Prospects of Liver Machine Perfusion

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The Netherlands
Disclosure

• I have no financial links with any commercial company

• Nor any other potential conflicts of interest
Prospects of Liver Machine Perfusion

• Why do we need machine perfusion?
• What do we know about machine perfusion?
  – Timing and temperature: Nomenclature
  – Available devices
• Clinical experience
  – Hypothermic (oxygenated) machine perfusion (HMP)
  – Controlled oxygenated rewarming (COR)
  – Normothermic machine perfusion (NMP)
• How to move forward?
The Incentive for Machine Perfusion

• **Shortage** of suitable donor livers

• Increased use of **extended criteria donors** (ECD) and **donation after circulatory death** (DCD) livers

• Conventional static cold storage does **not** provide optimal preservation of ECD and DCD liver grafts
  – Early graft dysfunction
  – **More** (biliary) complications
  – Retransplantation
  – Underutilization of the current donor pool
At the time of transplantation (n=128):

- **Biliary epithelial lining of the large bile ducts is severely injured in >90% of the donor livers**

- The degree of injury of the peribiliary glands and the peribiliary vasculature predicts the later development of biliary strictures
Machine Preservation: An Alternative For Static Cold Storage

• **Advantages:**
  - Reduces ischemia / reperfusion injury
  - Prolonged preservation times
  - Better *ex situ* assessment of graft viability
  - Potential of (pharmacological) preconditioning
  - Potential to restore / regenerate damaged tissue
  - Increase in numbers and quality of donor organs

• **Disadvantages:**
  - More complex
  - More expensive than static cold storage
Machine Preservation: An Alternative For Static Cold Storage

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**Disadvantages:**
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Machine Perfusion of Livers
- Still Unanswered Questions -

- What is the optimal temperature?
- Do we need to get rid of “the cold” completely?
- How long and when should we perfuse?
- What is the optimal perfusion pressure?
- What is the optimal oxygen carrier?
- What are good criteria for viability assessment?
  - Liver function
  - Bile duct viability


**Temperature (°C)**

- **Normothermic:** 35-37 °C
- **Hypothermic:** < 12 °C

**Percentage of metabolism (%)**

- NMP
- SNMP
- MMP
- HMP

**Graph:**

- X-axis: Temperature (°C)
- Y-axis: Percentage of metabolism (%)
# Type and Timing of Machine Perfusion

<table>
<thead>
<tr>
<th>Procurement</th>
<th>Pre-transport</th>
<th>During Transport</th>
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</tr>
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<tbody>
<tr>
<td>Cold flush out</td>
<td>Cold storage</td>
<td>Cold transport</td>
<td>Cold</td>
</tr>
</tbody>
</table>
Liver Machine Perfusion Devices

- Hypothermic
- No active oxygenation

- Normothermic

- Hypo- or normothermic (10 – 37 °C)
- Controlled rewarming
- Oxygenation

- Normothermic

- Normothermic
Prospect of Liver Machine Perfusion

• Why machine perfusion?
• What do we know?
• Timing and temperature: Nomenclature
• Available devices
• Clinical experience
  – Hypothermic (oxygenated) machine perfusion (HMP)
  – Controlled oxygenated rewarming (COR)
  – Normothermic machine perfusion (NMP)
• How to move forward?
# Rationale for Hypothermic Oxygenated Machine Perfusion

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<td><strong>Cold flush out</strong></td>
<td><strong>Cold storage</strong></td>
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</tbody>
</table>

**Cold flush out**, **Cold storage**, **Cold transport**, and **Hypothermic Oxygenated MP**
First Human Study of End-ischemic Machine Perfusion

Hypothermic Machine Preservation in Human Liver Transplantation: The First Clinical Series

J. V. Guerrera¹, *, S. D. Henry¹, B. Samstein¹, R. Odeh-Ramadan¹, M. Kinkhabwala², M. J. Goldstein¹, L. E. Ratner¹, J. F. Renz³, H. T. Lee⁴, R. S. Brown, Jr.¹ and J. C. Emond¹

