Hypothermic Oxygenated Machine Perfusion of Liver Grafts: Preliminary Experience in a Single Center

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Abbreviations:

ALT: Alanine aminotransferase

BMI: Body Mass Index

CCI: Comprehensive Complication Index

CIT: Cold ischemia time

DBD: Donation after brain death

DCD: Donation after circulatory death

EAD: Early Allograft Dysfunction

ECD: Extended Criteria Donor

HOPE: Hypothermic oxygenated machine perfusion

ICU: Intensive Care Unit

IRI: Ischemia-reperfusion injury

LT: Liver Transplantation

MELD: Model of end stage liver disease

MOF: Multi organ failure

NOMP: Normothermic oxygenated machine perfusion

POD: Postoperative day

PRS: Post-reperfusion syndrome

SCS: Static cold storage

PNF: Primary non function

SOMP: Subnormothermic oxygenated machine perfusion

Rezumat

Perfuzia mecanică oxigenată hipotermică a grefelor hepatice: experiența inițială a centrului nostru

Introducere: Necesitatea de a maximiza utilizarea grefelor și problema leziunilor ischemice-reperfuzionale a dus la utilizarea perfuziei mecanice oxigenate termoreglate care îmbunătățește funcția greii hepatice înainte de transplant. Printre aceste metode, protocolul HOPE (perfuzie mecanică hipotermică oxigenată) prezintă beneficii semnificative. Scopul lucrării este de a analiza experiența inițială în utilizarea unei astfel de proceduri într-un centru de referință de transplant hepatic.

Material și Metodă: Grefele hepatice standard cu timp de ischemie rece ≥ 6 ore, grefele marginale și grefele considerate improprii (dincolo de criteriile de grefă marginală) au fost perfuzate folosind HOPE. Criteriile de selecție pentru HOPE dual (perfuzia simultană a arterei hepatice și a venei porte) au fost steatoza hepatică, asocierea de cel puțin 3 criterii de grefă marginală și grefele considerate improprii pentru transplant. Principalele criterii pentru stabilirea ameliorării funcției greii au fost creșterea progresivă a debitelor arterial și portal, cu lactat sub 3 mmol/L sau, chiar dacă peste această valoare, cu o tendință de scădere pe parcursul perfuziei.

Rezultate: Grefele de ficat întregi recoltate de la 28 de donatori în perioada februarie 2016 și iunie 2021 au beneficiat de HOPE: 9 grefe considerate improprii au fost evaluate și confirmate ca fiind neutilizabile pentru transplant, în timp ce celelalte 19 au

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fost grefe marginale sau standard care au fost ulterior transplantate. HOPE dual a fost utilizat în 8 din cele 19 proceduri (42,1%). S-a obținut o creștere semnificativă a debitelor arterial și portal ($p = 0,005$ și respectiv $p = 0,001$). La recipienți s-au înregistrat îmbunătățiri semnificative ale valorilor AST, ALT, INR și lactat ($p < 0,001$, $p < 0,001$, $p < 0,001$ și respectiv $p = 0,05$). Rata complicațiilor majore postoperatorii (grad Dindo-Clavien ≥ 3) după LT a fost de 26,3%, în timp ce rata de disfuncție precoce a grefei a fost de 15,8%. Nu s-a înregistrat nici un caz cu sindrom sever de reperfuzie hepatică sau de rejet acut. Rata de mortalitate postoperatorii a fost de 15,8%. După o urmărire mediană de 9,3 luni (interval 2-44), rata tardivă a complicațiilor majore a fost de 15,8%, fără mortalitate.

Concluzie: Perfuzia mecanică oxigenată a grefelor este parte a practicii clinice actuale. În acest fel, grefele hepatice marginale pot fi utilizate în condiții de siguranță pentru transplant, îmbunătățind rezultatele postoperatorii și maximizând utilizarea grefelor disponibile. Pentru cele mai bune rezultate, considerăm că ambele tehnici ale HOPE (mono și dual HOPE) ar trebui utilizate, pe baza unor criterii de selecție specifice.

Cuvinte cheie: transplant hepatic, grefa marginală, perfuzie mecanică oxigenată hipotermică, dispozitiv LiverAssist

Abstract

Background: The need to maximize the use of donor organs and the issue of ischemia-reperfusion injury led to the use of thermoregulated oxygenated machine perfusion that improves the function of liver graft prior to transplantation. Among these methods, the HOPE (hypothermic oxygenated perfusion) protocol shows significant benefits. The aim of the paper is to analyze the early experience in using such procedure in a high-volume liver transplantation center.

Methods: Normal liver grafts with cold ischemia time ≥ 6 hours, marginal grafts and discarded (beyond ECD criteria) grafts were perfused using HOPE. Our selection criteria for dual HOPE (hepatic artery and portal perfusion) were steatosis, at least 3 associated ECD criteria, and discarded grafts. The main criteria to establish graft improvement were the progressive increase of arterial and portal flows, with lactate under 3 mmol/L or, even if over this value, with a decreasing trend during perfusion.

