BRIEF COMMUNICATION

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Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution

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Ex situ dual hypothermic oxygenated machine perfusion (DHOPE) and normothermic machine perfusion (NMP) of donor livers may have a complementary effect when applied sequentially. While DHOPE resuscitates the mitochondria and increases hepatic adenosine triphosphate (ATP) content, NMP enables hepatobiliary viability assessment prior to transplantation. In contrast to DHOPE, NMP requires a perfusion solution with an oxygen carrier, for which red blood cells (RBC) have been used in most series. RBC, however, have limitations and cannot be used cold. We, therefore, established a protocol of sequential DHOPE, controlled oxygenated rewarming (COR), and NMP using a new hemoglobin-based oxygen carrier (HBOC)-based perfusion fluid (DHOPE-COR-NMP trial, NTR5972). Seven livers from donation after circulatory death (DCD) donors, which were initially declined for transplantation nationwide, underwent DHOPE-COR-NMP. Livers were considered transplantable if perfusate pH and lactate normalized, bile production was ≥10 mL and biliary pH > 7.45 within 150 minutes of NMP. Based on these criteria five livers were transplanted. The primary endpoint, 3-month graft survival, was a 100%. In conclusion, sequential DHOPE-COR-NMP using an HBOC-based perfusion fluid offers a novel method of liver machine perfusion for combined resuscitation and viability testing of suboptimal livers prior to transplantation.

KEYWORDS
clinical research/practice, donors and donation: extended criteria, ischemia reperfusion injury (IRI), liver allograft function/dysfunction, liver transplantation/hepatology, organ perfusion and preservation, organ procurement, organ procurement and allocation

1 | INTRODUCTION

Ex situ machine perfusion is increasingly investigated as a tool to increase the number of donor livers for transplantation and to reduce posttransplant complications. Machine perfusion, was recently introduced in clinical practice using two different temperature protocols: hypothermic (4-12°C) or normothermic (37°C) machine perfusion.[1-5] (Dual) hypothermic oxygenated perfusion ([D]HOPE) can be applied to resuscitate the mitochondria and increase hepatic adenosine triphosphate (ATP) content, resulting in less cell injury, including less cholangiocyte injury. Normothermic machine perfusion (NMP) allows for ex situ functional testing of (extended criteria) donor livers prior to transplantation and suboptimal donor livers have been successfully transplanted after NMP.[2,6-8] (D)HOPE and NMP may therefore have a complementary effect when applied sequentially.[9,10] In preclinical studies using human donor livers, it was previously shown that a short period of DHOPE prior to NMP results in increased ATP concentrations, less hepatobiliary injury and improved function during the NMP phase, compared to direct end-ischemic NMP.[9,10]

Different perfusion solutions have been used for (D)HOPE and NMP. A perfusion fluid based on human red blood cells (RBC) is frequently used for NMP.[11-13] The use of RBC, however, has several drawbacks. Firstly, RBC are a relatively scarce human blood product.[14] Secondly, RBC may induce an immune reaction or cause an infection.[14,15] Lastly, RBC cannot be used during (D)HOPE due to increased stiffness of the erythrocyte lipid membranes and hemolysis at low temperatures. These drawbacks press the need for an alternative oxygen carrier, especially when hypothermic and normothermic machine perfusion are combined. Hemoglobin-based oxygen carriers (HBOC) are a suitable alternative for the use of RBC in ex situ liver machine perfusion. The bovine derived HBOC-201 (Hemopure) has previously been used successfully in experimental and preclinical studies of liver machine perfusion.[14,16,17]

Based on the presumed complementary effect of (D)HOPE and NMP we have combined these two techniques in a clinical machine perfusion protocol using an HBOC-201-based solution. The use of this perfusion solution eliminates the need to change the perfusion fluid during different temperature phases. Donor livers that were initially declined for transplantation nationwide were subjected to a combined protocol of DHOPE, controlled oxygenated rewarming (COR), and subsequent viability testing during NMP (DHOPE-COR-NMP Trial). This report describes the first transplantations of initially nation-wide declined livers that underwent ex situ machine perfusion with the HBOC-201-based perfusion fluid.

2 | MATERIALS AND METHODS

2.1 | Study protocol

Between August 2017 and April 2018, 20 livers were offered for inclusion in the DHOPE-COR-NMP study. All livers were declined for regular transplantation by the three liver transplant centers in the Netherlands. Thirteen livers were secondarily declined because of logistical reasons, long agonal phase (in case of donation after circulatory death), or macroscopic fibrosis/cirrhosis (Figure 1 and Figure S1). Seven livers were accepted to undergo DHOPE-COR-NMP. All seven livers were initially declined for transplantation because of a combination of risk factors, as described in Table 1. The median donor risk index was 2.82 (IQR 2.52-2.97), reflecting the suboptimal quality of these livers.

The study protocol was approved by the medical ethical review committee of our center (METc2016.281) and published in the national registry of clinical trials (www.trialregister.nl; NTR5972). The primary outcome parameter was 3-month graft survival. All recipients gave written informed consent.

