Machine perfusion of human donor livers with a focus on the biliary tree

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Opportunities for Scientific Expansion of the Deceased Donor Pool in Liver Transplantation

Alix P.M. Matton
Robert J. Porte

ABSTRACT

The shortage of suitable donor livers in combination with the growing demand of liver transplants has led to the transplantation of increasing numbers of suboptimal livers from extended criteria donors (ECD). These livers have suffered more injury, resulting in significantly higher rates of graft failure and biliary complications. Further expansion of the pool of donor livers from deceased donors can only be obtained by a more effective and successful utilization of ECD livers such as livers obtained from donation after circulatory death (DCD). In most countries, the number of livers after donation after brain death (DBD) has been stable or even declining during recent years. Although DCD donation is increasingly considered in several countries, the percentage of DCD livers that are declined for transplantation is also increasing as the risk of early graft failure or graft-related complications is often too high. The current method of cold preservation and static cold storage of donor organs, which has been successful in low risk and optimal donor livers in the past, is insufficient for ECD or DCD donor livers. Those livers require more sophisticated methods of organ preservation to avoid or minimize any additional injury. To this end, machine perfusion of donor livers is receiving increasing attention as an alternative for graft preservation.

Various methods of machine perfusion have been and are being explored in experimental studies and the first clinical trials have been reported. The preliminary results are very promising and machine perfusion technology is going through a rapid development. Current data suggest that machine perfusion will provide an important new tool to optimize the utilization of ECD livers, such as livers obtained from DCD donors.
KEY POINTS

1. The shortage of suitable donor livers in combination with the growing demand for liver transplants has led to the transplantation of increasing numbers of suboptimal livers from extended criteria donors (ECD).
2. Further expansion of the pool of livers from deceased donors can be obtained only with a more effective and successful utilization of ECD livers, such as livers obtained from donation after circulatory death (DCD).
3. Although DCD donation is increasing in several countries, the percentage of DCD livers that are declined for transplantation is also increasing because the risk of early graft failure or graft-related complications is often too high.
4. The current method of cold preservation and static cold storage of donor organs is insufficient for ECD or DCD livers. These livers require more sophisticated methods of organ preservation to avoid or minimize any additional injury.
5. Various methods of machine perfusion have been and are being explored in experimental studies, and the first clinical trials have been reported. The preliminary results are very promising, and machine perfusion technology is undergoing rapid development. Current data suggest that machine perfusion will provide an important new tool to optimize the utilization of ECD livers, such as livers obtained from DCD donors.

INTRODUCTION

Over the past decades, liver transplantation has become a successful treatment for patients with end-stage liver disease. A considerable number of patients awaiting a liver transplantation, however, die on the waiting list due to the significant global discrepancy between the demand and availability of suitable donor livers. In an attempt to expand the number of liver transplantations, physicians are currently pushing the limits by performing split and live liver donations, as well as accepting livers from extended criteria donors (ECD). In the Western hemisphere, the vast majority of livers used for transplantation, however, remain livers from deceased donors. Livers can be either donated after brain death (DBD) or circulatory death (DCD). While in most Western countries the number of DBD donations has remained steady or even declined over the last decade, the number of DCD donations has been increasing. The proportion of liver transplantations performed using DCD livers increased from 1.1% in 1995 to 11.2% in 2010 in the United States. In the United Kingdom, the percentage of DCD livers was 18% in 2012, while in the Netherlands it had increased to 38% in 2013. Simultaneously, however, the number of unused DCD livers has also been increasing over the past decade as a result of too many concomitant risk factors for graft dysfunction, such as older donor age, high BMI, and diabetes mellitus in the donor. It is not likely that expansion of the deceased donor pool will come from more DBD livers. The largest gain in the number of suitable deceased donor livers could potentially be obtained by maximizing the usage of DCD livers.
Other types of ECD livers that carry an increased risk of graft failure include steatotic livers, and livers from elderly donors. A common characteristic of DCD and other types of ECD livers is that they are at greater risk of developing significant ischemia/reperfusion injury, leading to parenchymal, endothelial and/or biliary injury and subsequent dysfunction (Table 1).

Table 1. The risk of donor livers from extended criteria donors.

