Evolving Trends in Machine Perfusion for Liver Transplantation

The imbalance between grafts available for transplantation and demands has moved the focus of many investigators on the search for novel strategies to rescue organs, previously considered to be unsuitable for transplantation. In this setting, machine perfusion is recognized as one of the most significant improvements in the field of transplantation over the past 20 years. Besides potentially improving organ shortage by repairing putative irreversible injuries, dynamic preservation strategies may offer an opportunity to test organ quality before implantation or to manipulate some functions; for example, by mitigating the immune response.

For livers, 2 main perfusion approaches are currently debated in the clinic: (a) perfusion with blood or alternative oxygen carriers at physiologic normothermic or subnormothermic conditions, or (b) perfusion with cooled oxygenated artificial fluids. The aim and mechanisms of both approaches differ greatly. Normothermic machine perfusion (NMP) is used to minimize the duration of cold storage, and is applied either in situ (eg, in donors before procurement), known as normothermic regional perfusion, or ex situ during or after organ transport to the recipient center. The first randomized NMP trial on predominantly donors livers donated after brain death (DBD) showed excellent 1-year graft survival (95%) by continuous application of NMP during the entire preservation period with, however, similar survival outcome in the control static preservation group. Hypothermic machine perfusion (HOPE) and dual hypothermic oxygenated perfusion (D-HOPE) are currently performed after cold storage, that is, just before implantation in a recipient. Recent observational studies reported a 5-year graft survival of 94% in HOPE-treated livers despite using higher risk livers with longer donor warm ischemia. Besides mortality, however, morbidity measured for ≤1 year after transplantation seems to be equally important. For example, a particularly distressful type of preservation injury occurs on the bile ducts, often weeks after transplantation, and this risk is mainly observed in livers donated after circulatory death (DCD). Of note, both upfront NMP or NMP after cold storage (eg, an end-ischemic normothermic perfusion) failed to protect significantly DCD liver grafts from such biliary injury. In contrast, end-ischemic HOPE has been shown to decrease significantly the histologic signs of bile duct injury after reperfusion and achieved a rate of <5% nonanastomotic strictures in recent reports on DCD livers. These results, however, should be interpreted with caution given the observational study design.

An important point, still poorly investigated, is whether machine perfusion techniques have any benefits in low-risk liver grafts, which are associated with excellent short- and long-term outcome using simple static preservation approaches. Such grafts include DBD livers without relevant steatosis or DCD livers with short warm and cold ischemia times. For example, transplantation with cold stored livers not associated with steatosis, fibrosis, or prolonged warm ischemic time achieved excellent 1-year graft survival of 92%. Correspondingly, the control group in the recent NMP trial showed a 1-year graft survival of 96% by simple cold storage, indicating that the trial was performed using mostly high-quality grafts and low-risk recipients. This issue might be also important in the ongoing HOPE trial, where HOPE targets exclusively DBD livers with no specific restriction on high-risk grafts. Therefore, a true benefit of machine perfusion in this trial would be linked to the inclusion of a relative high percentage of injured livers.

Despite the overwhelming enthusiasm for machine perfusion technologies, the routine replacement of cold storage by dynamic perfusion approaches is difficult to justify and certainly not cost efficient. A relevant benefit is to be expected only in conditions with increased risk of graft loss, such as advanced donor age, macrovesicular steatosis, and longer donor warm or cold ischemia. The current unsolved issue is at what threshold of risks we should use those novel strategies. There is a clear lack of data and consensus in this area, and this should be a high priority for added knowledge.

Current Randomized Machine Perfusion Trials: Do They Help?

