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Normothermic Machine Perfusion (NMP) of the Liver – Current Status and Future Perspectives

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A shortage of available organs for liver transplantation has led transplant surgeons and researchers to seek for innovative approaches in hepatoprotection and improvement of marginal allografts. The most exciting development in the past decade has been continuous mechanical perfusion of livers with blood or preservation solution to mitigate ischemia-reperfusion injury in contrast to the current standard of static cold storage. Two variations of machine perfusion have emerged in clinical practice. During hypothermic oxygenated perfusion the liver is perfused using a red blood cell-free perfusate at 2-10°C. In contrast, normothermic machine perfusion mimics physiologic liver perfusion using a red blood cell-based solution at 35.5-037.5°C, offering a multitude of potential advantages. Putative effects of normothermic perfusion include abrogation of hyperfibrinolysis after reperfusion and inflammation, glycogen repletion, and regeneration of adenosine triphosphate. Research in normothermic machine perfusion focuses on development of biomarkers predicting allograft quality and susceptibility to ischemia-reperfusion injury. Moreover, normothermic perfusion of marginal allografts allows for application of a variety of therapeutic interventions potentially enhancing organ quality. Both methods need to be subjected to translational investigation and evaluation in clinical trials. A clear advantage is transformation of an emergency procedure at night into a planned daytime surgery. Current clinical trials suggest that normothermic perfusion not only increases the use of hepatic allografts but is also associated with milder ischemia-reperfusion injury, resulting in a reduced risk of early allograft dysfunction and less biliary complications, including ischemic cholangiopathy, compared to static cold storage. The aim of this review is to give a concise overview of normothermic machine perfusion and its current applications, benefits, and possible advances in the future.

Keywords: **Cold Ischemia • Liver Transplantation • Organ Preservation • Warm Ischemia**

Abbreviations: **ALT** – alanine aminotransferase; **AST** – aspartate aminotransferase; **ATP** – adenosine triphosphate; **ECD** – extended criteria donors; **DCD** – donation after circulatory death; **HMP** – hypothermic machine perfusion; **MP** – machine perfusion; **NMP** – normothermic machine perfusion; **SCS** – static cold storage; **SNMP** – sub-normothermic machine perfusion

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Background

The shortage of organs available for liver transplantation in many countries has led transplant surgeons and researchers to seek for innovative approaches to increase the number of liver allografts suitable for transplant [1]. While split liver transplantation yielded a great success worldwide, potentially doubling the number of available allografts, technical and functional challenges as well as a shortage of grafts suitable for splitting still hinder its more widespread use [1].

Nowadays, increased donor age concomitant with relevant comorbidity enlarges the marginal organ donor pool. Especially, the global burden of obesity may result in more steatotic liver grafts. The ongoing discrepancy in many countries between organ demand and supply has moved the spotlight toward implementing rescue strategies for organs considered marginal or unsuitable for transplantation, or even extending the donor pool to deceased donors [2]. According to Eurotransplant, criteria of marginal donors are one or more of the following points: donor age >65 years, ICU stay with ventilation >7 days, body mass index >30, steatotic liver >40%, serum sodium >165 mmol/l, serum alanine aminotransferase (ALT) >105 U/l, serum aspartate aminotransferase (AST) >90 U/l, serum bilirubin >3 mg/dl, donation after cardiocirculatory death (DCD), and donation after euthanasia [https://www.eurotransplant.org/organs/liver/]. It is widely known that the current standard protocol "static cold storage and sudden rewarming" causes considerable damage to organs from extended-criteria donors (ECD), even when considered to be of "good quality", compared to healthy livers upon retrieval and reperfusion [3-5]. Problems associated with use of ECD liver grafts are a), higher susceptibility to ischemia-reperfusion injury, which may result in primary non-function, b) early allograft dysfunction, and c) non-anastomotic biliary strictures and ischemic cholangiopathy [6].

As early as in 1967, continuous perfusion of liver allografts after retrieval was first described by Brettschneider et al. However, since the introduction of cold storage after in-situ perfusion with University of Wisconsin or Histidine-Tryptophan-Ketoglutarate preservation solution, the strategy of dynamic organ perfusion was abandoned, mostly due to the excellent results of liver transplantation after static cold storage (SCS) [7].