2.2 | Procurement of donor livers

All donor livers were procured in a standard manner by a dedicated procurement team. After withdrawal of mechanical support,

FIGURE 1 Flow chart of livers offered in the context of the DHOPE-COR-NMP Trial. After initial decline by all Dutch liver transplant centers a total number of 20 livers were offered for inclusion in this trial. Thirteen livers did not undergo machine perfusion due to logistical reasons, long agonal phase, or macroscopic findings. Seven livers underwent machine perfusion for resuscitation and viability assessment. DHOPE, dual hypothermic oxygenated machine perfusion; COR, controlled oxygenated rewarming; NMP, normothermic machine perfusion [Color figure can be viewed at wileyonlinelibrary.com]
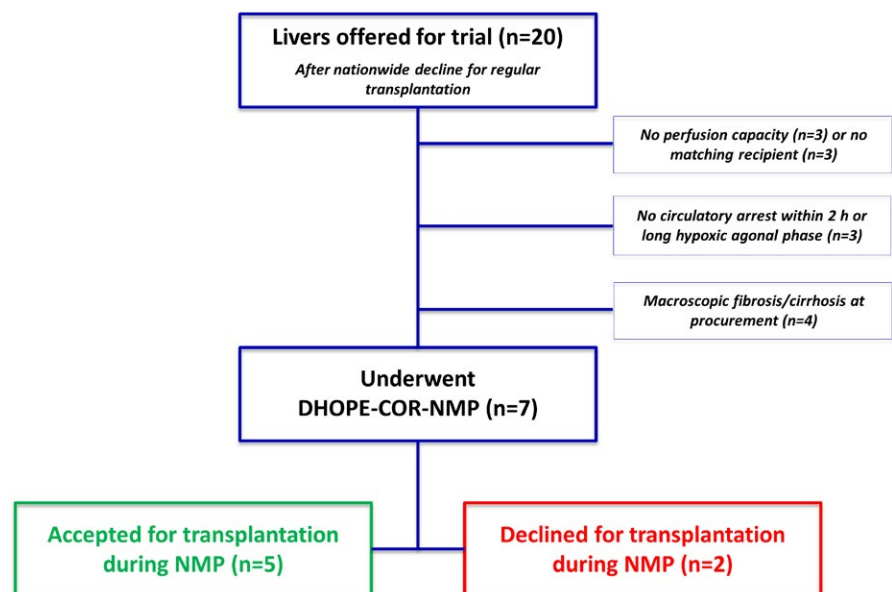
TABLE 1 Donor characteristics of livers that were accepted to undergo machine perfusion

	Liver 1	Liver 2	Liver 3	Liver 4	Liver 5	Liver 6	Liver 7
Age (y)	42	63	47	52	46	62	63
DBD/DCD donor	DCD	DCD	DCD	DCD	DCD	DCD	DCD
BMI / degree of steatosis	BMI 21	BMI 28	BMI 33, Histologically >60% steatosis	BMI 28	BMI 27	BMI 23	BMI 25
Notably increased laboratory values in the donor	Peak AST 1676 U/L, peak ALT 1375 U/L, peak γGT 166 U/L	–	–	Peak γGT 340 U/L	Peak AST 161 U/L, peak ALT 270 U/L, peak γGT 254 U/L	Peak AST 201 U/L, peak ALT 175 U/L	–
Intoxications	Binge drinking	–	–	Frequent alcohol consumption	Alcohol, heroin, speed cocaine, xtc	–	–
dWIT (min)[a]	29	23	30	33	27	35	25
CIT (min)[b]	289	306	525	294	256	278	221
Donor hepatectomy time (min)[c]	59	82	96	28	11	44	36
DRI[30]	2.53	2.82	2.46	2.92	2.50	3.75	3.03
ET-DRI[31]	2.65	2.92	2.47	3.31	2.85	2.88	2.87

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass index; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; dWIT, donor warm ischemia time; ET-DRI, Eurotransplant donor risk index; γGT, γ-glutamyltransferase.

[a]dWIT is defined as the time from withdrawal of mechanical support until the start of in situ cold perfusion.
[b]CIT is defined as the time from in situ cold perfusion until the start of machine perfusion.
[c]Donor hepatectomy time is defined as the time from in situ cold perfusion until the time of hepatectomy.

circulatory death was awaited, followed by a mandatory 5 minutes "no touch" period before procurement surgery was started. Cold in situ flush was performed with UW cold storage solution with the addition of 50 000 IU of heparin. After procurement, the livers were transported to our center using static cold storage. Upon arrival, the livers were prepared for machine perfusion, as described previously.[11]

2.3 | Machine perfusion settings

A combined machine perfusion protocol of 1 hour of DHOPE (resuscitation phase), 1 hour of COR, and subsequent NMP (viability testing phase) was established (Figure 2). For machine perfusion at different temperatures the Liver Assist (Organ Assist, Groningen, the Netherlands) perfusion device was used. DHOPE was performed at 10°C. During COR the temperature was gradually increased about 1°C per 2 minutes, to 37°C at the start of NMP. Portal vein and hepatic artery pressures were set at 5 and 11 mm Hg during DHOPE, and gradually increased during COR to 11 and 70 mm Hg at the start of NMP, respectively.

