Received: 4 July 2022 | Revised: 29 August 2022 | Accepted: 3 September 2022

DOI: 10.1111/cts.13409

ARTICLE

Controlled oxygenated rewarming as novel end-ischemic therapy for cold stored liver grafts. A randomized controlled trial

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Abstract

Abrupt return to normothermia has been shown a genuine factor contributing to graft dysfunction after transplantation. This study tested the concept to mitigate reperfusion injury of liver grafts by gentle warming-up using ex vivo machine perfusion prior to reperfusion. In a single center randomized controlled study, livers were assigned to conventional static cold storage (SCS) alone or to SCS followed by 90 min of ex vivo machine perfusion including controlled oxygenated rewarming (COR) by gentle and protracted elevation of the perfusate temperature from 10°C to 20°C. Primary outcome mean peak aspartate aminotransferase (AST) was 1371 U/L (SD 2871) after SCS versus 767 U/L (SD 1157) after COR ($p = 0.273$). Liver function test (LiMAx) on postoperative day 1 yielded 187 µg/kg/h (SD 121) after SCS, but rose to 294 µg/kg/h (SD 106) after COR ($p = 0.006$). Likewise, hepatic synthesis of coagulation factor V was significantly accelerated in the COR group immediately after transplantation (103% [SD 34] vs. 66% [SD 26]; $p = 0.001$). Fewer severe complications (Clavien-Dindo grade ≥3b) were reported in the COR group (8) than in the SCS group (15). Rewarming/reperfusion injury of liver grafts can be safely and effectively mitigated by controlling of the rewarming kinetics prior to blood reperfusion using end-ischemic ex vivo machine perfusion after cold storage.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Experimentally, evidence suggests that abrupt rewarming upon reperfusion after cold storage compromises graft recovery. This "rewarming injury" could be significantly alleviated by modifying the slope of temperature increase in the organ graft upon isolated machine perfusion. This controlled oxygenated rewarming

Trial Registration: The trial was registered with ISRCTN number 94691167.

gently restitutes metabolic turnover rates and prevents the abrupt reactivation of a not yet equilibrated cellular metabolism.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study is the first randomized controlled trial to address the question if and to which end the method of controlled oxygenated rewarming can be translated into clinical liver transplantation.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study shows that controlled oxygenated rewarming during ex vivo machine perfusion can be regarded as a safe and easily implementable novel tool in liver preservation, likely to improve early functional outcome after transplantation.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The addition of a brief machine perfusion protocol with a controlled rise in temperature is an easily implementable method in clinical routine and may contribute to improved organ recovery after transplantation.

INTRODUCTION

Increasing donor organ shortage represents a major challenge in transplantation medicine and presumably perpetuates in the future. As a consequence, the number of patients rises, waiting for an organ offer for long times, accompanied by an increasing mortality before actual organ transplantation.[1–3] In order to address the issue of organ shortage, the criteria for donor organ acceptance have been extended over time, including organs from older donors and less than optimal grafts.[4,5] Although use of those extended criteria donor organ grafts will likely expand in the future, "less than optimal" grafts are often related to a reduced functional reserve and less resilience against preservation/ reperfusion injury. Therefore, further developments of existing preservation technology are inevitable and should fulfill the requirements of the growing field of graft preservation and conditioning.

It could be indicated by previous research that only minor, reversible lesions manifest during vascular flush-out and cold preservation of the graft. In contrast, structural integrity is most affected at the time of warm, oxygenated reperfusion.[6,7] Experimental studies have shown, that irrespective of the adequate fulfillment of nutritional or energetic requirements, extensive exposure to hypothermic temperature induce vulnerability of tissue to an abrupt increase in temperature[8–11] and that warm reperfusion of a cold preserved organ is notably related to accompanying cellular injury. This "rewarming injury" can be considered as a genuine patho-mechanism, which contributes to graft dysfunction after transplantation.