Due to the above-mentioned increase of extended-criteria donors and marginal liver grafts, the decade-long paradigm of static cold storage may be currently shifting toward more complex approaches to organ preservation – Reintroduction of "machine perfusion" (MP) is considered the most notable evolution in liver transplantation of the past decade [2]. The term "machine perfusion" summarizes a variety of dynamic, continuous perfusion, and preservation techniques used to preserve (and possibly improve) ECD organs and assess graft viability prior to implantation [2].

Today, basically 2 variations of MP have emerged from the laboratory bench into clinical practice of liver transplantation: During ex vivo normothermic MP (NMP), perfusion of the liver with oxygenated red blood cell-based perfusion solution at normal temperature (35.5-37.5°C) mimics physiologic conditions. The perfusate can be enriched with nutritional compounds such as amino acids, vitamins, inorganic salt, and glucose, and is pumped via the portal vein and hepatic artery at different pressure levels [3]. Sub-normothermic (20-25°C) and hypothermic (2-10°C) machine perfusion (SNMP and HMP) of the liver require a red blood cell-free perfusate with physically dissolved oxygen, which can be delivered solely via the portal vein or via the portal vein and hepatic artery in combination (dual hypothermic oxygenated perfusion, HOPE) [3]. Most research in the field of NMP or HMP/SNMP is ultimately serving the goal to prevent decline of allograft quality following organ retrieval and ischemia-reperfusion injury. One of the first foci of this research was the development of clinical biomarkers that describe and predict allograft quality, susceptibility to ischemia-reperfusion injury, and, in consequence, the prediction of early allograft dysfunction. Pharmacologic measures to enhance graft performance (ie, excretory function and synthesis) and improve quality of livers during MP and ultimately after implantation are currently under investigation in preclinical models.

The aim of the present article is to give a concise overview of the current status of normothermic machine perfusion in clinical practice and to give a further outlook on what to expect in translational transplant medicine.

Current Status of Normothermic Machine Perfusion in Liver Transplantation

The goal of both SCS and HMP is to basically slow down liver graft metabolism after harvest and during transport to the recipient center. HMP, in contrast to SCS alone, thereby additionally ensures adequate supply with oxygen and nutrition. Several protective effects of HMP compared to SCS have been described, including less injury to the sinusoidal endothelium [8] and washout of accumulated substances [9]. While SCS and HMP aim at preserving allograft viability by slowing down metabolism and accumulation of toxic substances, NMP allows maintenance of near physiologic metabolism and synthetic liver function, offering optimal conditions for pre-transplant assessment of organ viability and integrity, in theory [5]. In an unprecedented feasibility study, Eshmuminov et al were able to perfuse hepatic allografts for 7 days, keeping livers functional at normothermic conditions throughout. Six of the 10

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Table 1. Indications for NMP.

Donor-related indications	Logistic-related indications	Recipient-related indications
• Age >65 years	• Combined or parallel organ transplantation	• Prolonged hepatectomy in highly complex recipients
• Risk for transmission of a disease (Hepatitis B/C) • Donor body mass index >30 kg/m2 total bilirubin >3 mg/dl serum sodium >165 mmol/l • Hospitalization on an intesive care unit >7 days • Hepatic steatosis >40% • Acute hemodynamic deterioration with risk of organ loss • DCD	• Overlap with other urgent surgerys	

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perfused human livers showed preserved bile production, synthesized coagulation factors, and restored cellular energy levels, hypothetically allowing transplantation. Of course, owing to the generally poor organ quality and the exploratory nature of the study, none of the grafts were considered for transplantation. Still, these results indicate that long-term NMP is safe and feasible [10]. Recent clinical studies and case series demonstrate the feasibility and safety of NMP preceding transplantation in humans. The first phase 1 trial of 20 livers successfully transplanted after NMP demonstrated reduced AST levels compared with controls and a 95% one-year patient survival rate [11]. Additionally, use of normothermic ex vivo liver perfusion in 10 human liver allografts perfused with albumin-based Steen solution as an alternative to a red blood cell-based perfusate with equivalent outcomes to traditional SCS post-liver transplantation was shown in 2016 [12,13]. In the largest prospective randomized clinical trial of 220 liver transplantations performed after NMP, a 50% reduction of graft damage compared to SCS in addition to a 50% reduction of discarded livers in the normothermic perfusion group was demonstrated. Moreover, the absolute rate of intrahepatic biliary strictures was lower in DCD livers after NMP (11.1%) compared to SCS (26.3%), although this finding did not reach statistical significance [13,14].

