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Hypothermic Machine Perfusion in Liver Transplantation — A Randomized Trial

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ABSTRACT

BACKGROUND

Transplantation of livers obtained from donors after circulatory death is associated with an increased risk of nonanastomotic biliary strictures. Hypothermic oxygenated machine perfusion of livers may reduce the incidence of biliary complications, but data from prospective, controlled studies are limited.

METHODS

In this multicenter, controlled trial, we randomly assigned patients who were undergoing transplantation of a liver obtained from a donor after circulatory death to receive that liver either after hypothermic oxygenated machine perfusion (machine-perfusion group) or after conventional static cold storage alone (control group). The primary end point was the incidence of nonanastomotic biliary strictures within 6 months after transplantation. Secondary end points included other graft-related and general complications.

RESULTS

A total of 160 patients were enrolled, of whom 78 received a machine-perfused liver and 78 received a liver after static cold storage only (4 patients did not receive a liver in this trial). Nonanastomotic biliary strictures occurred in 6% of the patients in the machine-perfusion group and in 18% of those in the control group (risk ratio, 0.36; 95% confidence interval [CI], 0.14 to 0.94; P=0.03). Postreperfusion syndrome occurred in 12% of the recipients of a machine-perfused liver and in 27% of those in the control group (risk ratio, 0.43; 95% CI, 0.20 to 0.91). Early allograft dysfunction occurred in 26% of the machine-perfused livers, as compared with 40% of control livers (risk ratio, 0.61; 95% CI, 0.39 to 0.96). The cumulative number of treatments for nonanastomotic biliary strictures was lower by a factor of almost 4 after machine perfusion, as compared with control. The incidence of adverse events was similar in the two groups.

CONCLUSIONS

Hypothermic oxygenated machine perfusion led to a lower risk of nonanastomotic biliary strictures following the transplantation of livers obtained from donors after circulatory death than conventional static cold storage. (Funded by Fonds NutsOhra; DHOPE-DCD ClinicalTrials.gov number, NCT02584283.)

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*A complete list of collaborators and their roles in the DHOPE-DCD trial is provided in the Supplementary Appendix, available at NEJM.org.

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Nonanastomotic biliary strictures are a major complication after liver transplantation, resulting in cholestasis and cholangitis and, frequently, in the use of biliary interventions or even retransplantation (i.e., transplantation of a new liver graft and removal of the first graft).[1,2] The incidence of nonanastomotic biliary strictures is approximately 3 times as high after the transplantation of livers obtained from donors after circulatory death as after the transplantation of livers obtained from brain-dead donors.[2,3] Nevertheless, liver grafts from donors after circulatory death are increasingly used for transplantation owing to persistent donor-organ shortage.[4,5]

Ischemia–reperfusion injury is a key mechanism in the pathogenesis of bile-duct injury and the subsequent development of biliary strictures after transplantation.[1,3] Although conventional static cold preservation provides some protection against ischemia–reperfusion injury, more-advanced preservation methods are needed to improve outcomes after transplantation of livers obtained from donors after circulatory death and to increase the frequency of their use.[4]

Oxygenated ex situ machine perfusion is a dynamic preservation method that has been developed to reduce the incidence and severity of ischemia–reperfusion injury and to improve outcomes after organ transplantation.[6-9] Preclinical studies have shown that a short period (1 to 2 hours) of hypothermic oxygenated machine perfusion restores mitochondrial function and reduces the production of radical oxygen species and damage-associated molecular patterns after transplantation.[10-12] This relatively simple technique can be performed after static cold storage.[6,8] The first clinical experiences suggested that this preservation method was safe, reduced the incidence of hepatobiliary preservation injury, and was associated with improved early graft function, as compared with static cold preservation alone.[13-16] Although these findings were promising and have increased the interest in machine-based preservation techniques, they were based on small single-center cohorts without a randomized control group. We conducted a multicenter, randomized, controlled trial to compare hypothermic oxygenated machine perfusion with static cold preservation in the transplantation of livers from donors after circulatory death, with the incidence of nonanastomotic biliary strictures as the primary end point.

