15]		Degli-Eposti *et al.* [16]		Jassem *et al.* [17]		Fuggle *et al.* [18]	
Duration of IPC	5 min		10 min		10 min		10 min		10 min	
Group	IPC	Ctl	IPC	Ctl	IPC	Ctl	IPC	Ctl	IPC	Ctl
No in group	34	28	50	51	26	24	9	14	19	16
1-year mortality	–	–	6	11	0	0	0	0	–	–
Incidence of PGNF	0	0	1	1	–	–	0	0	–	–
Incidence of retransplantation	0	1	1	1	1	1	–	–	–	–
Day 3 AST levels (iU/l)	183 (126–2311)	183 (108–316)	–	–	–	–	–	–	120 (91)	216 (137)
Length of ITU stay (days)	–	–	–	–	12/15	11/12	1	2.8	–	–
Length of hospital stay (days)	–	–	10	10	28/52	29/33	–	–	–	–
Incidence of episodes of acute rejection	–	–	6	11	4	11	2	7	7	5

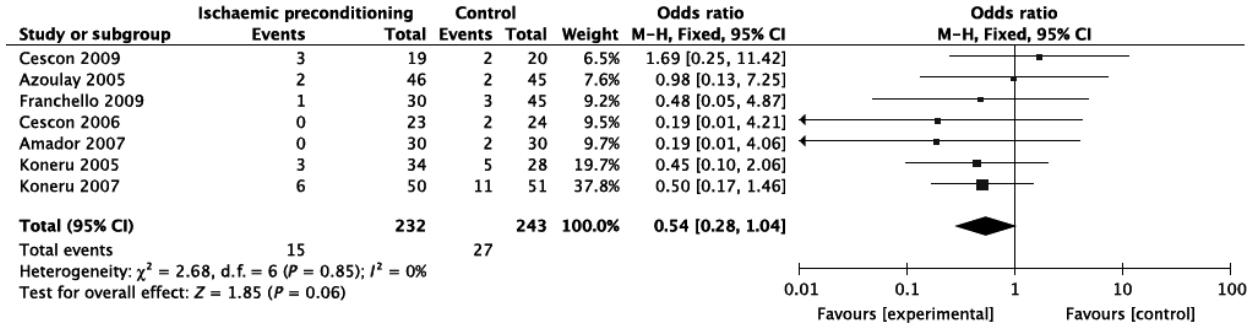

Figure 2 Forest plot comparing mortality between the groups.

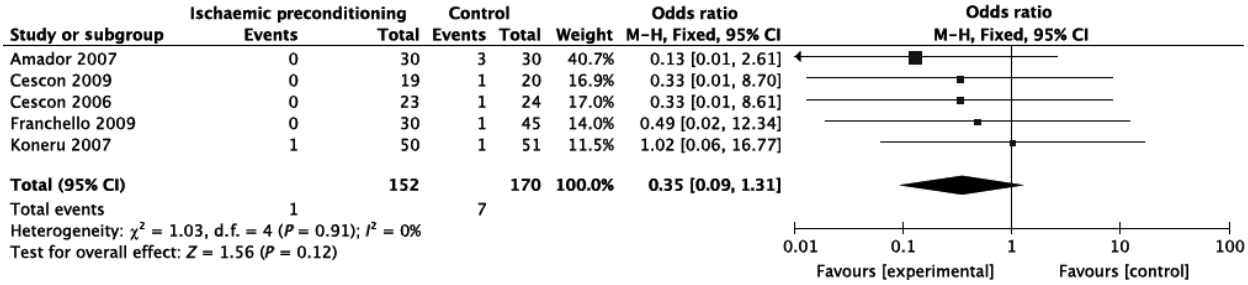

Figure 3 Forest plot comparing incidence of primary graft non-function (PGNF) between the groups.