Am J Transplant 2010

Non-oxygenated Cold Perfusion
After Static Cold Storage and Transportation
Hypothermic Oxygenated Machine Perfusion

HOPE for human liver grafts obtained from donors after cardiac death

Philipp Dutkowski¹, Andrea Schlegel¹, Michelle de Oliveira¹, Beat Müllhaupt², Fabienne Neff¹, Pierre-Alain Clavien¹,*

- Single portal perfusion (8°C)
- Restores mitochondrial O₂ debt
- Reduced reperfusion injury:
  - Less ROS production
  - Less apoptosis
  - Less nuclear injury
  - Less endothelial activation
  - Less Kupffer cell activation

Oxygen uptake during HOPE (Δ pO2 inflow-outflow)

Hypothermic machine perfusion time
Biomechanical Stimulation of Endothelial Cells
Liver Assist®
Oxygenated Dual Liver Perfusion

- Pressure controlled
- Temperature controlled (10 - 37°C)
- Pulsatile arterial flow
- Continuous portal flow
- Oxygenation
Hypothermic Oxygenated Machine Perfusion

Human Discarded Livers

Organ Preservation

n=12

Static Cold Storage

Reperfusion

NMP

n=6

Static Cold Storage

Westerkamp et al. Transplantation 2016
Hypothermic Oxygenated Machine Perfusion

Human Discarded Livers

Organ Preservation

Static Cold Storage

n=12

NMP

Reperfusion

Organ Preservation

Static Cold Storage

n=6

HMP

Westerkamp et al. Transplantation 2016
Hypothermic Oxygenated Machine Perfusion

Human Discarded Livers

Organ Preservation → Reperfusion

n=12

Static Cold Storage → NMP

n=6

Static Cold Storage → HMP → NMP

Westerkamp et al. Transplantation 2016
Hypothermic Oxygenated Machine Perfusion

Human Discarded Livers

Organ Preservation

Reperfusion

SCS alone

Static Cold Storage

NMP

SCS + HMP

Static Cold Storage

HMP

NMP

Westerkamp et al. Transplantation 2016
Hypothermic Oxygenated Machine Perfusion

ATP Regeneration

6 hr Cold Storage

6 hr Cold Storage

n = 12

n = 6

SCS + HMP

SCS only

HMP (hrs)

Viability testing (hrs)
Study in 18 human DCD donor livers

Cumulative Bile Production

Westerkamp et al. Transplantation 2016
Hypothermic Oxygenated Machine Perfusion

Glucose and Lactate Concentration in Perfusate

Westerkamp et al. Transplantation 2016
DHOPE in DCD Liver Transplantation - First in Man Study -

- Aim: Safety and feasibility
- Intervention: DHOPE (dual hypothermic oxygenated perfusion)
- Inclusion: 10 consecutive DCD liver transplantations (2014)
- Controls: 20 previous DCD liver transplantations (2008-2014)
  - Matched: donor age, MELD score, and asystole time
- Endpoints: Technical problems, microbiological tests, early graft function, complications, graft and patient survival

Van Rijn et al. Br J Surg 2017
ATP Regeneration Before Transplantation During Hypothermic Oxygenated Machine Perfusion (DHOPE)

11-fold increase of ATP during 2hrs of end-ischemic DHOPE

Van Rijn et al. Br J Surg 2017
Postoperative Serum ALT and Bilirubin

Van Rijn et al. Br J Surg 2017
Graft and Patient Survival After DCD Liver Transplantation

100% Actual Graft and Patient Survival at 1 Year

5 (25%) retransplants for biliary strictures in the control group vs 0 retransplants in the DHOPE group

<table>
<thead>
<tr>
<th>Number at risk</th>
<th>Baseline</th>
<th>1 month</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHOPE</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>16</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

Van Rijn et al. Br J Surg 2017
Histology of Large Bile Ducts

BEFORE REPERFUSION  AFTER REPERFUSION

DHOPE

CONTROL

Van Rijn, Gouw, Porte, et al. Unpublished data
Hypothermic Oxygenated Machine Perfusion
Clinical Trials in Europe

