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Current Status and Future Aspects

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Johannes Georg Laufhütte

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unter der Anleitung von

Dr. med. Helge Bruns

Assoz. Prof. Priv.-Doz. Dr. med. univ. Philipp Stiegler

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List of Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BMI	body mass index
CORM-3	carbon monoxide releasing molecule 3
DBD	donation after brain death
DCD	donation after cardiac death
DHOPE	dual hypothermic oxygenated machine perfusion
ECD	extended criteria donor
eGFR	estimated glomerular filtration rate
ET-1	endothelin-1
GST	glutathione S-transferase
H_2S	hydrogen sulphide
HMP	hypothermic machine perfusion
IRI	Ischemia reperfusion injury
KIM-1	kidney injury molecule 1
LDH	lactate dehydrogenase
L-FABP	liver-type fatty acid-binding protein
MMP	matrix metalloproteinase
NAG	N-acetyl-ß-D-glucosaminidase
NGAL	neutrophil gelatinase-associated lipocalin
NMP	normothermic machine perfusion
NO	nitric oxide
RNA	ribonucleic acid
ROS	reactive oxygen species
SCS	static cold storage
siRNA	small interfering ribonucleic acid
SMP	subnormothermic machine perfusion

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Zusammenfassung

Diese Diplomarbeit beschäftigt sich mit dem aktuellen Stand und den Zukunftsthemen der Konservierung der Organe Leber, Niere und Pankreas im Rahmen der Organtransplantation. Ein großes Problem im Bereich der Organtransplantation stellt aktuell der Mangel an Spenderorganen dar. Der Organkonservierung als einem zentralen Bestandteil des Transplantationsprozesses kommt in diesem Zusammenhang eine entscheidende Rolle zu. Durch Verbesserung bestehender, sowie Einführung neuer Konservierungsmethoden könnten zukünftig sowohl der Spenderpool erweitert als auch Komplikationen nach der Transplantation vermindert und das Patienten- und Transplantatüberleben verlängert werden. Aktuell sind zentrale Themen in diesem Zusammenhang insbesondere die Erforschung und die Ausweitung der Anwendung der maschinellen Perfusion zu konservierender Organe. In dieser Diplomarbeit wird daher der Fokus auf die verschiedenen Ansätze der maschinellen Perfusion gelegt. Dabei werden sowohl der aktuelle Stand als auch zukünftige Aufgaben in diesem Bereich dargelegt. Weiters werden von der breiteren Anwendung noch weiter entfernte Zukunftsthemen aus dem Bereich der Organkonservierung vorgestellt.

Abstract

This diploma thesis aims at presenting the current status and future aspects of the preservation of the organs liver, kidney and pancreas in the context of organ transplantation. Currently, a major problem in organ transplantation is the lack of donor organs. Organ preservation as a central component of the transplantation process plays a decisive role in this context. By improving existing preservation methods and introducing new ones, the donor pool could be expanded, complications after transplantation could be reduced and patient and graft survival could be extended. At present, central topics in this context are research on and expansion of the application of machine perfusion of grafts. Therefore, this diploma thesis focuses on the different concepts of machine perfusion. Both the current status and future aspects in this field are presented. Furthermore, future aspects in organ preservation are presented, which are even further away from a widespread application.

1 Introduction

Transplantation is the treatment of choice for end-stage liver and kidney disease, among others.(1-3) An optimization of organ transplantation can be realized in different steps of the transplantation process. According to Tavares-da Silva and Figueiredo, these include donor optimization, organ preconditioning before harvesting, improvements of the surgical harvesting procedures and improvements of organ preservation.(2) This thesis focuses on the latter of these four points. It presents the current status and further aspects of liver, kidney and pancreas preservation and demonstrates the potential for improvements in this field.

Organ preservation is an integral part of organ transplantation.(4) During organ preservation the organ should ideally be preserved in the best possible condition for as long as required.(5) Thus, organ preservation aims at bridging the period from organ retrieval from the donor to the transplantation of the organ into the recipient. By this means, it should provide time for both, finding a suitable recipient and transporting the organ from the donor to the recipient hospital.(4)

To give a brief overview of the history of organ preservation, Starzl mentions that organ preservation was not yet developed in the early 1950s, nor were immunosuppression and tissue matching.(6) Until the early 1980s organ transplantations were predominantly kidney transplantations according to Starzl. But as transplantation of other organs became more common, so-called flexible techniques were introduced. The first step of these flexible techniques is the in situ cooling of the organs by infusing a cold solution for averting warm ischemia. Afterwards the organs are quickly removed in a bloodless field. Thereafter the organs are dissected on a back table. In Starzl's opinion introduction of flexible techniques was a precondition for being able to harvest several organs from one donor.(6-8) Starzl reports that for the subsequent organ preservation two different approaches were invesigated. On the one hand ex vivo perfusion and on the other hand static cold preservation.(6) While static cold preservation has been the predominantly used approach for decades, there has been a rediscovery of machine perfusion and as a result extensive research on this technique in recent years.(9-11) This is mainly because machine perfusion is expected to be superior in preservation of higher risk organs. Thus, by using machine perfusion in organ

preservation, an expansion of the donor pool could be enabled which would contribute to counteracting the donor organ shortage.(9,12)

But so far “optimal” preservation strategies have not yet been found and organ preservation still remains a major difficulty in organ transplantation.(13) Hence, there is a lot of research in this field and this paper is intended to provide an overview of the many different developments in the field of liver, kidney and pancreas preservation.

2 Materials and Methods

A comprehensive literature search for published articles regarding organ preservation of donor livers, kidneys and pancreata was conducted using the PubMed database. The last date of search was October 11, 2020. The search was carried out using medical subject heading (MeSH) terms and free text terms “organ preservation”, “hypothermic machine perfusion”, “hypothermic machine preservation” “subnormothermic machine perfusion”, “subnormothermic machine preservation”, “rewarming machine perfusion”, “normothermic machine perfusion” and “normothermic machine preservation” combined with “liver”, “kidney” or “pancreas”. Further terms were “organ preservation kidney”, “liver preservation”, “pancreas preservation”, “kidney preservation future”, “kidney preservation trends”, “liver preservation future”, “liver preservation trends”, “history of organ preservation”, “ischemia reperfusion injury”, “reperfusion injury”, “polyethylene glycols organ preservation”, “cryopreservation”, “oxygen persufflation”. Regarding results, date limits were set in some cases to ensure the timeliness of the results. Additional search with different free text terms for websites, articles and books was performed using Startpage, Bing, Google, Google Scholar and Google Books.

The decision on whether to include articles was at first based on the relevance of the title. Only abstracts of articles with relevant titles were then evaluated regarding their relevance to the topic of this thesis. Articles with relevant abstracts were further assessed when they were published in scientific journals, fully accessible and written in English or German.

3 Results

3.1 Static Cold Storage

Static cold storage (SCS) is still the predominantly used method for preserving liver, kidney and pancreas grafts.(9-11,14-17) In SCS, the organ is cooled down to 4˚C(16), because hypothermia is used for considerably slowing down the metabolism.(4) A decelerated metabolism diminishes the energy consumption of the cells.(16) But the decreased metabolic activity of cells does not only have beneficial, but also harmful effects on the organ.(18) Since such a cooling process and the cold environment are not physiological for the cells, they react by swelling and lysis. To protect the cells from these harmful effects, preservation solutions are used.(16) After flushing the organ with such a preservation solution it is kept in the solution until it gets transplanted to the recipient.(19)

Static storage methods come along with ischemic conditions. In these an oxygen dyshomeostasis occurs. As soon as the organ gets oxygenated again, oxidative processes take place on a large scale. During these processes a large amount of reactive oxygen species (ROS) is formed. But at the same time the cells' ability for neutralizing ROS is severely limited, since main antioxidant pathways are suppressed during warm ischemia.(20) Thus, approaches for reducing oxidative stress in static storage could aim at either diminishing the production of ROS by inhibiting corresponding reactions or at fostering antioxidant pathways and boosting agents that are able to neutralize ROS, e.g. by adding chemical antioxidant agents.(20)

An important disadvantage of SCS is the lack of reliable methods for viability assessment. This is particularly disadvantageous in case of preserving marginal organs.(11,21,22) A further downside, especially in the context of a donor organ shortage, is that SCS is not well suited for preserving such lower quality organs.(11,16,19)

3.2 Machine Perfusion

Machine perfusion is not a novel approach. For example, trials using isolated kidney perfusion for experiments in physiology date back to the early 20th century.(10,11) Somewhat later, the technique of pulsatile machine perfusion has already been invented and investigated by Lindbergh and Carrel in the 1930s.(12,13) During the 1970s and 1980s, machine perfusion was an established

approach in kidney preservation (12,23,24), whereas in the past it did not go beyond experimental application in liver preservation.(9,25,26)

Machine perfusion is based on the principle of pumping a perfusate through the vasculature of the organ to be preserved. This is enabled by using a perfusion device. Perfusion is possible as soon as the organ has been cannulated and connected to the perfusion device.(27)

Potential benefits of machine perfusion might include the supply with substrates and oxygen for maintaining the cellular metabolism, the removal of metabolic waste products, the stimulation of a flow-dependent expression of genes resulting in protective effects for the endothelial lining, and the possibility to apply therapeutic agents during preservation.(28)

Becker et al. consider the rediscovery of machine perfusion in the past years and the enhancements in this approach made since then as the most important development in organ transplantation within the last decade.(29) But finding the most beneficial temperature and procedure of machine perfusion for preserving and reconditioning liver, kidney and pancreas adapted to the particular donor criteria and risk is still an important topic in organ preservation research.(17)

Machine perfusion protocols primarily differ in the vessels cannulated for perfusion, in the chosen temperature, in oxygenation of the perfusate, and in perfusion pressure.(30) Moreover, the timing of machine perfusion differs between the various studies.(27) Karangwa et al depict that machine perfusion can be applied in different moments of organ preservation. In some trials it was used during the whole preservation process from procurement until transplantation, in some it was used before SCS only, in others it was used after SCS as preparation before transplantation.(10) This is illustrated in the following figure showing different possibilities of the timing of machine perfusion during organ preservation:

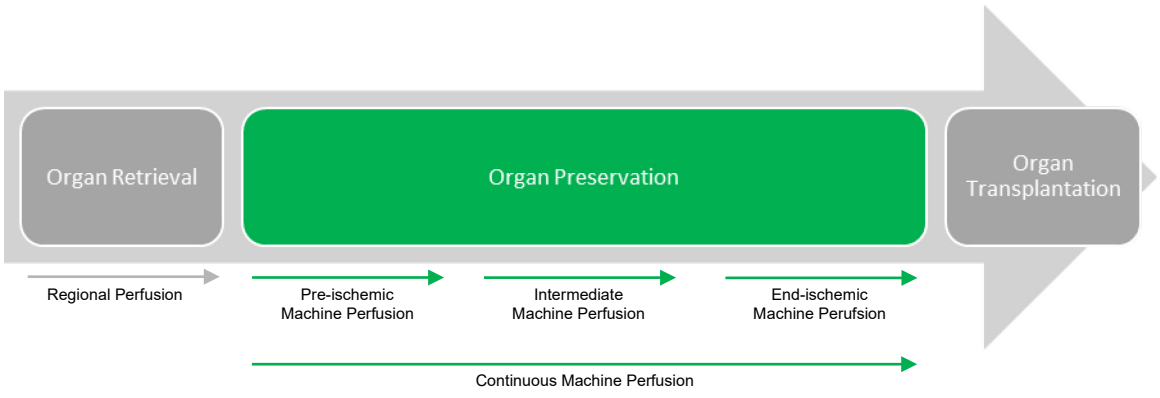

Figure 1: Different possibilities of timing the machine perfusion during organ preservation based on Jochmans et al.(27)