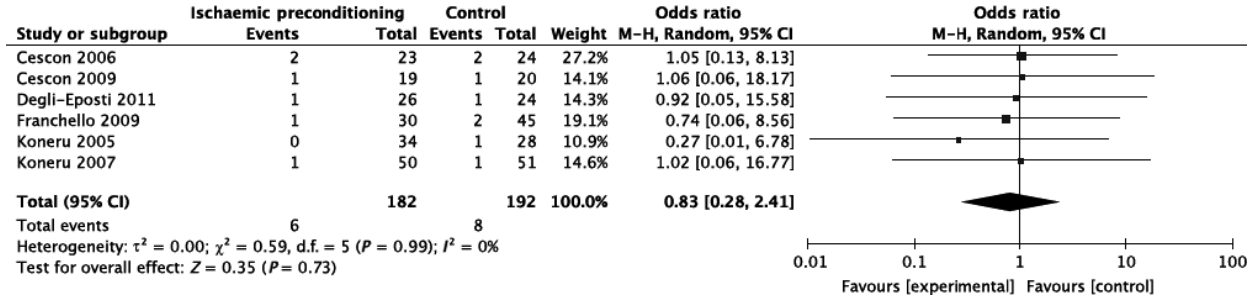

Figure 4 Forest plot comparing incidence of retransplantation between the groups.

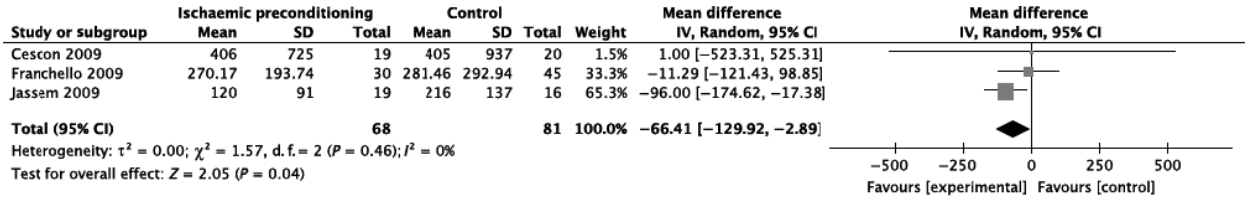

Figure 5 Forest plot comparing aspartate transferase (AST) levels on the 3rd postoperative day between the groups.

statistically significant MWD 1.21 (−1.02 to 3.45) days ($P = 0.29$).

Six studies [9–13,16] (362 patients: 174 IPC, 188 control) included data on length of hospital stay (Fig. 7). There was significant heterogeneity between the studies ($I^2 = 56\%$, $P = 0.06$). IPC was associated with an increase in length of total hospital stay, but this was not statistically significant MWD 0.56 (−4.77 to 5.9) days ($P = 0.84$).

Seven studies [9,12,13,15–18] (435 patients; 210 IPC, 225 control) included data on number of patients experiencing an episode of acute rejection (Fig. 8). There was significant heterogeneity between the studies ($I^2 = 37\%$, $P = 0.14$). IPC was associated with a

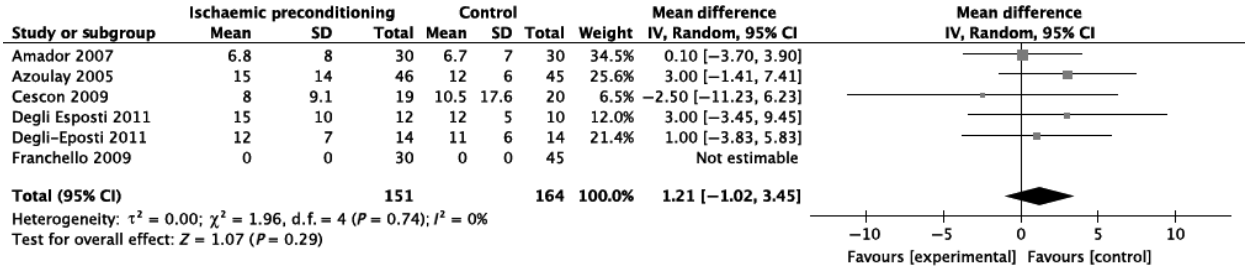

Figure 6 Forest plot comparing length of intensive therapy unit (ITU) stay between the groups.