• **Pilot Studies (Zürich and Groningen)**
  - End-ischemic (after static cold storage) (dual) hypothermic oxygenated machine perfusion [(D)HOPE] of DCD livers

• **Multi-center Randomized Clinical Trials (in progress)**
  - **HOPE-DBD Trial** *(PI: Dutkowski)*
    - End-ischemic hypothermic oxygenated machine perfusion versus static cold storage alone of DBD livers
    - Primary endpoint: Composite complications

  - **DHOPE-DCD Trial** *(PI: Porte)*
    - End-ischemic dual hypothermic oxygenated machine perfusion versus static cold storage alone of DCD livers
    - Primary endpoint: biliary strictures
Prospect of Liver Machine Perfusion

• Why machine perfusion?
• What do we know?
• Timing and temperature: Nomenclature
• Available devices

• Clinical experience
  – Hypothermic (oxygenated) machine perfusion (HMP)
    ➢ Controlled oxygenated rewarming (COR)
  – Normothermic machine perfusion (NMP)

• How to move forward?
## Controlled Oxygenated Rewarming

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<tr>
<td><strong>Cold flush out</strong></td>
<td><strong>Cheap, simple, safe</strong></td>
<td><strong>Cold storage</strong></td>
<td><strong>Restoration of energy content</strong></td>
</tr>
<tr>
<td><strong>Cold transport</strong></td>
<td><strong>Normothermic regional perfusion</strong></td>
<td><strong>Cold transport</strong></td>
<td><strong>Reducing of I/R injury</strong></td>
</tr>
<tr>
<td><strong>Controlled Oxygenated Rewarming</strong></td>
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Controlled Oxygenated Rewarming is a method that involves maintaining tissue in a hypothermic state to reduce the risk of ischemia-reperfusion (I/R) injury and maintaining energy content of cells. It is described as cheap, simple, and safe.
Controlled Oxygenated Rewarming

6 ECD livers transplanted in Essen
- Machine-assisted slow oxygenated rewarming to 20°C during 90 min
- 106 historical ECD controls

Results:
- 50% lower peak serum transaminases (AST and ALT)
- 6-months graft survival 100% vs 81% (p=0.24)
Prospect of Liver Machine Perfusion

- Why machine perfusion?
- What do we know?
- Timing and temperature: Nomenclature
- Available devices
- Clinical experience
  - Hypothermic (oxygenated) machine perfusion (HMP)
  - Controlled oxygenated rewarming (COR)
    - Normothermic machine perfusion (NMP)
- How to move forward?
# Two Types of Normothermic Machine Perfusion

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<tbody>
<tr>
<td>Cold flush out</td>
<td>Normothermic MP</td>
<td>Normothermic MP</td>
<td>Normothermic MP</td>
</tr>
<tr>
<td>Cold flush out</td>
<td>Cold storage</td>
<td>Cold transport</td>
<td>Normothermic MP</td>
</tr>
</tbody>
</table>
Sanguineous Normothermic Machine Perfusion Improves Hemodynamics and Biliary Epithelial Regeneration in Donation After Cardiac Death Porcine Livers

Qiang Liu,1 Ahmed Nassar,1 Kevin Farías,1 Laura Buccini,1 William Baldwin,2 Martin Mangino,3 Ana Bennett,4 Colin O’Rourke,1 Toshiro Okamoto,1 Teresa Diago Uso,1 John Fung,1 Kareem Abu-Elmagd,1 Charles Miller,1 and Cristiano Quintini1
Normothermic Machine Preservation

Liver Transplantation After *Ex Vivo* Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial

R. Ravikumar¹,²,†, W. Jassem³,†, H. Mergenthal⁴, N. Heaton³, D. Mirza⁴, M. T. P. R. Perera⁴, A. Quaglia³, D. Holroyd², T. Vogel¹, C. C. Coussios² and P. J. Friend¹,*