Results: Whole liver grafts harvested from 28 donors between February 2016 and June 2021 benefitted from HOPE: 9 otherwise discarded grafts were assessed and considered not fit for transplantation, while the other 19 were ECD or standard grafts that were subsequently transplanted. Dual HOPE was used in 8 out of the 19 procedures (42.1%). We obtained a significant increase of arterial and portal flow ($p=0.005$ and $p=0.001$, respectively). In recipients, significant improvement of AST, ALT, INR and lactate values were recorded ($p<0.001$, $p<0.001$, $p<0.001$, and $p=0.05$, respectively). The rate of major postoperative complications (Dindo-Clavien grade ≥ 3) after LT was 26.3%, while the rate of early graft dysfunction was 15.8%. No PRS or acute rejection was recorded. The postoperative mortality rate was 15.8%. After a median follow-up of 9.3 months (range 2-44), the late major complication rate was 15.8%, without mortality.

Conclusion: Machine perfusion is nowadays part of current clinical practice. This way, marginal liver grafts (DCD, ECD-DBD) may be safely used for transplantation improving the outcome, thus effectively enhance the use of a persistent scarce pool of donors. For best results, we believe that both techniques of HOPE (mono and dual HOPE) should be used based on specific selection criteria.

Key words: liver transplantation, marginal graft, hypothermic oxygenated perfusion, LiverAssist device

Introduction

The reduced number of available organs is a major challenge for liver transplant (LT) (1). A major source for organs used for transplant are the so-called marginal liver, representing low quality grafts that can still be used for LT, with an acceptable risk for complication after implantation by using the so-called extended criteria for donation (ECD) from donation after brain death (DBD) donors, and the grafts from donors after cardiac death (DCD). It is well known that ECD grafts, and especially DCD ones have a high risk for primary non-function / dysfunction (2).

The main preservation method for grafts is nowadays static cold storage (SCS). The major drawback of this method is that always injures graft in different degrees, mainly in function of the initial quality of the graft and of the preservation time. Good grafts tolerate well SCS times up to 12 hours. However, marginal grafts tolerate poorly the SCS, increasing the risk of graft failure after implantation, longer hospital stays, delayed biliary strictures, and increased treatment costs (3). To overcome this issues, a new method of preservation have been developed to increase the use of marginal grafts while optimizing the LT results (2), consisting in thermally controlled oxygenated perfusion of liver grafts that provides oxygen and nutrients to the graft, while removing the toxic waste (4). This procedure is carried out using a dedicated perfusion machine that controls the flow through portal vein and/or hepatic artery (simulating physiological flows), using dedicated pumps, and simultaneously oxygenates the graft, using an extracorporeal membrane oxygenation system. In the last decade, promising data regarding the liver graft perfusion using specially designed devices - OrganOx™, Organ Care System (OCS™) and Liver Assist® have been reported (5,6). In function of perfusion temperature, the procedure may be normothermic (36-37°C) (7), subnormothermic (20-28°C) (8) or hypothermic oxygenated mechanical perfusion (12-14°C) (9). The latter may involve dual perfusion (both

portal vein and hepatic artery) (dual HOPE) (10) or single perfusion (portal vein only) (11).

The aim of this paper is to analyse our early experience using the hypothermic oxygenated mechanical perfusion in a high-volume liver transplantation center.

Patients

The selection criteria for the recipients were adult patients (>18 years) with liver cirrhosis and/or hepatocellular carcinoma requiring orthotopic LT with whole liver grafts. Also, high MELD recipients were accepted, as we considered the perfused ECD grafts equivalent to standard grafts in terms of donor-recipient matching policy. The standard LT technique used in our center was previously described (12). The exclusion criteria were technical variants of LT, such as split, reduced graft, accessory, domino, or living donor LT (*Table 1*).

Data on the LTs performed with perfused grafts between February 2016 and June 2021 were recorded, including standard patient and recipient features, MELD score, transplant operation details, preservation time, use of machine perfusion (MP), and postoperative outcomes, were prospectively collected and retrospectively analyzed. Retransplants were excluded from the analysis. No DCD LTs were performed, as our current protocol does not include such donors. Patients signed an informed consent on receiving a graft treated with MP. Due to the retrospective design, no specific approval was requested from our Institutional Ethical Board.

Our study endpoints in recipient were: (1) levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (2) lactate level; (3) duration of hospital and intensive care unit stay; (4) rate of EAD (13); (5) significant post-reperfusion syndrome (PRS) rate; (6) acute rejection rate; (7) grade ≥ 3 complications rate (14); (8) biliary complications rate at 6 months; (9) patient and graft survival. Severe PRS was defined as persistent decrease in arterial blood pressure ($>30\%$ of the anhepatic level), asystole or hemodynamically significant arrhythmias (15). Biliary complications were classified as

Table 1. Criteria for ECD graft and recipient inclusion, exclusion criteria for HOPE

ECD criteria	Inclusion criteria	Exclusion criteria
• Donors ≥ 65 years	• Patients 18 years or older	• Split, reduced graft, accessory, domino, or living donor LT
• ICU stay ≥ 7 days	• Listed for LT	
• BMI > 30 kg/m ²	• Receiving ECD-allografts and/or normal grafts with CIT ≥ 6 h	
• Steatosis	• With end stage-liver disease and/or hepatocellular carcinoma	
• Serum-Sodium > 165 mmol/l	• With signed informed consent	
• Serum AST or ALT $> 3 \times$ upper limit of normal		
• Serum-Bilirubin > 3 mg/dl		
• Hypotension and inotropic support (≥ 2 pressors at any time, high-dose dopamine or epinephrine)		
• Resuscitated cardiac arrest		
• CIT > 12 hours		
• Viral infections: Positive serology for HBV hepatitis (AgHBs (+); AgHbc (+)) or for HCV hepatitis		

anastomotic (including strictures and fistulae) and extra-anastomotic.

