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The Risk of Microbial Transmission in Recipients of Donor Livers That Underwent Hypothermic or Normothermic Machine Perfusion

Chikako Endo, MD,[1] Bianca Lascaris, MD,[1] Isabel M. A. Brüggenwirth, MD, PhD,[1] Jan Roggeveld, MBO,[2] Hans Blokzijl, MD, PhD,[3] Vincent E. de Meijer, MD, PhD,[1] M. H. Edwina Doting, MD, PhD,[4] and Robert J. Porte, MD, PhD[1]

Background. Ex situ machine perfusion is increasingly used to preserve and assess donor livers before transplantation. Compared with traditional static cold storage (SCS), machine perfusion exposes livers to an additional risk of microbial contamination. However, information on the risk of microbial transmission during machine perfusion is lacking. **Methods.** All livers that underwent either hypothermic oxygenated machine perfusion (HOPE) or normothermic machine perfusion (NMP) in our center between September 2021 and September 2023, and during which samples were taken from SCS fluid and/or machine perfusion solution for microbiological examination, were included in this retrospective, observational clinical study. Microbial transmission was examined from SCS fluid to machine perfusion solution fluid and, subsequently, to recipients of these livers. **Results.** A total of 90 cases of liver machine perfusion were included: 59 HOPE and 31 NMP. SCS preservation fluid cultures before HOPE or NMP were positive for at least 1 microorganism in 52% of the cases. After HOPE, there were no cases of positive machine perfusion fluid or evidence of microbial transmission to the recipients. After NMP, in 1 (3%) patient *Escherichia coli* was grown from abdominal drain fluid, the same bacterial strain that was also grown from the SCS preservation fluid before NMP. This *E coli* was resistant to the antibiotics that are routinely added to the NMP perfusion fluid. **Conclusions.** The risk of microbial transmission after machine perfusion is very low but not absent. We recommend routine sampling of machine perfusion fluid at the end of the procedure for microbiological analysis.

(*Transplantation Direct* 2024;10: e1664; doi: 10.1097/TXD.0000000000001664.)

Ex situ machine perfusion of donor livers before transplantation is increasingly used to reduce posttransplant complications and to increase the number of suitable donor livers. Compared with traditional static cold storage (SCS), (dual or single) hypothermic oxygenated machine perfusion (HOPE) has been shown to reduce ischemia-reperfusion injury-related complications, such as nonanastomotic biliary strictures, post-reperfusion syndrome, and early allograft dysfunction.[1,2] Normothermic machine perfusion (NMP) can be used to extend the preservation time for up to 24 h[3] but is also used as a tool to assess the viability of high-risk donor livers before transplantation, thereby increasing the donor liver pool.[4]

Compared with traditional SCS, machine perfusion exposes livers to an additional risk of microbial contamination. However, information on the risk of bacterial transmission (transfer from the same microorganism from 1 source to the next) after ex situ machine perfusion is lacking. The reported incidence of microbial contamination (bacteria or fungi) of preservation fluid used for traditional SCS ranges between

Received 28 March 2024.

Accepted 19 April 2024.

[1] Department of Surgery, Section of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

[2] Department of Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

[3] Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

[4] Department of Microbiology and Infection Prevention, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

The authors declare no funding or conflicts of interest.

C.E., B.L., and R.J.P. participated in the writing of the article, performance of the research, data analysis, and research design. I.M.A.B. participated in the performance of the research. J.R. participated in the data analysis. H.B. participated in the performance of the research. V.E.d.M. participated in the performance of the research. M.H.E.D. participated in the research design, performance of the research, and data analysis.

C.E. and B.L. shared first author.

Correspondence: Robert J. Porte, MD, PhD, Division of HPB & Transplant Surgery, Department of Surgery, Erasmus MC Transplant Center, University Medical Center Rotterdam, P.O. box 2040, 3000 CA, Rotterdam, The Netherlands. (r.j.porte@erasmusmc.nl).

Current address for Dr Porte: Erasmus MC Transplant Institute, Department of Surgery, Division of HPB and Transplant Surgery, University Medical Center Rotterdam, Rotterdam, The Netherlands.

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001664

10% and 98%.[5,6] While transmission to recipients is low (0%–6%), graft loss and mortality after transmission from contaminated SCS preservation fluid have been reported.[5,6] Although performed under sterile conditions, machine perfusion introduces an extra step in the process of liver transplantation that might increase the microbial transmission rate to the recipient. Especially in NMP, where perfusate temperatures are between 35 and 37 °C and a red blood cell-based perfusion fluid is used,[4] microorganisms may easily survive and even grow during machine perfusion, despite the addition of antibiotics to the perfusion fluid. Hann et al[7] recently described a case of severe sepsis and early graft dysfunction in a liver transplant recipient because of bacterial contamination during NMP. Antibiotics are usually not administered during HOPE and data on the risk of microbial contamination and infection in larger series of liver machine perfusion is lacking.