During DHOPE, the perfusion fluid was oxygenated with 1 L/min 100% O_2, resulting in a perfusate pO_2 > 80 kPa, as described previously.[2] During NMP an air/oxygen mixture was used aimed to reach an arterial perfusate pO_2 of 10.0-13.3 kPa and a venous oxygen saturation of 55% to 75%. To obtain these targets, FiO_2 was varied between 21% and 40%.

Arterial perfusate samples were collected every half hour and analyzed using the ABL 90 Flex analyzer (Radiometer, Brønhøj, Denmark). In addition, venous perfusate samples were collected and analyzed every hour, to determine oxygen consumption. Oxygen consumption was calculated based on the difference between

arterial and venous oxygen content. The following equation was used, $([\{ApO_2-VpO_2\} \times K /760] \times \text{total flow}) + ([\{AsO_2-VsO_2\} \times Hb \times c \times 0.0001] \times \text{total flow}) / \text{Liver weight} \times 100$.[18] Where pO_2 was in mm Hg, sO_2 in %, Hb in g/dL, total flow (sum of arterial and portal flow) in mL/min and liver weight in g. K was a constant (0.0225) and c the oxygen binding capacity of HBOC (1.26).

Bile was collected from COR-NMP onwards and its quantity measured. Additionally, every half hour bile was collected under mineral oil to determine biliary pH and HCO_3^-, as described previously.[8,19] Mineral oil prevented exposure of bile to ambient air, thus preventing the exchange of CO_2 molecules, which influences biliary pH via HCO_3^-.

2.4 | Perfusion fluid

To facilitate perfusion at different temperatures, an acellular perfusion solution based on a bovine-derived HBOC (HBOC-201, HBO_2 Therapeutics, Souderton, PA) was used. In addition to HBOC-201, the perfusion fluid contained gelofusine, albumin, metronidazole, cefazolin, nutrients, glutathione, insulin, heparin, and $NaHCO_3$. Details of the perfusion solution composition are provided in Table S1. As of liver #6, taurocholate was added to the perfusion fluid (50 mg at baseline), followed by a continuous infusion of 7.7 mg/h during the NMP phase.[20] Taurocholate was produced according to GMP by our hospital pharmacy.

2.5 | Viability testing—assessment of hepatobiliary function

Liver viability and function were assessed during the NMP phase using predefined viability criteria, including sufficient bile production

FIGURE 2 Overview of the machine perfusion protocol. A, The machine perfusion protocol included 1 h of DHOPE, 1 h of COR, and subsequent NMP for at least 150 minutes. Each phase of machine perfusion served a different purpose as described in the upper part of the figure. Machine perfusion settings were adjusted according to the perfusion temperature. The temperature was kept at 10°C during DHOPE and was gradually increased to 37°C during the COR phase, after which the liver was functionally tested during NMP. PV and mean HA pressure were set at 5 and 25 mm Hg, respectively, during DHOPE and were gradually increased during COR to 10 and 70 mm Hg, respectively, at the start of NMP. DHOPE, dual hypothermic oxygenated machine perfusion; COR, controlled oxygenated rewarming; HA, hepatic artery; NMP, normothermic machine perfusion; PV, portal vein [Color figure can be viewed at wileyonlinelibrary.com]
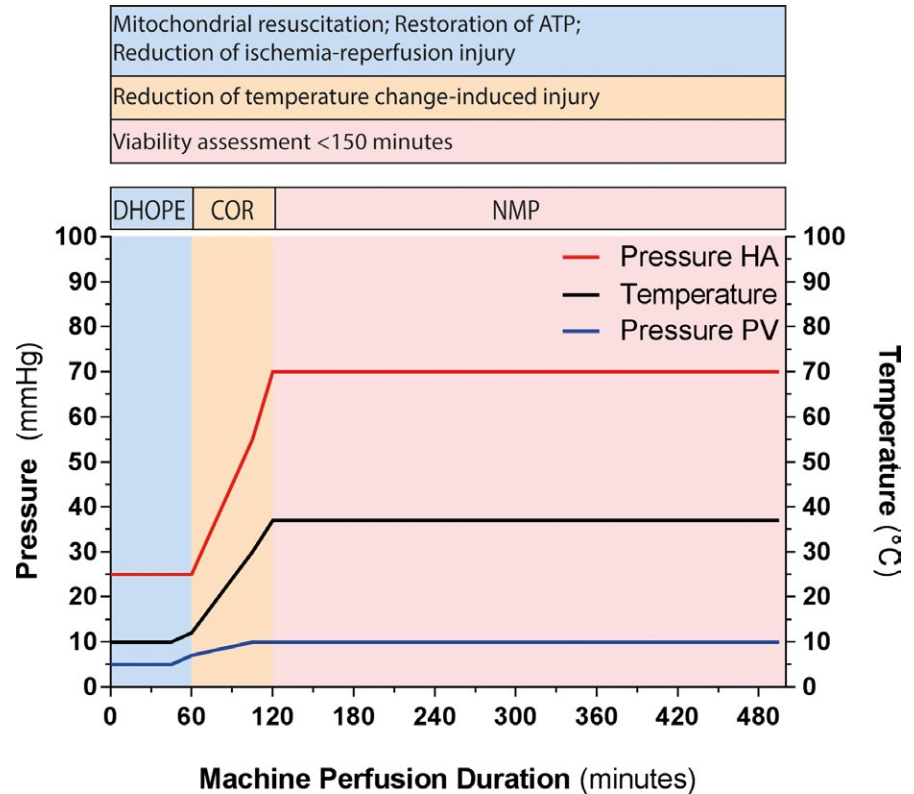
with a biliary pH of >7.45, and normalization of perfusate pH and lactate (Table 2).[5,7,8,21,22] The liver was considered acceptable for transplantation, if all criteria were met within the first 150 min of NMP. If the liver did not meet the predefined viability criteria, machine perfusion was terminated. If the liver did meet the predefined viability criteria, machine perfusion was continued and the recipient was brought to the operating room. When the hepatectomy of the native liver was almost complete, machine perfusion was terminated and the donor liver flushed out with 2 L of cold UW cold storage solution to remove the HBOC-based machine perfusion fluid. As routinely performed in our center, the first 400 mL of venous blood from the liver was drained and discarded to avoid spill of UW Cold Storage Solution into the recipient circulation.