Underlying mechanisms supporting the protective effect of NMP besides minimizing tissue ischemia and enhancing ex vivo metabolism have not been fully explained, although it is hypothesized that oxygenated perfusion exerts a beneficial effect to the sinusoidal endothelium and helps reloading of adenosine triphosphate (ATP) [15]. Furthermore, differences in gene expression of livers after SCS compared to NMP have been shown in pre- and post-reperfusion biopsies, with upregulation of genes involved in tissue regeneration and inflammation control after NMP in contrast to upregulation of genes with pro-inflammatory and pro-apoptotic function after SCS [15,16].

Since the field of machine perfusion is rather new, with only a small number of transplant centers having access to commercially available perfusion machines, there is no uniform agreement on the "right" indication for the use of machine perfusion to date [17]. In the majority of clinical studies, a heterogenous group of allografts derived from marginal donors were used with varying definitions of marginal or ECD and assigned to varying machine perfusion protocols. Therefore, a direct comparison of the efficacy of HOPE and NMP is impossible as of today. Because transplantation of livers in absence of steatosis, fibrosis or prolonged (warm) ischemia yields excellent results after SCS with 1-year graft survival rates exceeding 90%, as shown by a recent meta-analysis, the use of MP is usually still limited to clinical trials and transplantation of super-marginal organs [18]. As stated, grafts subjected to MP are most often derived from DCD and considered super-marginal livers. Features of super-marginal grafts may include and are not limited to: donor age >80 years, cold ischemia time >8-10 hours, warm ischemia time >30 minutes, graft macro-steatosis >30%, poor in-situ perfusion, prolonged retrieval, extensively elevated donor liver function tests, or livers technically transplantable, but declined for any other nonvascular reason [17,19]. Still, an early meta-analysis of 5 NMP-related clinical trials was able to delineate safety of NMP with favorable results concerning primary non-function and early allograft dysfunction [20]. Of note, NMP not only offers the ability to investigate organ viability prior to transplantation and potentially gives way for salvaging procedures, it also allows for postponing transplantation to daytime, transforming a nightly emergency procedure into "semi-elective" surgery. Most recently, the Innsbruck

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Group reported their algorithm as an SOP for decision-making and documentation of NMP (**Table 1**) [21]. Further prospective, randomized clinical trials of MP vs SCS and more specifically HOPE vs NMP are necessary to generate robust data on which an organ should be subjected to NMP or other MP strategies.

Pre-Transplantation Viability Assessment

Preservation of organs under almost physiologic conditions prior to transplantation offers a diagnostic window for assessment of organ function. This novum alone can be considered to be the most outstanding advantage of NMP compared to all other perfusion modalities. The decision to transplant a liver is hitherto based upon a rather superficial risk assessment, taking donor criteria (eg, age, laboratory values), recipient criteria and gross appearance of the liver into consideration. Objective, independent predictors and variables to predict graft function after transplant are lacking, a fact which may lead to the discarding of livers which could have potentially functioned. In the arguably very young clinical practice of NMP, steatotic organs and livers derived from elderly donors or DCD can now be objectively assessed, potentially increasing the ability to predict post-transplant outcome [22,23].