In our center, we perform HOPE alone for all livers obtained from donation after circulatory death donors and NMP in extended criteria donor livers that are considered too risky to be transplanted without prior ex situ viability testing. In our practice, NMP is always preceded by a short (1 h) period of HOPE. In this study, we aimed to assess the risk of bacterial and/or fungal transmission after both types of machine perfusion to determine if machine perfusion poses an extra risk to the recipient. In addition, we reviewed the efficacy of our antimicrobial policy and pharmacokinetics during NMP.

MATERIALS AND METHODS

Data Source and Study Population

We performed a retrospective, observational study, and reviewed all transplantations of a machine-perfused liver, including HOPE and NMP, performed in our hospital between September 2021 and September 2023. Transplantations were excluded when both samples from SCS fluid and machine perfusion solution (MPS) for microbiological testing were missing. Data on donor and perfusion characteristics were collected from our hospital registration database and our prospectively maintained perfusion database. Recipient data were derived from a post hoc analysis of an observational cohort study (www.trialregister.nl—Trial NL6334), which was approved by the Medical Ethics Committee (METc 2014/77).

Procurement and Organ Preparation

The process of liver procurement and organ preparation has been described previously.[2] In brief, donor livers were procured using a standardized procedure by one of the regional multiorgan procurement teams in the Netherlands. After procurement, the liver was preserved using SCS during transportation to our center. Upon arrival, cannulas were inserted in the portal vein alone (single HOPE) or in the portal vein and hepatic artery (dual HOPE and NMP) during the back table preparation. Before the start of machine perfusion, the liver was flushed with ≥1 L University of Wisconsin (UW) MPS (Carnamedica, Warsaw, Poland). A schematic presentation of the process of donor liver retrieval, machine perfusion, and transplantation is provided in Figure 1A for HOPE and Figure 1B for NMP.

Machine Perfusion

For all machine perfusion procedures, the Liver Assist device (XVIVO, Groningen, The Netherlands) was used. This is a pressure-controlled perfusion device that enables dual perfusion through the portal vein and the hepatic artery and can be used for both HOPE and NMP. To ensure

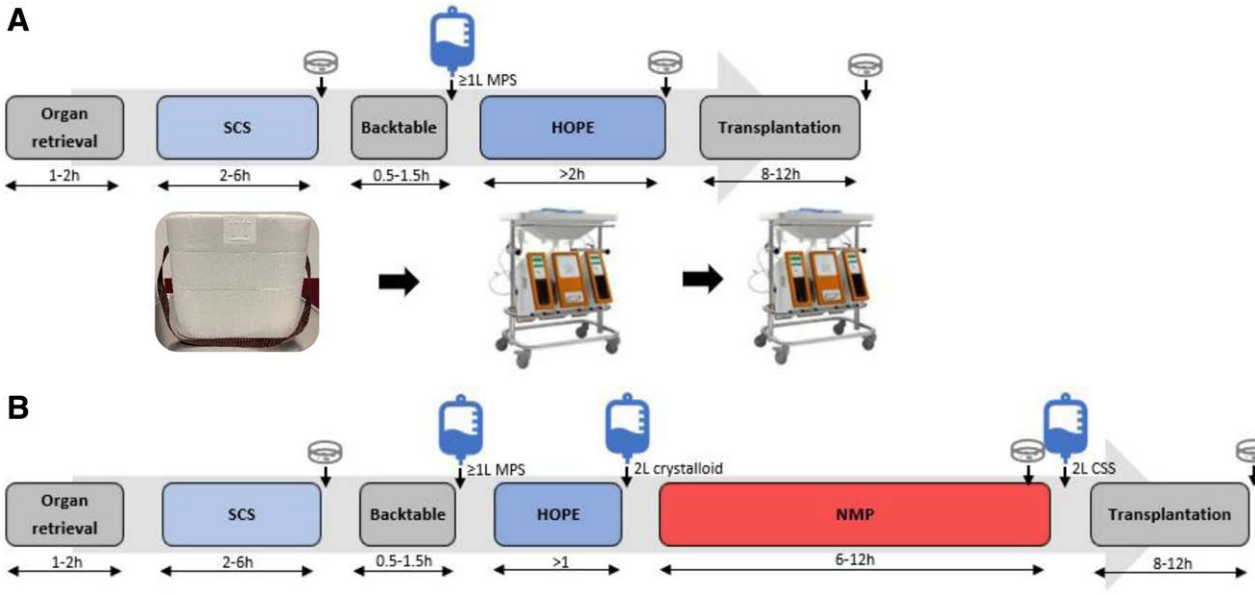

FIGURE 1. Overview of the machine perfusion procedure, including sample collection for microbiological analysis. A, Schematic presentation of the HOPE procedure. Samples for microbial analyses were taken from the preservation fluid after SCS, of the MPS the end of HOPE, and after transplantation of the recipient (blood or drain fluid). Sample points for microbial analysis are indicated by a petri dish. After the backtable preparation, livers were flushed with a minimum of 1 L UW MPS. B, Schematic presentation of the NMP procedure. Samples for microbial analyses were taken from the SCS preservation fluid, from the MPS at the end of NMP, and after transplantation from the recipient (blood or drain fluid). The liver was flushed after the backtable procedure with a minimum of 1 L MPS, with 2 L crystalloid between HOPE and NMP, and with 2 L cold storage solution after NMP before transfer to the recipient. HOPE, hypothermic oxygenated machine perfusion; MPS, machine perfusion solution; NMP, normothermic machine perfusion; SCS, static cold storage; UW, University of Wisconsin.

sterility during the whole process, all machine perfusions were performed in a dedicated Organ Preservation and Resuscitation unit with sterility conditions similar to an operating room.