There is no doubt that randomized controlled trials (RCTs) are needed to define the role of machine perfusion in liver transplantation. Such trials must secure not only safety and benefits, but also cost effectiveness, because some perfusion strategies are associated with exorbitant costs. Therefore, investigators must put particular attention in the study design to provide convincing information through sufficient sample sizes and clinically relevant endpoints. Optimally, to justify a change in organ preservation policies, novel approaches should demonstrate significant improvement in allograft function, liver use rates, the outcome of recipients including intensive care and hospital stays, graft and patient survivals, and at least 1-year post-transplant morbidity. In contrast, the use of surrogate markers of liver injury, such as the release of transaminases (aspartate aminotransferase [AST]/alanine aminotransferase) must be strictly avoided as a primary endpoint, because the correlation between AST or alanine aminotransferase release and outcome of a graft after transplantation is inconsistent, especially in DCD liver grafts. Serum transaminase levels after transplantation may confer some predictive value regarding graft survival only when markedly elevated (eg, >5000 U/L).
A recent large trial on NMP, unfortunately, chose peak serum AST levels as the primary endpoint, which not only poorly reflect whole graft injury, but these markers are washed out together with other cytoplasmic enzymes already during ex situ machine liver perfusion before implantation of the graft in the recipient. This bias is obvious, because washout of any liver enzymes is not part of cold stored grafts (eg, in the control group). Surprisingly, the authors failed to report AST concentrations in the perfusate.

Second, the availability of more sensitive markers of allograft function would be essential to demonstrate protective effects. Unfortunately, no such markers exist, and the most accurate clinical prediction for liver graft dysfunction after transplantation relies on lactate and bilirubin clearance together with the synthesis of coagulation factors. Importantly, dynamic changes of these laboratory parameters (eg, the slope of the serum international normalized ratio or bilirubin) are superior to any single peak values. Accordingly, a definition of liver graft dysfunction based on peak transaminases in combination with later (eg, 7-day) single levels of the international normalized ratio and bilirubin at arbitrary cut-off points must be avoided in perfusion trials. The only completed RCT, regretfully, focused on such definitions, and thereby failed to offer any valuable information about the role of NMP on liver graft function.

Third, rescuing damaged organs, otherwise discarded, is a major target of new technologies in this area. The policy to discard a graft for transplantation is, however, far from being standardized, greatly differs among centers and individual surgeons, and is prone to major selection biases in clinical trials. For example, in the recent perfusion RCT, one-quarter of accepted livers were discarded in the cold storage group by the respective recipient centers, although initially randomized because deemed transplantable by the donor surgeon. This figure contrasts with only 12% in the perfusion group. Such discrepancy between accepting grafts for the trial, but not for implantation, suggests an additional flaw in the study design. Future trials require a strict commitment in the decision to use or not a liver graft before randomization, regardless of whether perfusion or cold storage is used, to minimize differences in dropouts. In addition, the quality of all grafts need to be documented in terms of donor age, steatosis, and cold and warm ischemia times to increase comparability of discard reasons among centers.

Fourth, hospital stay after liver transplantation varies greatly among centers, despite adjusting risk, as illustrated in a recent benchmark analysis in large-volume centers recording a range of hospitalization between 6 and 24 days in low-risk transplants. The reason behind such considerable differences in hospital stay is mostly not treatment based, but reflects discharge policies of transplant centers, such as in-hospital waiting for rehabilitation places versus unrestricted transfer without rehabilitation. This difference indicates major limitations in interpreting such parameter in studies.

Fifth, graft and patient survivals are logically convincing parameters, but there is a need for adequate follow-up to assess safety or superiority in machine perfusion trials. A minimum observation of 1 year seems to be advisable, based on the fact that most graft losses related to preservation injury develop all along the first year after liver transplantation.

Finally, measuring morbidity after transplantation is crucial, because it affects the quality of life of patients and influences overall cost. Capturing complications after surgery, however, is challenging, because multiple definitions of postoperative complications exist. The Clavien-Dindo classification, which relies on the need for treatment to correct a negative event, or its surrogate metric, the comprehensive complication index, are currently widely used in surgery and transplantation with the availability of reference values provided in a recent multicenter benchmark study covering 1 year after transplantation.

In summary, most of the available endpoints in liver transplantation are weak and should be carefully selected in RCTs. Based on the fact that complications after liver transplant are frequent and have unequivocally the highest clinical relevance in terms of cost and quality of life, we suggest a cumulative assessment of post-transplant complications within 1 year, besides recipient and graft survival, as the preferred primary endpoints for future machine perfusion studies. This is the case, for example, in the 2 ongoing multicentric randomized machine perfusion trials on hypothermic liver perfusion (HOPE, D-HOPE). For convincing effectiveness against cold static preservation, any novel perfusion technology should prove superiority in relevant endpoints before claiming its general use (Table 1).