A case series of 6 livers which were declined for transplantation by all transplant centers in the United Kingdom, then subjected to NMP, was published by Mergental et al. Five livers showed sufficient functional criteria to allow transplantation. Viability criteria (applicable within 3 hours of perfusion) included either lactate clearance to <2.5 mmol/l or bile production and 2 of the following 3 criteria: pH-homeostasis >7.3, stable pressure/flow dynamics of the graft (ie, hepatic artery flow >150 ml/min, portal vein flow >500 ml/min), or homogenous graft perfusion with soft parenchymal consistency. These livers were transplanted and remained functional at follow-up after 7 (range 6-19) months postoperatively [24]. As potential markers for biliary viability more than actual bile production, biliary bicarbonate concentration greater than 18 mmol/l, biliary pH greater than 7.48, biliary glucose concentration less than 16 mmol/l a bile/perfusate glucose concentration ratio less than 0.67 and a biliary lactate dehydrogenase concentration less than 3.7 U/l where identified, which appeared to be linked to milder post-transplant cholangiopathy [25].

The predictive value of pH of excreted bile as a predictive marker for cholangiopathy after transplant and the importance of perfusing at physiological oxygen tensions was also described by Watson et al in their case series of transplanting 12 discarded livers following NMP and graft viability assessment [26]. In their later updated publication, a specific set of variables in the perfusate was associated with successful transplantation of perfused organs in a series of 47 livers undergoing NMP, 22 of which were transplanted: ALT <6000 IU/l at 2 hours; reduction in lactate ≥4.4 mmol/l/kg/h; falling glucose beyond 2 hours or perfusate glucose <10 mmol/l (180 mg/dl) with a subsequent reduction after a challenge with 2.5 g glucose; perfusate pH >7.2 (not requiring >30 mmol bicarbonate supplementation); bile glucose concentration ≤3 mmol/l (54 mg/dl) or ≥10 mmol less than perfusate glucose [27]. The most common predictive NMP-associated technical variables are summarized in **Table 2**.

Table 2. Markers of hepatocellular and biliary viability during perfusion.

- Lactate clearance in the perfusate
- pH homeostasis (without requiring ongoing bicarbonate supplementation)
- Stable hepatic artery and portal vein flow dynamics
- Decreasing transaminase levels
- Decreasing glucose
- Bile production
- Bile glucose <3 mmol/l or 10 mmol less than perfusate glucose
- Biliary bicarbonate concentration >18 mmol/lwith pH >7.5
- Biliary lactate dehyrogenase concentration <3.7 U/l

In addition to the aforementioned markers, there is evidence that ATP content in the liver may also serve as a viability marker. The absence of oxygen supply leads to a shutdown of adenine nucleotide metabolism, ultimately resulting in failure of ion transport through the cell membrane. Low ATP levels have been shown to correlate with poorer post-transplant outcomes. It is therefore suggested that assessment of so-called energetic recovery by measurement of differences in mitochondrial ATP content and oxygen-dependent phosphorylation of cellular ADP to ATP are possible viability markers during NMP [23,28,29]. Moreover, recent evidence from 12 liver allografts perfused under normothermic conditions suggests that microRNAs in bile and perfusate might also serve as biomarkers for injury and function. Although the allografts examined in this study were not transplanted, it was shown that HDmiR-122 and CDmiR-222 correlate significantly with classic markers of bile duct and hepatocyte injury [30].

In this evolving field, all parameters used as potentially discriminative biomarkers for liver injury and function still require validation in larger prospective cohort studies before introduction into daily clinical practice. Still, the potential to non-invasively evaluate liver function measuring biomarkers in the perfusate during NMP is a major advantage over for the invasive "histologic assessment" only and seems profoundly more reliable than the subjective, far-from-standardized evaluation by the transplanting surgeon [28].

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Therapeutic Intervention During Ex Vivo Liver Preservation