The different ways of machine perfusion are usually classified according to the temperature because the metabolic activity of the graft is mainly influenced by temperature.(22) Regarding temperature Karangwa et al. recommend a classification of machine perfusion as follows:(10)

1) hypothermic machine perfusion from 0°C–12°C
2) midthermic machine perfusion from 13°C–24°C
3) subnormothermic machine perfusion from 25°C–34°C
4) normothermic machine perfusion from 35°C–38°C

Bellini et al. mention that the best temperature for organ preservation has still remained a secret.(13) One reason for this is that a temperature suitable for a specific organ might not be ideal for another one. For example, the best temperature for liver preservation might not be the best for kidney preservation. Among others, this is because of different types of cells in different organs.(13,17) In addition to these differences between several kinds of organs there might be differences between individual organs as well. This means that also organs of the same kind, like different livers, should be preserved applying different techniques or temperatures depending on organ quality. This includes aspects like whether the organ to be preserved stems from an extended criteria, a donation after brain death (DBD) or a donation after cardiac death (DCD) donor.(13,31) This means, that choosing the right setting of organ preservation based on the individual case might be the most successful way in the future.(13,31)

A further controversial question in machine perfusion research, besides the choice of temperature, are the potential necessity and advantages of an oxygenation of the perfusate.(17)

Apart from these unclarified questions, there is consensus that examining and determining the viability of grafts ex vivo during preservation is possible when machine perfusion is applied.(10,12,16,32) This is beneficial in general and especially for grafts derived from DCD donors.(12) As a result it could allow to diminish discard rates and thus to use a higher proportion of donor organs for transplantation.(16) Moreover, machine perfusion could allow for the application of therapeutic agents during preservation which might have beneficial effects on

posttransplant graft function.(10,19) By this means, for instance, the de-fatting of livers with hepatic steatosis might be enabled.(10)

3.3 Ischemia Reperfusion Injury

In general, posttransplant organ function is strongly influenced by quality, injury and ischemia reperfusion injury (IRI) of the transplanted organ according to Hoyer et al.(33) IRI is a major limitation on organ transplantation, since it can cause severe posttransplant complications and restricts the donor pool.(34)

At this juncture it has to be determined that the mechanisms and consequences of IRI during ex vivo machine perfusion are not yet sufficiently known.(22) Identifying the mechanism of IRI is key for the following aspects: (17,35)

- finding ways to detect IRI
- discovering interventions to reduce IRI
- developing preservation protocols to recondition the organ during machine perfusion.

Main aspects of IRI are inflammatory processes and the formation of ROS triggered by the reperfusion with blood after prolonged ischemia. This again damages the cells and can lead to cell death.(35) Wu et al. describe different aspects of IRI. One out of these is the anaerobic metabolic pathway and the disturbance of the mitochondrial electron transport chain caused by ischemic conditions and the subsequent hypoxia.(35) As a consequence the cellular ATP formation and ATP level drops which induces cell swelling and a perturbation of cytoplasmic enzyme function since ion channels can’t work properly under conditions of ATP deficiency.(35) Linked to that, among others a calcium overload develops in cells and mitochondria.(36) In addition to that, the anaerobic metabolism caused by the lack of oxygen during prolonged ischemia results in rising intracellular lactate levels. This again leads to a drop of the intracellular pH.(36) Moreover, mitochondrial injuries and electrolyte disorders during reperfusion stimulate the occurence of oxidative stress via the NADPH oxidase system, the NOS system and the xanthine oxidase system. Finally, retention of ROS induces cellular injuries and results in cell death.(35)

Another aspect of cold ischemia influencing the preserved kidney was investigated by Le Pape et al. It is the endoplasmic reticulum’s unfolded protein response that seems to be involved in relevant cellular alterations. Further investigating it may

help identifying markers for assessing the transplantability of preserved kidneys or finding therapeutic interventions aiming at a reduction of preservation-related injuries.(37)

Moderate and mild IRI should not be regarded as harmless according to De Beule et al.(22) Andersson et al. reported that in rat kidneys mild IRI altered the glomerular charge barrier and by that diminished glomerular charge selectivity. Additionally, size selectivity was slightly decreased.(38) The decrease in size selectivity was particularly evident after prolonged ischaemic reperfusion.(39) Regarding potential injuries during organ preservation it should be mentioned that the renal tissue consists of many different types of cells. Some of them can't be regenerated after getting injured while others, like proximal tubular epithelial cells can be regenerated, at least to a certain extent and under the right conditions.(22,40)

During ischemia reperfusion a sterile inflammation of the tissue takes place.(41) Infiltrating leukocytes as wells as vascular endothelial cells are involved in the inflammation.(41) The effects of the different machine perfusion techniques on the endothelial cells are still unknown.(22) But known is that endothelial cells exposed to inflammatory stimuli have procoagulant properties.(42) Regarding leukocytes, the following can be said: In machine perfusion of the kidney circulating leukocytes are commonly eliminated. The effects of this are unknown.(22) Nonetheless, it is believed that the elimination of circulating leukocytes, such as in using red blood cell based or acellular perfusates instead of whole blood, reduces inflammatory processes during machine perfusion.(22) This should not hide the fact that there are resident myeloid and lymphoid immune cells present in renal tissue.(43) But it is also unclear what consequences their presence has on the renal tissue during machine perfusion and on posttransplant outcomes.(22)

In the renal tissue, a release of proinflammatory cytokines is observable during machine perfusion, according to de Beule et al., but measurable ones with a predictive value for posttransplant renal function have not yet been identified.(22) Nonetheless it has been shown that apoptosis of renal cells was diminished during ischemia reperfusion in kidneys preserved by hypothermic machine perfusion (HMP) compared to kidneys preserved by SCS.(44)

Also in liver transplantation IRI is a major challenge. During ischemia certain metabolic products are formed which activate an inflammatory immune response

provoking damage to liver cells when the organ is reperfused.(16) A main problem in liver transplantation is that cold as well as warm ischemia harm the organ and it's possible that this injury manifests only after reperfusion in the recipient.(45) There is evidence of harmful effects of hypothermia. In liver cells of rats, Rauen et al. observed an iron-dependent mechanism injuring liver cells. This mechanism appeared in hypothermia at a temperature below 16 ˚C and reached its maximum at a temperature of 4 ˚C – 8 ˚C.(46) Therefore, Hoyer et al regard 16 ˚C as a temperature threshold for subnormothermic and hypothermic organ preservation.(47) Furthermore, Rauen et al. also found iron-independent cell damage in cells stored at temperatures below 13 ˚C. This mainly appeared during rewarming of the cells.(46) While usual preservation solutions like Euro-Collins and University of Wisconsin solution were not able to prevent this cold-induced cellular damage, adding an iron chelator to the solution inhibited the iron-dependent injury. In addition to that, a low content of chloride in the solution inhibited the iron-independent damage.(46)

During cold ischemia and warm reperfusion liver cells die and get harmed by endoplasmic reticulum stress and mitochondrial dysfunction. For example, a loss of the mitochondrial membrane potential is observable during hypothermia and progresses over time. Thus, strategies protecting endoplasmic reticulum and mitochondria could reduce IRI in livers preserved under hypothermic conditions according to Duval et al.(48)

In a rat model, the change from hypothermia (4 ˚C) to normothermia (37 ˚C) was associated with a very fast decrease of the liver's ATP level. In the course of this a weaker response to the cellular ATP demand was observed. This was caused by a disfunction of the phosphorylation subsystem in injured mitochondria, according to Leducq et al.(49) Hoyer et al blame the sudden rewarming upon causing these issues and therefore recommend a slow controlled rewarming of hypothermic liver grafts before transplantation for avoiding this sudden rewarming.(47)

3.4 Liver Preservation

A main challenge in liver transplantation is the donor liver shortage(9), as can be recognised by the following numbers from the United States: According to the "OPTN/SRTR 2018 Annual Data Report: Liver" of the Organ Procurement and Transplantation Network 11,844 patients were added to the waiting list for a liver transplantation in 2018, but only 8,250 patients received a donor liver. These

numbers illustrate the necessity of expanding the donor pool.(50) Relevant in this context is that in a lot of countries there is a rising quantity of deceased organ donors according to Nasralla et al., but this is especially because of a higher number of elderly donors with multimorbidity and DCD donors.(51,52) At the same time the number of DBD donors has shrunk due to improvements in several fields like road traffic safety, treatment of cerebrovascular accidents and the management of intracranial vascular malformations.(53,54) That's why nowadays livers of poorer quality, so called extended criteria donor (ECD) livers, are more often transplanted.(51) For instance, such ECD livers comprise livers of non-heart beating donors, steatotic livers and livers of older donors according to Vekemans et al.(19) Since the transplantation of livers of these donor groups is associated with an increased risk for posttransplant complications like primary non-function and the development of biliary strictures, the number of transplantable organs did not increase although there are more deceased organ donors, following Nasralla et al.(52) This is also caused by the fact that the regenerative potential of ECD livers from injuries during the whole transplantation process is worse and they are less tolerant of ischemia.(55) Thus, they are more susceptible to and suffer from stronger IRI.(9,55)

This underlines that a precondition for expanding the donor pool towards higher risk donors is the optimization of organ preservation.(9) For preserving ECD livers, simple cold storage is non-satisfying.(19) Machine perfusion might be superior in preserving ECD livers and therefore machine perfusion is a very important topic in organ preservation research.(19) But regarding studies on preservation of ECD livers it has to be taken into account that the definitions of ECD livers differ between transplant centres in some cases which impedes comparisons.(51)

An important group of higher risk donors are DCD donors. This higher risk is illustrated by the fact that the long term patient- and graft-survival of recipients of DCD livers compared to recipients of DBD livers was significantly lower according to a study published in 2011.(56) But in expanding the donor pool DCD donors could play an important role. Non-heart beating donors, unlike heart beating donors, undergo warm ischemia because the blood supply is not maintained until the procurement process is initiated.(19) As a result, DCD grafts suffer from two periods of warm ischemia. Furthermore, the time of warm ischemia of DCD grafts is in total longer compared to DBD grafts. This is caused by the warm ischemia

during the time span from cardiac arrest to the beginning of organ preservation.(57,58) Abt et al. suspect this period of warm ischemia as a conceivable risk for developing intrahepatic strictures.(57)