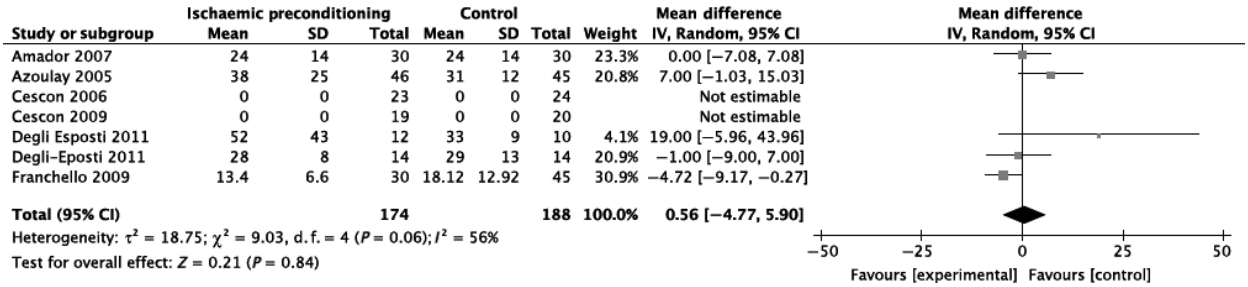

Figure 7 Forest plot comparing length of hospital stay between the groups.

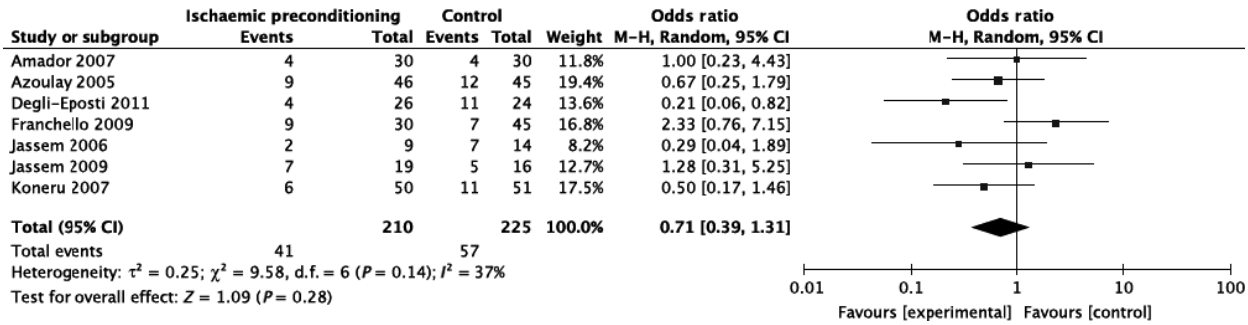

Figure 8 Forest plot comparing incidence of acute rejection episodes between the groups.

reduction in number of patients experiencing an episode of acute rejection (20% vs. 25%), but this was not statistically significant (OR 0.71 95% C.I. 0.39–1.31, $P = 0.28$).

Discussion

Ischaemic preconditioning is an inexpensive intervention that has been shown to ameliorate hepatic IR injury in small animal models [19–21]. Several small human trials have investigated IPC of donor livers prior to recovery of organs. The majority of these trials have been pilot feasibility trials, and none have been adequately powered to determine a significant benefit in terms of the most important clinically relevant outcomes of patient morbidity and mortality following liver transplantation. An audit of liver transplant activity in the UK demonstrated that 90-day patient and graft survival are 90.8% and 89.3%, respectively [22], and as such designing a trial around, these end points would be difficult due to the required trial size and associated cost. A meta-analysis investigating the benefit of this intervention in liver transplantation is therefore of importance as a meta-analysis of underpowered trials may reveal significant results.

A Cochrane review involving a literature search performed in early 2007 included 3 RCTs and failed to show any clinical benefit from IPC [23]. Several further RCTs have been performed since this time justifying a further review of the literature.