METHODS

TRIAL DESIGN AND OVERSIGHT

The DHOPE-DCD (Dual Hypothermic Oxygenated Perfusion of DCD Liver Grafts in Preventing Nonanastomotic Biliary Strictures after Transplantation) trial was investigator-initiated and was designed as a multicenter, prospective, two-group, randomized, controlled, clinical trial. The trial was conducted in six liver-transplantation centers in Europe. Centralized balanced-block randomization (in blocks of six) was computer-generated, with stratification according to trial center and primary sclerosing cholangitis as an indication for transplantation (yes or no). Patients were randomly assigned in a 1:1 ratio to receive a liver preserved either with hypothermic oxygenated machine perfusion after static cold preservation during transportation (machine-perfusion group) or with static cold preservation alone (control group). Randomization took place immediately after a donor liver had been deemed to be suitable and had been accepted by the transplantation surgeon for a recipient. The trial did not interfere with the regular process of organ allocation or acceptance.

The trial protocol, which is available with the full text of this article at NEJM.org, has been published previously.[17] The protocol was approved by research ethics committees at each trial site and medical-device regulatory bodies in each country. Patients and the organ-procurement teams were unaware of the trial-group assignments. The authors designed and implemented the trial and collected and analyzed the data. The first and last authors wrote the first draft of the manuscript, and all the authors contributed to the subsequent versions. All the authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. Access to the data was not restricted by confidentiality agreements.

Fonds NutsOhra supported this trial. Bridge to Life provided the machine-perfusion fluid (Belzer MPS UW machine-perfusion solution) free of charge. Each participating center covered the costs for the purchase of a machine-perfusion device, and training of perfusionists was pro-

vided by the manufacturer (Organ Assist) as part of their regular after-sales responsibilities. The funding organization and the companies had no role in the trial design; the collection, management, analysis, or interpretation of the data; or the writing of the manuscript and the decision to submit it for publication.

TRIAL PATIENTS

Patients 18 years of age or older who were undergoing liver-only transplantation with a graft from a donor after circulatory death (in controlled circumstances) were eligible for inclusion in the trial. Patients were excluded if the body weight of the donor was less than 40 kg or if the donor was positive for the human immunodeficiency virus or hepatitis B or C virus. Patients were also excluded if they were undergoing transplantation for fulminant liver failure or for primary nonfunction after a previous transplantation, were incapable of providing informed consent, were positive for the human immunodeficiency virus, or had a contraindication to undergoing magnetic resonance cholangiography. All the patients provided written informed consent.

Donor livers were obtained, preserved, and transported to the transplantation centers according to standard practice, with the use of conventional static cold preservation. The transplantation surgery and postoperative care were performed according to standard local practice.

HYPOTHERMIC OXYGENATED MACHINE PERFUSION

The Liver Assist device (Organ Assist) was used for ex situ machine perfusion of the liver. The device enables pressure-controlled, dual perfusion through the portal vein and the hepatic artery with the use of two centrifugal pumps, providing continuous portal flow and a pulsatile arterial flow at 60 beats per minute. The perfusion device was primed with 4 liters of cold Belzer machine-perfusion solution (Bridge to Life), supplemented with 3 mmol of glutathione per liter of solution (Biomedica). The perfusion pressure was 25 mm Hg for the hepatic artery and 5 mm Hg for the portal vein. The temperature of the perfusion fluid was 10°C. Oxygenation was provided by 500 ml per minute of 100% oxygen flow to each oxygenator.[15] The minimum protocol-stipulated duration of machine perfusion was 2 hours, a duration that is considered to be sufficient to restore mitochondria and intrahepatic ATP and to protect organs against ischemia–reperfusion injury.[10,11,15] Additional details are provided in the Supplementary Appendix, available at NEJM.org.