Matsuno et al. state that the outcomes after transplantation of livers derived from DCD donors are not always favourable in the beginning. In this context one main issue is the development of ischemic biliary strictures.(12) Biliary strictures are among the most frequent kinds of biliary complications after liver transplantation according to Wojcicki et al.'s review.(59) Particularly the high rate of non-anastomotic biliary strictures seems to restrict the use of DCD livers, because they are associated with a high rate of graft failure.(52) The results of a meta-analysis by Jay et al. published in 2011 show that recipients of a DCD liver graft had an overall rate of biliary complications of 29% compared to 17% in the group of patients who had received a DBD liver graft. The odds ratio was 2.42.(60) Especially the ischemic cholangiopathy rate in the DCD group was much higher with 16% in comparison to only 3% in the DBD group. The authors calculated an odds ratio of 10.81.(60) Ischemic cholangiopathy has been regarded as an important restriction on the use of liver grafts derived from DCD donors.(61) Jay et al. also report higher rates of graft failure, retransplantation and mortality in DCD liver recipients. They advocate to bear these facts in mind when discussing about the transplantation of DCD liver grafts.(60) Furthermore, DCD livers seem to be more susceptible for developing peribiliary vascular plexus injury. In a sample of liver grafts preserved by SCS, op den Dries et al. observed an absence of peribiliary vascular plexus injury in a significantly higher proportion of DBD livers than DCD livers.(62) Preservation by normothermic machine perfusion (NMP) might have beneficial effects on the outcomes of DCD liver transplantation. This is underlined by a trial of Nasralla et al.(52) Although warm ischemia time was prolonged in NMP, they didn't find a significant difference in the rate of non-anastomotic biliary strictures between the preservation of DCD liver grafts by SCS (26.3%) versus preservation by NMP (11.1%). But it has to be added that the study did not have enough power for evaluating this outcome.(52)

Since the beginning of liver transplantation SCS has been the standard technique for liver preservation. SCS is a simple method for liver preservation and thus, in the first decades there hasn't been much interest and research in machine perfusion as preservation method for liver grafts, although Brettschneider et al.

firstly described the preservation of canine livers by machine perfusion in 1967.(9,25) Also Starzl et al. have already used a hypothermic perfusion system for liver preservation back in the late 1960s.(26) Higher costs and more challenging logistics were disadvantages compared to SCS which turned out to be sufficient for preserving standard criteria allografts with low risk. That's why research on machine perfusion was mainly interrupted for many years.(63) But a few years ago the topic received more attention with the increasing donor liver shortage.(9) The necessity of expanding the donor pool for counteracting the donor liver shortage further emphasises the requirements for an organ preservation technique, as they have an even higher importance for marginal organs. These requirements are: (19)

- a prolongation of the acceptable preservation time
- a possibility of viability assessment before transplantation
- a reduction of posttransplant primary graft nonfunction.

Machine perfusion might be superior to SCS in meeting these requirements.(19) Since first results of machine perfusion in liver preservation were promising, the interest in it was rising which is reflected in an increasing number of studies on machine perfusion in liver preservation that have been published since the mid-2000s.(9,10)

3.4.1 Static Cold Storage in Liver Preservation

According to Matsuno et al. a few years ago most transplantation centres used simple cold storage for preserving organs since it is easier to handle and cheaper than machine perfusion.(12) Nowadays SCS is still the predominantly used technique of liver preservation.(9,10,15-17) At the same time there haven't been any major changes of this method for decades.(64)

SCS of liver grafts mainly aims at reducing the metabolism of the organ.(12) But nevertheless, under hypothermic conditions like in SCS there still is a metabolic activity and this activity results in interstitial acidosis.(65) The main problem with interstitial acidosis is that the restitution to a physiological pH during reperfusion is associated with an increase of cellular damage.(66) As Perera et al. found significantly higher lactate levels in DCD livers compared to DBD livers during SCS. They suspect that a higher level of acidosis prior to preservation causes this phenomenon. And at the same time common preservation solutions might be

unable to buffer this higher level of acidosis. Therefore, they suggest further research on reducing lactate acidosis in DCD livers.(65)

SCS further aims at mitigating IRI harming the liver cells. Since SCS, of course, can't prevent IRI completely, the posttransplant outcomes depend mainly on the quality of the liver grafts.(16) Ceresa et al. mention that a low-risk liver preserved by SCS usually shows satisfying posttransplant outcomes, because it is able to deal with the extent of IRI occurring in the course of organ transplantation. In contrast to that, marginal livers do not only have a lower quality before organ preservation begins, but their quality is usually also further diminished by an even heavier extent of IRI caused by the transplantation process. That worsens posttransplant outcomes distinctly.(16) As a consequence, since cold storage and IRI cause specific impairments of preserved livers, there are certain requirements regarding the quality of liver grafts if a preservation by simple cold storage should be done. These requirements limit the number of transplantable liver grafts following Ravikumar et al.(11) It can therefore be concluded that SCS is not the optimal method for preserving marginal organs.(19)

The application of simple cold storage has a further limitation. It is the lack of an appropriate method for viability assessment. This is particularly disadvantageous in case of preserving marginal livers according to Ravikumar et al.(11)

A further disadvantage of SCS is that the epithelial cells of the common bile duct get harmed during cold ischemia.(67) Injuries of this epithelium are yet detectable at the end of cold storage.(67) Reperfusion even worsens these injuries.(67) Reperfusing an ischemic organ immediately with oxygenized blood does not seem to be a satisfying solution, since it might boost the formation of ROS and might contribute to necrosis.(17,68)

3.4.2 Machine Perfusion in Liver Preservation

The mode of operation of machine perfusion is based on a continuous perfusion of the preserved organs blood vessels, which is the key difference to static preservation techniques.(19) Machine perfusion, according to Vekemans et al., maintains the oxygen and nutrient supply and helps protecting the livers microcirculation.(19) For example, an important goal of machine perfusion is the maintenance of endothelial functionality by providing moderate shear stress which is caused by the continuous perfusion.(63) Contrary to this, simple cold storage leads to a constriction of the liver sinusoids. This hinders the distribution of the

preservation solution inside the liver. Subsequently, a disruption of microcirculation might occur as a result of simple cold storage.(19)

Furthermore, machine perfusion maintains the viability of the liver graft and may even enhance its function, which is sometimes referred as “resuscitation” and “reconditioning” of the organ.(10,63) For example, the metabolic energetic state in mitochondria can be restored. On the one hand, this includes a reduction of ROS and inflammatory cytokines that are produced as part of IRI. On the other hand, it includes the resynthesis of tissue adenosine triphosphate (ATP).(17) Organ resuscitation and reconditioning are beneficial as especially ECD livers could profit from that.(10) Thus, machine perfusion could contribute to an expansion of the donor pool because the transplantation of lower quality organs like livers from DCD donors could be enabled. Such an expansion is important for being able to meet the demand for donor grafts in a more sufficient way, since this demand is higher than the actual supply.(12) To sum it up, the potential for resuscitating and reconditioning the organ during preservation is an important advantage of machine perfusion compared to SCS which primarily aims at confining ischaemic damages and not at enhancing the organ function during preservation.(63)

But it is important to mention that investigated machine preservation methods and protocols in liver preservation, so far, vary especially in terms of temperature and oxygen supply and concerning the aspect, whether they are flow or pressure controlled.(10) Furthermore, considerable differences regarding the description of methodology and nomenclature are observable, what impairs the comparability of different methods and their outcomes. This makes it more complicated to carry out meta-analyses.(10) That’s why Karangwa et al. call for a standardized nomenclature in machine perfusion.(10) Standardizing the description of methodology would finally enable objective comparisons between different trials and simplify meta-analyses.(10) Karangwa et al. have published a list of methodological aspects they would like to be mentioned as part of the description of methodology in every publication on machine perfusion trials. Among others they suggest mentioning:(10)

- timing and duration of machine perfusion
- perfusion temperature
- ex situ or in situ perfusion
- pressure- or flow-controlled perfusion

- single or dual vessel perfusion
- used perfusate
- oxygenated or non-oxygenated perfusate
- pharmacological interventions

So far it is still unclear and controversially discussed which preservation techniques are most appropriate for which liver grafts.(17) But as there is a trend away from the one standard preservation method for all grafts towards more individualized solutions in organ preservation, Schlegel et al. issued recommendations for choosing a suitable preservation method based on the risk of the individual liver graft.(18)

Lai et al. mention that research in the field of machine perfusion in liver preservation has mainly been focused on demonstrating feasibility and safety of the chosen methods so far. Therefore they call for studies going beyond this by focusing on selection criteria and selection process for identifying transplantable ECD organs with the help of machine perfusion.(69) Moreover, in their review published in 2017, Marecki et al. mention that until then there was no proof of superiority of machine perfusion over simple cold storage in liver preservation.(32)

Nevertheless, machine perfusion might allow to mitigate detrimental effects of warm ischemia on DCD livers. By this means, among others, it may diminish typical posttransplant complications associated with ECD livers, like biliary strictures, delayed graft function and primary nonfunction of the organ. Thus, Marecki et al. deem machine perfusion as a method with potential to improve liver preservation.(32)

Marecki et al found that two ideas dominate the discussion of liver preservation: on the one hand there are supporters of HMP because of its maximum preservation effect and on the other hand there are supporters of NMP because this method is much closer to the physiological situation.(32) But there is also a growing number of studies concerning alternatives to hypothermic and normothermic machine preservation, namely rewarming machine preservation and subnormothermic machine preservation.(32)

3.4.2.1 Hypothermic Machine Perfusion in Liver Preservation

HMP joins characteristics of dynamic methods like removal of metabolites, oxygen transport, ATP recharging and controlled shear stress-mediated gene activation

with the safety of hypothermic conditions, which come along with a decreased cellular metabolism.(63) This reduced metabolic activity of the cells also reduces their demand for oxygen and nutrients.(13,19) At the same time, ATP synthesis is enabled by providing necessary substrates in the perfusate.(19,64) But the machine perfusion does not only provide substrates, it also enables to wash away metabolites, toxins and cytokines which are involved in damaging the organ. Thus, it might contribute to decreasing organ damage caused by preservation.(64) HMP enables a limitation of IRI according to Quillin et al.(9) But on the other hand hypothermia might also aggravate IRI by modifying structures and bonds of lipids and proteins.(13)

In the context of hypothermic preservation, it should be noted that injuries of the endothelial cells of the sinusoids are an issue that is boosted by hypothermic conditions. This is because of shear stress because hypothermia causes a constriction and increased rigidity of the liver sinusoids and the perfusate is more viscous in hypothermic conditions. Both aspects increase shear stress to the endothelium. Shear stress is probably further increased by the dysfunction of cellular sodium/potassium-pumps in hypothermic conditions which in turn causes a swelling of endothelial cells in the sinusoids and results in a further constriction of the sinusoidal lumen.(19)

In HMP research the optimum perfusion pressure is a topic because the homogeneity of the liver perfusion is influenced by perfusion pressure.(19) In a study on hypothermic oxygenated perfusion after warm ischemia in pig livers after warm ischemia it was shown that low pressure hypothermic perfusion has protective effects on the endothelium, while the application of higher pressure resulted in activation of Kupffer cells and harmed the endothelium. The study also indicates that oxygenation diminishes reperfusion injury, while Kupffer cells were activated and hepatocytes were damaged when oxygen was absent.(70)

It is also important to compare HMP and NMP in terms of their advantages and disadvantages. An advantage of HMP compared to NMP is that a failure of the perfusion device is not as fatal, because in this case the situation of SCS would be imitated under hypothermic conditions.(9) A further advantage of HMP is that it is simpler and less costly.(71) But on the other hand, an ex situ viability assessment like in NMP is not possible in HMP.(71)

In their review, Matsuno et al. deem hypothermic preservation in general inferior to ex vivo perfusion methods using subnormothermic or normothermic oxygenated preservation solutions regarding liver grafts donated after cardiac death. This is because liver grafts derived from DCD donors also go through severe tissue damage secondary to hypoxia and hypoperfusion before the initial period of warm ischemia. They state that cold storage then further harms the organ, since it could impair the capability to enhance cell function, as metabolic activity is reduced during cold storage. That's why they recommend continuous subnormothermic or normothermic ex vivo perfusion for livers donated after cardiac death.(12)

So far, the use of HMP in liver preservation has not been subject to many clinical trials.(63) But several different HMP protocols have already been investigated. These include end-ischemic HMP without oxygenation, end-ischemic oxygenated HMP and end-ischemic dual hypothermic oxygenated machine perfusion.