Ischaemic preconditioning was associated with 45% reduction in recipient mortality post liver transplantation (6% vs. 11%). This large reduction in mortality from a single intervention did not prove to be statistically

significant. Similarly, IPC was associated with an 82% reduction in the incidence of grafts loss secondary to PGNF (0.7% vs. 4%) although this again this was not significant ($P = 0.12$). This data demonstrating lower recipient mortality and graft loss post liver transplant in patients who had received a graft from donors undergoing IPC was not statistically significant but would suggest that a larger prospective randomized trial is required which is powered to demonstrate a reduction in these clinically significant end points. A power calculation was performed with an alpha error of 0.05 and a beta error of 0.2 for both PGNF and 1-year mortality. This calculated that total sample size of 660 patients with 330 in each arm would be required to adequately power a trial to demonstrate a significant reduction in PGNF and a sample size of 974 with 487 patients in each arm would be necessary to demonstrate a significant reduction in 1-year mortality. Such a multicentre trial would be feasible in the UK.

Recent work has shown that AST levels on the 3rd day following liver transplant, rather than peak transaminase levels, correlate with recipient mortality rate and the incidence of graft loss, need for organ support and incidence of postoperative complications and infections [8]. Day 3 AST levels were therefore included as a secondary end point for this meta-analysis. Three studies measured AST levels on day three. Patients that received a graft that underwent IPC had significantly lower AST levels on the 3rd postoperative day.

A significant reduction in an important surrogate marker for post-transplant outcomes would again suggest a beneficial effect to donor IPC and would also support the need for a further clinical trial.

Patients who received grafts from donors who underwent IPC spent on average 1 day longer in ITU and in 0.6 days longer in hospital. Neither of these were significant, but both of these variables are associated with adverse outcomes following liver transplant including a greater need for organ support or the development of complications post-transplantation. A large randomized clinical trial is further supported to ensure that donor IPC is not associated with any adverse outcomes.

All of the included trials were small and underpowered to detect a significant reduction in important clinical end points which could lead to inconclusive results from this meta-analysis. Eight studies were randomized controlled trials with little evidence of bias and a low dropout rate post randomization.

There was significant variability between trials. There is no consensus regarding the optimal preconditioning stimulus in humans. Of the 10 trials included, nine trials performed a single IPC stimulus of 10 min whilst only one trial performed a single IPC stimulus of 5 min [14]. Furthermore, there is a lack of validated end points in clinical trials of liver transplantation and as such the end points measured in individual trials varied significantly making comparison of the trials difficult. Very few trials reported on the incidence of specific postoperative complications including infections. The incidence of these complications reflects underlying graft function; however, we were unable to comment on the effects of IPC on these important end points.

Two trials which were included were cohort studies [16,17], comparing a cohort of patients undergoing IPC to a historical matched cohort. Although cohort studies provide less robust evidence than a randomized clinical trial, neither of these trials were included in the analysis involving AST levels which were significantly reduced by IPC.

Only one trial included marginal grafts in a subgroup analysis [16]. This cohort study demonstrated a reduction in IR injury both in normal and marginal grafts following IPC and a reduction in incidence of acute rejection in marginal grafts that underwent IPC when compared to controls. This study raises the important question of whether IPC is more efficacious in grafts subjected to a more significant IR injury. It was the only study to perform a subgroup analysis of extended criteria grafts. A trial investigating the value of IPC or RIPC specifically with extended criteria donors including DCD donors prior to withdrawal of circulatory and ventilator support would be warranted.

No trial investigated recipient outcome longer than 1 year postoperatively, and as such, the long-term effect of IPC on post-transplant outcomes remains unknown. Furthermore, data regarding recipient quality-of-life post-transplantation were not included. This is an important end point in liver transplantation as the aim of transplantation is not only to improve survival but also to improve quality of life.

This analysis has shown that donor IPC prior to graft recovery results in a reduction in acute liver injury as indicated by reduced day 3 AST levels and a major but not statistically significant reduction in one-year mortality and incidence of PGNF. An adequately powered multicentred RCT is required to confirm the harms and benefits of donor ischaemic preconditioning to recipients undergoing liver transplantation.

Authorship

FPR designed the study, collected the data, performed the analysis and wrote the final manuscript. LJM

helped collect the data and perform the analysis and reviewed the final manuscript. GW/BF/BRD help design the study and reviewed the final manuscript.

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Conflict of interest

The authors have declared no conflicts of interest.

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