Our group systematically investigated the role of temperature during reconditioning of cold preserved organ grafts in functional recovery upon later sanguineous reperfusion.[8]

Studies using 18 h cold preserved isolated pig livers could demonstrate the beneficial effect of a brief hypothermic machine perfusion (HMP) prior to warm sanguineous reperfusion. By contrast, little evidence was found for a therapeutic effect of a reconditioning perfusion at constant 20°C.

Notably, it could be shown that during reconditioning perfusion an integration of a slow and controlled warming up of the organ disclosed significant advantages, even compared to the other reconditioning protocols. Perfusion starts with an extended period in hypothermia and is gradually increased to room temperature.[8,11] This reconditioning method, termed "controlled oxygenated rewarming" (COR) seems to promote restitution of cellular homeostasis and reduces rewarming injury through an adapted upregulation of metabolism. During the transient phase of cold to mid-thermic perfusion temperature a gentle restitution in mitochondrial function at limited workload is enabled, which was proven to facilitate a restitution of liver cell function in comparison to hypothermic or subnormothermic perfusion. It seems that COR alleviates the trigger for mitochondrial dysfunction upon normothermic reperfusion. Similar results were then described by Matsuno and co-workers.[12] Using a pig liver transplantation model, they evaluated the transaminases released after perfusion with gradual rewarming. Likewise, Banan et al. could show significantly reduced Kupffer cell activation upon isolated reperfusion of cold stored porcine livers after slowing down the rewarming process.[13] The principle of COR is also adapted to other organs. In a porcine renal reperfusion model, it could be demonstrated that COR induces revitalization of critically preserved donor organs and also improves well preserved organs after hypothermic reconditioning.[14] Moreover it was supported that "rewarming

injury" may occur even after optimal cold preservation (e.g., by continuous HMP).[14]

Comparing both reconditioning protocols, normothermic perfusion and COR, gentle rewarming of pig livers resulted in superior functional outcome upon reperfusion, which is probably related to better protection of mitochondrial function, improved energetic recovery, and a mitigation of mitochondrial induction of cellular apoptosis.[15]

The present trial now aims at scrutinizing this novel concept in a monocentric feasibility study using a controlled randomized setting. It is intended to gather systematic data that will also allow for an estimation of sample size required for a potential secondary large multicentric phase II study.

The benefit of the trial is expected to be the clinical establishment of a new and putatively beneficial adjunct in preservation /transplantation that will help to improve the outcome of marginal donor livers and thus contribute to enhance the amount of successfully transplantable donor organs.

PATIENTS AND METHODS

Study design

The randomized controlled study was conceived in a single blinded monocentric parallel group design. This was intended as a screening approach for the safety, feasibility, and presumed benefit of the new concept in a controlled fashion while keeping the expenses at an acceptable level.

The intervention under investigation is considered as adjunct to the standard procedure in use.

Therefore, the control group consists in pursuing the conventional standard care without any additional modification.

The research ethics board of the University Duisburg-Essen approved this study (approval number 16-7110-BO). Trial protocol was registered before recruitment (ISRCTN94691167). All transplants were performed between April 2019 and April 2021 at the University Hospital of Essen, Germany.

Eligibility and consent

The study was performed on less than optimal (as reflected in the liver inclusion criteria) organ grafts that are likely to take most benefit from the treatment. Donor livers fulfilling the extended criteria as defined by the German Medical Chamber, were enrolled in the study. At least one of the following criteria had to be fulfilled:

- Donor age >65 years,
- Intensive therapy including assisted ventilation >7 days,
- Obesity of donor (body mass index > 30),
- Serum sodium >165 Mol/L.
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× of normal.
- Serum bilirubin >3 mg/dl.
- Liver steatosis (histologically proven) >40%.

All patients listed for a liver transplant at the University hospital Essen with a minimum age of 18 years and residency in Germany were eligible to participate in the study. Written informed consent had been obtained from all participants. However, patients listed as "high urgency," re-transplantations, and patients that simultaneously participated in another preservation trial were excluded from the study.

Randomization

Whenever an eligible liver graft did arrive at the hospital, an experienced transplant surgeon made the primary decision if the liver is accepted for the allocated patient or has to be declined due to quality deficits. Only if the liver was accepted for transplantation and the allocated recipient had consented to participate in the study, then the graft was randomized to the treatment arm or the standard arm.