3.4.2.1.1 End-ischemic Hypothermic Machine Perfusion without Oxygenation

Guarrera et al. conducted the first clinical series on liver preservation using HMP after a period of SCS. Their results were published in 2010 and showed the safety of this method.(64) It included 20 patients receiving liver grafts preserved by HMP after SCS. This group was matched to 20 patients who received liver grafts only preserved by SCS.(64) The liver grafts were perfused via hepatic artery and portal vein with Vasosol® as preservation solution. An active oxygenation was not used.(64) The one-year patient and graft survival was the same in both groups. Furthermore, primary nonfunction of the transplanted liver was observed neither in the HMP group nor in the SCS group. But in the SCS group five cases of early allograft dysfunction occurred compared to only one case in the HMP group. Biliary complications occurred in 4 patients of the SCS group and in 2 patients of the HMP group.(64) Significant differences between the two groups were found in lower peak serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and total bilirubin as well as in the shorter mean hospital length of stay in the HMP group.(64) In Guarrera et al.'s opinion, their results hint at a decrease of preservation injury due to the use of HMP which might result in better posttransplant outcomes.(64)

Guarrera et al. also investigated the effect of hypothermic machine preservation on 31 ECD livers. For comparison a group of simple cold storage preserved ECD

livers was matched to these livers. As in their clinical trial mentioned above, the organs were perfused via portal vein and hepatic artery after arrival. Failures of the perfusion system did not appear.(51) There were non-significantly less cases of early allograft dysfunction and primary non-function in the HMP group. 1-year graft and patient survival were only slightly higher.(51) Further clinical trials with higher power are necessary for identifying possible significant differences.(51) Serum levels of creatinine, total bilirubin, aspartate aminotransferase and ALT were lower in the HMP group during the first days posttransplant, but the only significant differences were a lower ALT level on the first day and a lower serum creatinine on the fifth day.(51) Further significant differences were found in the lower rate of biliary complications and the shorter hospital stay in patients who had received a liver preserved by HMP.(51) Regarding viability assessment, according to Guarrera et al., there might be a hint that high values of effluent aspartate aminotransferase and an increased portal pressure might indicate that the liver is threatened by serious reperfusion injury. But, of course, further research on this is necessary.(51) In their conclusion, Guarrera et al. stress that hypothermic machine preservation is a promising way for preserving ECD livers and might also increase the organ utilization.(51)

3.4.2.1.2 End-ischemic Oxygenated Hypothermic Machine Perfusion

In end-ischemic HMP, the organ is preserved by SCS during transport. Only after arrival in the recipient hospital it is perfused. This simplifies the logistics of organ transport compared to a continuous machine perfusion, because the perfusion device does not have to be transported from the place of organ retrieval to the place of transplantation.(72) Thus, single hypothermic oxygenated perfusion via the portal vein is characterised by a certain simplicity which also makes the application of this technique comparatively cheap.(73)

In 2014 Dutkowski et al. published the results of the first trial of preserving DCD livers (Maastricht III) by hypothermic oxygenated machine perfusion after an initial phase of SCS. The perfusion system was connected to the portal vein. As perfusate they used University of Wisconsin gluconate solution.(74) Eight livers of DCD donors were preserved by hypothermic oxygenated machine perfusion and afterwards transplanted. The transplant recipients were matched to recipients of DBD liver grafts preserved by SCS only.(74) In both groups no primary non-function and no delayed graft function were observed. Moreover, a special aspect,

according to Dutkowski et al., is that contrary to previous experience in DCD livers no intrahepatic cholangiopathy was found in the DCD group.(74) The duration of ICU- and hospital-stay was similar in both groups, whereas the hospital stay caused significantly lower costs in the DCD group.(74) Dutkowski et al. conclude that the use of hypothermic oxygenated machine perfusion in clinical practice is possible and safe. They also stress the good outcomes and the small extent of reperfusion injury observed in the DCD group, which might be due to protective effects of hypothermic oxygenated machine perfusion.(74)

In 2015 further results were published by Dutkowski et al. 25 DCD livers (Maastricht III) had been transplanted after hypothermic oxygenated machine perfusion preservation and, for comparison, matched to 50 DCD livers preserved by SCS.(72) The preservation process was carried out as in Dutkowski et al.'s trial mentioned above.(72,74) Length of intensive care unit and hospital stay were similar. In primary non-function there was no significant difference. But the rate of early allograft dysfunction was significantly lower in the hypothermic oxygenated perfusion group, as was the rate of biliary complications. Peak serum levels of aspartate aminotransferase and ALT were also significantly lower.(72) The 1 year graft survival was significantly higher than in the SCS group.(72) In Dutkowski et al.'s opinion, hypothermic oxygenated perfusion might enable an expansion of the donor pool because it results in an improved function of DCD livers by reducing reperfusion injury.(72)

Schlegel et al. showed that hypothermic oxygenated machine perfusion applied as end-ischemic perfusion is a promising preservation technique for DCD livers.(75) The hypothermic oxygenated machine perfusion was carried out via the portal vein.(75) They compared the outcomes of 50 DCD liver grafts preserved by hypothermic oxygenated machine perfusion to the outcomes of 50 matched DBD livers and to the outcomes of 50 DCD livers that had only been preserved by simple cold storage prior to transplantation.(75) In the five-year follow-up the graft survival, excluding tumour-related graft loss, was significantly higher in the HMP group compared to the simple cold storage group (94% to 78%), despite a significantly higher donor-recipient risk.(75) While seven SCS liver grafts were lost due to primary non-function and ischaemic cholangiopathy, it were zero in the hypothermic oxygenated machine perfusion group. A difference that reached significance.(75) Moreover, the similarity of outcomes between hypothermic

oxygenated perfusion of DCD livers and standard low-risk DBD livers, showed the effectiveness of the investigated approach.(75) Thus, they regard hypothermic oxygenated machine perfusion after SCS as suitable method for preserving high risk liver grafts.(75) According to Schlegel et al., these results are a further proof of effectivity of this method and underline its potential role for enlarging the donor pool by an improved utilization of DCD livers.(75)

Ravaioli et al. published an abstract including some recent results of a clinical trial concerning hypothermic oxygenated machine perfusion of ten livers and kidneys. The organs were derived from ECDs and the results were compared to results of matched organs preserved by SCS.(76) In livers preserved by hypothermic oxygenated machine perfusion, both, the early allograft dysfunction and the peak aspartate aminotransferase during the first seven days after transplantation were significantly lower. In Ravaioli et al.'s opinion, these results show the capability of hypothermic oxygenated machine perfusion to diminish organ damages and injuries caused by ischemia during preservation.(76)

Currently, a phase II study concerning hypothermic oxygenated machine perfusion on human liver grafts is carried out at the University of Zurich (NCT01317342). The planned completion date was in April 2019, but since 2016 there has not been any update regarding this trial on clinicaltrials.gov.(74,77)

3.4.2.1.3 End-ischemic Dual Hypothermic Oxygenated Machine Perfusion

A third method of HMP is the so-called end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) which van Rijn et al. applied for preserving ten DCD livers (Maastricht III) prior to transplantation.(71) This method is based on both, active oxygenation of the perfusate and perfusion via hepatic artery and portal vein.(71) Westerkamp et al. have already demonstrated that end-ischemic oxygenated HMP via portal vein and hepatic artery is able to restore the energy resources of the preserved liver.(78) Furthermore, the idea behind this method, according to Westerkamp et al., is that arterial blood supply is crucial for providing bile ducts enough nutrients and oxygen. Thus, a dual perfusion via portal vein and hepatic artery might have a protective effect on the epithelium of the bile ducts and might improve its preservation compared to perfusion only via portal vein.(78) That's why van Rijn et al. believe it might be necessary to use such a dual perfusion for protection of the bile ducts during preservation in DCD livers

particularly.(71) But the cannulation and perfusion of the hepatic artery also carries an increased risk for the development of a hepatic artery thrombosis caused by mechanical injuries of the hepatic artery.(71) In this clinical trial no case of hepatic artery thrombosis was observed in the DHOPE-group.(71) In van Rijn et al.'s clinical trial, the livers were preserved by SCS during transport. In the transplant centre, the liver has been perfused by DHOPE for a minimum of 2 hours prior to transplantation.(71) For comparing the outcomes, the recipients of the ten DHOPE-preserved livers were matched to 20 recipients of DCD liver grafts exclusively preserved by SCS.(71) In the DHOPE group both, the 1-year graft and 1-year patient survival rate were 100%. These rates were lower in the SCS group, having a 1-year graft survival rate of 67% and a 1-year patient survival rate of 85%.(71) Moreover, during the first seven days posttransplant, in the DHOPE-group significantly lower peak serum levels of ALT were observed.(71) On the seventh day posttransplant also the serum bilirubin concentration was significantly lower in the DHOPE-group.(71) Furthermore, there were lower median serum levels of bilirubin, alkaline phosphatase, ALT and γ-glutamyl transferase in the DHOPE-group 30 days posttransplant.(71) In addition, the study showed a smaller proportion of ischemic cholangiopathy, especially of non-anastomotic biliary strictures, in the DHOPE-group, but the small number of patients necessitates a verification of these results by a randomized controlled trial involving a higher number of patients.(71) Such a trial is currently being carried out (NCT02584283) (71), and the study completion date was in January 2020 according to ClinicalTrials.gov.(79)