END-POINT MEASURES

The primary end point was the incidence of symptomatic nonanastomotic biliary strictures at 6 months after transplantation. The occurrence of nonanastomotic biliary strictures was assessed primarily by the medical teams of the participating centers on the basis of the presence of the following prespecified criteria: any irregularity or narrowing of the lumen of the intrahepatic or extrahepatic donor bile ducts, excluding the biliary anastomosis, diagnosed with the use of cholangiography (preferably, magnetic resonance cholangiography), in combination with clinical symptoms (e.g., jaundice or cholangitis) or an elevation of cholestatic laboratory variables, in the presence of a patent hepatic artery. All clinical data, including data from cholangiographies, were submitted to the central data center for review. To avoid reporting bias, magnetic resonance cholangiography was performed after 6 months, in accordance with the study protocol, to detect radiologic evidence of cholangiopathy (nonanastomotic strictures) in patients who had not already received a diagnosis in the preceding time period. Nonanastomotic biliary strictures are typically detected 3 to 4 months after transplantation, and an observation period of 6 months was therefore considered to be appropriate for the detection of clinically meaningful events.[1,18] All the cholangiographies, both in patients who were symptomatic and in those who were asymptomatic, were reviewed by two independent radiologists who were unaware of the preservation method and clinical symptoms. In the case of discordant readings, a third radiologist was consulted for cases that could not be settled by consensus.

Secondary end points included intraoperative postreperfusion syndrome,[19,20] defined as a decrease of more than 30% in the mean systemic arterial blood pressure within 10 minutes after reperfusion, with or without a doubling of the norepinephrine dose; primary nonfunction, defined as liver failure, without an identifiable

cause, that necessitated retransplantation or led to death within 7 days after transplantation; early allograft dysfunction, assessed according to the Olthoff criteria[21]; and durations of stay in the intensive care unit and hospital. Other secondary end points included thrombosis of the hepatic artery or portal vein, anastomotic biliary strictures or leakage, and use of renal-replacement therapy within 6 months after transplantation. Serum markers of hepatobiliary injury and function were recorded daily during the first week and at 1 month, 3 months, and 6 months after transplantation. Patient and graft survival were recorded up to 1 year after the transplantation.

STATISTICAL ANALYSIS

The trial was powered to detect a clinically relevant difference in the incidence of symptomatic nonanastomotic biliary strictures between the two trial groups. On the basis of previous reports about the transplantation of livers obtained from donors after circulatory death, we presumed an incidence of 29% among livers that had been preserved by static cold storage, and we expected that the incidence with machine perfusion would be 11% (proportional reduction, 60%).[22-25] On the basis of a power of 80% and a 5% significance level (two-sided test) in two independent groups, we calculated that 77 livers would be needed in each trial group. We aimed to include 1 additional patient per trial group, resulting in 78 patients per group.

All end-point analyses were prespecified in the protocol and statistical analysis plan, which was finalized before the database was locked. The primary end point was analyzed with the use of a chi-square test, as well as in a log-binomial regression model with calculation of risk ratios. Prespecified covariates in this model were based on relevant literature and included stratification factors (trial site and primary sclerosing cholangitis) and donor-specific risk factors (donor risk index and donor warm-ischemia time, defined as the time period between circulatory arrest and in situ cold flush-out in the donor).[26,27] For consistency with the original protocol, we also analyzed the results using logistic-regression modeling and report them in the Supplementary Appendix. Time-to-event outcomes were analyzed with the use of Kaplan–Meier curves with a log-rank test and Cox proportional-hazards regression model with the calculation of hazard ratios. Secondary binary end points were assessed by means of a chi-square test or log-binomial regression to adjust for stratification factors. Continuous (log-transformed) outcomes were compared with the use of an independent Student's t-test. Missing data were assumed to be missing at random, and multiple imputation was performed when more than 10% of all the patients had missing data for a specific variable. There was no adjustment for multiplicity in analyses of secondary end points, and these analyses should be considered to be exploratory. Tests were two-sided, and results are reported with 95% confidence intervals. A P value of less than 0.05 was considered to indicate statistical significance. Analyses were performed with the use of SPSS software, version 23.0 (SPSS).

RESULTS

PATIENTS

From January 2016 through July 2019, we assessed a total of 245 patients for eligibility, of whom 160 underwent randomization. After randomization, four transplantations were canceled before any trial procedure was started. In one case, the liver was intended to undergo machine perfusion, and in three cases, the liver had been assigned to the control group. The reasons for cancelation were massive steatosis in two livers and a nonreconstructable damaged artery in another liver. These three livers had initially been deemed transplantable and had been accepted; they were secondarily rejected on the basis of this new information. In one patient, pseudomyxoma peritonei was detected after laparotomy; the transplantation was canceled and the liver was allocated to another patient outside the trial. This resulted in the inclusion of 78 patients in the machine-perfusion group and 78 patients in the control group (Fig. S1 in the Supplementary Appendix).