Am J Transplant 2016

- Normothermic machine perfusion from donor to recipient
- Organox Metra device
- 20 Liver transplants (Kings and Birmingham)
  - 16 DBD liver grafts
  - 4 DCD liver grafts
- Matched 1:2 with cold stored livers
# Liver Transplantation After *Ex Vivo* Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial


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### Table 2: Clinical outcomes of normothermic machine perfusion (NMP) and control livers

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>NMP (n = 20)</th>
<th>Control (n = 40)</th>
<th>Risk ratio/effect size (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-day graft survival, n (%)</td>
<td>20 (100)</td>
<td>39 (97.5)</td>
<td>1.03 (0.98–1.08) RR</td>
<td>1.00</td>
</tr>
<tr>
<td>PNF, n (%)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>EAD, n (%)</td>
<td>3 (15)</td>
<td>9 (22.5)</td>
<td>0.67 (0.20–2.19) RR</td>
<td>0.734</td>
</tr>
<tr>
<td>Peak AST within 7 days (IU/L), median (range)</td>
<td>417 (84–4681)</td>
<td>9021 (218–3786)</td>
<td>−0.44 (−0.98 to 0.11) ES</td>
<td>0.034</td>
</tr>
<tr>
<td>Bilirubin on day 7 (µmol/L), median (range)</td>
<td>25 (8–211)</td>
<td>301 (9–221)</td>
<td>−0.23 (−0.77 to 0.32) ES</td>
<td>0.203</td>
</tr>
<tr>
<td>INR on day 7, median (range)</td>
<td>1.05 (0.88–1.40)</td>
<td>1.03 (0.90–2.22)1</td>
<td>−0.16 (−0.70 to 0.38) ES</td>
<td>0.922</td>
</tr>
<tr>
<td>ALP on day 7 (U/L)</td>
<td>245 (81–568)</td>
<td>243 (76–743)1</td>
<td>−0.11 (−0.65 to 0.43) ES</td>
<td>0.798</td>
</tr>
<tr>
<td>ITU stay (days), median (range)</td>
<td>3 (1–8)</td>
<td>3 (1–41)1</td>
<td>−0.42 (−0.96 to 0.13) ES</td>
<td>0.459</td>
</tr>
<tr>
<td>Hospital stay (days), median (range)</td>
<td>12 (6–34)</td>
<td>14 (8–88)1</td>
<td>−0.44 (−0.98 to 0.11) ES</td>
<td>0.100</td>
</tr>
<tr>
<td>30-day mortality (%)</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>6-month survival, n (%)</td>
<td>20 (100)</td>
<td>39 (97.5)</td>
<td>1.03 (0.98–1.08) RR</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Am J Transplant 2016*
The first reported study on normothermic perfusion of human livers

Proof of technical feasibility of NMP

6 hours of normothermic, oxygenated perfusion

Perfusion fluid: RBC, FFP, nutrients, vitamins, trace elements, antibiotics

Allows functional assessment of the liver before transplantation

Potential: Therapeutic intervention and (re)conditioning
Histology After 6 hr of Normothermic Perfusion

Before

After 6 hr of perfusion

Liver Parenchyma

Extrahepatic Bile Duct
End-ischemic Normothermic Machine Perfusion

Case Report

Preimplant Normothermic Liver Perfusion of a Suboptimal Liver Donated After Circulatory Death

C. J. E. Watson¹,*, V. Kosmoliaptsis¹, L. V. Randle¹, N. K. Russell¹, W. J. H. Griffiths¹, S. Davies², H. Mergental³ and A. J. Butler¹

Cambridge, UK

First Human Liver Transplantation Using a Marginal Allograft Resuscitated by Normothermic Machine Perfusion

Perera T, Mergental H, et al.