Additionally, van Rijn et al. investigated bile duct biopsies of the DCD liver grafts. These biopsies were conducted at the end of SCS and after graft reperfusion in the recipient.(80) The biopsies obtained at the end of SCS didn't show significant differences between the liver grafts of the DHOPE-group and the SCS group, but all of them have already shown substantial bile duct injury in histology.(80) But after reperfusion the severity of histological bile duct injuries aggravated in the SCS group whereas it didn't in the DHOPE-group.(80) According to van Rijn et al.'s results IRI of the bile ducts was less in the DHOPE-group compared to the SCS group.(80) This was not achieved by curing previously existing injuries, but by preventing further damage to the bile ducts after reperfusion, since this is what DHOPE aims at.(80) For example, the peribiliary glands were preserved in a

superior way in the DHOPE group.(80) This is important, since the development of posttransplant biliary strictures is associated with pretransplant damage of the peribiliary glands.(62) The extrahepatic and large intrahepatic bile ducts are the location of these peribiliary glands.(81,82) It has previously been assumed and shown that the peribiliary glands contain stem and progenitor cells like “biliary tree stem/progenitor cells”.(82,83) Damage to the peribiliary glands during preservation might result in loosing biliary progenitor cells located in the peribiliary glands.(62) This loss again might restrict the bile ducts’ capability of regeneration after injury and might thereby contribute to the development of non-anastomotic biliary strictures.(62,84) Van Rijn et al. hypothesize that an improvement of the bile ducts’ capability of regeneration and thereby a lower incidence of non-anastomotic strictures might be a result of the protective effect of DHOPE on the peribiliary glands.(80)

Also Patrono et al. used DHPOE for preserving livers. They published the results of their first 4 cases of liver transplantation after hypothermic oxygenated machine perfusion. The DBD liver grafts were perfused for 150 to 200 minutes via hepatic artery and portal vein using a modified University of Wisconsin solution after SCS.(85) Since it was only a series of a small number of cases without any control group, it can only be mentioned that the technique was safe, simple and applicable. Therefore, Patrono et al. call for further studies on the outcomes of hypothermic oxygenated machine perfusion for evaluating its benefits and finding a suitable range of application.(85) Also comparisons to NMP would be interesting, since hypothermic oxygenated machine perfusion in contrary to NMP does not allow for viability assessment of the graft function during preservation in a way NMP does.(85)

While van Rijn et al. consider this dual oxygenated perfusion via portal vein and hepatic artery as beneficial(71), Kim et al. regard it as sufficient to use either a dual vessel perfusion via portal vein and artery or a combination of a single vessel perfusion via the portal vein and external oxygenation of the perfusate as the cellular oxygen consumption is decreased in hypothermia.(86) Similarly, Dutkowski et al. are of the opinion that perfusion of the portal vein only is adequate in hypothermic oxygenated perfusion.(72) Furthermore, it is known that not only the hepatic artery is of importance for the blood flow and oxygen supply of the common bile duct, but especially also the portal vein.(87) According to Schlegel et

al, the portal vein enables a sufficient oxygen supply for the whole perfused liver graft and thereby also for the extrahepatic biliary tree.(73,88) Thus, Schlegel et al. are not convinced that there would be an increased availability of oxygen to the biliary tract by perfusing both, portal vein and hepatic artery. They don't see an extra benefit in doing so.(73) Nevertheless, Brüggenwirth et al. stress that, in their opinion, additional perfusion via the hepatic artery is important since it enables a more physiologic flow. They are worried whether a perfusion via portal vein only can preserve the peribiliary vascular plexus(89), which is responsible for the arterial blood supply to the biliary tract(82,90,91), and thereby the whole biliary tract in the best way.(89) The importance of preserving the peribiliary vascular plexus well was shown by op den Dries et al.(62,89) Otherwise these structures may get harmed and this might cause posttransplant biliary strictures.(62,90) However, Schlegel et al. and Brüggenwirth et al. share the view that randomized clinical trials comparing the different approaches of liver preservation are necessary.(73,89)

3.4.2.2 Midthermic Machine Perfusion in Liver Preservation

Ishikawa et al. mention that organs can be preserved in hypothermic conditions only for short time, since otherwise, when the preservation time is prolonged, the cellular metabolism decompensates.(92) This is illustrated by the following results: While the cellular ATP level determined by ATP consumption and formation was balanced at storage temperatures of 22˚C, 33˚C and 37˚C, the energy metabolism of the cell was characterized by ATP consumption outweighing ATP formation at 4˚C and 10˚C.(92) Ishikawa et al. advocate an oxygenated perfusion at about 22˚C to put the liver into a "static low activated state", which enables a basal cellular metabolism and a restoration of ATP formation which as a result facilitates an ATP level at steady state.(92) Such a preservation at 22˚C could allow cells to survive without proliferation according to Ishikawa et al.'s study on a perfusion system tested in preservation of rat livers.(92)

3.4.2.3 Subnormothermic Machine Perfusion in Liver Preservation

Bruinsma et al. used subnormothermic machine perfusion (SMP) after SCS for preserving 22 previously discarded human liver grafts.(93) It should be noted that according to Karangwa et al.'s nomenclature the selected perfusion temperature of 21°C should actually be described as midthermic machine perfusion. Bruinsma et

al. however called it SMP.(10,93) The reduced metabolism at 21°C doesn't require an oxygen carrier in the perfusate because actively oxygenating the perfusate is sufficient for meeting the liver's oxygen demand. Nonetheless, the remaining metabolism necessitates a nutrient-rich perfusate.(93) SMP enabled a preservation with only very little injury to the preserved livers according to ALT-levels and histological findings.(93) The preserved livers were not transplanted. Hence, clinical outcomes were not assessed.(93)

Moreover, in an experimental model comparing the metabolomic profiles of steatotic human livers preserved by either subnormothermic (20-22°C) or normothermic machine perfusion, differences between these preservation methods could be detected. While a significant depletion of available glutathione was found in the SMP group, a superior ATP restoration was observable at the same time.(94) Hence, preservation protocols combining both methods in terms of "sequential temperature variation, guided by evidence-based metabolite replacement" may have even more beneficial effects on steatotic liver grafts.(94)

3.4.2.4 Normothermic Machine Perfusion in Liver Preservation

Imitating physiological conditions by maintaining a physiological temperature and by providing sufficient nutrients and oxygen for meeting the high demand at body temperature is the idea behind NMP. This should enable a balanced metabolism of the cells close to the physiological one.(12,15,63,95) This enables liver cells to maintain an aerobic metabolism.(96) Thus, the reduction of the so called „cellular energy charge" can be avoided which is important for enhancing graft quality. In general, that mechanism is the opposite of simple cold storage as simple cold storage considerably diminishes the metabolic processes of the cell by reducing the temperature. Simple cold storage therefore is not about avoiding, but about delaying the reduction of the „cellular energy charge". As a result the preservation period should be as short as possible in simple cold storage while it could be prolonged in NMP.(15)

Jassem et al. demonstrated, that the benefits of NMP of liver grafts are not limited to maintaining metabolism, but NMP also contributes to an inhibition of inflammatory processes and decreases cell death.(96) Thus, IRI is diminished.(96) Jassem et al. suppose that the down-regulation of several "pathways such as allograft rejection, graft versus host disease, and platelet/coagulation" potentially has a part in the reduction of IRI.(96) They also found differences in gene activity

between simple cold storage and NMP: while there was more immune-related gene activity in livers preserved by cold storage, livers preserved by NMP showed more gene activity in areas of growth, metabolism and tissue repair mechanisms.(96) To sum it up, these beneficial effects of NMP might help expanding the donor pool to DCD livers and other marginal livers since their posttransplant outcomes could be improved.(55,96) At the same time, according to Ceresa et al. it remains unclear, whether NMP does only have advantageous effects on liver grafts from high-risk donors or also on liver grafts from low-risk donors.(55) But in general, Ceresa et al. would already consider NMP a success, if it enabled an improved organ utilization, because this could decrease the number of patients dying while waiting on a suitable liver graft.(16) According to Ceresa et al. nomothermic machine perfusion could improve the utilization of donor livers not only by enabling an ex vivo viability assessment and a superior preservation, as mentioned above, but also by longer maximum preservation times. Such longer preservation times would provide a larger time frame for optimizing logistical processes linked to organ distribution and transplantation.(16)

In general, an important aspect in NMP is the question which perfusate is best for preservation. Currently, perfusing livers with whole blood has become established in NMP according to di Francesco et al.(95) Selzner et al. see that critically. They are of the opinion that the use of fresh whole human blood is not favourable to organ perfusion because of its limited availability and since it contains mediators of reperfusion injury.(97) In addition to that, Matsuno et al. mention that there could be a higher risk of microvascular failure, sinusoidal plugging and bacterial growth when perfusates based on blood are applied.(12) Nevertheless, during normothermic preservation, it is necessary to provide the cells a sufficient oxygen supply for their metabolism. Blood is able to transport enough oxygen and was predominantly used for normothermic and subnormothermic perfusion according to Kim et al.(86)

While in HMP it is being discussed whether a combined perfusion via portal vein and hepatic artery is beneficial(71,72,86), in NMP the organ has to be perfused via both, portal vein and hepatic artery.(63)

A further important aspect is that adding pharmaceuticals like antibiotics or antithrombotics to the perfusate during NMP supports the reconditioning of the graft, if required, and has already found its way into liver preservation. For

determining whether an organ needs pharmaceutical interventions and whether an organ is suitable for transplantation, assessment of liver function is an opportunity coming along with NMP.(95) Although liver function can be well observed during normothermic liver perfusion, viability assessment of the liver remains difficult in case of a diminished liver function.(98)

NMP also has drawbacks. One disadvantage might be the high demand of ATP because the metabolism is not slowed down. More anaerobic glycolysis might be a result of this.(17) A further downside is that the application of NMP comes along with a risk for bacterial contamination according to Vekemans et al.(19)

Another drawback are the consequences of a failure of the perfusion system during transport. If an organ is transported under normothermic conditions and the perfusion machine fails during transport, the organ might be lost and can't be transplanted.(9) This is because warm ischemia occurs in case of technical errors of the perfusion system and when the perfusion system is operated incorrectly. Warm ischemia results in considerably more severe damages to the preserved organ compared to the effects caused by failures in HMP.(12,15,63,97) That's why the failure-free operation is a key precondition for safe application of NMP as preservation method.(15) Additionally, this underlines that the intricacy of logistics in NMP is higher compared to HMP.(19) A potential solution of this problem could be end-ischemic NMP as the logistics are less complicated when the liver is stored under hypothermic conditions during transport and NMP is started after arrival in the transplant centre.(55)

Moreover, normothermic liver preservation also comes along with a higher complexity and high costs as the organ must be metabolically completely maintained.(12,15)

For gleaning which effects NMP has on survival of grafts and patients and whether a decrease of biliary complications is possible, clinical trials involving more patients and including a long-term follow-up are necessary according to Ceresa et al.(16) Currently there are several clinical trials going on regarding NMP.(16) An aspect to consider in this context is that selection bias can occur in prospective randomized controlled clinical trials on machine perfusion, because it's complicated to standardise the process of accepting or discarding liver grafts.(63)

Furthermore, Nasralla et al. suggest an examination of the economic aspects of NMP as well because economic aspects are also important for the acceptance of

a preservation technique. They suppose possible economic benefits not only in a better early graft function and logistic aspects, but also in earlier transplantation due to an improved organ utilization, as this is associated with lower costs.(52)
Several different NMP protocols have already been investigated. These include: end-ischemic NMP, continuous NMP and ischemia-free NMP.