<table>
<thead>
<tr>
<th>Parenchymal injury</th>
<th>Endothelial injury</th>
<th>Biliary injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher rate of primary non-function</td>
<td>Higher rate of early hepatic artery thrombosis</td>
<td>Higher rate of ischemic cholangiopathy (non-anastomotic biliary strictures)</td>
</tr>
<tr>
<td>Higher rate of Initial poor function</td>
<td>Microvascular/sinusoidal thrombosis</td>
<td></td>
</tr>
</tbody>
</table>

Biliary injury, in particular, is a significant problem in the transplantation of DCD livers. Bile duct injury can result in leakage and fibrosis of the larger bile ducts, leading to so called non-anastomotic biliary strictures (NAS; also known as ischemic-type biliary lesions or ischemic cholangiopathy). The development of NAS has been reported in up to 30% of DCD livers, of which 50% of patients die or require re-transplantation. The pathophysiology of NAS is not yet fully understood, however ischemia-related injury, immune-mediated injury, bile salt toxicity and a lack of regenerative capacity of the bile ducts are thought to be responsible for the development of NAS. Ischemia-related injury plays the largest role as biliary epithelial cells are very susceptible to ischemia and are mainly dependent on the oxygen supply through the hepatic artery. As a result of the increased rates of graft failure and biliary complications, the costs of DCD transplantations are about 30% higher compared to DBD transplantations.

It has become evident that the current method of organ preservation, which is based on cooling, is not good enough to protect suboptimal donor livers such as those from ECD and DCD donors. The current standard method of organ preservation is static cold storage (SCS), in which the organ is flushed with ice-cold preservation fluid and stored at low temperature (0-4°C) in a box with melting ice during transportation from the donor hospital to the transplant center. The advantages of preserving livers using SCS are that it is easily executable, transportable and cheap. However, SCS also causes damage to the organ, frequently resulting in an unacceptably low quality liver graft in suboptimal ECD livers (Figure 1). During SCS livers are not oxygenated, resulting in adenosine triphosphosphate (ATP) depletion, and cold-induced damage occurs. Furthermore, there is no means of assessing the functionality and
viability of the organ short before implantation. Therefore, optimization of the utilization of ECD livers should come from novel organ preservation methods. To this end, machine perfusion is the most promising technique.

Figure 1. Schematic presentation of the decline in liver graft quality and viability during static cold storage (SCS) versus machine perfusion. In extended criteria donor (ECD) liver grafts, SCS results in a rapid decline in organ quality below a level at which it can still be transplanted with acceptable outcome. Machine perfusion has the potential to slow down the rate at which this decline in quality occurs, resulting in better organ viability after a given time period of preservation and potentially allowing for prolongation of the preservation time. In addition, machine perfusion may potentially allow for the resuscitation of liver grafts. Abbreviations: DBD: donation after brain death; DCD: donation after circulatory death.

MACHINE PERFUSION AS AN ALTERNATIVE PRESERVATION METHOD OF DONOR LIVERS

Experimental research has indicated that machine perfusion is superior to SCS in the preservation of donor livers. Machine perfusion leads to less ischemia / reperfusion injury, allows for prolonged preservation of the organs, and has the potential to restore and/or stimulate regeneration of damaged tissue. Moreover, machine perfusion also allows for the ex vivo assessment of graft viability and provides the potential of (pharmacological) preconditioning. In such a way, machine perfusion has the potential to increase the number and quality of donor organs. Disadvantages of machine perfusion, however, are that it is more complex and expensive to perform than SCS (Table 2).
Table 2. Advantages and disadvantages of static cold storage versus machine perfusion of donor livers.

<table>
<thead>
<tr>
<th>Static Cold Storage</th>
<th>Machine Perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td></td>
</tr>
<tr>
<td>Easy to execute</td>
<td>Reduced ischemia / reperfusion injury</td>
</tr>
<tr>
<td>Easy transportation</td>
<td>Prolonged preservation times</td>
</tr>
<tr>
<td>Low costs</td>
<td>Better <em>ex vivo</em> assessment of graft viability</td>
</tr>
<tr>
<td></td>
<td>Potential for (pharmacological) preconditioning</td>
</tr>
<tr>
<td></td>
<td>Potential to restore / regenerate damaged tissue</td>
</tr>
<tr>
<td></td>
<td>Increase in numbers and quality of donor organs</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td></td>
</tr>
<tr>
<td>No functional assessment</td>
<td>More complex</td>
</tr>
<tr>
<td>No oxygenation</td>
<td>More expensive than static cold storage</td>
</tr>
<tr>
<td>Cold induced injury</td>
<td></td>
</tr>
<tr>
<td>Not good enough for ECD livers</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: ECD, extended criteria donor.*