Methods

Standard liver grafts with cold ischemia time (CIT) ≥ 6 hours, ECD criteria liver grafts and discarded (beyond ECD criteria) liver grafts were perfused using the hypothermic oxygenated mechanical perfusion method. Standard liver grafts with CIT ≥ 6 hours were perfused to prevent becoming marginal grafts, the ECD grafts were perfused to improve their function prior to LT, while the discarded grafts were tested on the machine to evaluate their function and availability for LT. The ECD criteria are depicted in *Table 1* (16,17).

Following cross-clamping (in situ flushing with in cold Wisconsin® preservation solution of the abdominal organs and begin of CIT), the grafts were removed and transported in conventional SCS on packed ice (4-6°C). The back table preparation was performed prior or after transportation to the transplant center. Grafts were weighed and flushed with a supplementary 1 L of preservation solution at the end of back table preparation. As our center protocol does not include pre-transplant routine graft biopsy, graft macroscopic appearance and donor BMI were mainly used as surrogates for

diagnosis of steatosis, pre-transplant biopsies being performed only in some cases. Graft macro- and microsteatosis assessment by biopsy was usually performed during MP or after implantation.

At transplantation site, the grafts were prepared for mounting on the LiverAssist® perfusion device, by cannulating the portal vein only (for the HOPE procedure) or both portal vein and hepatic artery (for dual HOPE procedure); to avoid the risk of damaging the hepatic artery due to cannulation, the supra-truncal aorta was used. In case of aberrant hepatic artery, the appropriate vascular reconstruction was performed before MP. The standard grafts with CIT ≥ 6 hours and marginal grafts with less than 3 ECD criteria for donation were allocated to HOPE procedure, while marginal grafts with steatosis and/or with at least 3 ECD criteria, and discarded grafts (beyond ECD DBD grafts) were allocated to dual HOPE. This policy was based on the concept that severely impaired parenchyma benefits most from dual perfusion of both artery and portal systems.

Prior to the connection to the device, the liver was flushed via the portal vein cannula with 1000 mL cold (0-4°C) Belzer MP® solution (Bridge-to-Life, Ltd., Northbrook, IL) until the caval effluent was clear. Simultaneously with

the back table procedure, the Liver Assist device was prepared for use. The disposable kit was mounted on the machine, and the LiverAssist® device (OrganAssist®, Groningen, The Netherlands) was primed with 3L of Belzer MPS® solution (Bridge to Life Europe Ltd, Wandsworth, London, UK). After the system pressure was equalized to the atmospheric value, the perfusion was pressure controlled with the arterial and portal inflow limited to a mean of 27 mmHg (pulsatile pumping), and 4 mm Hg (continuous pumping), respectively. The temperature of the perfusion fluid was set to 12°C, and the thermoregulator was continuously filled with crushed ice placed in the dedicated reservoir of the cooling unit to achieve the designated temperature. After woods, the priming procedure was performed, consisting in eliminating the air bubbles present inside the disposable tubing, followed by sensor pressure nulling. The oxygen flow was set at 500 mL/min of 100% oxygen on each of the two membrane oxygenators, and the solution was oxygenated for at least 15 minutes prior to graft attachment to the device. The Liver Assist device was set once the temperature was achieved. These settings during procedure were based on previous studies and were lower than physiological pressures to avoid shear stress of the cold endothelium of the hepatic vasculature (7,18, 19). The oxygenation was performed in such manner to obtain an adequate pO₂, which has been proved to increase the ATP, while being safe for the graft (20,21).

Once the device was set, the grafts were mounted via the cannulas, without prior flushing with Belzer MP solution. The surgeon connected the cannulas to the disposable tubing of the machine after which the arterial and portal pumps of the device were started. Even when only the portal vein was perfused, the arterial pump was activated to better recirculate the perfusate in the system, insuring optimal oxygenation and cooling. Perfusion fluid and graft were continuously cooled to 12°C during the whole procedure. The device continuously registered flow rates and

temperature and gave alarms in case of high flow or temperature. Perfusate was sampled every 30 minutes, including gas analyses, AST, ALT, lactate, and base deficit. A surgeon supervised the machine and graft throughout the entire procedure. The transplant operation, consisting exclusively in whole graft LT, was coordinated to allow for a minimum of 90 minutes of MP, which was concluded at the end of recipient hepatectomy, when the graft was disconnected from the device and transferred to for implantation. The grafts were flushed with chilled 5% albumin before implantation in the recipient.