3.4.2.4.1 End-ischemic Normothermic Machine Perfusion

An approach that has been applied in studies several times is the combination of simple cold storage and NMP. In this context it is important to mention that cooling down the liver graft results in injuries and it has not been clarified yet whether normothermic perfusion after cold storage can reduce these injuries and enable outcomes comparable to outcomes using continuous NMP for liver preservation.(55)
Advantages of cold storage combined with NMP are lower costs and less complicated logistics compared to continuous NMP.(55) On the one hand this is, because the device used for machine perfusion does not have to be transported, because the organ is preserved by SCS during the transport. On the other hand this is, because the initiation of NMP in the donor hospital might take more time than SCS and might therefore prolong the procedure of organ retrieval.(55) This assumption is supported by the observation of a prolongation of the organ retrieval time by a minimum of 2 hours, when NMP was started at the donor hospital.(97) To sum it up, the advantages regarding logistics and costs might favour the adoption of this preservation method in clinical practice.(55)
Perera et al. were first to transplant a DCD liver after prolonged warm ischemia time. The liver was stored by SCS before it was perfused and resuscitated by NMP. They had chosen a red cell-based fluid as perfusate.(99) The patient recovered well after liver transplantation.(99) A main reason for using NMP was the opportunity to evaluate the organ function prior to transplantation. According to Perera et al. this opportunity is the key advantage of NMP over HMP since they regard NMP as the only preservation technique which allows for assessment of graft viability.(99) Perera et al. evaluated the liver function by lactate level and bile production during NMP.(99) In their opinion this case showed that viability assessment during NMP can enable a successful transplantation of a primarily declined liver.(99)

Mergental et al. transplanted five livers which had previously been rejected by all transplant units in the UK. These high risk organs were transplanted to low risk recipients. All livers were preserved by both, SCS in the beginning and NMP afterwards. Four of the five livers were derived from DCD donors. Before transplantation a viability assessment was performed during NMP using markers like bile production, perfusate lactate level, perfusate pH, homogenous graft perfusion, arterial flow and portal venous flow.(53) Mergental et al. did not observe any case of delayed graft function.(53) Of course, the power of the study was limited by the small number of transplanted livers, but in Mergental et al.s' opinion it showed the feasibility of using NMP for preserving high risk livers. Applying this technique might thus increase the utilization of high risk liver grafts.(53)

In 2017 a study of Watson et al. was published. They transplanted 12 previously declined livers that had been preserved by both, cold storage and NMP. 9 of these livers were derived from DCD donors and 3 from DBD donors.(45) Markers for viability assessment were the capability of the liver to maintain pH and also changes in glucose, transaminase concentrations, and lactate.(45) The first 6 livers were perfused using high oxygen tension. Since five of the 6 recipients developed a hemodynamic instability after reperfusion which was potentially related to the hyperoxic circumstances during NMP. Thus, low instead of high oxygen tension was used for preserving the following 6 livers. No hemodynamic instability was observed in this group, but it remains unclear whether that was because of the different oxygen tensions.(45) Cholangiopathy was found in three recipients of DCD livers. Moreover, in one patient the development of a primary nonfunction of the liver was observed.(45) Based on the results, Watson et al. concluded that viability assessment during NMP is possible. Furthermore, for prevention of postreperfusion syndrome and vasoplegia it might be important to avoid high oxygen tension during machine perfusion.(45)

An experiment on NMP by Vogel et al. involving 13 human livers was published in 2017. The quality of these organs was not high enough for being accepted at a UK liver transplant centre. The livers were preserved by NMP for 24 hours. The used NMP system was a prototype with automatic oxygenation, temperature-, flow- and pressure-regulation. For perfusion Vogel et al used a packed red blood cell- based liquid combined with a crystalloid solution preheated to 37˚C.(15) With their study, Vogel et al. demonstrated that a 24-hour-lasting NMP of liver grafts of minor

quality is possible in general. A main advantage of this method is the possibility of monitoring functional parameters aiming at evaluating the viability of the liver graft.(15) Furthermore, this method might enable an extended tolerable preservation time for liver grafts of minor quality and might also permit therapeutic applications for enhancing the quality of the organ.(15,52) Vogel et al are of the opinion that further research on NMP is necessary, even if using this method in clinical practice is already possible.(15)

Ghinolfi et al. carried out a randomized, prospective pilot trial comparing the outcomes of 10 liver transplantations after cold storage followed by ex situ NMP prior to transplantation to 10 liver transplantations after cold storage only. Their results were published in 2019.(100) For cold storage at 4˚C, Celsior solution was used, while a perfusate based on blood was used for normothermic perfusion.(100) The liver grafts were derived from DBD donors aged 70 years or older.(100) Primary endpoints of the trial were patient survival and graft survival at the 6th month after transplantation. Due to the small number of liver grafts, the statistical power of the trial was limited. That could be the reason why significant differences regarding the primary endpoints couldn't be found. As a consequence, advantages of NMP on the clinical outcome could not be demonstrated by this trial.(100) Ghinolfi et al. did not find substantial advantages of NMP on liver histology, but mitochondrial swelling was significantly diminished.(100) Moreover, a strong activation of autophagy in biopsies of NMP livers was detected. Ghinolfi et al. suppose that NMP might reduce cellular injuries acquired during cold storage by inducing cellular repair mechanisms.(100) Thereby IRI might be diminished.(100) Interesting regarding autophagy is that age-related changes of autophagy in liver cells of older donors aggravate the liver cells' susceptibility to IRI according to Wang et al.(101) It is therefore presumed that the stronger activity of autophagy during NMP might contribute to diminishing IRI and increasing the quality of liver grafts of older donors.(100,101) Nonetheless, it should be noted, that autophagy during ischemia reperfusion does not only have beneficial effects on liver cells and study results are conflicting according to Cursio et al.'s review. Thus, more information on this topic is needed.(102)

Ceresa et al.'s randomized trial involving 30 transplanted livers focused on evaluating safety and feasibility of NMP after SCS. The pool of transplanted organs consisted not only of DBD, but also of DCD livers. For liver preservation

NMP was applied for at least 4 hours after SCS at 4˚C.(55) The results were compared to a sample of livers that had been transplanted after continuous NMP in the context of another study.(55) 30-day graft survival, the primary outcome of the trial, was 94%. This underlines that the chosen preservation method of NMP after simple cold storage is safe and feasible.(55) A 12-month graft survival rate of 84% and a 12-month patient survival rate of 90% were observed.(55) While there were no significant differences between both groups regarding clinical outcomes, some significant deviations were observed in regard to perfusion parameters. The end-ischemic NMP group showed a significantly lower lactate concentration in the perfusate at the beginning of preservation and it showed significantly higher hepatic arterial flow rates.(55) Nevertheless, Ceresa et al. regarded the small number of transplanted livers as a limitation of their study and claim more powered studies for evaluating this preservation technique.(55)

Mergental et al. transplanted several previously discarded DBD and DCD livers after NMP. NMP over 4 to 24 hours was applied after SCS.(103) Of 31 included liver grafts 22 could be transplanted after meeting the viability criteria which were as follows: (103)

- the liver had to metabolise perfusate lactate to levels ≤ 2.5 mmol/L within 4 h after the start of NMP
- additionally, at least 2 of the following criteria had to be met:
    - evidence of bile production
    - maintenance of perfusate pH ≥ 7.30
    - metabolism of glucose
    - maintenance of stable arterial and portal flows (≥150 and ≥500 mL/min, respectively)
    - homogenous perfusion with soft consistency of the parenchyma.

Posttransplant 90-day graft and patient survival was 100%. According to Mergental et al.'s conclusion a higher proportion of high-risk livers will be transplanted if viability assessment during NMP is used.(103)

A recent innovation in NMP was shown by di Francesco et al. This innovation aims at diminishing IRI. Di Francesco et al. published a case report of a patient who received a liver preserved by end-ischemic NMP. The liver was not flushed with cold solution after NMP immediately before transplantation. This cold flush is usually done for washing out several substances like waste products and

potassium.(95) According to them the cold flush immediately before transplantation is the commonly performed approach and they claim to have been the first team to omit this step. They omitted it because they assume that this cold flush leads to a hypothermic shock having deleterious effects on the liver in the sense of contributing to IRI.(95) According to di Francesco et al. the cold flush might be obsolete for several reasons. First, the sudden cooling harms the tissue. Second, the potassium concentration was not elevated, but already under control during graft reconditioning and third, the blood used as perfusate during reconditioning is laced with anticoagulants which can avert thromboembolic events.(104)

3.4.2.4.2 Continuous Normothermic Machine Perfusion

Another approach in NMP is continuous normothermic perfusion during the whole preservation. In Selzner et al.'s study 12 livers were perfused by normothermic ex vivo liver perfusion. Of them 2 were excluded, since they were not transplanted in the end because of insufficient quality. The remaining 10 graft recipients were matched with 30 recipients who had received livers which had been preserved by cold storage.(97) Selzner et al. showed the safety of normothermic ex vivo liver perfusion regarding technical aspects.(97) Steen solution was used but it was still unclear which solution suited best for the use in portable normothermic ex vivo liver perfusion devices.(97) It has to be stressed that Selzner et al.'s study was not about the reduction of preservation injury in marginal grafts. They only selected livers which they had also chosen for cold storage as preservation method.(97) Normothermic ex vivo liver perfusion provided more time for evaluating appearance and function of the livers what they considered as advantageous.(97) Furthermore Selzner et al. reported a reduction of time pressure to finish the recipient hepatectomy fast and a reduced instability upon reperfusion subjectively perceived by the transplant teams when normothermic ex vivo liver perfusion was used.(97) Nevertheless, Selzner et al. emphasise that the organ retrieval process took about 2 hours longer and costs for normothermic ex vivo liver perfusion were higher compared to cold storage. This in fact doesn't include a statement about cost effectiveness of NMP, since this was not assessed in their study.(97)

Ravikumar et al. conducted a phase 1 clinical trial for transplantation of 20 human liver grafts preserved by NMP. NMP has already been started at the donor hospital and has been continued during transport. Their results were published in 2016 and

showed a similar 30-day graft survival compared to the matched simple cold storage cases. All 20 grafts in the NMP group survived the first 30 posttransplantational days. A significant difference between the two groups was found in the lower median peak AST-level during the first seven days in the NMP group.(11) The use of NMP in a clinical setting is feasible and safe according to Ravikumar et al. and that's what they wanted to demonstrate. However, in this clinical trial they didn't investigate the patient survival.(11) Furthermore, Ravikumar et al. suggest a noninferiority trial. Such a trial should compare the results of preserving marginal livers by NMP to the results of preserving standard criteria donor livers by simple cold storage. Satisfying results of such a trial would allow to enlarge the donor pool by using NMP for preserving marginal livers.(11)

Bral et al. conducted a clinical trial on normothermic ex vivo liver perfusion. They preserved ten liver grafts, of which four were derived from DCD donors. Nine of these ten liver grafts were transplanted in the end.(105) They report non-significantly higher peak AST-levels in the NMP group compared to the SCS group during the first seven posttransplant days.(105) Furthermore, there were no statistical differences between the two groups regarding 30-day graft survival.(105) But the patients of the NMP group had to stay significantly longer in intensive care unit and hospital, which caused high costs. That's why Bral et al. favour a randomized controlled trial for investigating benefits and costs of NMP in clinical practice, because lower costs could be a crucial point for the adoption of this technique in clinical practice.(105)