Hypothermic Oxygenated Machine Perfusion

Before the start of HOPE, the perfusion machine was primed with 3 L UW MPS. No antibiotics were added to the machine perfusion fluid during HOPE. The hepatic artery perfusion pressure was set at ≤25 mm Hg, and the portal vein perfusion pressure at ≤5 mm Hg. During the whole procedure, the temperature of the MPS was kept ≤12 °C. For the oxygenation, a gas flow of 1 L/min of 100% oxygen was used. The duration of a HOPE perfusion was a minimum of 2 h (Figure 1A). At the end of perfusion, the liver was disconnected from the machine and placed in a sterile bowl with UW cold storage solution (Bridge to Life, London, United Kingdom) and ice for transfer to the recipient's operating room.

Normothermic Machine Perfusion

The NMP procedure has been described previously.[4] In brief, NMP was preceded by ≥1-h HOPE and 1-h controlled oxygenated rewarming. Between HOPE and controlled rewarming, the liver was temporarily removed from the machine to exchange the acellular UW MPS in the machine for a perfusate containing red blood cells to provide oxygen transport to the liver. In between HOPE and NMP, the liver was flushed with 2 L crystalloid (saline or Ringer's lactate solution [Baxter, Utrecht, The Netherlands]) to remove UW MPS (Figure 1B). The NMP perfusion solution was supplemented with nutrients and medications, including 400 mg cefazolin and 220 mg metronidazole, as described previously.[4] During rewarming, the temperature was gradually increased from 20 to 37 °C, and the portal vein and hepatic artery pressures were increased to a maximum of 11 and 70 mm Hg, respectively. Throughout rewarming and NMP, an air/O_2 mixture was used to maintain adequate oxygenation of the liver. After 150 min of NMP, the decision to transplant the liver or not was based on previously established viability criteria.[4] When the liver was accepted, the liver remained on the machine until the recipient hepatectomy was completed. At the end of perfusion, the liver was disconnected from the machine, and flushed with 2 L UW cold storage solution before transfer to the recipient.

Antimicrobial Prophylaxis

Before the surgery, all recipients received 1000 mg cefotaxime and 500 mg metronidazole intravenously as antibiotic prophylaxis, which was repeated every 6 and 8 h, respectively, for 48-h posttransplantation. In addition, recipients received prophylaxis with valaciclovir for 28 d postoperatively, fluconazole until the removal of the abdominal drains, and fungizone until the day of discharge. When indicated (eg, known allergies), another antibiotic regime was provided.

Sample Collection for Microbiology

During both types of machine perfusion, samples for microbiological testing (bacteria and fungi) were taken from (1) the SCS preservation fluid before the start of the back table procedure, (2) the MPS fluid at the end of perfusion (ie, at the end of HOPE alone, or at the end of NMP), and (3) recipient abdominal drain fluid (ascites) and/or blood (if clinically indicated) up to postoperative day 7 (Figure 1). During the NMP procedures, there was no sample taken between HOPE and NMP. All samples were sent to the microbiology laboratory for microbial identification and matching of the microorganisms by antibiotic susceptibility and DNA profiles, when indicated.

Microbiological Analyses

Microbial Identification

All samples were processed by the BACTEC 9240 method (Becton-Dickinson Microbiology Systems, Sparks, MD). The inoculated bottles were incubated for 5 d at 35 °C before being discharged. Microbial identification of positive cultures of SCS preservation fluid, the MPS fluid, and recipient abdominal drain fluid and/or blood was performed using Vitek (Biomerieux, Marcy-L'Etoile, France) and matrix-assisted laser desorption ionization-time of flight (Bruker Daltonik, Bremen, Germany). Antibiotic susceptibility was tested using disc diffusion or the microdilution method of Vitek. The European Committee on Antimicrobial Susceptibility Testing criteria were used to define susceptibility or resistance to antimicrobial agents.[8]

Whole Genome Sequencing

For genotyping, DNA was extracted directly from colonies using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Using a Qubit 2.0 fluorometer (Life Technologies, Bleiswijk, The Netherlands), the DNA concentrations were determined. Library preparations were performed using the Nextera XT v3 kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Sequencing of the DNA libraries was performed with an Illumina MiSeq platform (Illumina), generating paired-end reads of 250 base pairs. Trimmed reads ($Q \geq 20$) were de novo assembled with word size 30 using CLC Genomics Workbench, Version 20.0.4 (Qiagen).

The obtained FASTA files (DNA and protein sequence alignment software) were uploaded in Ridom SeqSphere (Ridom GmnH, Munster, Germany). For typing, core genome multilocus sequence typing comparison was performed, based on reference *Escherichia coli* 042 (NC_017626.1).