The main selection criteria for MP were the ECD criteria (*Table 1*). Particularly, graft steatosis was usually evaluated by the retrieval surgeon, as biopsy was not routinely available. Logistic issues determining expected ischemia time ≥ 6 hours of normal grafts were also a selection criteria. Steatosis, more than 3 associated ECD criteria, and discarded grafts (beyond ECD DBD grafts) were the selection criteria for dual HOPE procedure.

Our endpoints for the MP procedure were: (1) peak levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (2) lactate level in the recipient at the end of transplant operation; (3) perfusate gases content (O₂ and CO₂); (4) arterial and portal flow debit.

The main criteria to establish that graft improvement was obtained after the procedure and the graft is fit for LT were the progressive increase of arterial and portal flows, while the lactate remained under 3 mmol/L or, even if over this value, decreased during perfusion below this threshold.

All data were collected by trained members on specific paper case report forms and used as source data. Continuous variables were compared with the Student t test. All p-values were based on 2-sided testing and considered statistically significant if $p < 0.05$. Values were displayed as median and range for metric parameters, and numbers and (percent) for nominal data. Statistical analysis was performed using SPSS Statistics (IBM Corp., NY).

Results

Donor, Graft and Perfusion Features

Whole liver grafts harvested from 28 donors between February 2016 and June 2021 benefitted from HOPE: 9 otherwise discarded grafts were assessed and considered not fit for LT, while the other 19 were ECD or standard grafts that were subsequently transplanted. The median set-up time for the procedure was 35 minutes (range 25-50).

For donors that provided grafts for LT, the median age was 58 years (range 22-81), with a male/female ratio of 8/11. The main cause of brain death was cerebrovascular event (42% of donors). Out of these grafts, 17 (89.5%) had ECD criteria. The criteria for ECD grafts are depicted in *Table 2*, with multiple criteria recorded in 76.5% of donors. CIT, perfusion duration, warm ischemia time, total preservation time (perfusion and CIT), total ischemic time (CIT and warm ischemia time), and graft weight are depicted in *Table 3*.

Table 2. Donors used for liver transplantation with perfused grafts (N=19)

Donors	Median (range) / n (%)
Age (years)	58 (22-81)
Gender (male / female)	8 / 11
ECD criteria n (%)	
- Donors ≥ 65 years	7 (41.2)
- ICU stay ≥ 7 days	4 (23.5)
- BMI > 30 kg/m ²	1 (5.9)
- Steatosis	5 (29.4)
- Serum-Sodium > 165 mmol/l	7 (41.2)
- Serum AST or ALT $> 3 \times$ upper limit of normal	3 (17.6)
- Serum-Bilirubin > 3 mg/dl	0 (0.0)
- Hypotension and inotropic support (≥ 2 pressors at any time, high-dose dopamine or epinephrine)	8 (47.1)
- Resuscitated cardiac arrest	7 (41.2)
- CIT > 12 hours	0 (0.0)
- Viral infections: Positive serology for HBV hepatitis (AgHBs (+); AgHbc (+)) or for HCV hepatitis	2 (11.8)
Number of ECD criteria n (%)	
- 1 out of 7	4 (23.5)
- 2-3 out of 7	10 (58.8)
- > 3 out of 7	3 (17.7)
Cause of death n (%)	
- Cerebrovascular accident	8 (42.1)
- Trauma	7 (36.8)
- Others	4 (21.1)

Table 3. Perfused grafts features

Perfused transplanted grafts (N=19)	
Graft types n (%)	
- ECD grafts	17 (89.5)
- Normal grafts with CIT > 6 h	2 (10.5)
CIT (min) median (range)	543 (291-820)
HOPE duration (min) median (range)	122.5 (90-310)
Warm ischemia time (min) median (range)	35 (10-77)
Total preservation time (HOPE and CIT) (min) median (range)	635 (292-1106)
Total ischemic time (cold and warm ischemia time) (min) median (range)	586 (313-897)
Graft weight (g) median (range)	1670 (1245-2022)

Parameters related to the procedure are depicted in *Table 4*. We used dual HOPE in 8 out of the 19 procedures (42.1%). During all procedures we obtained a significant increase of arterial and portal flow ($p=0.005$ and $p=0.001$, respectively). The median arterial and portal flow improvement rate, defined as (end-flow – start-flow) $\times 100$ /end-flow, was 36% (range 11-71) and 53% (range 8-87), respectively. pH values of the perfusate did not exceed 7.3, while lactate values in the perfusate exceeded 3 mmol/L in 2 procedures, but always decreased after the peak value; only one of these 2 grafts had an end-value of the lactate above 3 mmol/l (3.6).

We also assessed 9 grafts beyond ECD criteria. The criteria for discarded grafts were: severe steatosis (N=2), significant fibrosis (N=4) and resuscitation after long period of cardiac arrest (N=3), respectively. None of these grafts were considered for LT after testing on the LiverAssist[®] using dual HOPE procedure due to poor flow parameters and high lactate values.

Recipient and Intraoperative Features

The median age of recipients was 58 years (range 44-67), with a male/female ratio of 14/5. All had cirrhosis, with a median MELD score of 15 (range 9-26), while 10 (53%) also had hepatocellular carcinoma. The median blood loss was 2000 ml (range 1000-8500). The median operative time was 385 min (range 232-678).