Regarding the previously mentioned trials of Ravikumar et al.(11), Bral et al.(105) and Selzner et al.(97) it has to be added that Ceresa et al. stress that the studies didn't have enough power to show differences in outcome and that they were not randomized.(16)

Nasralla et al. were first to publish the results of a randomized trial of using NMP in liver preservation. 220 liver transplantations were carried out using either NMP or SCS.(52) Nasralla et al.'s trial also underlines that NMP is safe and feasible in clinical practice, albeit the organ retrieval process might have to be adapted to the use of this preservation method.(52) In the NMP group Nasralla et al. found significantly lower peak serum-AST-levels, which had been defined as primary outcome of the trial. Early allograft dysfunction was also significantly decreased in this group.(52) In contrast to Bral et al., Nasralla et al. did not find any differences

in length of intensive care unit- and hospital-stay.(52,105) Furthermore, Nasralla et al. report prolonged preservation times in NMP which according to them didn't seem to affect the outcomes negatively. This might facilitate logistical changes like mainly transplanting during daytime and thereby enhancing outcomes and cut costs as well as optimizing the organ utilization.(52) Moreover, Nasralla et al. observed a more than 50% lower discard of liver grafts in the NMP group. Hence, it was possible to transplant 20% more livers in this group compared to the SCS group. But they believe, that using NMP could enhance the organ utilization even further, if it enabled to transplant liver grafts which are currently considered untransplantable.(52) Either way, a higher number of transplantable livers would help to reduce the waitlist mortality.(52) Nasralla et al.'s opinion is that a trial including considerably more patients is necessary for determining, whether there are differences between the two groups in graft or patient survival, because in their trial no differences in these measures were observed.(52)

3.4.2.4.3 Ischemia-free Normothermic Machine Perfusion

Zhao et al. assume that avoiding ischemia during liver procurement by using an ischaemia-free approach might be beneficial for the outcome of liver transplantation.(106) Such an ischemia-free approach for liver transplantation using NMP was first described by He et al. in a study published in 2018. Their intention was not to interrupt the blood supply of the liver aiming at preventing ischemia, because ischemia occurring during the transplantation process causes IRI.(34) Thus, NMP was applied continuously during procurement, preservation and implantation of the liver.(34) He et al. are convinced that it is more beneficial to circumvent IRI than to treat it. That's why they are convinced that establishing an ischemia free approach would be an important innovation in organ transplantation.(34) This is underlined by van Leeuwen et al. They consider He et al.'s publication as "a milestone in the history of organ transplantation".(107) Especially ECD livers could profit from an ischemia-free approach, which could contribute to an expansion of the donor pool.(107) He et al.'s hypothesis is supported by the fact that inflammatory cytokine levels were considerably lower in the ischemia-free approach than in the conventional approach.(34) Nevertheless, van Leeuwen et al. suggest a simplification of the surgical procedure for fostering the implementation of the ischemia-free approach in other transplant centres.(107) A liver with macrovesicular steatosis to an extent of 85-95% derived from a brain

death donor was transplanted by He et al.(34) Preservation-related and posttransplant damage to vascular endothelium, biliary endothelium and hepatocytes was minimized according to histological findings and functional tests. Furthermore, neither biliary nor vascular complications were observed and the liver was not rejected.(34) Of course, this was only a single case and therefore there is a limitation regarding the evaluation of the outcome, which necessitates studies with larger numbers of transplanted livers preserved by using the ischemia-free approach.(34) It has to be added that the used perfusion device is a limitation of the ischemia-free approach because it is not suitable for transportation between different hospitals. Therefore, this specific approach is not yet applicable to a setting including the necessity of transporting the grafts from one hospital to another, which is very common in organ transplantation.(107) He et al.'s approach relies on a donor iliac vein graft that is anastomosed end-to-side to the donor portal vein for being able to cannulate and perfuse the portal venous system without an interruption of the blood flow.(34,108) This end-to-side anastomosis might increase the risk for development of a thrombosis.(108) For superseding this end-to-side anastomosis, van Leeuwen et al. developed an approach based on a surgically reopened umbilical vein for cannulating and perfusing the portal venous system. In a trial on human livers they demonstrated the feasibility of their approach of a continuous oxygenated portal venous machine perfusion via the umbilical vein.(108)

3.4.2.5 Rewarming Machine Perfusion in Liver Preservation

Marecki et al. consider rewarming machine preservation to be a compromise combining several positive characteristics of other methods. In the end it might combine a relatively long maximum duration of storage, a comparatively convenient handling and the possibility to predict outcomes reliably before transplanting the liver.(32) The commonly applied prompt rewarming of the liver during reperfusion by blood immediately after SCS is problematic in Minor er al.'s opinion. This is because the cellular metabolism during cold ischemia considerably differs from the normal state in vivo and this rapid change of conditions stresses the cells severely.(109) By slowly raising up the perfusate's temperature a gradual activation of the cellular metabolism can be realized.(109) This contributes to an energetic re-equibrilation of the cells before transplantation because the

mitochondria can be restored gradually at a limited workload. That seems to result in superior liver function after transplantation.(47,109)

Based on this Hoyer et al. investigated the safety and feasibility of controlled oxygenated rewarming prior to transplantation in a clinical series of 6 patients.(47) The transplanted livers were preserved by SCS using University of Wisconsin or Custodiol solution and then transported to the transplantation centre. After arrival they underwent a macroscopic inspection and were connected to the perfusion system. This device was used for controlled oxygenated rewarming of the livers with Custodiol-N lasting for 90 minutes. The hepatic artery was perfused with pulsatile perfusion pressure whereas the portal vein was perfused with a continuous perfusion pressure. The perfusion started at a temperature of 10˚C. After 30 minutes the temperature was raised to 12˚C, after 45 minutes to 16˚C and after 60 minutes to 20˚C, which was the maximum temperature of the perfusate in this trial. The livers were transplanted after 90 minutes of rewarming perfusion.(47) The outcomes of these 6 patients were compared to the outcomes of a historical control cohort of patients with similar profiles. The controlled oxygenated rewarming group showed not only a survival rate of 100% after 6 months, but also no case of early allograft dysfunction. In the control group 36% of patients suffered from early allograft dysfunction. Furthermore, the posttransplant levels of aspartate aminotransferase were surprisingly low what might indicate low hepatocellular damage.(47) For investigating the outcomes and benefits of controlled oxygenated rewarming compared to other preservation methods, randomized controlled trials are necessary.(47)

3.4.2.6 Combined Hypothermic and Normothermic Machine Perfusion

Boteon et al. investigated the effects of a combination of hypothermic oxygenated perfusion and normothermic perfusion on the functional parameters of ECD livers. Their proof of concept study was able to show the feasibility of this approach.(110) The liver grafts were not transplanted after preservation, since in the UK, this approach has not yet been an accepted transplant protocol.(110) The approach is based on the assumption that hypothermic oxygenated perfusion may restore the metabolism of the liver graft before NMP is applied, which then enables an ex vivo viability assessment.(110) Additionally, NMP may be capable to stop the loss of quality of the liver graft during perfusion.(110) In contrast, according to Boteon et al., hypothermic oxygenated machine perfusion is evidently able to protect the

quality of liver grafts during preservation and not only to stop the loss of quality.(110) In their opinion, this aspect seems to be an advantage of HMP, while NMP seems to be appropriate for ex vivo viability assessment.(98,110) Thus, they investigated the potential benefit of a preservation protocol of 2 hours of hypothermic oxygenated perfusion followed by 4 hours of normothermic perfusion in comparison to a protocol of NMP lasting for 6 hours.(110) The machine perfusion was carried out after a period of SCS.(110) The livers were perfused via portal vein only in case of hypothermic oxygenated perfusion and via hepatic artery and portal vein in normothermic perfusion.(110) In hypothermic oxygenated perfusion Belzer MPS® University of Wisconsin Machine Perfusion Solution was used, whereas in normothermic perfusion a perfusate based on Hemopure, “an acellular, polymerised bovine haemoglobin-based oxygen carrier” and “human albumin solution” was applied.(110) While the liver grafts were perfused at hypothermia, the ATP levels in the tissue grew.(110) This suggests a positive effect of hypothermic oxygenated perfusion on ATP storage and the recovery of mitochondrial function.(110) In this study, markers used for ex vivo viability assessment were “stable vascular flows”, “homogeneous parenchymal perfusion”, bile production and a reduction of the lactate levels below 2.5mmol/L in the perfusate within 6 hours.(110) Both groups showed similar bile production and similar lactate levels after the perfusion.(110) All five livers which underwent hypothermic oxygenated perfusion and normothermic perfusion were considered viable. This is a non-significantly higher number compared to only three viable livers out of five after normothermic perfusion only.(110) Moreover, in livers only perfused by normothermic perfusion more signs for inflammatory processes and oxidative injury were found.(110) To sum it up, the functional recovery of ECD livers might be enhanced by the approach combining hypothermic oxygenated perfusion and normothermic perfusion.(110)

3.4.2.7 Combined Hypothermic, Rewarming and Normothermic Machine Perfusion

Van Leeuwen et al. created a perfusion protocol consisting of 1 hour of DHOPE for resuscitating the organ followed by 1 hour of controlled oxygenated rewarming followed by about 5 hours of NMP.(111) During NMP viability assessment was carried out based on perfusate pH, perfusate lactate, bile production and bile pH. Only if all predefined criteria regarding these four measures were met, the liver

graft was accepted for transplantation. Of 16 previously declined DCD livers preserved according to Van Leeuwen et al.'s protocol, 11 could be transplanted. Graft and patient survival at 3 and 6 months after transplantation was 100%. Based on their results they estimated that applying their preservation protocol could raise the total amount of transplantable livers by about 20% by making previously declined livers transplantable.(111)

3.4.2.8 Regional Perfusion

Machine perfusion can also begin in situ before organ procurement. The abdominal regional perfusion used for perfusing abdominal organs is based on a cannulation of the aorta or the iliac arteries and the vena cava or the iliac veins. A clamp on or a balloon in the descending thoracic aorta then insulates the abdominal compartment from the thorax.(27)

In 2016 de Carlis et al. presented a case of liver preservation combining normothermic regional perfusion with HMP aiming at diminishing the negative effects of prolonged warm ischemia. In their case, normothermic regional perfusion was applied for 8 hours.(112)

3.4.3 Markers for Viability Assessment of Liver Grafts

For profiting from the opportunity of viability assessment prior to transplantation, which is enabled during machine perfusion, reliable markers are required and there is broad consensus that it's necessary to find and introduce reliable markers which are able to predict posttransplant outcomes.(16,32,105) The acceptance of markers for viability assessment in clinical practice depends on their sensitivity, specificity and the simplicity regarding their application.(30)

The ex vivo viability markers assumed to be relevant in HMP on the one hand and NMP on the other hand are not identical and it is not possible to equate hypothermic with NMP regarding the use of a specific marker.(86) According to Watson et al., hypothermia has heterogeneous effects on the different parts of liver metabolism. The assessment of metabolic markers at hypothermia, therefore, might not reflect the liver function at normothermic conditions. This hinders a pretransplant ex vivo viability assessment of the liver graft during HMP.(98)