Birmingham, UK
End-ischemic Normothermic Machine Perfusion

A Word of Caution

- NMP is complex and requires experience

- Both the Cambridge and Birmingham team have reported the loss of a patient due to PNF of a ECD liver that was transplanted after end-ischemic NMP

- A high percentage of biliary complications has been noted after transplantation of DCD livers that were treated with end-ischemic NMP
  - Watson, oral communication at the Groningen Workshop on Liver Machine Perfusion 2015
Normothermic Machine Preservation

• Single center report from Edmonton, Canada
• 10 patients
  – 4 DCD, 6 DBD
• Matched 1:3 with a control group of SCS livers
• Device: Organox Metra
• NMP from donor to recipient

Results:
• 6-month graft survival 80% in NMP group vs 100% in SCS group (p=0.01)
• ICU and hospital stays were significantly more prolonged in the NMP group
• One liver was lost (not transplanted) due a technical problem during NMP

Hypothermic versus Normothermic

- Hypothermic Oxygenated Machine Perfusion
  - Resuscitation of the mitochondria
  - Restoration of ATP, recovery of oxygen debt
  - Reduction of I/R injury
  - Relatively simple and safe
  - Relatively low costs

- Normothermic Oxygenated Machine Perfusion
  - The liver is metabolically active
  - Enables ex situ assessment of liver function (end-ischemic)
  - Benefit of continuous NMP not yet proven
  - Potential: Therapeutic interventions and longer preservation time
  - Potentially riskier?
  - Expensive

0-12°C
### What Is The Best Strategy?

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<tr>
<td>Resolution of oxygen debt</td>
<td>Cheap, simple, safe</td>
<td>Restoration of energy content</td>
<td>Restoration of energy content</td>
</tr>
<tr>
<td><strong>ECOPS system</strong></td>
<td></td>
<td>Viability testing</td>
<td>Viability testing</td>
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</table>

#### Oxygenated perfusion

- Normothermic regional perfusion
- Slow oxygenated cooling
- Static cold storage and transport
- Oxygenated HMP/Controlled rewarming/NMP
The Groningen Organ Preservation & Resuscitation Unit

Central facility for:
- Back table preparation
- Simultaneous machine perfusion of: Lungs, liver, two kidneys
Potential Future Applications of Normothermic Machine Perfusion

- Gen therapy
- Stem cell therapy
- Decellularization and development of a liver scaffold for creation of a new (autologous) organ

Decellularized liver scaffold
Summary - 1

• Liver machine preservation techniques are rapidly developing and entering the clinical arena

• Clinical experience with liver machine perfusion
  – Hypothermic (oxygenated) machine perfusion (HMP, HOPE, DHOPE)
  – Controlled oxygenated rewarming (COR)
  – Normothermic machine perfusion (NMP)

• Hypothermic oxygenated MP and COR
  – Restore hepatic ATP content and reduce I/R injury
  – Relatively simple and safe
  – Can be performed after static cold storage in the transplant center
Summary - II

• Normothermic oxygenated machine perfusion
  1. **Normothermic Machine Perfusion Preservation**
     • From donor to recipient
  2. **End-ischemic Normothermic Machine Perfusion**
     • After static cold storage and arrival at the transplant center

• Expensive and potentially risky
• Preclinical experience is necessary!
• The only way to really increase the pool of donor livers?
  – Application of gene and stem cell therapies

• Optimal clinical application of machine perfusion technology may consist of a **combination of different modalities?**

S. A. Karangwa¹, ², P. Dutkowski³, P. Fontes⁴, ⁵, P. J. Friend⁶, J. V. Guerrera⁷, J. F. Markmann⁸, H. Mergental⁹, T. Minor ¹⁰, C. Quintini¹¹, M. Selzner¹², K. Uygun¹³, C. J. Watson¹⁴ and R. J. Porte¹ ²

SCS ≤ 3 hr

Procurement

Pre-SCS MP

Preservation MP

Donor hospital

Transportation

Transplantation

Time

SCS

Post-SCS MP

Transplant center
The Effect of Machine Preservation of Donor Livers

- Static cold storage
- Machine perfusion
- Minimal quality

End-ischemic machine perfusion