3 | RESULTS

3.1 | Machine perfusion characteristics

Five of the seven livers (liver #1, and #4-#7) that underwent DHOPE-COR-NMP were identified as transplantable based on functional assessment during NMP (Table 3). Median cold ischemia time of all livers was 289 minutes (IQR 256-306 minutes). Machine perfusion times per liver are provided in Table 4. Median total duration of machine perfusion was shorter for the non-transplanted livers due to termination of machine perfusion after these livers did not meet all predefined viability criteria within 150 min of NMP.

Both portal vein and hepatic artery flow remained low during DHOPE and increased during COR. At 150 minutes of NMP, median portal vein flow was 1680 mL/min (IQR 1460-1740 mL/min) (Figure 3A). Overall, resistance in the portal vein decreased towards NMP, but remained relatively high in liver #3 (Figure 3B). At 150 minutes of NMP, median hepatic artery flow was 547 mL/min (IQR 240-737 mL/min). Furthermore, hepatic artery flow was variable between the livers (Figure 3C). Resistance in the hepatic artery, generally remained <0.2 mm Hg*min/L/g, except for resistance in liver #7 (Figure 3D). During COR, total flow increased as well, to a median of 2512 min (IQR 2133-2570 min) at 150 minutes of NMP (Figure 3E).

3.2 | Hepatobiliary function and damage during machine perfusion

Perfusate pH normalized within 150 minutes of NMP of liver #1, #2, and #4 to #7, but not in liver #3, despite the addition of 25 mL 8.4% $NaHCO_3$ (Figure 4A). Perfusate lactate normalized within 150 minutes of NMP in all livers, except for liver #3 (Figure 4B). Furthermore, all livers produced sufficient amounts of bile. Bile production of liver #6 appeared lower, however, this was caused by a cannulation problem of the bile duct (Figure 4C). Alanine aminotransferase (ALT) concentrations in perfusate of the transplanted livers were <2000 U/L. In the two non-transplanted livers ALT concentrations were >2000 U/L, with a peak ALT concentration of 8460 U/L in liver #3 (Figure 4D). Livers #1 and #4 to #7 produced bile with a
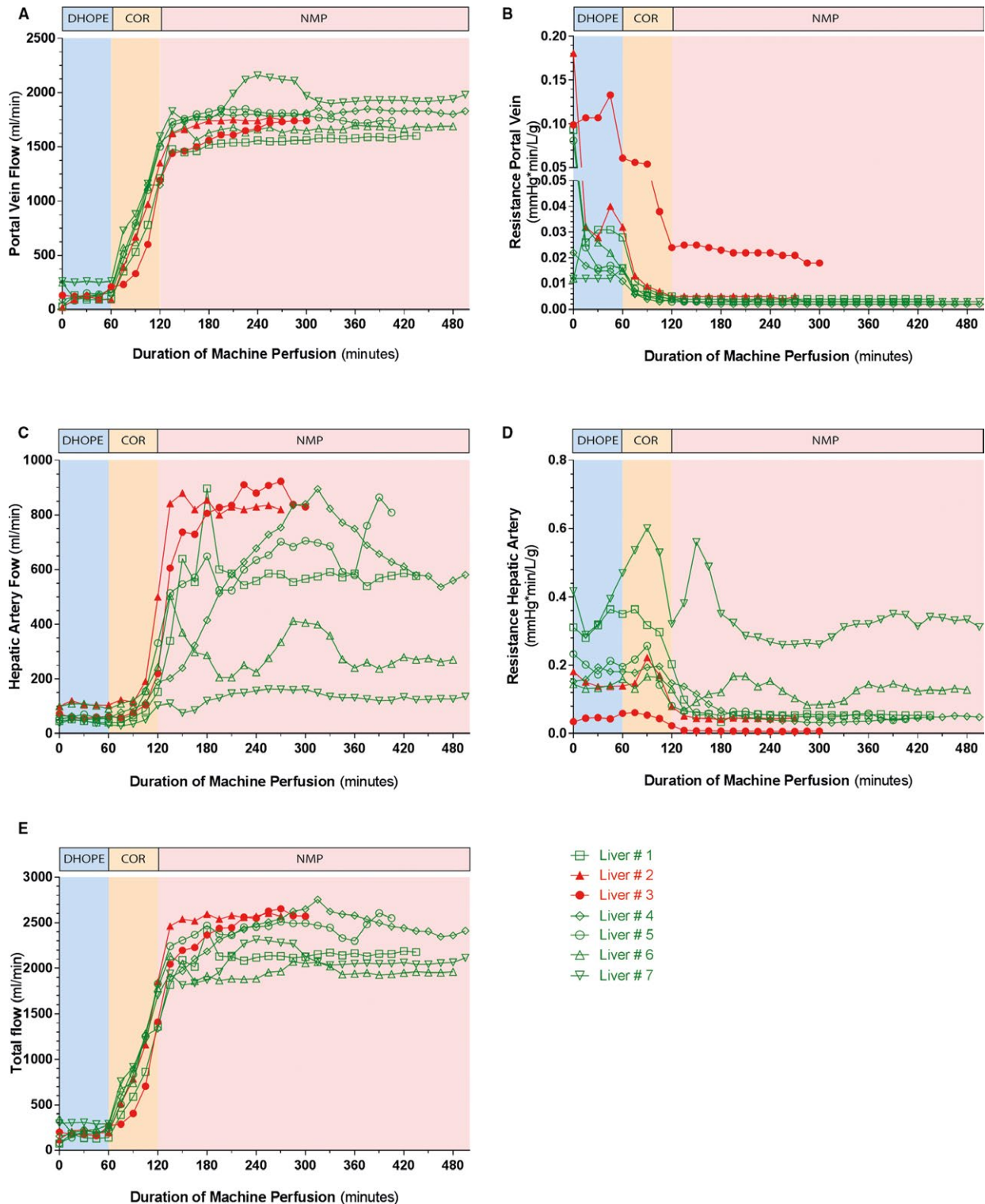
FIGURE 3 Flows and resistance during machine perfusion. A, PV flows were low during DHOPE. After 150 min of NMP, median portal vein flow was 1680 mL/min (IQR 1460-1740 mL/min). B, Resistance in the portal vein was low, except for liver #3. C, Hepatic artery (HA) flows were low during DHOPE and COR. During NMP hepatic artery flows varied between 100 and 900 mL/min. At 150 min of NMP, median