NMP theoretically provides a platform for organ repair during perfusion by the addition of therapeutic substances into the perfusate [31]. So far, anti-inflammatory agents, mesenchymal stem cells, gene therapy agents, and defatting substances have all been investigated in animal and translational human models of liver perfusion [32]. For reconditioning of marginal liver allografts during MP, defatting agents are of special interest, since steatosis of the liver is associated with a higher rate of ischemia-reperfusion injury and primary graft non-function. Defatting properties of NMP as such have already been shown in porcine and murine models of liver perfusion [33,34]. Unfortunately, the experimental decrease in tissue steatosis after NMP could not be reproduced in human livers [35]. It has recently been shown that pharmacological modulation of lipid metabolism (using a combination of PPARα ligands GW7647 and GW501516, PXR ligand Hypericin, the constitutive androstane receptor ligand scorparone, the glucagon mimetic cAMP-activator forskolin, and the insulin-mimetic adipokine visfatin [33]) during normothermic perfusion of formerly discarded human livers can induce defatting of the organ, which improved the metabolic status and functional recovery [36]. However, although some livers in that study met functional criteria which would potentially have allowed for transplantation, these allografts were discarded and further safety evaluation of these substances is required, since some of the agents used may exhibit cytotoxic and carcinogenic properties [6].

Immunomodulation of perfused liver allografts is an exciting new field in liver transplant medicine. NMP alone appears to exert immunomodulatory effects via increase of CD4posCD25highCD127negFOXP3pos regulatory T cells (Treg) in the liver [16]. A higher proportion of interferon-gamma and interleukin 17-producing T cells was visible with less necrosis of liver parenchyma [16,37]. Still, immunophenotyping and immune cell analysis of liver tissue samples in an RCT setting has yet to be investigated [6]. In animal studies, gene silencing using RNA interference to mitigate ischemia-reperfusion injury is currently being investigated. Cell therapy and extra-cellular vesicles are also being explored as enhancers of the positive immunomodulatory effects, aiming at dampening of ischemia-reperfusion injury and potential long-term reduction of immunosuppressive medication [38-40]. NMP offers the necessary therapeutic window to directly infuse these agents into the graft, allowing for the delivery of immune cells (such as T cells or mesenchymal stem cells) or extra-cellular vesicles directly into the liver prior to transplant [6]. In 2020, the Birmingham Group published a series of 6 discarded human livers perfused under normothermic conditions with infusion of multi-potent adult progenitor cells into the right hepatic artery during perfusion. Trans-endothelial migration of infused multi-potent cells was shown with subsequent secretion of anti-inflammatory and immunomodulatory agents, possibly resulting in less systemic inflammation upon reperfusion in-situ [41].

The usage of gene modulating agents such as anti-sense oligonucleotides and small interfering RNA (siRNA) demonstrated the potential to silence the virulence of hepatitis c virus in a porcine model of liver perfusion [42]. For example, the Fas-receptor, whose activation promotes mediator cascades that significantly contribute to ischemia-reperfusion injury, can be targeted by siRNA. Several other putative targets like p53, RelB, TNFα, and pro-apoptotic caspases may be silenced as well for hepatoprotection during perfusion/reperfusion [32,43]. Successful infiltration of the liver with siRNA during NMP was most recently demonstrated in rodents, paving the way for translational studies in human liver allografts [44].

Modulation of vascular resistance has been thoroughly investigated using dilatating agents such as prostaglandin E1, prostacyclin, and BQ123 (an endothelin receptor agonist) and verapamil (a calcium channel blocker) with promising results in rodents and porcine models of liver perfusion [45-49]. Interestingly, in a very recent clinical trial using prostacyclin for vasodilatation of livers during NMP before transplantation, hyperperfusion was visible in the first few allografts, leading to introduction of norepinephrine into the perfusion protocol to counteract low arterial resistance and maintain acceptable perfusion pressure [50]. This highlights the necessity of further clinical trials evaluating the effect of vasoactive substances on perfusion hemodynamics and resulting organ function in the future.

Conclusively, next to putative effects of NMP like abrogation of hyperfibrinolysis after reperfusion and inflammation [16,51], glycogen repletion and ATP regeneration (mostly linked to the absent or mitigated ischemia-reperfusion injury) [22], NMP offers a window for a variety of future therapeutic options, which all need to be subjected to thorough translational investigation [23].