The technique of machine preservation and perfusion is still evolving and several questions remain unanswered (Table 3). It remains to be determined what is the optimal temperature at which organs should be perfused, whether or not an oxygen carrier should be added to the perfusion fluid, how long and at what pressure livers should be perfused, and finally what is the optimal timing of machine perfusion in the time period between procurement and transplantation. Furthermore, reliable criteria for the viability assessment of donor livers have yet to be confirmed in the clinical setting. With respect to the timing, machine perfusion can be performed in the donor (normothermic regional perfusion)\(^{19}\), immediately after procurement, and/or during or after the storage and transportation of the organ (Figure 2).
Table 3. The various temperatures and timing of liver machine perfusion.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothermic perfusion</td>
<td>0 – 15°C</td>
</tr>
<tr>
<td>Subnormothermic perfusion</td>
<td>15 – 35°C</td>
</tr>
<tr>
<td>Normothermic perfusion</td>
<td>37°C</td>
</tr>
</tbody>
</table>

**Timing**
- In the donor (normothermic regional perfusion)
- Immediately after procurement
- During or after storage and transportation

Figure 2. Schematic overview of the various combinations and types of liver machine perfusion that have been described. The optimal combination of different machine perfusion techniques remains to be determined and may very per type of donor livers.

A large number of animal experiments have been performed to explore the feasibility and potential benefits of machine perfusion. In one study, hypothermic oxygenated machine perfusion of porcine DCD livers has been shown to prevent arteriolonecrosis of the peribiliary vascular plexus, potentially reducing posttransplant biliary ischemia and leading to faster and more efficient regeneration of the biliary epithelium. Another study recently suggested that
normothermic machine perfusion also improves biliary epithelial regeneration in a pig model of DCD livers.\textsuperscript{21} Moreover, there is evidence from an experimental study that gradual warming up of DCD liver grafts is superior to SCS and hypothermic machine perfusion.\textsuperscript{22}

The first clinical application of liver machine perfusion was reported by Guarerra \textit{et al.} in 2010.\textsuperscript{23} This study in 20 patients involved dual (portal vein and hepatic artery) non-oxygenated hypothermic machine perfusion of the donor liver prior to transplantation. This method resulted in lower cellular damage markers and less ischemia/reperfusion injury after transplantation.\textsuperscript{24,25} A second clinical trial has been reported by Dutkowski \textit{et al.} in 2014.\textsuperscript{26} These investigators have reported on the feasibility and safety of hypothermic oxygenated machine perfusion through the portal vein in DCD livers and reported excellent early outcome after transplantation in eight patients. Our group has recently initiated a pilot study on hypothermic oxygenated machine perfusion using dual perfusion of both the portal vein and hepatic artery in DCD livers (Netherlands Trial Registry, NTR4493; www.trialregister.nl). This trial is still ongoing, but the initial results are encouraging.

More clinical trials will be needed to elucidate whether the different methods of machine perfusion are beneficial in the prevention of graft failure and biliary complications after transplantation, especially in DCD liver grafts. A multi-center randomized controlled clinical trial will soon be initiated by our group to compare hypothermic dual oxygenated machine perfusion with SCS in DCD liver grafts. Primary endpoint in this trial will be the development of NAS. Another randomized controlled clinical trial has been initiated to evaluate the effects of hypothermic oxygenated perfusion through the portal vein alone in DBD livers (ClinicalTrials.gov, ID: NCT01317342). In addition, a randomized controlled clinical trial on normothermic machine perfusion (Controlled-Trials.com, ID: ISRCTN39731134) will soon be launched, and a pilot study of normothermic regional perfusion in DCD organ donors was recently completed.\textsuperscript{19}