This was done concealed by central web pre-operative 1:1 block randomization, independently organized by the Center for Clinical Trials Essen. Recipients were stratified according to LabMELD categories <20, 20–30, and > 30.

Enrollment of patients is shown in Figure 1. Each patient who was registered and randomized became part of the intention to treat (ITT) analysis set. Based on the sample size calculation with a power of 80% and mean values expected to be equal to 1900 (SCS) and 700 (COR) with SDs of 1550 (SCS) and 400 (COR), a total of 40 patients were randomly assigned to one of the following groups.

Static cold storage

Study control arm: Procedure according to usual clinical standards without any experimental treatment.

Controlled oxygenated rewarming by ex vivo machine perfusion

The controlled oxygenated rewarming was performed on a CE-certified liver perfusion device, which allows for a

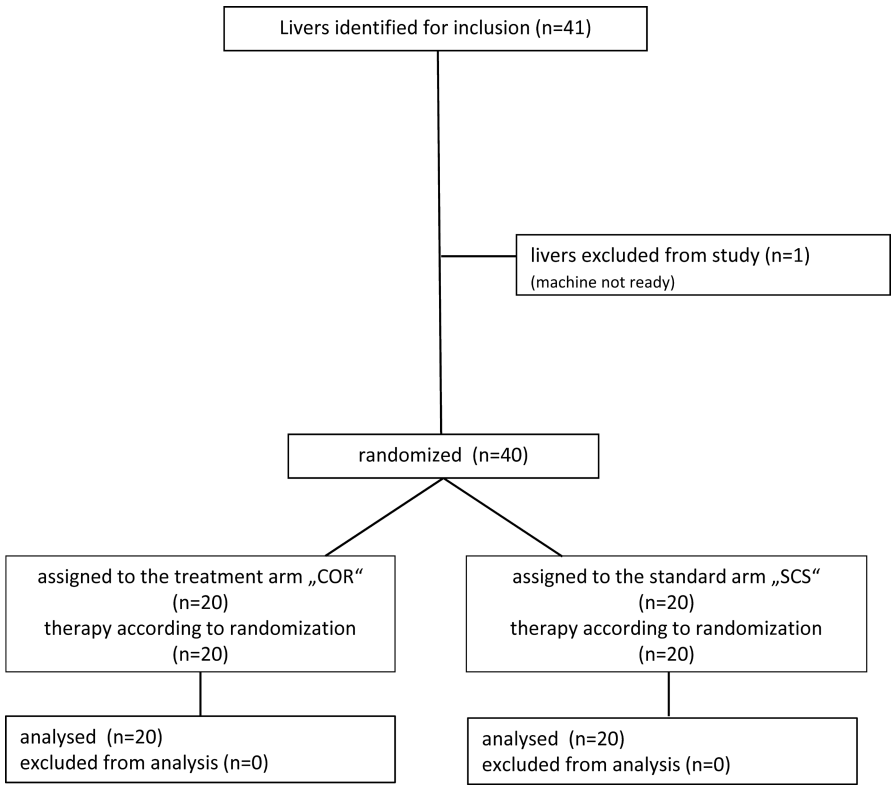

FIGURE 1 Consolidated Standards of Reporting Trials (CONSORT) diagram illustrating the study enrollment. COR, controlled oxygenated rewarming; SCS, static cold storage

dual perfusion via the arteria hepatica and the vena portae in a closed circuit (Liver Assist, Fa. Organ Assist, The Netherlands; cf. figure).[16] The Liver Assist system provides two separate perfusion lines, each comprising a rotary perfusion pump, membrane oxygenator with integrated heat exchanger, and pressure sensor used for perfusion of the portal vein and hepatic artery, respectively. The hepatic artery is perfused via a pulsatile set perfusion pressure, the portal vein via a continuous pressure. A total of 2 L of preservation solution is recirculated through the liver at ~8°C. The solution is oxygenated with 100% oxygen, been used based on previous findings on hypothermic rat liver perfusion,[17] and in order to allow for sufficient oxygenation of the liver tissue during the rewarming process in absence of oxygen carriers.