Table 4. Parameters of HOPE procedures

Mechanical perfusion	Median (range)/n (%)				
HOPE / dual HOPE n	11 / 8				
	at start	at end			
Arterial flow (ml) median (range)	58.5 (44-160)	131 (53-202)			
Portal flow (ml) median (range)	230 (40-550)	500 (230- 670)			
	Arterial	Portal			
Flow improvement (%) median (range)	36.5 (11.3-70.9)	53.1 (8-87.1)			
	At start	At 30 min	At 60 min	At 90 min	At 120 min
pH median (range)	7.1 (7-7.3)	7.1 (7-7.1)	7.1 (7-7.1)	7.1 (7-7.3)	7.1 (7-7.3)
lactate median (range)	1.6 (0.3-2.6)	2.3 (1.4-4.1)	2.4 (1.4-3.6)	2.2 (1-3.6)	2.1 (1.3-3.6)

Graft and Recipient Outcome

Significant improvement of AST, ALT, INR and lactate values were recorded between POD 1 and POD 7 ($p<0.001$, $p<0.001$, $p<0.001$, and $p=0.05$, respectively), without significant improvement of total bilirubin and albumin (Table 5).

The rate of major postoperative complications (Dindo-Clavien grade ≥ 3) after LT was 26.3% (5 cases). The rate of early graft dysfunction was 15.8% (3 cases). No PRS or acute rejection was recorded. There was one case with early stenosis and fistula at the level of the biliary anastomosis. The postoperative mortality rate was 15.8% (3 cases), the cause of death being primary non function in one case and pulmonary sepsis in 2 cases. The median ICU stay was 7 days (range 1-51), while the median hospital stay was 26 days (range 11-60).

The median follow-up was 9.3 months (range 2-44). The late major complication rate was 15.8% (3 cases), with late hepatic artery thrombosis requiring retransplantation (one

case), severe neurological impairment (one case), and HBV reactivation. No death was recorded during the follow-up.

First Case with Liver Assist® in Romania (February 2016)

The donor was a 22-yr old female with polytrauma after car incident (including liver hematoma in segment 7), with multiple criteria of ECD liver graft: resuscitated cardiac arrest; high doses of noradrenaline (1 microg/kg/min the first 12 hours, followed by 0.5 microg/kg/min until harvesting) associated in the last 24 hours with adrenaline (1 microg/kg/min); hypernatremia (172 mEq/L). According to our protocol, we performed a dual HOPE procedure of 2 hours (Fig. 1). The recipient was a 64-yr old male with HCC outside Milan Criteria (2 nodules – one of 5 cm with previous TACE, and one of 2 cm) on alcohol-related liver cirrhosis. The transplant was performed according to our standard technique previously described (12), with uneventful intra- and postoperative course

Table 5. Postoperative outcome after liver transplantation with perfused grafts

Postoperative parameters	POD1	POD3	POD5	POD7
AST (U/l) median (range)	235.5 (92-877)	85.87 (45-280)	43 (22-143)	32 (4.8-81)
ALT (U/l) median (range)	242.5 (55-649)	145.5 (24-447)	74.8 (25-290)	47 (16-173)
Total bilirubin (mg/dl) median (range)	1.8 (0.7-6.4)	2 (0.9-10.1)	2.1 (0.9-9.2)	1.8 (0.7-8.7)
INR median (range)	2.1 (1.3-4)	1.5 (1.2-1.8)	1.4 (1.1-1.7)	1.3 (1.1-1.5)
Albumine (g/l) median (range)	3 (2.1-4.2)	3.3 (1.5-4.4)	3.5 (2.6-4)	3.5 (2.7-4.3)
	POD1	POD2	POD3	POD4
Lactate (mmol/L) median (range)	1.2 (0.8-3.9)	1.1 (0.5-2.3)	0.9 (0.6-1.6)	0.9 (0.4-1.6)

Figure 1. Liver graft during dual HOPE procedure. Note that both hepatic artery and portal vein are cannulated and perfused

(Fig. 2). The patient was discharged in POD 14 and had a regular follow-up at 66 months.

Discussions

History

In the 1930s, whilst solid organ transplantation was becoming a clinical reality, Carrel and Lindbergh used for the first time the MP of the organs to be transplanted, in normothermy, with blood as perfusate (22), while in the 1960s hypothermic MP was introduced using Belzer's solution (23). However, due to technical issues, the method was abandoned in favor of the SCS method introduced by Collins et al. in 1969 (24). Later, in the 2000s,

the technological advancements allowed the revival of the MP technique (25), needed to overcome the well-known disadvantages of the SCS.