Antibiotic Concentration Measurements

Cefazolin was separated on an Accucore C18 column (Thermo Fisher Scientific, Sunnyvale, CA). Detection was performed on a Thermo Scientific Quantiva tandem quadrupole mass spectrometer. The method was validated according to the requirements for bioanalytical methods issued by the US Food and Drug Administration and European Medicines Agency.

Statistical Analyses

Continuous data were presented as median and interquartile range. Categorical data were expressed as numbers and percentages. Statistical analyses were performed using SPSS, version 28 (IBM, Armonk, NY).

RESULTS

Sample Inclusion

Between September 2021 and September 2023, 59 HOPE and 31 NMP procedures were performed in our center where at least a SCS fluid or a MPS fluid was collected for examination. Two HOPE and 1 NMP procedures were excluded

because there was not at least 1 sample taken for microbial testing. In the group of livers that underwent HOPE (n = 59), SCS preservation fluid samples were available in 56 cases, and a MPS sample was collected at the end of HOPE in 45 cases. In the group of livers that underwent NMP, preservation fluid samples were examined after SCS in all 31 cases and machine perfusion samples were available in 25 of these. Samples of abdominal drain fluid were routinely obtained within the first week after transplantation and available for all recipients. Samples for blood culture were only taken when clinically indicated.

The characteristics of the donors, recipients, and machine perfusion procedures are presented in Table 1.

Microbial Contamination of Preservation Fluid After SCS

Of the 56 SCS preservation fluid samples taken before HOPE, 29 (52%) were positive for a microorganism, and of the 31 SCS preservation fluid samples taken before NMP, 16 (52%) were positive for a microorganism. In total, 74 different microorganisms were detected (Table 2), with a maximum of 5 different microorganisms in 1 sample. The most frequently found microorganisms were bacteria (97%), typically from the skin (84%) or gut (16%), but also cases of contamination with yeast (3%) were detected. The most frequently detected bacteria were *Staphylococcus epidermidis* (27%) and *Staphylococcus warneri* (15%).

Microbial Transmission During HOPE and to the Recipient

After HOPE, all microbial organisms detected in the SCS fluid disappeared (Figure 2A). Unfortunately, 14 (24%) MPS fluid samples after HOPE were missing. None of the recipients of a HOPE-preserved liver had a positive abdominal drain fluid culture or showed signs of infection by a microorganism found in the SCS preservation fluid. As a result, none of the recipients developed graft failure or died because of microbial transmission from either SCS or MPS.

Microbial Transmission During NMP and to the Recipient

After NMP, all microorganisms detected in the SCS fluid disappeared after NMP (Figure 2B). However, 6 (19%) MPS fluid samples were not available for examination. In one of the recipients, *E coli* was grown from the SCS preservation fluid as well as from the abdominal drain fluid postoperatively. Unfortunately, in this case, no sample was taken from the perfusate at the end of NMP. However, DNA sequencing confirmed that the *E coli* found in the recipient's drain fluid was the same as the one grown from the SCS preservation fluid, indicating the transmission of the bacterium during NMP.

This *E coli* appeared to be an extended-spectrum beta-lactamase (ESBL) *E coli*, which was resistant to the antibiotics added to the NMP solution (cefazolin and metronidazole). In the surveillance cultures of throat and rectum, taken 10 wk before liver transplantation, no resistant Gram-negative rods were found in this recipient. For this reason, transmission of *E coli* ESBL from donor to the recipient appeared to be the most likely cause.

Based on the microbiology results, the recipient was treated with intravenous meropenem for 14 d. The patient never showed any clinical or laboratory signs of an active infection and was discharged from the hospital in good condition on postoperative day 14. Altogether, evidence of microbial transmission after NMP was observed in 1 of the 31 (3%) recipients. In addition, none of the recipients developed graft failure or died because of microbial transmission from either SCS or MPS.

Antibiotic Policy and Pharmacokinetics During NMP

Of the 74 microorganisms that were grown from the SCS preservation fluid before NMP, 48 (65%) were sensitive to cefazolin and/or metronidazole (the antibiotics present in the NMP perfusion fluid), 22 (29%) were resistant, and in 4 (6%) microorganisms it remained unknown, because the microorganism was considered to be low pathogenic and sensitivity analysis was not performed.

Concentrations of cefazolin were analyzed in a subset of perfusate and bile samples obtained during NMP (Figure 3). At all-time points, cefazolin concentrations in the NMP perfusate and bile samples were within or slightly above the therapeutic window used for concentrations in human blood (8–200 mg/L). During NMP, the concentration of antibiotics in the perfusate dropped slowly but remained within the therapeutic window for up to 12 h of NMP.