Perfusion pressures are 4 mmHg at the portal vein and 25 mmHg (60 bpm) at the hepatic artery. Temperature is kept hypothermic at 8°C during the first period of perfusion and then gradually increased to 12, 16, and 20°C after 30, 45, and 60 min, respectively.[8] Temperature control of the perfusion solution is provided by thermoelectric elements (Peltier-type) in combination with a water pump connected to the integrated heat exchanger of the oxygenators. Temperature, flow rates, and perfusion pressures in the arterial and portal circuits are displayed on the monitor of the device in real time.

Upon arrival of the donor liver in the hospital, back-table preparation of the organ was performed by an experienced surgeon according to regular standards. Single use cannulas are inserted into both vessels and the liver is connected to the perfusion circuit. During ongoing perfusion, perfusate samples are taken at regular intervals for timely measurement of metabolic parameters, like lactate, pH, potassium, and glucose on an acid base laboratory (Radiometer). The perfusion was continued until the recipient operation has advanced to the point that the liver could be implanted. At that time, the liver was taken out of the machine, re-flushed via the portal vein, and transplanted in standard technique.

Surgery

Orthotopic liver transplantation was performed with vena cava replacement and end-to-end-anastomosis of portal vein, hepatic artery, and bile duct. All patients were treated at the intensive care unit (ICU) after transplantation. The peri-operative care was similar in both groups as well as the concept of immunosuppression. Intravenous corticosteroids (1000 mg methylprednisolone) were applied intra-operatively. Postoperatively, tacrolimus (adjusted in accordance to the trough level of the drug) in combination with corticosteroids and mycophenolate mofetil were utilized.

Objectives and end points

All patients were observed for 7 days following transplantation on a daily basis. Follow-up included additional

observations on the day of discharge and 3 months after transplantation.

Serum peak value of aspartate aminotransferase AST during the first 3 days after transplantation was defined as the primary end point. During this time, measurement of AST was done at least twice and at most three times a day, with an interval of at least 6 h between measurements. Only the values obtained in these measurements were used for study purposes. The choice of the end point has been made after extensive study of the literature dealing with follow-up parameters after liver transplantation.[18]

Secondary end points were maximal liver function capacity (LiMAx test[19]) on day 1 after transplantation, 3 months after graft survival, early allograft dysfunction (EAD), ICU stay, as well as post-operative surgical complications according to Dindo-Clavien Classification Grade >3b. Laboratory data on liver integrity were also evaluated.

Liver function after transplantation

One day after transplantation, functional recovery of the livers was approximated by the postoperative measurement of themaximum liver function capacity, as evaluated by the metabolism of 13C-Methacetin as dynamic functional parameter of postischemic liver function.[20]

Early allograft dysfunction

EAD was defined as bilirubin ≥10 mg/dl on postoperative day 7 and/or International Normalized Ratio ≥1.6 on postoperative day 7 and/or AST or ALT >2000 IU/L within the first 7 days.[21] Each case was classified as "EAD" or "no-EAD."

Morbidity

All postoperative surgical complications classified according to Clavien-Dindo and all serious adverse events occurring within 30 days of transplantation have been documented.

Complications with a Clavien-Dindo grading IIIb or above were considered serious adverse events.[22]

Monitoring the data safety monitoring board

Patient recruitment, data completeness, and accuracy, including source data verification and proper conduct of the study according to regulatory requirements, had been controlled periodically by external monitoring. Confidential safety reports were reviewed at regular intervals by an independent data safety monitoring board.

Statistical analysis

Data are reported as ITT set, which includes all randomized patients.

Continuous variables were expressed as mean ± SD; and categorial variables as absolute numbers or percentage frequencies.

The primary study end point was the maximum absolute AST value (U/L) on one of the first 3 postoperative days. As the AST distribution is highly skewed, data were analyzed with a nonparametric statistical procedure, the two-sided exact Mann–Whitney *U* test.