hepatic artery flow was 547 mL/min (IQR 240-737 mL/min). D, Resistance in the hepatic artery was <0.2 mm Hg*min/L/g, except for liver #7. E, Total flow increased to a median of 2512 min (IQR 2133-2570 min) at 150 minutes of NMP. The red lines represent the non-transplanted livers and the green lines represent the transplanted livers. DHOPE, dual hypothermic oxygenated machine perfusion; COR, controlled oxygenated rewarming; HA, hepatic artery; IQR, interquartile range; NMP, normothermic machine perfusion; PV, portal vein; Tx, transplantation [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Viability criteria for donor liver assessment during NMP phase

Viability criteria
• Cumulative bile production of ≥10 mL at 150 min of NMP and ≥4 mL in the preceding hour.[8]
• Lactate concentration in perfusate is within "normal" values (0.5-1.7 mmol/L) within 150 min of NMP.[5,7]
• Perfusion fluid pH is within normal values (7.35-7.45) within 150 min of NMP, without the need for repeated addition of NaHCO₃.[5,7]
• Biliary pH of >7.45 within 150 min of NMP.[21,22]

All predefined viability criteria had to be met in order to consider a liver transplantable.
NMP, normothermic machine perfusion.

pH > 7.45, whereas livers #2 and #3 did not (Figure 4E). Bile duct biopsies of these two livers revealed signs of substantial histological injury (Figure S2).

Median oxygen consumption was 0.14 mL O_2/min/100 g liver weight during DHOPE and increased during COR to a median peak value of 2.77 mL O_2/min/100 g liver weight during NMP.

3.3 | Pre- and postoperative outcomes

Median follow up after transplantation was 197 days (IQR 152-307 days). Laboratory values of the recipients are shown in Figure 6. Serum ALT levels rapidly decreased during the first week after transplantation and values were (nearly) normal at 30 and 90 days after transplantation. The recipients of liver #1 and #7 had remarkably low peak serum ALT concentrations of 201 and 337 U/L, respectively (Figure 5A). Serum total bilirubin levels rapidly decreased during the first postoperative days in all recipients. However, a temporary peak in serum bilirubin was noted at the end of the first week in recipients of liver #4 and #6 (Figure 5B). None of the transplanted livers classified for early allograft dysfunction.[23]

Thus far we have observed a 100% patient and graft survival. None of the recipients has developed clinically evident non-anastomotic strictures of the biliary tree during the median follow-up of 197 days (6.5 months).