The baseline characteristics of the donors and recipients were well matched in the two trial groups (Table 1). Inherent to the intervention, the static cold-ischemia time was slightly shorter in the machine-perfusion group than in the control group (6 hours 11 minutes vs. 6 hours 49 minutes) and the total preservation time was longer (8 hours 44 minutes vs. 6 hours 49 minutes).

Table 1. Characteristics of the Donors and Recipients at Baseline.*

Characteristic	Machine Perfusion (N=78)	Control (N=78)
Donor characteristics		
Age — yr		
Median	52	49
Interquartile range	43–57	37–59
Male sex — no. (%)	52 (67)	51 (65)
Donor risk index†		
Median	2.12	2.12
Interquartile range	1.84–2.38	1.86–2.42
Body-mass index‡		
Median	25	25
Interquartile range	23–27	21–28
Preservation characteristics		
Time from withdrawal of life support to aortic flush-out — min		
Median	29	27
Interquartile range	22–33	21–35
Time from circulatory arrest in the donor to aortic flush-out — min		
Median	11	11
Interquartile range	8–13	8–15
Static cold-ischemia time§		
Median	6 hr 11 min	6 hr 49 min
Interquartile range	5 hr 16 min–6 hr 55 min	5 hr 56 min–7 hr 57 min
Machine-perfusion time		
Median	2 hr 12 min	NA
Interquartile range	2 hr 00 min–2 hr 33 min	NA
Total preservation time¶		
Median	8 hr 44 min	6 hr 49 min
Interquartile range	7 hr 46 min–9 hr 16 min	5 hr 56 min–7 hr 57 min
Recipient characteristics		
Age — yr		
Median	60	60
Interquartile range	52–65	52–65
Male sex — no. (%)	55 (71)	52 (67)
Laboratory MELD score‖		
Median	14	16
Interquartile range	10–19	10–22
Renal-replacement therapy — no. (%)	3 (4)	2 (3)

* Data on additional characteristics, including causes of death of the donor and indications for transplantation, are provided in Table S1. NA denotes not applicable.

† The donor risk index is a scoring system that was developed to quantitatively predict the risk of post-transplantation graft failure in liver transplantation, on the basis of donor risk factors.[27]

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

§ The static cold-ischemia time was defined as time between aortic cold flush-out in the donor to reperfusion in the recipient, minus the machine perfusion time. P<0.001 for the comparison of the two groups.

¶ The total preservation time was defined as the time between aortic cold flush-out in the donor to reperfusion in the recipient. P<0.001 for the comparison of the two groups.

‖ The laboratory Model of End-Stage Liver Disease (MELD) score ranges from 6 to 40, with higher scores indicating more advanced disease. The laboratory MELD score is based on original laboratory variables; MELD exception points, which are used to assign increased priority on the waiting list to patients whose severity of illness or risk of complications is not captured by the laboratory MELD score, are not included in the scores shown here.