Secondary end point analyses were done with Mann–Whitney *U* test for independent categorial variables and Fisher's exact test for categorial variables. Any *p* values of <0.05 were considered indicative for statistical significance. The secondary analyses are exploratory, so that no adjustment for multiplicity is needed.

RESULTS

Recruitment and follow-up

According to the study protocol, 20 patients were randomized into the treatment group and 20 patients randomized into the standard group.

Enrollment of patients is shown in Figure 1. One eligible patient was excluded from the study as the machine had not been ready to be used. Follow-up was completed 3 months after transplantation.

Donor and recipient characteristics

Donor and recipient demographics are outlined in Table 1. There were no remarkable differences between the groups in any of the donor or recipient demographics.

Liver function during COR

All livers in the COR group underwent controlled oxygenated rewarming without any complications, and all grafts were successfully transplanted. Total perfusate flow on the machine is depicted in Figure 2, along with a representation of temperature kinetics upon controlled rewarming of the graft. Lactate levels were rather low

TABLE 1 Donor and recipient data; data given as mean ± SD, or frequency and percentage as appropriate

Donor characteristics	COR ($n = 20$)	SCS ($n = 20$)	Total
Donor age, years	63.65 ± 12.75	63.50 ± 16.18	63.58 ± 14.38
Donor gender, M/F (%)	9/20 (45.0)	5/20 (25.0)	14/40 (35.0)
Donor BMI, kg/m^2	27.15 ± 3.96	27.91 ± 6.13	27.53 ± 5.11
Donor ICU stay, days	5.70 ± 4.54	4.90 ± 4.09	5.30 ± 4.29
Donor cause of death *n* (%)			
Cerebrovascular	16/20 (80.0)	12/20 (60.0)	28/40 (70.0)
Hypoxia	3/20 (15.0)	6/20 (30.0)	9/40 (22.5)
Trauma	1/20 (5.0)	1/20 (5.0)	2/40 (5.0)
Others	0/20 (0.0)	1/20 (5.0)	1/40 (2.5)
Donor risk index	1.80 ± 0.31	1.90 ± 0.29	1.85 ± 0.37
Macrosteatosis (≥20%)	15/20 (75)	14/20 (70)	29/40 (72.5)
Storage solution HTK/UW, *n*	20/0	20/0	40/0
Cold ischemia time, min	485 ± 82	454 ± 83	469 ± 81
Warm ischemia time, min	29.5 ± 5.3	27.1 ± 6.3	28.3 ± 5.9
Recipient characteristics			
Recipient age, years	57.50 ± 6.97	48.65 ± 14.47	53.08 ± 12.07
Recipient gender, M/F (%)	14/20 (70.0)	15/20 (75.0)	29/40 (72.5)
Recipient BMI, kg/m^2	26.90 ± 5.31	24.30 ± 3.59	25.60 ± 4.66
Underlying disease no./total no. (%)			
Viral hepatitis	3/20 (15.0)	4/20 (20.0)	7/40 (17.5)
Cholestatic disease	3/20 (15.0)	5/20 (25.0)	8/40 (20.0)
ASH	3/20 (15.00)	3/20 (15.0)	6/40 (15.0)
NASH	3/20 (15.0)	1/20 (5.0)	4/40 (10.0)
Others	8/20 (40.0)	7/20 (35.0)	15/40 (37.5)
Laboratory MELD score	16.20 ± 8.30	18.70 ± 8.12	17.45 ± 8.20

Abbreviations: ASH, alcoholic steatohepatitis; BMI, body mass index; COR, controlled oxygenated rewarming; HTK, histidine-tryptophan-ketoglutarate; ICU, intensive care unit; MELD, Model for End-stage Liver Disease.; NASH, nonalcoholic steatohepatitis; SCS, static cold storage; UW, University of Wisconsin.