DISCUSSION

In this study, we have analyzed the risk of microbial transmission and infection during HOPE and NMP of donor livers. In a cohort of 90 machine perfusion procedures (59 HOPE and 31 NMP), samples were taken for microbiological analysis from the cold preservation fluid at the end of SCS, from the machine perfusion fluid, and from the recipient's abdominal drain fluid up to 1 wk after transplantation. In the recipients, samples from abdominal drain fluid were taken routinely, while blood samples were only taken when clinically indicated. Despite a relatively high rate of positive cultures of the SCS preservation fluid (52% before HOPE and NMP), the rate of infections in these recipients was very low. None of the 59 recipients of a HOPE-preserved liver and only 1 of the 31 (3%) recipients of a NMP-preserved liver became contaminated with a microorganism grown from either the SCS preservation solution or the machine perfusion fluid.

About half of the cultures obtained from the SCS preservation fluid were positive for at least 1 microorganism. This rate is similar to what has been reported in the literature, with contamination percentages varying between 10% and 98%.[5,6] Without the use of machine perfusion, the reported rate of microbial transmission from SCS preservation solution to the recipients, causing a clinically relevant infection, is very low and varies between 0% and 6%.[5,6] The current study indicates that this risk is not increased after ex situ machine perfusion of the donor liver before transplantation. Contamination of the SCS preservation solution may occur during organ procurement or during packing of the organs. Microorganisms most frequently found in SCS preservation fluid are skin flora with a relatively low pathogenicity. Nevertheless, contamination with gut-derived pathogens such as *E coli* or *Enterococci* may cause severe infection in the recipient. Antimicrobial prophylaxis consisting of anti-bacterial and anti-fungal medication is, therefore, routinely given to transplant recipients in most centers.

TABLE 1.

Donor, recipient, and machine perfusion characteristics

Characteristics	NMP (n = 31)	HOPE (n = 59)
Donor Characteristics		
Age, y	65 (62–70)	47 (36–58)
Sex		
Male	22 (71%)	40 (68%)
Female	9 (29%)	19 (32%)
Donor type		
DBD	0 (0%)	31 (53%)
DCD	31 (100%)	28 (47%)
Cause of death		
Anoxia	17 (55%)	10 (17%)
Cerebrovascular accident	8 (25%)	31 (53%)
Trauma	3 (10%)	12 (21%)
Other[a]	3 (10%)	5 (9%)
Length of ICU stay, d	3 (3–6)	3 (2–5)
Perfusion characteristics		
Static cold ischemia time, min	227 (190–266)	255 (232–298)
Machine perfusion time, min	641 (563–779)	232 (149–611)
Recipient characteristics		
Age, y	59 (46–66)	49 (34–65)
Sex		
Male	17 (55%)	35 (59%)
Female	14 (45%)	24 (41%)
MELD score	12 (9–18)	14 (11–20)
Body mass index, kg/m^2	27.0 (25. 4–30.8)	23.6 (20. 9–28.5)
Underlying liver disease		
MASLD	4 (13%)	7 (12%)
Alcoholic	7 (23)	5 (8%)
Biliary diseases[b]	8 (26%)	23 (39%)
Malignancy[c]	2 (6%)	4 (7%)
Other[d]	10 (32%)	20 (34%)
Blood loss during surgery, mL	2000 (1225–3750)	2475 (1500–3588)
RBC, mL	560 (0–1400)	840 (560–1680)
FFP, mL	0 (0–0)	0 (0–620)
Platelets, mL	0 (0–0)	0 (0–0)
Postoperative ICU stay, d	1 (1–2)	2 (1–4)
Postoperative hospital stay, d	13 (9–20)	15 (10–21)

Continuous data are presented as median (IQR), and categorical data are presented as number (percentage).

[a]Medical/postoperative complications and euthanasia.

[b]Primary sclerosing cholangitis, primary biliary cholangitis, ischemic-type biliary lesions, Caroli disease, biliary atresia, progressive familial intrahepatic cholestasis, and Stevens-Johnson syndrome with vanishing bile duct syndrome.

[c]Cholangiocarcinoma, hepatocellular carcinoma, epithelioid hemangioendothelioma, neuroendocrine hepatic metastases, and colorectal carcinoma hepatic metastases.

[d]Polycystic liver disease, cryptogenic cirrhosis, post-viral cirrhosis, autoimmune hepatitis, alpha-1 antitrypsin deficiency, congenital liver fibrosis, nodular regenerative hyperplasia, recurrent/uncurable cyst infections, methylmalonic aciduria, hereditary hemochromatosis, cardiac cirrhosis, Budd-Chiari syndrome, and hepatic artery thrombosis.

DBD, donation after brain death; DCD, donation after circulatory death; FFP, fresh frozen plasma; HOPE, hypothermic oxygenated machine perfusion; ICU, intensive care unit; IQR, interquartile range; MASLD, metabolic dysfunction-associated steatotic liver disease; MELD, Model for End-Stage Liver Disease; NMP, normothermic machine perfusion; RBC, red blood cell.

TABLE 2.