Potential Disadvantages of NMP

Despite the most promising results of the first clinical trials of NMP in liver transplantation, it has to be clearly noted that malfunction of the device, technical difficulties, and human errors during perfusion of the liver can result in immediate and irreversible damage to the graft, with considerably more detrimental effects under normothermic and sub-normothermic conditions, than under hypothermic conditions [19]. In short, an already cooled organ might still be preserved on ice, but an organ perfused under normothermic conditions suffers immediate warm ischemia, with a high likelihood of subsequent graft loss. Furthermore, in personal discussion with different

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transplant centers in Germany and Europe, it appeared that perfusion with red blood cell-based perfusate under normothermic conditions is thought to be technically more challenging than continuous perfusion with cold perfusate and requires a more in-depth training and greater expertise.

Apart from these technical aspects, the first evidence on microvascular occlusion of sinusoidal capillaries by rouleaux-like aggregations of red blood cells in 7 liver allografts perfused under normothermic conditions was given by Tingle et al in reply to a larger study, in which the same phenomenon was described in marginal kidney allografts [52,53]. This is of special interest, as the sinusoidal and portal capillaries supply the biliary system, which is considered to be most sensitive to ischemia. Whether this phenomenon results in more ischemic-type biliary lesions after NMP compared to HMP or SNMP is yet to be explored in long-term results of current and future clinical trials. Of note, in the recently published first results of the VITTAL-Trial (ClinicalTrials.gov number NCT02740608) of 22 primarily declined allografts transplanted after end-ischemic NMP, a significantly higher rate of non-anastomotic biliary strictures was observed after NMP compared to a contemporary matched control group. A study to test the effects of end-ischemic NMP versus end-ischemic HOPE in a multicenter prospective randomized controlled setting on ECD liver grafts in DBD liver transplantation is about to begin recruitment. Human whole-liver grafts will be submitted to either NMP or HOPE directly before implantation and will be compared to a control group of ECD liver allografts transplanted after SCS (NCT04644744). We believe that the short- and long-term results of this multicenter clinical trial will be of great value in investigating possible superiority of MP protocols over SCS and to elucidate differences between normothermic and hypothermic perfusion techniques.

Cold-to-Warm Perfusion

The discrepancy between demand for livers and donor supply led to the emergence of dynamic preservation techniques. Firstly, HMP was introduced, offering cold perfusion solution to the explanted organ, slowing down metabolism, and assuring adequate oxygen supply to inhibit ischemic injury, thereby resuscitating the mitochondria and increase ATP content, resulting in less cell injury, including less cholangiocyte injury [54]. Still, the abrupt increase in temperature upon reperfusion exerts a considerable injury to hepatocytes and the biliary system. Although HMP has been shown to permit transplantation of marginal grafts with considerable functional and energetic recovery, viability assessment of marginal allografts is not equally possible during HMP compared to normothermic protocols. NMP immediately after retrieval mitigates ischemia-reperfusion injury and is able to provide more information regarding liver function [5]. Unfortunately, machine perfusion immediately after retrieval has major logistical and infrastructural implications of transport and expertise, possibly hindering its further application. Subjecting an organ to SCS after in-situ cold perfusion and transporting the organ on ice appears to be more feasible for many reasons. On the other hand, subjecting an organ to SCS for transport with end-ischemic NMP at the recipient center potentially also results ischemia-reperfusion injury comparable to in-situ perfusion after implantation [5]. Sequential use of oxygenated HMP preceding NMP hypothetically combines "the best of both worlds", with few considerations to be made. Of note, while NMP depends on red blood cell-based perfusate, HMP and sequential hypothermic to normothermic machine perfusion would require a red blood cell-free perfusate due to the risk of clotting and hemolysis under hypothermic conditions [54]. A change of perfusate during the perfusion process has been described before [55], but was considered disadvantageous due to the unnecessary additional cold ischemia time. A hemoglobin-based oxygen carrier solution has most recently been shown to function under hypothermic and normothermic conditions without detrimental effects on hepatic tissue and ATP regeneration [56]. In addition to the advantage of being able to sequentially perfuse liver allografts without changing the perfusion solution, the immunogenic effect of third-party-derived red blood cells may be significant.