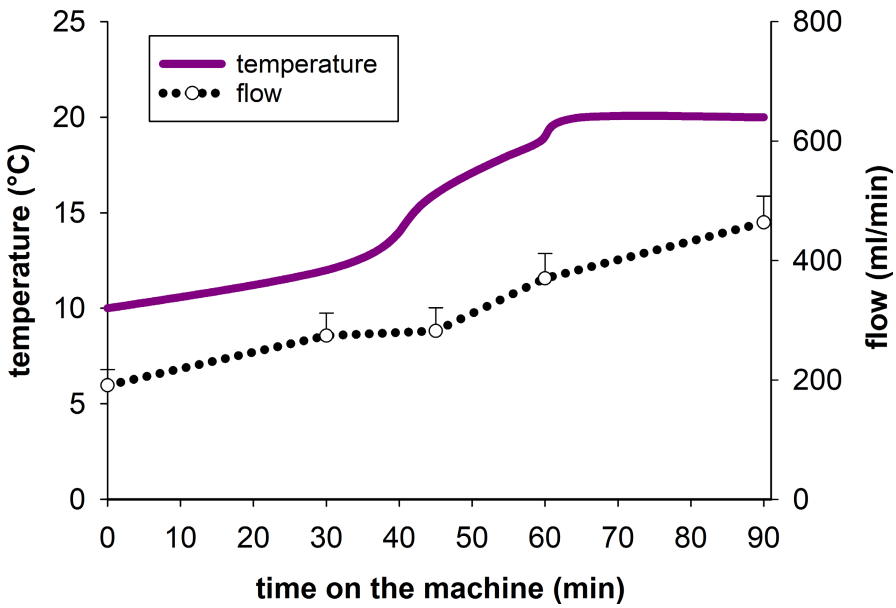

FIGURE 2 Time courses of temperature (solid line) and total liver flow during controlled oxygenated rewarming perfusion prior to transplantation

during the initial hypothermic period (1.0 ± 0.2 mmol/L) and slightly rose up on transition to the metabolically more active mid-thermic period. Mean lactate concentrations after 90 min of perfusion were 3.3 ± 0.4 mmol/L. Bile production during 90 min of cold to mid-thermic perfusion was mostly not detectable and did not qualify to be used as the prognostic parameter.

Postoperative outcome

Peak AST values within the first 3 days after liver transplantation were reduced by ~45% in the COR group compared to standard treatment, and this difference remained prominent until postoperative day 7 (cf. Table 2). However, the parameter was associated with a rather high SD and the difference between the two groups did not reach statistical significance.

Functional liver recovery after transplantation was evaluated by the LiMAx test, performed on the first postoperative day, while the patient was still intubated. Liver function was found to be significantly increased after COR treatment by more than one third of the values obtained in the cold storage group (Figure 3).

In line with this, serum concentrations of the coagulation factor V, which is synthesized exclusively in the liver, showed also significantly higher values (area under the curve) in the COR group than in the cold storage group during the observation period along the first 3 days after transplantation (Figure 3).

Early allograft dysfunction

EAD occurred in 20% after COR and 30% after SCS with no significant differences between the groups. Only serum bilirubin values were consistently found to be significantly higher in the SCS group until postoperative day 7.

Other secondary outcome parameters, like length of ICU stay, length of hospital stay, or 3 months graft survival did not show statistically significant differences among the groups.

One patient in the SCS group developed arterial thrombosis 1 day after transplantation and was successfully retransplanted. This patient is now well and alive.

Adverse events

The proportion of patients with postoperative complications (Clavien-Dindo grades I-V) was 14 of 20 after SCS and 10 of 20 after COR. A greater total number of postoperative complications was also observed in the SCS arm than after COR. The incidence of complications greater than or equal to grade IIIb according to Clavien-Dindo were 15 in the SCS group and eight after COR. No significances were calculated for this parameter.