List of microorganisms identified in the cold storage preservation fluid

Organism	Origin	Pathogenic[a]	Frequency, n (%)
Candida spp.	Skin/gut/oral	Yes[b]	2 (2.7)
Clostridium perfringens	Gut	Yes	1 (1.4)
Corynebacterium spp.	Skin		1 (1.4)
Cutibacterium acnes	Skin		1 (1.4)
Enterobacter cloacae	Gut		1 (1.4)
Enterococcus spp.	Gut	Yes[b]	2 (2.7)
Escherichia coli	Gut	Yes	1 (1.4)
Klebsiella pneumoniae	Gut	Yes	1 (1.4)
Lactobacillus spp.	Gut		1 (1.4)
Other CNS	Skin		20 (27.0)
Prevotella salivae	Gut		1 (1.4)
Propionibacterium spp.	Skin		1 (1.4)
Serratia marcescens	Gut	Yes	1 (1.4)
Staphylococcus epidermidis	Skin		20 (27.0)
Staphylococcus warneri	Skin		11 (14.9)
Streptococci spp.	Skin/gut/oral		8 (10.8)
Veillonella atypica	Gut		1 (1.4)

[a]All microorganisms can be potentially pathogenic when they enter the bloodstream.

[b]Pathogenic in immunocompromised patients.

CNS, coagulase-negative staphylococci; spp., species.

After HOPE, none of the microorganisms detected in the SCS preservation fluid were found in the MPS fluid at the end of HOPE, despite the 52% contamination rate of the SCS preservation solution. During HOPE, no antibiotics were added to the machine perfusion fluid. These findings, therefore, suggest that the process of HOPE, including the organ flush between SCS and HOPE, might contributes to a mechanical clearing of any microbiological contamination. In addition, continuous flow with low temperature solution may prevent microorganism from surviving during HOPE. The formal proof of a presumed cleansing effect of HOPE would require a head-to-head comparison with SCS alone in a future study. Based on the current experience, we have no reason to change our current policy and start adding antibiotics during HOPE, also when it is applied for several hours to extend the preservation time for logistical reasons.[8]

After NMP, no microorganisms were grown from the machine perfusion fluid samples. However, in 1 recipient of a NMP-preserved liver abdominal drain fluid was positive for an *E coli* that was also grown from the SCS preservation fluid. In this patient, laboratory blood tests revealed increased inflammation parameters, but the patient remained asymptomatic. Immediately after the positive SCS preservation fluid and abdominal drain fluid cultures were noted, the patient was treated with intravenous meropenem and the patient could be discharged from the hospital without infectious symptoms. Unfortunately, in this particular case, no sample for culture was taken from the NMP fluid, which could have confirmed that the *E coli* had indeed "survived" the NMP procedure. However, this is very likely, because DNA sequencing confirmed that the *E coli* found in the recipient's drain fluid was the same as the one grown from the SCS preservation fluid. Moreover, this *E coli* was an *E coli* ESBL, known to be resistant to cefazolin that (together with metronidazole) is routinely added to the NMP perfusion fluid.

In our series, end-ischemic NMP was always preceded by 1 h of dual HOPE, to minimize ischemia-reperfusion injury at the start of NMP. Given the observed cleansing effect of HOPE, it might be that the rate of microbial transmission would have been higher if NMP was not preceded by HOPE. This topic requires further research, but it may indicate another benefit of HOPE before NMP.

Even with a low contamination and transmission rate during the current clinically used methods of machine perfusion, the transmission of microorganisms from contaminated