Static Cold Storage

SCS causes injury to the liver graft. The hypoxic conditions during SCS favors the anaerobic metabolism leading to accumulation of metabolites that induce ischemia-reperfusion injury (IRI) that induces graft dysfunction and systemic inflammatory response syndrome in recipient (Fig. 3). The main modifications are cell swelling and loss of cytoplasmic processes, destruction of sinusoidal endothelial cells, sinusoidal constriction, inflammatory reaction, platelet aggregation and formation of microthrombi (20). The first event in the induction of pathological ischemia-reperfusion injury appears to be a burst of mitochondria-derived superoxide (O_2^-), leading to the production of other reactive oxygen species, like hydrogen peroxide (H_2O_2). Reverse electron transfer through mitochondrial complex I is the main mechanism of superoxide production as a result of the high protonmotive force that is created upon reperfusion (26). This leads to specific inflammation, Kupffer cells activation, dendritic cells maturation, endothelial expression of adhesion molecules, neutrophils infiltration, platelet aggregation and, finally, impairment of the microcirculation (27). These injuries are more severe in marginal

Figure 2. Implanted liver graft. Note the liver hematoma in segment 7

Figure 3. The ischemia-reperfusion injury (IRI) leading to graft dysfunction, systemic inflammatory response syndrome (SIRS), and even shock

grafts, which are grafts coming from donors after circulatory death (DCD) or from donation after brain death (DBD) donors with extended criteria for donation (ECD), such as older donors (over 65), donor with mild steatosis, donors with a long stay in the ICU unit before liver retrieval, high liver enzymes and cold ischemia >12 hours(28). These injuries are very detrimental on early and late organ function, affecting the parenchyma (graft dysfunction) and the biliary tree (late biliary stenosis). To limit these adverse effects, the preservation period using SCS is limited to a maximum of 12 h, for a normal liver graft, and to a maximum of 6 h in case of extended criteria donor (ECD) grafts.

Besides the ischemia due to cold ischemia, a second type of ischemia occurs consisting in rewarming ischemia, with different mechanisms. Rewarming ischemia is encountered during back-table procedure and during the implantation (when the vascular anastomoses are done). In this stage the cellular energy stores decrease. Afterwards the ischemic liver tissue is exposed to oxygen at normothermic temperature (37 °C) and the reperfusion injury appears. Quality grafts tolerate well these modifications, but the marginal ones are

significantly affected, with graft dysfunction frequently occurring in these cases.

Machine Perfusion

The machine perfusion (MP) comes as better method compared to the SCS, improving the quality of grafts, especially in case of marginal ones. Moreover, it also overcomes the effects of rewarming ischemia. It provides nutrients to the liver and removes toxic substances.

MP can increase the use of marginal grafts in the context of a serious shortage of organs. This requires the development of new techniques to minimize the extent of ischemia/reperfusion injury and to increase the quality of marginal grafts and their life (29,30).

The aims of the organ perfusion is to treat and assess the graft, to prolong the graft preservation time, while protecting the recipient against the IRI effects.

Treating the liver graft to improve its quality prior to LT is carried out by the standard perfusion protocol, potentiated by adjuvants like forskolin and glucagon (to increase the cAMP-PKA), and / or FFA, glycerol, polyethylene glycol 35 (to increase the cell metabolism, mitochondria and

peroxisomes activity) (31,32). The graft may also be assessed during perfusion (especially in case of DCD and beyond ECD-DBD grafts) by evaluating the liver function while on the machine, in order to make decision about its viability and suitability for LT, thus avoiding dismal transplantations. The parameters commonly used are (6):

- the bile production and output (in NOMP and SOMP);
- the markers of cell injury in the perfusate: base excess, lactate, AST, ALT, hyaluronic acid, and, more recently, the released mitochondrial flavoproteins (flavin mononucleotide - FMN) (cofactor in complex-1 mediated mitochondrial respiration) by fluometric analysis (33);
- the flow parameters during MP: portal and arterial pressure and resistance.

MP protects the recipient against potentially life-threatening complications by significantly improving the parenchyma function and graft hemodynamics after LT, leading to increased lactate clearance, lower INR values, increased synthesis of factor V, decreased values of AST, ALT and bilirubin, and increased platelets count.

The dynamic preservation method using MP may consist in organ (graft) or regional (in donor) oxygenated mechanical perfusion using special solutions at 12-14°C (hypothermic perfusion) or at 20-28°C (sub-normothermic perfusion), or blood at 36-37°C (normothermic perfusion). Various additives, as medications and nutrients, could be added in the perfusate

in order to improve the efficacy of the procedure. The dynamic preservation is clearly superior to SCS, enabling the normal cellular metabolism, recovering the cellular energy status, allowing the repair of reversible injury and the functional testing of the graft before LT.

The normothermic oxygenated regional perfusion is especially used in donation after cardiac death (DCD) donors with better results in comparison to super-rapid organ retrieval in terms of graft loss and biliary complications (34). In this setting, normothermic seems to be better than hypothermic perfusion (34,35).