TABLE 2 Clinical outcome parameters: values given as mean ± SD

	COR ($n = 20$)	SCS ($n = 20$)	p value
Primary end point			
Peak AST (U/L; POD1-3)	767 ± 1157	1371 ± 2871	0.273
Secondary end points			
Liver function (LiMAx; µg/kg/h)	294 ± 106	187 ± 121	0.006
Factor 5 (%; POD 1)	103 ± 34	66 ± 26	0.001
NH3 (mg/dl; POD 1)	60 ± 27	73 ± 28	0.113
AST (POD 7)	48 ± 62	90 ± 134	0.058
Bilirubin (POD 7)	1.6 ± 2.1	4.2 ± 4.5	0.006
INR (POD 7)	1.0 ± 0.1	1.1 ± 0.3	0.152
EAD, *n*	4	6	0.48
ICU stay, days	14.3 ± 11.2	16.6 ± 25.5	0.28
Hospital stay, days	30.3 ± 14.9	38.8 ± 26.6	0.261
3 month graft survival death censored, *n* (%)	20 (100%)	19 (95%)	>0.999
Re-transplantation, *n*	0	1	>0.999
Rejection episodes within 3 months, *n*	2	1	>0.999
Number of postoperative complications	12	23	
Clavien-Dindo Grades, *n*			
Grade I	0	1	
Grade II	1	4	
Grade III a	3	3	
Grade III b	2	7	
Grade IV a	2	5	
Grade IV b	0	0	
Grade V	4	3	

Abbreviations: AST, aspartate aminotransferase; COR, controlled oxygenated rewarming; EAD, early allograft dysfunction; ICU, intensive care unit; INR, International Normalized Ratio; LiMAx, Liver function test; SCS, static cold storage.

DISCUSSION

This is the first randomized controlled trial (RCT) which evaluates the therapeutic potential of a controlled

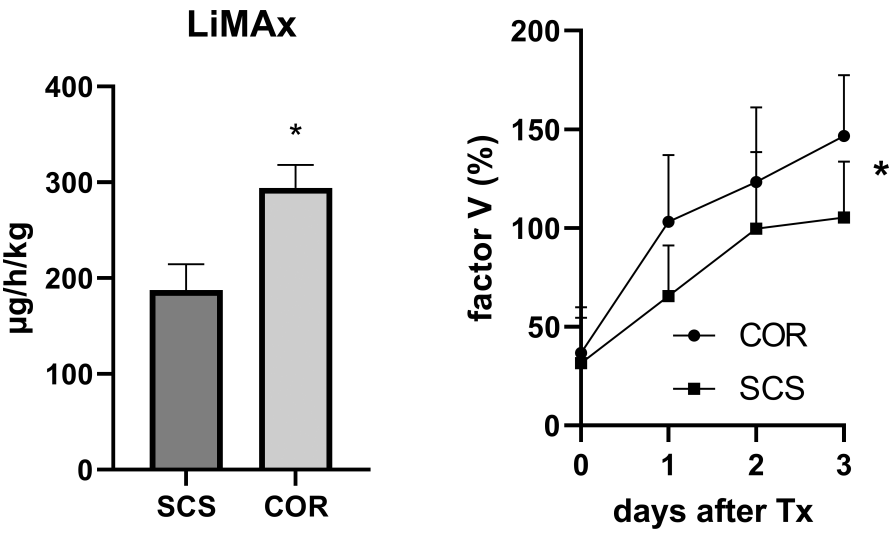

FIGURE 3 Postoperative recovery liver function after controlled oxygenated rewarming (COR) and after static cold storage (SCS). Left: Liver function test (LiMAx) on postoperative day 1 (*: $p < 0.05$ Mann–Whitney *U* test). Right: Recovery of serum activity of coagulation factor V during the first 3 days after transplantation *: $p < 0.05$ (area under the curve [AUC] Mann–Whitney *U* test). Tx, transplantation

transition from hypo- to mid-thermia by ex vivo machine perfusion prior to engraftment. The primary end point had been defined as the maximum serum concentration of AST during the first 3 days after transplantation, the mean value of which was found to be reduced by over 40% in the treatment group in comparison to the control arm. However, this difference did not reach statistical significance due to a high variance in the present study. On the other hand, functional evaluation of early liver recovery gave more consistent results. Maximal liver function as tested by the metabolism of ^{13}C labeled methacetin (LiMAx test)[23] on postoperative day 1 revealed an over 50% improved performance of the COR-treated livers, which was significant and in line with an accelerated synthesis of coagulation factor V over the first 3 days.