4 | DISCUSSION

The clinical series described in this report provides two novel findings that are relevant for the further development of machine perfusion technology in organ transplantation. First, we have successfully used a combination of hypothermic and normothermic machine perfusion to resuscitate and select initially declined suboptimal livers for transplantation. The 100% graft survival at 3 months, which was the primary end point, indicated the safety and feasibility of the procedure. Secondly, we have shown that an HBOC-based machine perfusion solution can be safely used in clinical liver transplantation.

Several groups have described the successful use of NMP to select suboptimal and initially declined donor livers.[7,12] Although the optimal parameters for viability assessment are still under debate, most groups have been using a combination of bile production, perfusate lactate levels, and pH as markers of hepatocellular function. We have previously suggested to use biliary pH and bicarbonate as markers of biliary epithelial (cholangiocellular) viability.[22] Biliary epithelium actively modifies bile composition by the secretion of bicarbonate, resulting in an alkalotic biliary environment. This alkalotic environment protects biliary epithelial cells against the detergent effects of hydrophobic bile salts, a phenomenon known as the "bicarbonate umbrella."[24] In a clinical series of liver NMP, Watson et al have recently confirmed the potential usefulness of biliary pH as marker of biliary viability.[12] We have, therefore, added biliary pH as a bile duct viability criterion to our protocol. Of the four predefined selection criteria, the criterion for biliary pH was the most frequent reason for secondary discard in these clinical series.

Although, favorable outcomes after NMP regarding graft and patient survival have been reported, it has not yet been demonstrated that NMP protects the cholangiocyte compartment.[7,12,25] (D)HOPE in DCD liver grafts has, on the other hand, been described to reduce histological signs of biliary ischemia-reperfusion injury and the incidence of posttransplant cholangiopathy.[2,3,26] Furthermore, DHOPE has been shown to reduce ischemia reperfusion injury via resuscitation of the mitochondria and the increase in hepatic ATP content, thereby also protecting the hepatocytes.[2,3,6] The latter might explain why postoperative peak ALT was lower in our recipients than in the studies that compared SCS to end-ischemic NMP alone.[7,12] However, definitive conclusions on this topic cannot be drawn from the current study due to the lack of a control group.

In this study we combined the presumed benefits of DHOPE and NMP to resuscitate and select high-risk donor livers that can be safely transplanted, despite initial nationwide decline. Besides a 100% graft survival at a median follow up of 197 days, none of the recipients has developed clinical signs of posttransplant cholangiopathy so far. The development of posttransplant cholangiopathy was, however, not the primary outcome of this study and was based on clinical symptoms and laboratory findings, rather than on imaging studies. Therefore, subclinical cases of cholangiopathy may have been missed and final conclusions on the efficacy of combined DHOPE, COR, and NMP in the prevention of posttransplant cholangiopathy require longer follow-up in a larger series.

We applied sequential DHOPE and NMP linked by a controlled rewarming phase, as sudden temperature shifts may contribute to cellular injury.[27,28] A previous clinical study has indicated that a short period of COR prior to implantation of donor livers results in less hepatocellular injury, compared to direct implantation of a cold stored donor liver, as evidenced by lower postoperative peak transaminases and a higher graft survival rate at 6 months after transplantation.[28] Based on the relative small number of livers included in the current study and the absence of a control group, we cannot draw conclusions on the value of the COR phase. Previous preclinical studies have shown that (D)HOPE and NMP can also be combined without a COR phase.[9,10]

TABLE 3 Viability criteria overview and transplantation decision per liver

	Liver #1	Liver #2	Liver #3	Liver #4	Liver #5	Liver #6	Liver #7
Perfusate pH	+	+	−	+	+	+	+
Perfusate latate	+	+	−	+	+	+	+
Bile production	+	+	+	+	+	+	+
Biliary pH	+	−	−	+	+	+	+
Transplantation (yes/no)	Yes	No	No	Yes	Yes	Yes	Yes

+, the liver met this viability criterion; −, the liver did not meet this viability criterion.

TABLE 4 Machine perfusion times per liver

	Liver #1	Liver #2	Liver #3	Liver #4	Liver #5	Liver #6	Liver #7
Total duration of NMP (min)	347	163	180	397	301	373	391
Duration of NMP from viability assessment onwards (min)	197	–	–	247	157	223	241
Total machine perfusion time (DHOPE-COR-NMP) (min)	467	283	300	517	421	493	511

DHOPE, dual hypothermic oxygenated machine perfusion; COR, controlled oxygenated rewarming; NMP, normothermic machine perfusion.

For the application of this combined machine perfusion protocol we have developed a perfusion fluid that can be used at various temperatures and eliminates the use of a third party human blood product. While during hypothermic machine perfusion, oxygen can be dissolved in the perfusion solution, during NMP an oxygen carrier is necessary. Perfusion solutions based on RBC, which are mostly used for NMP, cannot be used at low temperatures due to increasing lipid membrane stiffness and the risk of hemolysis. In contrast to RBC, HBOC can be used at low temperatures. In addition, HBOC-201, used in this study, has a lower oxygen affinity than human Hb in erythrocytes and thus gives off the oxygen more easily. In the cold, the affinity of HBOC-201 for oxygen increases, similar to that of human Hb in erythrocytes, but is still less.