The normothermic oxygenated machine perfusion (HOPE) of graft reduces the CIT, maintains liver function with minimal injury, allows bile production before implantation, allows use of perfusate scavengers / cytokines filters, maintains or even improves the various hepatobiliary parameters post-implantation (36), with extensive data currently available in the literature (*Table 6*). The sub-normothermic oxygenated machine perfusion (SOMP) of the graft allows bile production, as NOMP, maintains liver function with minimal injury, maintains and even improves the various hepatobiliary parameters post-implantation (37); however, scarce data are currently available in the literature. HOPE effectively maintains liver function with minimal injury, maintains or improves the various hepatobiliary parameters post-implantation while avoiding the disadvantages of blood use (when compared to NOMP), with extensive published data (*Table 7*). The

Table 6. Clinical trials on NOMP

STUDIES	Year	RCT	DBD	DCD	+ HOPE	Technique
COMPLETED						
Bral et al (45)	2017		✓	✓		NOMP (Organox)
De Carlis et al (46)	2018			✓	✓	NRP+HOPE
Hessheimer et al (47)	2018			✓		NORP
Nasralla et al (48)	2018	✓	✓	✓		NOMP (Organox)
Ravikumar et al (49)	2016		✓	✓		NOMP
Liver Revive trial (50)	2016	✓		✓		NOMP (OCS)
Watson et al (42)	2018			✓		NORP
Watson et al (51)	2018		✓	✓		NOMP (Liver Assist)
ONGOING						
Liver Protect trial (52)	ongoing	✓	✓	✓		NOMP (OCS)

Table 7. Clinical trials on HOPE

STUDIES	Year	RCT	DBD	DCD	+ NOMP	Technique
COMPLETED						
De Carlis et al (46)	2018			✓	✓	NRP+HOPE
Dutkowski et al (41)	2015			✓		HOPE
Guarrera et al (40)	2010		✓			HMP
Schlegel et al (53)	2018			✓		HOPE
Van Rijn et al (54)	2017			✓		D-HOPE
Van Rijn et al (11)	2018			✓		D-HOPE
Porte et al (55)	2021	✓		✓		D-HOPE
Czigany et al (56)	2021	✓	✓ (ECD)			HOPE
ONGOING						
Dutkowski et al (57)	ongoing	✓	✓			HOPE
University of Cincinnati et al (58)	ongoing	✓	✓			HOPE

liver may be perfused only via the portal vein (HOPE method) (10) or via both the hepatic artery and portal vein (dual HOPE) (38). Particularly, HOPE increases the ATP in the graft and protects from mitochondrial injury before LT (39). The devices that are more often used are OrganOx™ and Organ Care System (OCS™) for NOMP and Liver Assist® (single or dual pump), VitaSmart® and LifePort® Liver Transporter for HOPE and SOMP.

Currently, there are no conclusive comparative clinical data to support the superiority of

one of these perfusion techniques. However, there are some preliminary data demonstrating the superiority of HOPE and SOMP over NOMP in terms of preventing the IRI and biliary complications (*Table 8*) (40-43). It is most probable that a combination of these procedure would provide the best results (38) (*Fig. 4*). For example, normothermic regional perfusion and HOPE in DCD achieved similar post-LT results as in standard DBD LT (44).

Recently, an new method of preservation was introduced, the deep cold preservation method, consisting in the perfusion of graft with protective chemicals to combat ice formation (a cocktail of cryoprotectant chemicals, including trehalose and glycerol) and sub-

Table 8. Comparison between SCS, normothermic and hypothermic or sub-normothermic oxygenated machine perfusion

Parameters	SCS	Oxygenated MP	
		Normothermia	Hypothermia Subnormothermia
Portability	✓✓	✓~	✓~
Simplicity	✓✓	~	✓~
Cost	✓✓	~	✓~
Viability testing	-	✓✓	✓
DCD (biliary complications)	✓✓	✓	✓✓
Reperfusion reduction	~	✓	✓✓
NADH	✓✓	✓	✓
Succinate	✓✓	✓	✓
ADP	✓	✓	✓✓
AMP	✓	✓	✓
Hypoxanthine	✓		
Electron flow	0	reverse	forward
Adhesion mediators	✓✓	-	✓✓
Pro-inflammatory injury cytokines	✓✓	-	✓✓
ATP	✓✓	✓	✓✓

Figure 4. Combinations of preservation methods. HOMP - Hypothermic oxygenated machine perfusion (HOPE)

sequent storing at subzero temperatures (-4°C) (supercooling), maintaining the graft viability for about three times (27 hours) as the SCS (59). However, this method is in a very early stage of experimentation.

Hypothermic Oxygenated Mechanical Perfusion

HOPE is particularly effective after 2 hours of perfusion (when the O_2 consumption decreases) in restoring the cellular ATP, that represents the energy that the organ needs to face aggressions (inflammation, ischemic/reperfusion syndrome) and to reinstate its normal function (41). In the experimental setting, HOPE has shown to improve liver graft preservation as compared to SCS through continuous shear stress over sinusoidal endothelial cells, washout of metabolites produced during ischemia, increase of cellular adenosine triphosphate content, better peri-biliary vascular plexus perfusion, and decreased mitochondrial release of reactive oxygen species at reperfusion by reverse electron transfer through mitochondrial complex (60,61). Particularly, HOPE-treated liver grafts, as opposed to the ones after SCS, release potassium in the perfusate during MP and take up potassium after reperfusion in the recipient, preventing the development of the acute hyperkalemia that is frequently associated with severe PRS (62).