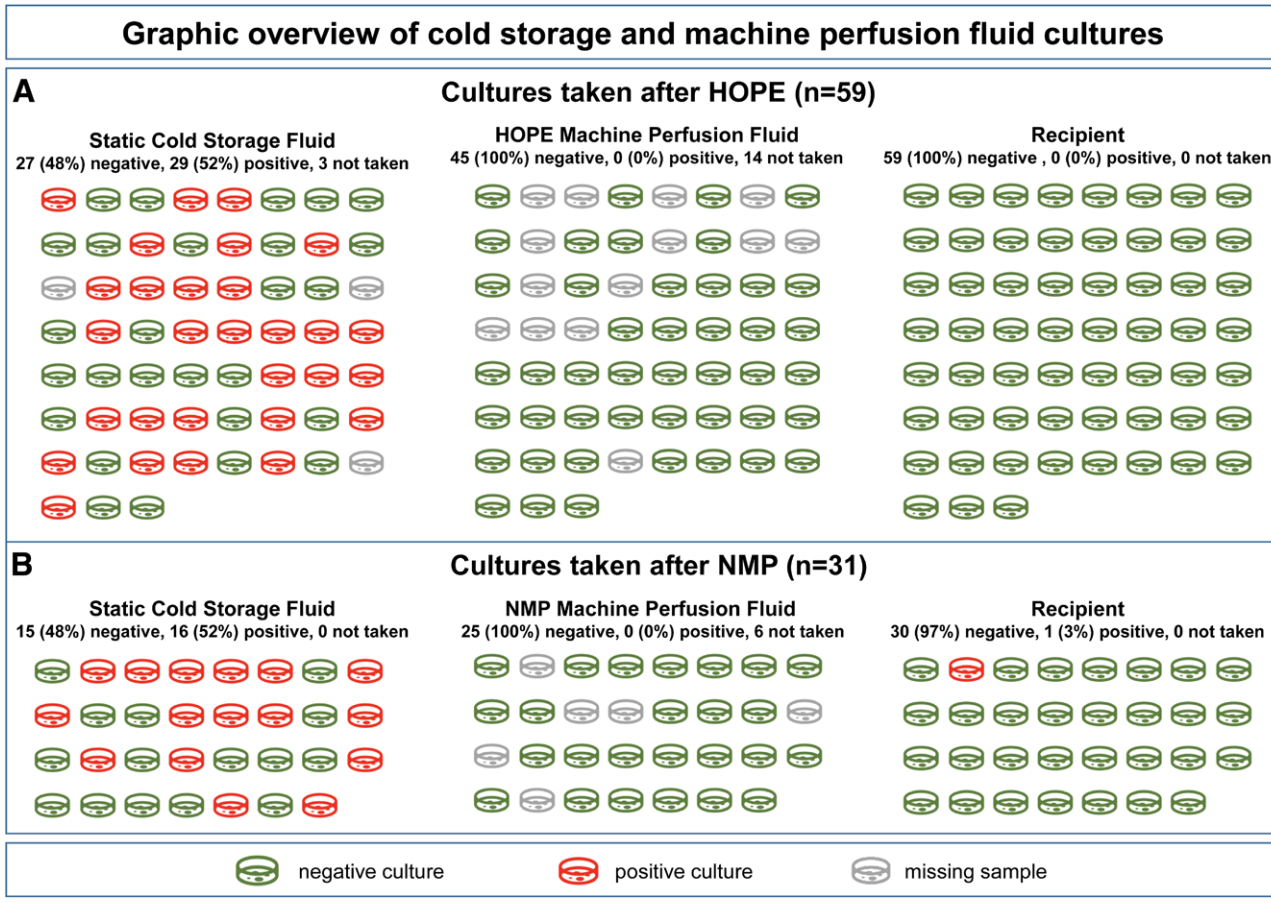

FIGURE 2. Graphic overview of cold storage and machine perfusion fluid cultures. Graphic overview of all samples available for microbiological analysis after HOPE (A) or NMP (B). Each petri dish reflects a single case in chronological order and represent the same procedure within the 3 columns. Green petri dishes represent a negative microbial culture, red represent a positive culture, and gray represent a missing sample. A, Overview of the samples analyzed after 59 HOPE perfusions. Although 29 of the 56 (52%) available SCS preservation samples were positive for a microorganism, none of the 45 machine perfusion samples after HOPE were positive. Also, none of the recipients of a HOPE preserved liver had a positive culture of an abdominal drain fluid sample with a microorganism that had been grown from either the SCS or HOPE fluid. B, Overview of the samples analyzed after 31 NMP perfusions. Although 16 of the 31 (52%) available SCS preservation samples were positive for a microorganism, none of the 25 available machine perfusion samples after NMP were positive for a microorganism. One of the 31 (3%) recipients of a NMP preserved liver had a positive culture of an abdominal drain fluid sample with a microorganism (*Escherichia coli*) that was also grown from the SCS preservation fluid. Unfortunately, no sample from the NMP fluid was taken in this case. HOPE, hypothermic oxygenated machine perfusion; NMP, normothermic machine perfusion; SCS, static cold storage.

preservation fluid can result in severe morbidity, including sepsis, graft loss, and even mortality.[5] Currently, there is no consensus on which antibacterial and antifungal medication should be added during machine perfusion, and centers have developed their own antibiotic and antifungal regime.[9] The temperature and machine perfusion fluid composition (eg, red blood cells) used for NMP provide an ideal condition for bacterial growth.

Hann et al[7] have reported a case of *E coli* infection in a liver recipient after NMP with a strain that was resistant to the antibiotics in the NMP perfusion fluid. Based on this case, the authors advocated to use meropenem as a prophylactic antibiotic during NMP. Although we also observed 1 case of transmission of cefazolin-resistant *E coli* after NMP, we do not advocate the routine use of meropenem as antibacterial prophylaxis during NMP, because of the risk of selecting resistant bacteria. In the Netherlands, it is a general policy not to use last-resort antibiotics, such as meropenem, for routine prophylaxis, but only for the treatment of a proven infection.[10] We, therefore, continue to use only cefazoline and metronidazole during the NMP, because most bacteria found in the SCS preservation fluid are skin and gut flora. Also, skin flora is the most likely source of contamination during organ retrieval and/or packing and typically of low pathogenicity. No antifungal medication is administrated because transmission of yeasts is low, and the recipients already receive antifungal prophylaxis. However, based on our experience presented here, we do advocate to take routine cultures of the machine perfusion fluid after either HOPE or NMP. As illustrated by the missing samples, especially in our early experience, culturing of the machine perfusion fluid has not always been standard practice in our center. However, with the current knowledge and experience, we now routinely take samples for microbiological culture at the end of ex situ machine perfusion. Routine biweekly culturing of abdominal fluid drains in the recipients has already been standard surveillance practice in our center. Together with the machine perfusion samples, this helped us to timely identify patients who are at risk of developing a symptomatic infection and may benefit from targeted antibiotic treatment.