Table 2. Primary and Secondary End Points.*

Outcome	Machine Perfusion (N=78)	Control (N=78)	Treatment Effect (95% CI)	P Value
Primary end point†				
Nonanastomotic biliary strictures — no. (%)	5 (6)	14 (18)		0.03
Unadjusted risk ratio			0.36 (0.14 to 0.94)	0.03
Adjusted risk ratio			0.35 (0.14 to 0.92)	0.03
Secondary end points				
Postreperfusion syndrome				
>30% decrease in systemic mean arterial pressure — no./total no. (%)	9/72 (12)	19/70 (27)	0.43 (0.20 to 0.91)‡	
>30% decrease in systemic mean arterial pressure or ≥100% increase in norepinephrine dose — no./total no. (%)	20/72 (28)	33/72 (46)	0.59 (0.38 to 0.92)‡	
Serum potassium after reperfusion — mmol/liter§	4.1±0.7	4.4±1.1	−0.4 (−0.1 to −0.6)	
Graft-related complication — no. (%)				
Early allograft dysfunction¶	20 (26)	31 (40)	0.61 (0.39 to 0.96)	
Primary nonfunction	0	1 (1)	NA	
Hepatic-artery thrombosis	2 (3)	2 (3)	0.94 (0.12 to 7.19)‡	
Portal-vein thrombosis	0	2 (3)	NA	
Biliary anastomotic stricture	23 (29)	22 (28)	1.07 (0.52 to 2.20)‡	
Biliary anastomotic leakage	6 (8)	8 (10)	0.69 (0.22 to 2.13)‡	
Renal failure leading to dialysis — no. (%)	7 (9)	7 (9)	0.79 (0.27 to 2.34)‡	
Median duration of stay (interquartile range) — days				
In the intensive care unit	2 (2 to 5)	2 (1 to 4)	NA	
In the hospital	15 (12 to 20)	15 (12 to 26)	NA	
Retransplantation within 6 mo — no. (%)	3 (4)	6 (8)	0.49 (0.12 to 1.94)‖	
Primary nonfunction — no.	0	1		
Hepatic-artery thrombosis — no.	2	1		
Severe liver laceration — no.**	0	2		
Nonanastomotic biliary strictures — no.	0	2		
Secondary liver dysfunction in the context of multiorgan failure of unknown origin — no.	1	0		
Death of patient within 6 mo — no. (%)	6 (8)	4 (5)	1.46 (0.41 to 5.21)‖	
Multiorgan failure — no.	2	0		
Sepsis — no.	0	3		
Respiratory failure — no.	2	1		
Anoxic brain injury — no.	1	0		
Hemophagocytic syndrome — no.	1	0		

* Plus–minus values are means ±SD. Because of an absence of events in one group or an obvious lack of difference, some treatment differences were not assessed (NA). The widths of the confidence intervals have not been adjusted for multiplicity, and so the inferences drawn from them may not be reproducible.

† The P value for the first assessment of the primary end point is from a chi-square test. The other two P values are based on the unadjusted and adjusted log-binomial regression analysis. For the adjusted analysis, the risk ratio and 95% confidence interval were adjusted for prespecified covariates, including stratification factors (transplantation center and primary sclerosing cholangitis) and established donor risk factors (donor warm-ischemia time and donor risk index).

‡ The treatment effect is expressed as risk ratio and 95% confidence interval, with adjustment for stratification factors.

§ Data were available for 54 patients in the machine-perfusion group and for 60 in the control group. The results of statistical testing are after multiple imputations. The treatment effect is expressed as the mean difference and 95% confidence interval.

¶ Early allograft dysfunction was defined as any one of the following clinical indicators: a bilirubin level of at least 171 μmol per liter (10 mg per deciliter) on postoperative day 7; an international normalized ratio of at least 1.6 on postoperative day 7; or alanine aminotransferase and aspartate aminotransferase levels of more than 2000 U per liter within the first 7 postoperative days. Data were available for all patients. The treatment effect is expressed as a risk ratio and 95% confidence interval, with adjustment for stratification factors.

‖ The treatment effect is expressed as a hazard ratio and 95% confidence interval, with adjustment for stratification factors.

** Liver laceration occurred during the donor hepatectomy and caused severe bleeding and subcapsular hematoma after reperfusion in the recipient, necessitating gauze packing and listing for retransplantation.

PRIMARY END POINT

Symptomatic nonanastomotic biliary strictures occurred in 5 of 78 patients (6%) in the machine-perfusion group and in 14 of 78 (18%) in the control group (risk ratio, 0.36; 95% confidence interval [CI], 0.14 to 0.94; P=0.03). When the analysis was adjusted for stratification factors and prespecified donor risk factors in the log-binomial regression model, the result remained essentially the same (Table 2). These findings were confirmed in the time-to-event analyses that used the Kaplan–Meier method and Cox regression analysis (hazard ratio, 0.32; 95% CI, 0.11 to 0.89; P=0.03; P=0.03 also by the log-rank test) (Fig. 1).

All 19 patients who had symptomatic nonanastomotic strictures received the diagnosis before the trial magnetic resonance cholangiography was performed at 6 months after transplantation, and all had clinical symptoms or cholestatic laboratory tests (or both) that supported this diagnosis (Table 3). Blinded review of the cholangiograms in symptomatic patients confirmed radiologic evidence of nonanastomotic strictures.