In so far, COR reduced early reperfusion injury to a similar extent than recently reported for continuous normothermic machine perfusion (NMP) from a European multicenter RCT, where graft injury was found reduced by ~50%.[18]

Likewise, the first North American results on NMP instead of static cold storage disclosed an albeit nonsignificant 35% reduction of peak AST by continuous machine perfusion and a reduction of serum bilirubin after 7 days by 45%.[24] A common denominator of both techniques can be seen in the mitigation if not prevention of abrupt rewarming after extended periods of hypothermia, along with the concomitant cellular injury. Taken into account that limited periods of hypothermic storage do not instantly promote irreversible tissue damage it is thus conceivable that potentially reversible disturbances of cellular homeostasis may trigger the exacerbation of cellular injury upon abrupt rewarming of tissue still subjected to hypothermic torpor.[11]

Preclinical experimental studies have systematically shown that NMP by far works best, only if executed without prior periods of cold preservation.[25,26] By contrast, slow elevation of perfusate temperature during postponed machine perfusion after initial cold storage significantly mitigates the impediments incurred during abrupt normothermic machine perfusion.[8,15,27] The underlying mechanism responsible for reperfusion injury being in large part linked to the abrupt rewarming process have been extensively studied in systematic experimental investigations.[11]

After several hours[28,29] of adaptation to hypothermia below ~13°C,[9] an abrupt rise in temperature ensues in adaptive dysfunction of subcellular components predominantly on the mitochondrial level. The pivotal part of the rewarming injury takes place while the temperature rises between 10°C and 20°C and results in abundant generation of oxygen free radicals, mitochondrial transition pore opening, and induction of apoptosis[29] and respiratory chain uncoupling.[30]

The protection provided by mitigation of the abrupt thermal transit after cold storage has been found already in preclinical studies to be at least equal to continuous normothermic perfusion during the whole preservation period.[27]

In terms of practicability, the end-ischemic approach of controlled rewarming at the implantation clinic appears to be very attractive. It precludes the necessity of perfusion devices being timely available at the retrieval site. Current procurement techniques do not need to be changed or made more complex. Trafficking of machines or continuous surveillance of the transported graft, as required for continuous normothermic perfusion techniques, are not necessary.

The pilot character of the present trial, however, goes along with several limitations.

By design, this investigation has concentrated only on livers that were deemed well transplantable to the surgeon, and were accepted for the recipient, irrespective of the potential use of the machine. It hence remains open to debate if minimization of rewarming injury would allow for further extension of eligibility criteria for liver transplantation.

The limited number of patients included in this primary trial argues for a judicious interpretation of the results in one or the other direction. Nonetheless, valuable data have been provided for the first time under controlled conditions which do represent a solid base for the indication and planning of a larger confirmatory study.

In summary, this study has demonstrated feasibility of a rewarming machine perfusion in a controlled setting following arrival at the implantation center and improved early graft function after transplantation. In how far mitigation of immediate rewarming/reperfusion injury eventually also translates into enhanced long-term results, remains to be seen in the years to come.

AUTHOR CONTRIBUTIONS

T.M., C.v.H., M.G., H.Z., F.S., B.L., E.-M.H., N.K., and A.P. wrote the manuscript. T.M. designed the research. T.M., C.v.H., H.Z., F.S., B.L., and M.G. performed the research. C.v.H., H.Z., F.S., and E.-M.H. analyzed the data.

FUNDING INFORMATION

Else Kröner Fresenius Stiftung, Key research project. This Article reports independent research, funded by an unrestricted grant of the Else Kröner Fresenius Stiftung given to Thomas Minor. Open Access funding enabled and organized by Projekt DEAL. [Correction added on 20 October 2022, after first online publication: Projekt Deal funding statement has been added.]

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Minor T, von Horn C, Zlatev H, et al. Controlled oxygenated rewarming as novel end-ischemic therapy for cold stored liver grafts. A randomized controlled trial. *Clin Transl Sci*. 2022;15:2918-2927. doi:10.1111/cts.13409

DOI: 10.1111/cts.13409
URN: urn:nbn:de:hbz:465-20230925-144136-5