HOPE prolongs the preservation time on machine to at least 6-7 hours, and total CIT to up to 19 hours, without affecting in any way the graft function, allowing to optimize the transplantation activity in difficult settings, such as difficult total hepatectomy in recipient and/or various organizing issues (*Table 9*). Recently, early experience on preservation time of 1 week was proved feasible (63).

Moreover, MP may facilitate the ex-situ split of liver grafts during HOPE, significantly facilitating this type of LT and improving its results (64,65).

HOPE has already proven able to decrease the costs due to reduced morbidity and hospital stay (69), decreased early dysfunction rate (5%

vs 25% in case of SCS) (67), decreased biliary complication rate (10% vs 20% in case for SCS) (67). However, it does not influence primary non-function (67). Indeed, we had a case of primary non function, even though the graft behaved within parameters during MP. Moreover, DCD after HOPE provides similar results to DBD in terms of 5-yr graft survival (53). Additionally, it safely increases the CIT to up to 20 hours (66). As a note of caution, HOPE induces reperfusion hypokalemia instead of hyperkalemia as would normally result after implantation of grafts preserved using SCS (62).

No differences were showed in terms of early and 5-year outcomes post-LT between LT performed with DCD and DBD after dual HOPE (53). It is recommended to reduce cold preservation time to maximum 9h also in the setting of dual HOPE, particularly for marginal grafts (70).

In this paper, we show that the use of HOPE in ECD grafts from DBD donors leads to a significant improvement of AST, ALT, INR and lactate values after LT, with no PRS syndrome and a significant reduction of 90-day major complications. In what PRS is concerned, the data available in the literature confirm that occurs rarely or even anecdotally in LT with HOPE grafts, with a rate no higher than 4% (71). The recorded rate of major complications of 26% is in line with recently published data that reported a rate of 44%, in comparison with 74% after SCS alone (56). Moreover, it was also recently shown a trend towards reduced EAD after HOPE in comparison to SCS only (17% vs. 35%) (56). Indeed, we also recorded a low EAD rate of 15.8%. None of the early or late compli-

Table 9. Clinical studies on preservation time using the mechanical perfusion

STUDIES	HOPE Mean duration (hours)	Total CIT (HOPE + SCS) (h)
De Carlis et al. (66)	7	19
Guarrera et al. (40)	4	9
Guarrera et al. (67)	4	9
Dutkowski et al. (41)	5	8.5
De Carlis et al. (68)	3 HOPE + 8 NOMP (11)	7
van Rijn et al. (54)	2	8.5
Dutkowski et al. (10)	2	4.5

cations were related to the use of MP. No late biliary complications were recorded. Of note, we accepted also high MELD recipients with a score up to 26, as we considered that perfused marginal grafts should behave as standard grafts and therefore considered as such when matching the graft to recipient. In our opinion this is the second most valuable benefit, along the improvement of LT outcome. However, this policy is currently uncommon, as other centers tend to allocate the perfused grafts to low-MELD recipients; a recent study on HOPE reported the highest MELD score of 18 (56).

In our experience, the most obvious benefit of HOPE was the improvement of rheologic features during the perfusion and hemodynamic stability at graft reperfusion in the recipient. Improved hemodynamic stability is consistently observed when MP is used (normothermic or hypothermic), most probably due to the reduction of ischemia-reperfusion injury, the washout of metabolites produced during ischemia and to the “vascular bed recruitment” effect of MP (53).

Currently, our center is the only one in Romania performing mechanical perfusion of grafts, and, to our knowledge, the only one worldwide that uses both types of HOPE, HOPE and dual HOPE.

This study is a retrospective analysis of an early experience at a single center and has therefore a series of limitations, including its retrospective nature, limited number of cases. Another limitation is in case of HOPE for steatosis, as the diagnosis was mainly based on subjective criteria rather than biopsy.

Further scientific and technological developments in organ perfusion are needed for establishing the optimal temperature, perfusate composition and O₂ protocols, increasing the preservation time to up to 24 hours (72), use of effective healing chemicals / biological agents, as stem cells, to restore normal liver function (73,74), and even prevention of HCC recurrence in the implanted graft (75). Precise markers for function assessment and transplant suitability by analyzing the perfusate, including metabolomics and proteomics perfusate drugs to

treat graft diseases (steatosis, HCV, HBV, etc.) (76), and, why not, preconditioning xenografts.

Conclusions

Machine perfusion is nowadays part of current clinical practice. Hypothermic oxygenated machine perfusion is a useful and safe method that improves liver graft function, decreases the reperfusion graft injury and the risk of graft dysfunction, thus allowing the use of marginal grafts with low post-LT morbidity. This way, marginal liver grafts (DCD, ECD-DBD) and maybe some of the beyond ECD-DBD liver grafts may be used for LT improving the outcome, thus effectively enhance the use of a persistent scarce pool of donors. Moreover, we believe that perfused marginal grafts could be matched to recipients as the standard ones in terms of MELD score. For best results, we consider that both techniques of HOPE (mono and dual HOPE) should be used based on specific selection criteria.

Conflict of Interest

The authors declare no conflicts of interests.

Ethics Approval

All procedures performed were in accordance with the ethical standards of the 1964 Helsinki Declaration.

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