Future Randomized Trials: From Marketing to Science

Future machine perfusion trials should compare the different competing techniques using clinically relevant endpoints. To properly conduct such studies, an objective and adequate knowledge of the various perfusion technologies is mandatory, and thus should optimally be restricted to few experienced centers. We would urge the transplant community, including the industry, to rather focus on supporting current research in highly engaged perfusion teams, instead of suggesting widespread use of one specific perfusion technology. Accessible registries to allow research in long-term outcome should also be implemented.

Mechanism of Different Machine Perfusion Techniques: What Is Currently Known?

The precise underlying mechanism for protection of liver grafts by machine perfusion remains under debate. For NMP, the current explanation is a reduction of cold ischemia by minimizing the anaerobic metabolism with less accumulation of citric acid cycle products and improved ATP restoration. The extreme of such strategy is an ischemia-free organ transplantation, where donor livers are put on a
normothermic circuit even before procurement, similar to normothermic regional perfusion, but with continuing normothermic perfusion until implantation. It might be more efficient to develop repair strategies of pretransplant or procurement injuries, which are common in the clinic (eg, fatty liver, prolonged cold or warm ischemia times). End-ischemic NMP in discarded human livers with longer periods of cold ischemia failed to protect against biliary injury, the most prevalent cause of graft loss after DCD liver transplantation. Continuous or endischemic NMP after ischemia triggers an inflammatory cascade, mostly related to mitochondrial reactive oxygen species (Figure 1), which resemble in vivo conditions. Evidence for an activation of “danger” signals by normothermic perfusion techniques is also well-described in normothermic perfusion of other organs including heart, kidney, and lung. The target of all ex situ normothermic interventions is, therefore, to capture reactive oxygen species and “danger” molecules (danger associated molecular patterns and proinflammatory cytokines), for example, by perfusion scavengers or by the implementation of cytokine filters into the circuit. In contrast, hypothermic perfusion techniques mitigate oxidative stress despite high availability of oxygen in the cold, probably owing to changes in mitochondrial electron transfer (eg, down-regulated electron transfer at temperatures below the Arrhenius break temperature of 15°C). At the same time, metabolism of accumulated electron donors, such as NADH and succinate, leads to ATP resynthesis with surprisingly high efficiency, compared with normothermic conditions, possibly owing to less electron and proton leakage in the cold. End-ischemic cold oxygenated perfusion, therefore, allows for a higher upload of cellular energetic levels in DCD liver grafts, compared with normothermic perfusion (Figure 1). These observations support possible complementary effects of, for example, combining first hypothermic and afterwards normothermic perfusion, or a period of cold ischemic storage after a period of normothermic circuit even before implantation.

Table 1. Current observational studies and randomized trials (RCT) on human machine liver perfusion