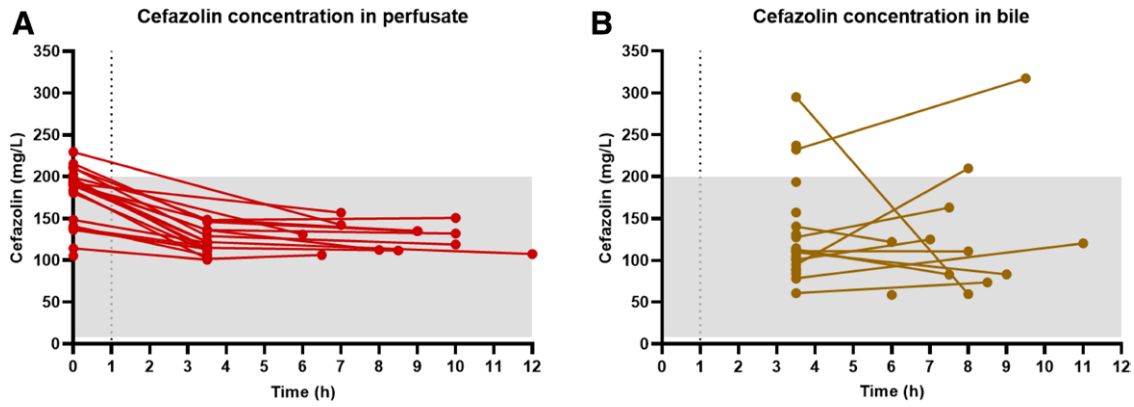

FIGURE 3. Cefazolin concentrations in the perfusate and bile during normothermic machine perfusion (NMP). A, The concentration of cefazolin in the machine perfusion solution was analyzed at baseline in 25 NMP procedures, at the time of viability assessment (2.5 h of NMP) in 17 NMP procedures, and at the end of NMP in 12 NMP procedures. B, The concentration of cefazolin in bile was determined after 2.5 h of NMP (time of viability assessment, when all livers had bile production to analyze) in 23 NMP procedures and at the end of NMP in 12 NMP procedures. The gray area represents the therapeutic window for cefazolin concentration in human blood. The dotted line indicates the start of NMP (after 1 h of rewarming). The cefazolin concentrations in the NMP perfusate and bile samples were within or slightly above the therapeutic window at all-time points.

In the current series, machine perfusion was applied for several hours. We and others are developing new perfusion devices that enable ex situ NMP for days and even up to a week.[11,12] When long-term (>24 h) NMP becomes clinically available, the risk of microbial contamination may increase.[13] Lau et al[14] recently reported a high rate of bacterial contamination (50%) during long-term NMP in an experimental, preclinical setting. Although this percentage appears relatively high, and this may have resulted from contamination during ex situ splitting of the liver and from insufficient sterility precautions in a preclinical research setting, it indicates that more research is needed to assess the efficacy and optimal dosing of antimicrobial prophylaxis during long-term NMP.

In our protocol, cefazolin and metronidazole were added to the NMP fluid at the start of the procedure, and no extra doses of antibiotics were added during NMP. Analysis of the cefazolin concentrations revealed stable levels in the perfusion fluid of up to 12 h of NMP and within the therapeutic range used for blood samples in patients (Figure 3). We were also able to measure cefazolin in samples of bile that were produced during NMP. In vivo, cefazolin is not metabolized by the liver and is mainly eliminated by the kidneys into the urine. However, our data indicate that some of the cefazolin is lost from the perfusion fluid through excretion into the bile. The consequence of this for long-term machine perfusion beyond 12–24 h will need to be determined in future research. Unfortunately, in our hospital, we were not able to measure concentrations of metronidazole in either perfusion fluid or bile samples.

The main limitation of this study is its retrospective design. At the start of our machine perfusion program, we did not routinely collect machine perfusion samples for culture in our clinical protocols. This explains why, in a number of cases, no samples were taken from the machine perfusion fluid for microbiological analysis. On the other hand, we have routinely taken samples for culture from the SCS preservation fluid (before machine perfusion) and from the abdominal drain fluid after the transplantation. This enabled us to identify 1 case of transmission of a cefazolin-resistant *E coli* from the SCS preservation fluid to the recipient of a NMP-preserved liver, despite the lack of a machine perfusion sample. Another limitation is that we did not taken samples between HOPE and NMP. However, only the presence of bacteria or fungi at the end of the entire perfusion procedure would be potentially clinically relevant.

In conclusion, this study performed in a cohort of ex situ machine perfusion procedures indicates that microbiological transmission is possible when using this novel preservation technique, yet the risk of a symptomatic infection in the recipient is very low. We observed no main difference in the risk of microbial transmission during either HOPE or NMP, despite the fact that NMP has an intrinsically higher risk because of the perfusate composition (eg, red blood cells) and the applied temperature of 35–37 °C. We do not advocate the prophylactic use of last-resort antibiotics, such as meropenem, because of the risk of resistant bacteria selection. Based on our experience, we now routinely collect samples of machine perfusion for microbiological analysis at the end of the procedures. This enables timely detection of a possible microbial transmission after ex situ machine perfusion and tailored administration of antibiotics to the recipient.

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