HBOC-201 has previously been used in experimental and preclinical studies on ex-situ liver machine perfusion, but not in clinical practice.[14,16,17] In a study with discarded human livers, NMP with an HBOC-201-based perfusion fluid resulted in similar outcome compared to NMP with RBC, indicating HBOC-201 as a suitable alternative for RBC.[14] Our group reported higher ATP concentration, and cumulative bile production in discarded human livers undergoing NMP with an HBOC-201-based solution, compared to perfusion with an RBC-based perfusion fluid.[16] Altogether these preclinical studies and the currently presented first, clinical application indicate that HBOC-201 can be used as a substitute for RBC in fluids for machine perfusion of donor organs.

A limitation of this series is a lack of a control group. However, livers of suboptimal quality with a perceived high risk of primary nonfunction or early allograft dysfunction were included in the current study, making it unethical, in our opinion, to transplant these livers without resuscitation and functional assessment. Furthermore, this study cannot discriminate between the beneficial effects of DHOPE, COR, and NMP separately. Finally, extrahepatic bile duct biopsies of the two livers that were secondary declined for transplantation, based on their failure to produce bile with a pH > 7.45 during NMP, were taken. Yet, biopsies of higher level bile ducts were not taken. Although we have previously shown that the degree of histological injury of the extrahepatic bile duct of a donor liver after cold storage is representative for the degree of injury of the proximal biliary tree, including larger intrahepatic ducts,[29] we do not formally know whether this is also true after NMP.

A potential limitation of HBOC is its susceptibility to a conversion into methemoglobin, especially in the venous phase with low oxygen saturation. In contrast to erythrocytes, HBOC do not contain NADH-dependent enzyme methemoglobin reductase, which is responsible for converting methemoglobin back to hemoglobin. We have noted a gradual increase in methemoglobin during NMP, but not during DHOPE when the perfusion fluid was oxygenated with an FiO_2 of 100% (Figure S3). In a separate experiment, we have noted that the percentage of methemoglobin can be corrected or slowed down by the addition of extra HBOC-201, glutathione, or vitamin C to the perfusion fluid (Figure S3). However, we do not prefer the use of vitamin C due to its effects on the pH and osmolality of the perfusion fluid.