<table>
<thead>
<tr>
<th>Year</th>
<th>RCT</th>
<th>Graft type</th>
<th>Technique</th>
<th>Endpoints</th>
<th>Clinical Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bral et al</td>
<td>2017</td>
<td>No</td>
<td>DBD + DCD</td>
<td>NMP (Organox)</td>
<td>Graft function and survival</td>
</tr>
<tr>
<td>De Carli et al</td>
<td>2018</td>
<td>No</td>
<td>DCD</td>
<td>NRP + HOPE</td>
<td>Graft function, biliary complications, 1-year survival</td>
</tr>
<tr>
<td>Dutkowski et al</td>
<td>2015</td>
<td>No</td>
<td>DCD</td>
<td>HOPE</td>
<td>Graft function, 1-year survival, biliary complications</td>
</tr>
<tr>
<td>Guarerra et al</td>
<td>2010</td>
<td>No</td>
<td>DBD</td>
<td>HMP</td>
<td>PNF, EAD; 1-year graft and patient survival, biliary complications</td>
</tr>
<tr>
<td>Guarerra et al</td>
<td>2015</td>
<td>No</td>
<td>DCD</td>
<td>HMP</td>
<td>PNF, EAD; 1-year graft and patient survival, biliary complications</td>
</tr>
<tr>
<td>Hessheimer et al</td>
<td>2018</td>
<td>No</td>
<td>DCD</td>
<td>NRP</td>
<td>Graft function, biliary complications, 1-year graft survival</td>
</tr>
<tr>
<td>Nasralla et al</td>
<td>2018</td>
<td>No</td>
<td>DBD + DCD</td>
<td>NMP (Organox)</td>
<td>Peak AST, EAD, graft use</td>
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<tr>
<td>Ravikumar et al</td>
<td>2016</td>
<td>No</td>
<td>DBD + DCD</td>
<td>NMP</td>
<td>Peak AST, EAD</td>
</tr>
<tr>
<td>Liver Revive trial</td>
<td>2016</td>
<td>Yes</td>
<td>DCD</td>
<td>NMP (OCS)</td>
<td>Number of donor livers preserved in a near physiological state, serious adverse events</td>
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<tr>
<td>Schlegel et al</td>
<td>2018</td>
<td>No</td>
<td>DCD</td>
<td>HOPE</td>
<td>5-year graft survival, biliary complications</td>
</tr>
<tr>
<td>Van Rijn et al</td>
<td>2017</td>
<td>No</td>
<td>DCD</td>
<td>D-HOPE</td>
<td>Histology of bile ducts during implantation</td>
</tr>
<tr>
<td>Van Rijn et al</td>
<td>2018</td>
<td>No</td>
<td>DCD</td>
<td>D-HOPE</td>
<td>Liver function, liver ATP, biliary complications, 1-year graft and patient survival</td>
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<tr>
<td>Watson et al</td>
<td>2018</td>
<td>No</td>
<td>DCD</td>
<td>NRP</td>
<td>Peak ALT, graft function, 90-day survival</td>
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<tr>
<td>Watson et al</td>
<td>2018</td>
<td>No</td>
<td>DBD + DCD</td>
<td>NMP (liver assist)</td>
<td>Postreperfusion syndrome, PNF, bile duct histology, biliary complications, graft survival</td>
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<tr>
<td>Ongoing RCTs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dutkowski et al</td>
<td>Ongoing</td>
<td>Yes</td>
<td>DBD</td>
<td>HOPE</td>
<td>1-year cumulative Clavien grade ≥II, CCI</td>
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<tr>
<td>Porte et al</td>
<td>Ongoing</td>
<td>Yes</td>
<td>DCD</td>
<td>D-HOPE</td>
<td>6-month biliary complications</td>
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<tr>
<td>Lurje et al</td>
<td>Ongoing</td>
<td>Yes</td>
<td>ECD DBD</td>
<td>HOPE</td>
<td>Peak ALT</td>
</tr>
<tr>
<td>Liver Protect trial</td>
<td>Ongoing</td>
<td>Yes</td>
<td>DBD + DCD</td>
<td>NMP (OCS)</td>
<td>EAD, serious adverse events</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; CCI, comprehensive complication index; DBD, donation after brain death; DCD, donation after circulatory death; D-HOPE, dual hypothermic oxygenated perfusion; HOPE, hypothermic oxygenated perfusion; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; OCS, organ care system; PNF, primary nonfunction.

*Clinical relevance not yet available.
Future Outlook

Future investigations on perfusion should include the search for protective mechanisms and evaluation of effective repair of injured organs. Thereby, perfusion technologies convey a great potential to influence cellular metabolism before implantation, modulating inflammation, immune response and perhaps triggering anticancer pathways. It is central that expensive and cumbersome RCTs in the field of organ perfusion select exclusively clinically relevant endpoints. Additionally, the prediction of organ function during machine perfusion will likewise be possible by detailed perfusate analysis including metabolomics and proteomics, leading to safer use of marginal grafts. We anticipate that within the next few years a far better understanding will be available about which perfusion strategies should be applied best and for which liver graft.

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Conflicts of interest
The authors disclose the following: PD, JdJ, RP, and PAC are involved in a European RCT of Hypothermic Oxygenated PErfusion (HOPE) in DBD liver transplant recipients. JdJ and RP are involved in a European RCT of dual Hypothermic Oxygenated PErfusion (D-HOPE) in DCD liver transplant recipients. JVG is involved in Research Support and Consulting, Lifeline Scientific-Organ Recovery Systems. PAC and PD have filed patents on long-term ex situ perfusion technology along with ETH (Eidgenössische Technische Hochschule).

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