A sensitivity analysis that involved all the patients who completed 6 months of follow-up, including the trial magnetic resonance cholangiography, did not change the conclusion (Table S2). Given the small between-group difference in the static cold-ischemia time, we conducted a post hoc sensitivity analysis with this variable as a covariate in the log-binomial regression model; the conclusion did not change.

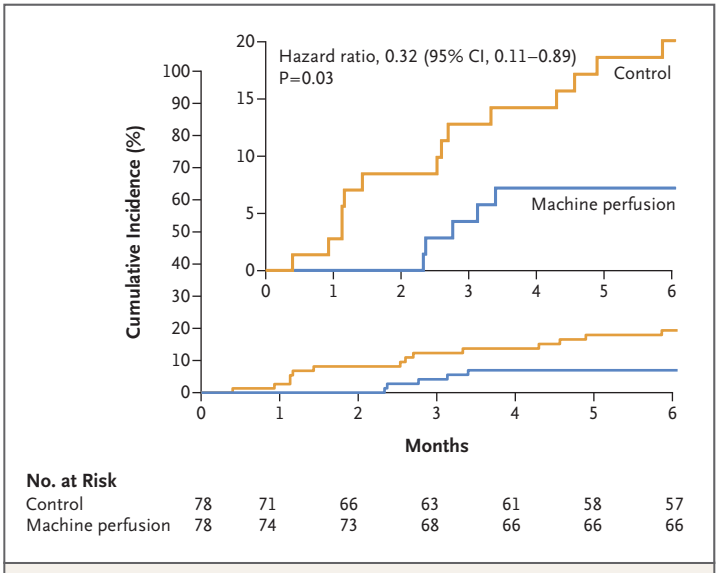

Figure 1. Cumulative Incidence of Symptomatic Nonanastomotic Biliary Strictures.

Shown are the time-to-event Kaplan–Meier curves for symptomatic nonanastomotic biliary strictures within 6 months after liver transplantation (primary end point). The hazard ratio was adjusted for stratification factors (transplantation center and primary sclerosing cholangitis) and for prespecified, established donor risk factors (donor warm-ischemia time and donor risk index); the P value is from a Cox regression analysis. P=0.03 also by the log-rank test. The inset shows the same data on an enlarged y axis.

SECONDARY END-POINT MEASURES

Intraoperatively, the postreperfusion syndrome, which was defined as a decrease of more than 30% in the mean arterial blood pressure, occurred less frequently in recipients of a machine-perfused liver than in those in the control group (12% vs. 27%; adjusted risk ratio, 0.43; 95% CI, 0.20 to 0.91). This difference remained when we included increased inotropic support in the definition (Table 2). In line with this, the mean (±SD) serum potassium levels immediately after transplantation were lower in the machine-perfusion group than in the control group (4.1±0.7 mmol per liter vs. 4.4±1.1 mmol per liter; mean difference, −0.4 mmol per liter; 95% CI, −0.1 to −0.6).

Early allograft dysfunction occurred in 20 machine-perfused livers (26% of the patients), as compared with 31 control livers (40% of the patients) (adjusted risk ratio, 0.61; 95% CI, 0.39 to 0.96). There were no cases of primary nonfunction in the machine-perfusion group, but one case was observed in the control group.

The cumulative number of treatments for nonanastomotic biliary strictures and related complications within 6 months after transplantation was lower by a factor of almost 4 in the machine-perfusion group than in the control group (Table 3). Two patients, both in the control group, underwent retransplantation because of severe nonanastomotic strictures. There were no between-group differences in the incidence of anastomotic biliary leakage or strictures (Table 2). Results of the blinded review of all cholangiograms are presented in Tables S3 through S7.