In conclusion, this first clinical experience demonstrates the feasibility of combined hypo- and normothermic machine perfusion after traditional static cold storage of suboptimal liver grafts. The combination of oxygenated hypothermic and normothermic perfusion protects livers against ischemia-reperfusion injury and enables hepatobiliary viability assessment prior to transplantation. The use of a novel HBOC-201-based perfusion fluid eliminated the need to
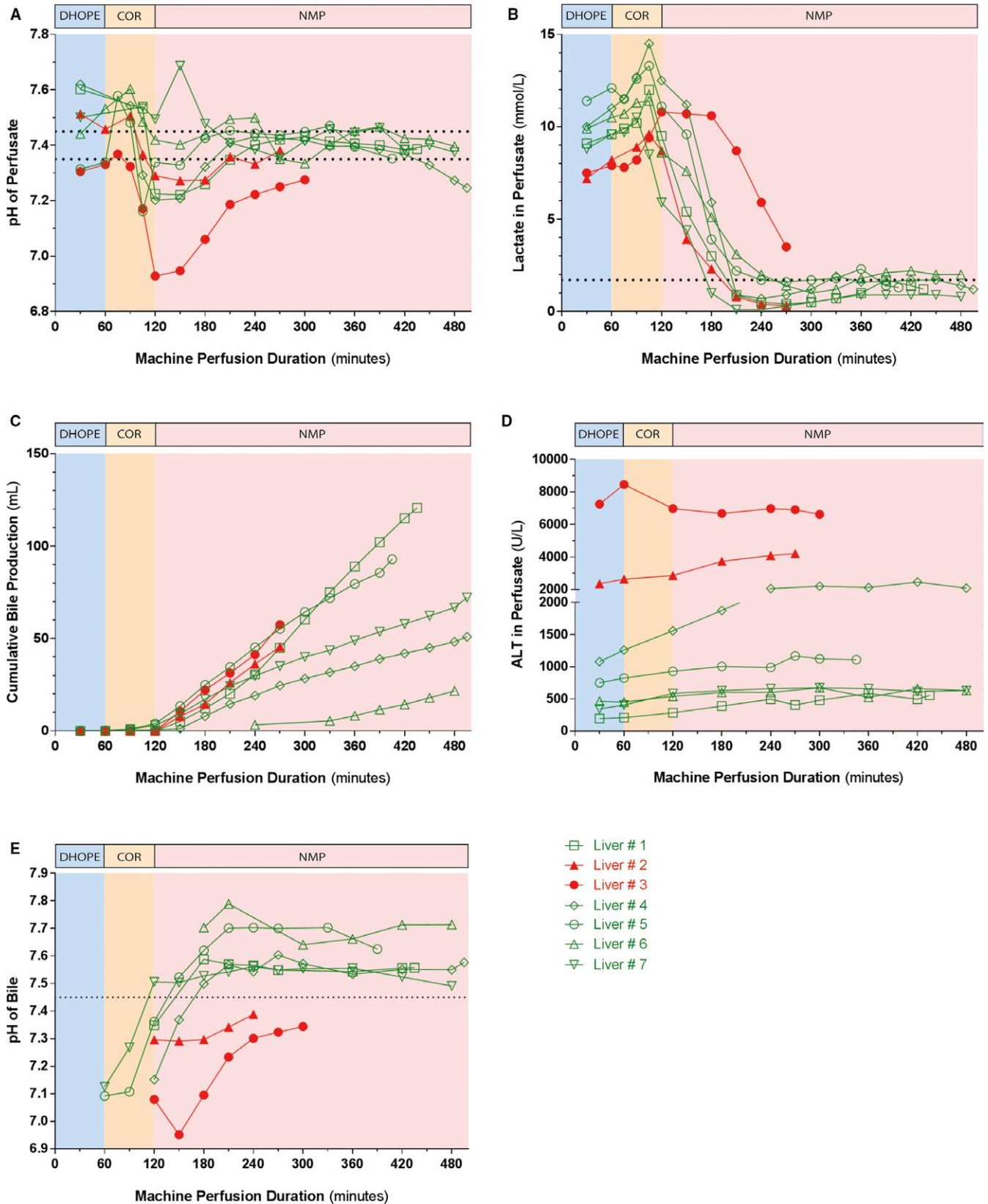
FIGURE 4 Machine perfusion fluid biochemistry. A-B, Biochemical parameters used for viability assessment of the liver. In all but one liver, perfusate pH and lactate values normalized within 150 minutes after start of the NMP. C, All livers produced sufficient amounts of bile. Liver #6 seemingly produced less bile due to a cannulation problem of the bile duct. D, ALT perfusate levels were <2000 U/L in the transplanted livers and >2000 U/L in the nontransplanted livers. E, Biliary pH, a marker of biliary epithelial viability, increased to >7.45 in all livers that were transplanted, whereas biliary pH remained <7.45 in the livers that were not transplanted livers. The red lines represent the nontransplanted livers and the green lines represent the transplanted livers. ALT, alanine aminotransferase; NMP, normothermic machine perfusion [Color figure can be viewed at wileyonlinelibrary.com]
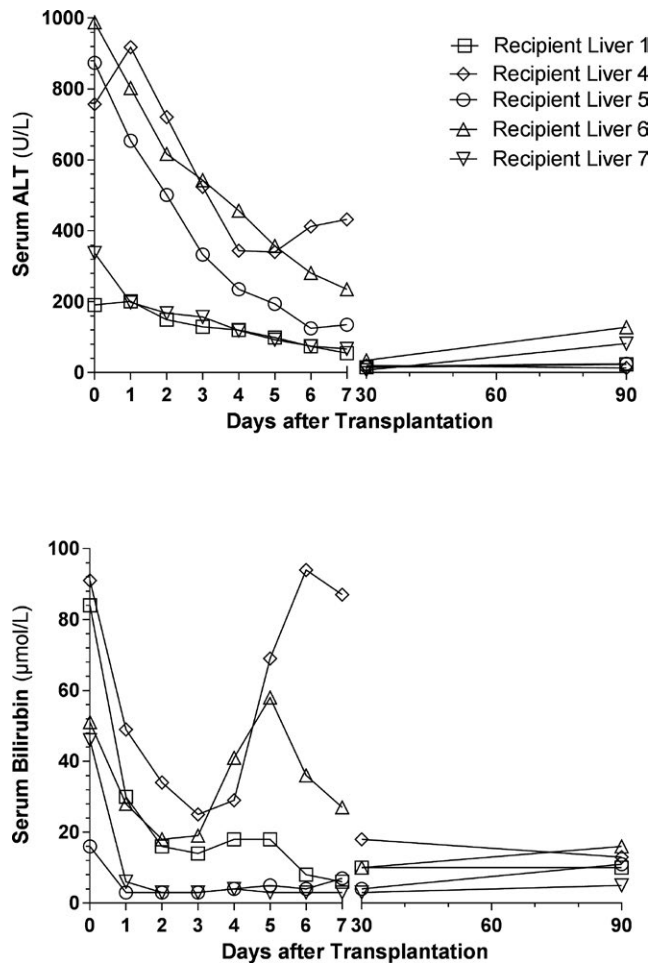
FIGURE 5 Posttransplantation serum ALT and total bilirubin. Laboratory values were recorded at postoperative day 0 until 7, and at 1 and 3 months. Postoperative day 0 was defined as the time from reperfusion in the recipient until midnight of the same day. A, Postoperative serum ALT concentrations rapidly decreased during the first week. The recipients of liver #1 and #7 had low peak serum ALT concentrations of 201 and 331 U/L, respectively. B, Postoperative total bilirubin concentration likewise decreased during the first week, except for a transient increase in the recipients of livers #4 and #6 at the end of the first week. Bilirubin levels of these livers, however, normalized during the weeks thereafter. ALT, alanine aminotransferase

change perfusion fluid during various temperature phases and appeared to be a safe alternative for RBC as oxygen carrier in ex situ donor organ machine perfusion. This new protocol of ex situ machine perfusion provides a tool to safely expand the pool of organs for liver transplantation.

ACKNOWLEDGMENTS

DISCLOSURE

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.