Moreover, the abrupt temperature shift from hypothermic to normothermic perfusion may again trigger mitochondrial dysfunction. With the exception of the seldom-applied normothermic regional- or direct-machine perfusion, all end-ischemic perfusion techniques have the same drawback of ischemia-reperfusion injury in one way or another. Evidence from animal studies in pigs suggests that stepwise, controlled oxygenated rewarming of cold-stored livers prior to normothermic perfusion results in improved cellular enzyme activity, reduction of gene expression and perfusate activities of inflammatory molecules, and improved portal vein flow dynamics compared to HMP or SNMP [57]. A number of exploratory clinical studies arose from these promising preclinical models. Controlled oxygenated rewarming alone was shown to be feasible and safe, with no early allograft dysfunction and 100% 6-month survival rate in 6 patients in a first-in-humans trial [58]. With controlled rewarming after SCS prior to NMP at the recipient center, superior results were found concerning mitochondrial function, with enhanced energetic recovery and mitigated hepatocellular injury resulting in superior function of allografts compared to NMP after SCS alone in 18 liver transplant recipients [58,59]. Most recently, excellent long-term clinical outcomes after application of stepwise controlled oxygenated rewarming were demonstrated [60]. The Groningen Group reported on 16 livers undergoing a hypothermic perfusion–controlled oxygenated rewarming–normothermic perfusion protocol, 11 of which were transplanted after meeting the group's

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criteria for hepatocellular and biliary function. All recipients were alive with functioning grafts at 6-month follow-up, with only 1 patient suffering from biliary strictures 4 months after surgery. The authors delineated a 20% increase in utilization of donor livers at their center.

In short, applying a combined, sequential perfusion technique which includes controlled rewarming may circumvent not only physiologic (ie, ischemia-reperfusion injury) but also infrastructural and personnel-related obstacles in liver transplantation: allograft retrieval and transport can be realized as SCS "on ice" in standard fashion. Subsequently, the transplant center would utilize data-based SOPs to determine whether to immediately transplant the liver or subject the allograft to controlled rewarming and NMP for further function assessment and potential modification [5]. There is no question that multiple RCTs are needed to disseminate MP techniques and define the place for more even more complex combined perfusion techniques in liver transplantation.

Perspectives and Conclusion

A variety of different ex-situ machine perfusion techniques exist for human liver transplantation. It appears that end-ischemic HMP, (D)HOPE, and NMP are currently the most commonly applied perfusion techniques in human liver transplantation. Current clinical trials usually focus on comparison of different perfusion techniques against the current criterion standard of cold storage. These trials are important to determine whether and how MP holds advantages over SCS, but it has to be kept in mind that these trials are performed with a certain selection bias and also put patients in danger of receiving an organ formerly considered unsuitable for transplantation based on parameters that have yet to be validified in larger meta-analyses. Still, the necessity of such trials remains undisputable.

One might hypothesize that marginal organs in the sense of steatotic livers or allografts derived from elderly donors benefit more from controlled rewarming and normothermic perfusion to mitigate reperfusion injury than allografts considered marginal due to cardiopulmonary reanimation, long cold ischemia time, or long-term stay on an intensive care unit. It would be highly interesting to know whether allografts considered marginal for different reasons also benefit differently from the various perfusion modalities. Of note, effects of machine perfusion on low-risk liver allografts are not addressed at all in the scope of current clinical trials. Due to the excellent results of liver transplantation of "healthy" allografts after SCS, such trials remain hard to justify, bearing in mind that MP also exerts considerable financial stress on transplant centers [2].

The introduction of machine perfusion and especially normothermic machine perfusion into daily clinical practice seems indispensable due to its proven capacity to expand the donor pool and decrease the rate of organ discard. The ability to assess organ viability prior to transplantation along with the current development of repair strategies of preservation or procurement injuries and the promising concept of combined sequential perfusion techniques to mitigate ischemia-reperfusion injury are the underlying reasons that will possibly overcome even financial caveats. It has to be kept in mind though that potential detrimental effects of normothermic perfusion, like obstruction of sinusoidal capillaries through red blood cell-based perfusates, possibly resulting in late biliary complications, are under investigation and have yet to be reported on in detail. Reports on long-term outcome of liver transplantation after machine perfusion are expected to be published in the near future. The rate of biliary complications after NMP alone should be of great interest.

Conflicts of Interest

None.

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