**SUMMARY, FUTURE PERSPECTIVE AND CHALLENGES**

The largest potential gain to be obtained in expanding the deceased donor pool lies in the utilization of ECD livers, as there is an increasing number of unused DCD livers compared to a stable or even declining number of DBD livers. It is crucial that measures are taken to improve the quality of ECD donor livers, especially of livers that are obtained from DCD donors. DCD livers form already a substantial proportion of all liver transplantations performed in countries such as the United Kingdom and the Netherlands. Increased utilization of DCD livers may contribute significantly to the number of available deceased donor livers in other countries as well. Moreover, improving the quality of DCD livers could lead to a substantial reduction in the rate of early graft failure after transplantation. Assessing the viability of livers, in particular suboptimal ECD livers, prior to transplantation would also lead to a more careful selection of transplantable livers. This would theoretically not only result in better outcomes after
transplantation, but also to the expansion of the number of available donor livers. A common characteristic of DCD and other types of ECD livers is that these livers have suffered a higher degree of injury prior to transplantation, explaining the higher risk of early graft failure after transplantation. It has become evident that the current method of organ preservation, which is based on cooling and static cold storage, is not sufficient to adequately preserve these preinjured ECD and DCD livers. If we want to improve the numbers and success rate of transplantation of livers from DCD and ECD donors, we have to introduce more sophisticated methods of organ preservation. Machine perfusion is receiving increasing attention as an alternative preservation method (Figure 1). Experimental studies have indicated that machine perfusion provides better protection of DCD livers and the first clinical trials have been initiated and reported. The potential role of machine perfusion in expanding the deceased donor pool is two-fold. Firstly, machine perfusion can be used for the resuscitation of liver grafts prior to transplantation, thereby not only improving the quality of DCD transplants but also increasing the number of transplantable ECD livers. Secondly, machine perfusion can be used to assess the function and viability of liver grafts prior to transplantation, thereby allowing for the careful selection of transplantable livers out of a pool of currently discarded ECD livers. Various protocols of machine perfusion have been described, but it remains to be established which method provides the best protection of DCD livers (Figure 2). The optimal and most cost-effective strategy of liver preservation based on machine perfusion technology may be a combination of different techniques for the different phases of organ preservation and transportation (Figure 3). An important outcome parameter to determine the efficacy of machine perfusion will be the degree of biliary injury and the rate of biliary complications (i.e. NAS) after DCD livers transplantation.
Figure 3. The optimal and most cost-effective strategy of liver preservation based on machine perfusion technology may be a combination of different techniques for the different phases of organ preservation and transportation, as depicted in this figure.

<table>
<thead>
<tr>
<th>Procurement</th>
<th>Pre-transport</th>
<th>During Transport</th>
<th>Pre-implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold flush out</td>
<td>Cold storage</td>
<td>Cold transport</td>
<td>Cold</td>
</tr>
<tr>
<td>Cold flush out</td>
<td>Cold storage</td>
<td>Cold transport</td>
<td>Hypothermic (oxygenated)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>perfusion</td>
</tr>
<tr>
<td>Cold flush out</td>
<td>Cold storage</td>
<td>Cold transport</td>
<td>Controlled oxygenated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rewarming perfusion</td>
</tr>
<tr>
<td>Cold flush out</td>
<td>Oxygenated perfusion</td>
<td>Cold transport</td>
<td>Controlled oxygenated</td>
</tr>
<tr>
<td></td>
<td>(1-2 hr)</td>
<td></td>
<td>rewarming perfusion</td>
</tr>
<tr>
<td>Cold flush out</td>
<td>(Sub) Normothermic</td>
<td>(Sub) Normothermic</td>
<td>(Sub) Normothermic</td>
</tr>
<tr>
<td></td>
<td>perfusion</td>
<td>perfusion</td>
<td>perfusion</td>
</tr>
<tr>
<td>Normothermic regional</td>
<td>Slow oxygenated</td>
<td>Cold transport</td>
<td>Controlled oxygenated</td>
</tr>
<tr>
<td>perfusion</td>
<td>cooling perfusion</td>
<td></td>
<td>rewarming perfusion</td>
</tr>
</tbody>
</table>

**Figure 3.** The optimal and most cost-effective strategy of liver preservation based on machine perfusion technology may be a combination of different techniques for the different phases of organ preservation and transportation, as depicted in this figure.
REFERENCES