Laboratory analyses of serum liver-function tests are presented in Figure S2. In accordance with the higher percentage of patients with symptomatic nonanastomotic biliary strictures in the control group than in the machine-perfusion group, serum cholestasis markers in the control group were higher than those in the

Table 3. Clinical Symptoms, Laboratory Abnormalities, and Biliary Interventions in Patients with Symptomatic Nonanastomotic Biliary Strictures (NAS).*

Variable	Time from Transplantation to First Signs of NAS	Cholestatic Laboratory Tests at Time of Detection of NAS†			NAS-Related Biliary Treatment or Intervention			
		γ-Glutamyl-transferase	Alkaline Phosphatase	Bilirubin‡	Antibiotics for Cholangitis	Endoscopic or Percutaneous Stenting	Readmission	Reoperation
	no. of days	*U/liter*		*μmol/liter*	*no. of events*			
Machine-perfusion group								
Patient no.								
1	94	149	316	30	1	—	1	—
2	70	219	222	157	1	1	1	—
3	83	91	217	30	—	2	2	—
4	102	48	139	14	1§	—	1	—
5	71	743	547	9	1	2	1	—
Total no. of events					4	5	6	—
Control group								
Patient no.								
6	34	991	754	40	1	—	—	—
7	28	338	962	320	1	—	1	Retransplantation for NAS
8	12	63	157	8	—	—	—	Resection of bile duct¶
9	81	832	1150	31	1	2	1	—
10	176	618	650	30	—	1	1	—
11	34	1091	250	48	1	2	3	—
12	129	695	653	30	1	1	1	—
13	147	226	733	22	1	1	1	—
14	137	1430	1065	77	1	5	1	—
15	43	366	323	19	4	1	4	—
16	76	189	144	9	—	2	2	—
17	35	370	272	19	1	2	—	—
18	100	560	317	200	1	4	1	—
19	78	2801	4017	376	1	1	1	Retransplantation for NAS
Total no. of events					14	22	17	3

* All the patients had radiologically confirmed nonanastomotic strictures of the donor bile ducts that were detected before the trial magnetic resonance cholangiography at 6 months after transplantation and in the presence of a patent hepatic artery. Cholangiographic details are provided in the Supplementary Appendix. Interventions for other types of biliary complications, such as anastomotic strictures, are excluded from this overview.

† Laboratory results were based on the nearest available prespecified sample point at 1 month, 3 months, or 6 months after transplantation.

‡ To convert the values for serum bilirubin from micromoles per liter to milligrams per deciliter, divide by 17.1.

§ The patient had clinical evidence of bacterial cholangitis. Cholangiography revealed multiple nonanastomotic strictures of the intrahepatic donor bile ducts. Despite treatment with antibiotic agents and ursodeoxycholic acid, the values on the cholestatic laboratory tests worsened; at 6 months after transplantation, the alkaline phosphatase level was 378 IU per liter and the γ-glutamyltransferase level was 263 U per liter.

¶ The patient underwent resection of a necrotic extrahepatic bile duct. There were multiple strictures of the intrahepatic bile ducts on cholangiography.

machine-perfusion group at 3 months (alkaline phosphatase and bilirubin) and 6 months (alkaline phosphatase). There were no relevant differences between the two groups in the use of renal-replacement therapy, in the durations of stay in the intensive care unit or hospital, or in graft and patient survival at 1 year (Table 2 and Fig. S3).

SAFETY AND ADVERSE EVENTS

The distribution of patients for whom adverse events were reported was similar in the two groups (Table 4). There was no relevant clinical difference between the two groups in the severity of adverse events (Table S8).

Table 4. All Reported Adverse Events within 6 Months after Transplantation.*

Event	Machine Perfusion (N=78)	Control (N=78)
Total no. of events	644	694
Infection — no. (%)	131 (20)	162 (23)
Rejection of transplanted liver — no. (%)	9 (1)	16 (2)
Renal event — no. (%)	47 (7)	36 (5)
Hepatic event — no. (%)	91 (14)	111 (16)
Cardiovascular event — no. (%)	52 (8)	52 (7)
Respiratory event — no. (%)	36 (6)	29 (4)
Neurologic event — no. (%)	62 (10)	55 (8)
Gastrointestinal event — no. (%)	43 (7)	51 (7)
Hematologic event — no. (%)	39 (6)	41 (6)
Dermatologic event — no. (%)	19 (3)	12 (2)
Endocrine event — no. (%)	20 (3)	25 (4)
Cancer — no. (%)	3 (<1)	1 (<1)
Miscellaneous adverse event — no. (%)	91 (14)	103 (15)
Device error — no. (%)†	1 (<1)	0

* The data shown are the numbers of reported adverse events; the percentages are based on the total number of reported adverse events (rather than on the total number of patients). Patients could have had more than one event, and no statistical test was applied to these data. Percentages may not total 100 because of rounding.

† Leakage of the disposable tubing set was reported in one case before machine perfusion was started. After replacement of this disposable set, high flows were noted owing to a malfunctioning pressure sensor caused by a user error. This was immediately corrected without injury to the liver.

DISCUSSION

In this trial involving patients receiving a liver graft from a donor after circulatory death, those who had been randomly assigned to receive the liver graft after hypothermic oxygenated machine perfusion had a risk of symptomatic nonanastomotic biliary strictures within 6 months after transplantation that was approximately two thirds lower than those who had been randomly assigned to receive the liver graft after conventional static cold preservation alone. The lower incidence of this type of cholangiopathy was both statistically and clinically significant.

Nonanastomotic biliary strictures are a result of incomplete recovery from biliary ischemia–reperfusion injury, resulting in fibrotic narrowing of the bile-duct lumen and obstruction of bile flow.[1,3] Although some patients can be treated with endoscopic or percutaneous interventions, strictures are often resistant to dilatations and stenting, and retransplantation may remain the only definitive therapy.[18,22,24] In the present trial, the cumulative number of interventions for nonanastomotic biliary strictures and antibiotic therapy for related cholangitis was lower by a factor of almost 4 among machine-perfused livers than among control livers. Two patients in the control group underwent retransplantation within 6 months because of severe cholangiopathy.

The protective effect of machine perfusion was also shown by the lower risk of postreperfusion syndrome and early allograft dysfunction. Graft reperfusion is often accompanied by hepatic release of potassium and circulatory instability.[28] In a clinical pilot study,[29] a reduction in serum potassium levels was observed after the transplantation of hypothermic machine-perfused livers, and this benefit was confirmed in the current prospective trial.

Important advantages of hypothermic machine perfusion over other dynamic preservation methods, such as normothermic machine perfusion, are its relative simplicity and intrinsic safety. Technical malfunction leading to insufficient hepatic perfusion would not immediately be detrimental because the organ is maintained at low temperature. This situation differs from normothermic machine perfusion, in which device or operator errors result in warm ischemia and may lead to organ loss.[30-32] Another advantage of hypothermic machine perfusion is that it is effective after static cold storage. Although a transportable hypothermic perfusion device is currently under clinical investigation (ClinicalTrials.gov number, NCT03484455), it is still undetermined whether this provides additional benefit.

Despite the restoration of ATP, hepatic metabolism remains suppressed and livers do not produce bile during this type of machine perfusion. Although the release of mitochondrial flavin mononucleotide into the perfusate has been correlated with hepatic function after transplantation, it remains unknown whether this also predicts the risk of cholangiopathy.[33] In contrast to normothermic machine perfusion, hypothermic machine perfusion is, therefore, currently not considered to be a tool for viability testing before transplantation; rather, it is a method to reduce the incidence of ischemia–reperfusion injury. This makes it suited for donor livers with an increased risk of development of ischemia-related complications, such as livers obtained from donors after circulatory death. To this end, hypothermic and normothermic machine perfusion serve different goals and are not competing techniques. The two techniques can be applied sequentially with complementary benefits.[34-36] Whether hypothermic machine perfusion is also beneficial in the transplantation of livers obtained from brain-dead donors is the subject of ongoing clinical trials (NCT01317342 and NCT03124641).

In the present trial, machine perfusion did not have an effect on patient or graft survival. Given the high percentage of patients who survive after liver transplantation and the relatively low risk of graft loss, much larger trials would be needed to detect an effect on these outcome measures.

Reimbursement of this new technology by health care funders will involve a health-economic evaluation. Costs for transplantation of a liver from a donor after circulatory death are 25 to 30% higher than those for transplantation of livers from brain-dead donors, mainly because of the higher incidence of biliary complications.[37,38] The prevention of post-transplantation cholangiopathy may not only increase the acceptance for transplantation of liver grafts obtained from donors after circulatory death but may also make the use of machine perfusion cost-effective.

In this randomized trial involving patients who underwent transplantation of a liver obtained from a donor after circulatory death, we found that hypothermic oxygenated machine perfusion led to a lower incidence of symptomatic nonanastomotic biliary strictures than conventional static cold preservation.

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APPENDIX

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