Kim et al. stress that there are no verified markers for viability assessment so far.(86) For NMP this statement is supported by Nasralla et al. They emphasised

that far more transplantations using NMP are necessary for identifying specific markers.(52)

Verhoeven et al. distinguish between markers indicating organ injuries and markers representing organ function.(30) While the majority of markers belonged to the first group, bile production was the only marker for organ function, at the time their review was published in 2014.(30) However, markers for organ function are crucial. They might predict posttransplant organ function in a better way, because the predictive value of severe ischemic injury and thus also markers indicating organ injuries for posttransplant organ function might not be satisfying.(30) But markers for organ function necessitate a sufficiently active metabolism and this again requires normothermic or subnormothermic preservation.(30) Since the cellular metabolism is altered in hypothermia, viability assessment might be more complicated using HMP compared to viability assessment in NMP which tries to imitate the physiological conditions for maintaining the regular cellular metabolism.(19) Watson et al. therefore even go so far as to claim, that hypothermic perfusion doesn't enable a reliable viability assessment.(98)

There are several different markers that are used for viability assessment in trials on machine perfusion in liver transplantation. But, in general, there has only been a small number of trials on this topic and particularly the used markers sometimes differed between the trials. Furthermore, meta-analyses can't be done properly because the outcome variables often vary between the trials. As a result there is only very limited evidence for the validity of these markers so far.(30) Regarding markers measured in the perfusate it has to be added that a potential limitation might be possible accumulation because of the recirculation of the perfusate in most machine perfusion systems.(30)

3.4.3.1 Histological Assessment

Tissue haemorrhage and cell necrosis can be assessed by a histological examination.(30) A histological assessment in liver preservation can also be used for identifying the degree of graft steatosis of steatotic livers prior to transplantation.(30) But it has to be considered that this histological evaluation is distinctly limited by observer dependent differences in histological evaluation of hepatic steatosis.(113)

In general, a drawback of histological markers besides invasiveness and observer dependency is that biopsies only show a small part of the tissue. Therefore, a biopsy does not always provide reliable information on the situation of the organ as a whole, because the degree of injury and histological changes can differ throughout the organ.(30) As opposed to that, markers measurable in the perfusate have the advantage that they are probably able to represent the state of the whole organ.(30) Furthermore those markers in the perfusate can be gauged noninvasively.(30)

3.4.3.2 Adenosine Triphosphate

Not only in livers but in all organs tissue level ATP indicates organ function and viability. Thus, it is an approved marker for assessing organ function and viability according to Bellini et al.(17) In 2019 Bellini et al. suggested to re-establish tissue ATP levels as a marker for viability assessment in liver, kidney and pancreas grafts.(17) Low levels of ATP might indicate an increased risk for early allograft dysfunction and primary nonfunction.(30)

3.4.3.3 Transaminases

The levels of the transaminases AST and ALT represent damage of liver cells, while ALT is more specific for hepatocytes.(30) Ghinolfi et al. regard transaminase levels as an adequate indicator for organ function or dysfunction during NMP.(100) According to Watson et al.'s review, transaminase levels in the perfusate can not only be used as a marker to determine injuries of hepatocytes during normothermic, but also during HMP.(98) Eisenbach et al. have demonstrated that lower peak AST-levels during the first posttransplant days are associated with a superior outcome.(114)

3.4.3.4 Bile

Bile production and composition of bile as markers for viability assessment are perhaps more useful in normothermic and subnormothermic settings and less useful in hypothermic settings, since hypothermia considerably limits the metabolic activity and with that the bile production.(30)

Bile production is regarded as adequate indicator for organ function during NMP.(100) But Watson et al. conclude from their study that bile production itself might not be a reliable marker for viability assessment. According to them biliary pH might be a superior marker, since in their trial the "inability to produce an alkali

bile" during ex vivo perfusion was associated with the posttransplant development of a cholangiopathy.(45) pH regulation and bile chemistry are currently used as markers during NMP.(98)

3.4.3.5 Lactate

Ghinolfi et al. regard lactate clearance as suitable indicator for organ function during NMP.(100) It is currently used as marker indicating actual liver function during NMP.(98)

3.4.3.6 Hyaluronic Acid and Thrombomodulin

Hyaluronic acid and thrombomodulin could be used as markers for endothelial injury.(30)

3.4.3.7 Flavin Mononucleotide

For assessing the viability of human liver grafts during hypothermic oxygenated machine perfusion Muller et al. used fluorimetry for quantifying flavin mononucleotide in the perfusate, which is indicating mitochondrial injury. A correlation of the flavin mononucleotide level with early graft loss and severe allograft dysfunction could be detected.(115) According to Muller et al., these results are of importance, since reliable methods for predicting liver function during ex situ machine perfusion have not been described before.(115)

3.4.4 Therapeutic Interventions and Agents in Liver Preservation

3.4.4.1 Liver Defatting

An important problem in liver transplantation is hepatic steatosis in donor organs, not least since it aggravates organ damages caused by IRI and hypothermia.(21) A systematic review showed that transplanting livers with severe steatosis elevates the risk of developing primary nonfunction, while transplanting livers with moderate to severe steatosis was associated with diminished graft survival.(116) Despite potential benefits of machine perfusion in liver preservation, steatotic livers are usually often still not considered as transplantable.(94) Thus, more sophisticated ways of predicting posttransplant liver function of steatotic livers could contribute to a better utilisation of steatotic liver grafts and reduce posttransplant complications.(117) Furthermore, according to Kron et al., hypothermic oxygenated machine perfusion might have protective effects on steatotic livers.(118) But for using steatotic livers as grafts methods beyond usual

machine perfusion seem to be required.(94) For instance, it may be possible to recondition steatotic livers during machine perfusion and to modify the degree of steatosis by that, although there is no clear evidence for this.(117)

As the prevalence of hepatic steatosis is high, the defatting of steatotic liver grafts during machine perfusion could increase the percentage of transplantable livers considerably.(21) Such a reduction of the hepatic fat content could be realized by applying a “defatting cocktail” consisting of several therapeutic agents. Animal models on that method have already been conducted with varying results.(94,119,120) Moreover, Boteon et al. have already published a study on the application of a defatting therapy consisting of a combination of several drugs manipulating lipid metabolism during end-ischemic NMP. The therapy was applied on 5 human liver grafts, previously discarded because of macrosteatosis. In these livers levels of tissue triglycerides could be reduced significantly compared to the control group within a few hours. This was accompanied by improved metabolic parameters and less vascular resistance and reperfusion injury.(121)

3.4.4.2 Anti-inflammatory and Vasodilating Agents

The addition of the anti-inflammatory and vasodilating agents sevoflurane, carbon monoxide, prostaglandine E1 and acetylcystein to the perfusate in SMP showed advantageous outcomes in an animal model. Posttransplant bilirubin, ALT and AST levels could be reduced.(122)

Also Echeverri et al. compared the effects of several vasodilators on hepatic artery flow during NMP of porcine livers. Hepatic artery flow was significantly increased under all tested vasodilators compared to perfusion without a vasodilator. The application of BQ123, an endothelin-1 antagonist, and verapamil resulted in a decreased damage of liver cells and an increased flow in the hepatic artery compared to the application of epoprostenol.(123)

3.4.4.3 Hormones

Hormones might also have beneficial effects as ingredients of machine perfusion solutions.(1) According to a review on the therapeutic use of several hormones in organ preservation potential beneficial effects in liver preservation could be found for adding melatonin, dopamine, prolactin, glucagon, relaxin, prostaglandine E1 and several growth factors like insulin like growth factor 1.(1) The main challenges in using hormones as therapeutic agents in organ preservation are: (1)

1. pharmacokinetics and bioavailability of these hormones as ingredients of preservation solutions in the organ preservation setting
2. physiochemical stability of hormone containing preservation solutions during storage and application

Conceivable solutions for these challenges might be found in using hormone analogues with longer half times and in a distribution of hormones or hormone analogues by nanocarriers.(1)

3.4.4.4 Polyethylene glycol

Panisello Rosello et al. propose to further investigate the addition of polyethylene glycol 35 to the perfusate in hypothermic oxygenated machine perfusion of liver grafts, because of its potential protective effects on mitochondria and the glycocalyx. By doing so an enhancement of graft viability might be possible.(124)

3.4.4.5 Small Interfering RNA

A further conceivable technique of organ reconditioning by therapeutic agents during machine perfusion might be the application of small interfering RNA (siRNA).(17) Gillooly et al. regard the therapeutic application of siRNA during machine perfusion preservation as an important future aspect in organ preservation with the potential of beneficial effects on transplant outcomes.(125) For transporting siRNA to its target nanoparticles could be used according to DiRito et al.(126)

3.4.4.6 Miravirsen

Acute reinfection with the hepatitis C virus during reperfusion of the transplanted liver is an important topic in liver transplantation since a considerable proportion of liver recipients has got an infection with the hepatitis C virus and since hepatitis C recurrence after transplantation causes a cirrhosis of the transplanted liver in about 25 percent of infected patients within 5 years after transplantation.(127,128) A potential future strategy for preventing a hepatitis C infection of the liver graft after transplantation has been demonstrated in an animal model. Goldaracena et al. pretreated porcine liver cells with miravirsen, an inhibitor of hepatitis C virus replication, during normothermic ex vivo liver perfusion.(127) But whether this could really prevent the hepatitis C recurrence in human liver grafts after transplantation still needs to be demonstrated.(127)

3.4.4.7 Addition of Oxygen Carriers

For addressing the lack of oxygen during SCS, the addition of oxygen carriers like M101 to the preservation solution could enable an oxygen supply during SCS.(129)

3.4.4.8 Oxygen Persufflation

Oxygen persufflation is intended to provide oxygen to organs preserved at hypothermic conditions. It is based on perfusing the organ with a humidified oxygenated gas via its own vasculature. In doing so, the oxygen can be distributed throughout the whole organ.(130,131) This aims at reducing organ injuries caused by ischemia.(132) An advantage of oxygen persufflation is its simplicity combined with low cost.(131) It is not a novel approach but hasn't become a standard in liver preservation yet.(130,131) Recently, the results of a randomized controlled trial on reconditioning human liver grafts by venous oxygen persufflation immediately before transplantation were published. Beneficial effects on some higher risk organs could be detected.(132)

3.4.5 Future Aspects in Liver Preservation

An important topic in liver transplantation – as in the field of organ transplantation in general – is the expansion of the donor pool.(133) Pinezich and Vunjak-Novakovic are convinced that ex vivo machine perfusion can make decisive contributions to organ transplantation - especially by a huge expansion of the donor pool.(21) For optimizing the outcome of machine perfusion, in Marecki et al's opinion further research should determine: (32)

- limits of recoverable warm ischemia time
- the time span, for how long liver grafts can be exposed to cold storage before perfusion is applied
- the optimum duration of perfusion

One component of a strategy for reaching an expansion of the donor pool could be an increased utilisation of donor livers derived from donors aged 70 and older. Ghinolfi et al. found that this was not associated with an increased risk for biliary or vascular complications. Solely, the necessity of retransplantation caused by graft dysfunction was somewhat higher. Especially livers of older donors without risk factors showed very good long-term graft survival.(133)

Additionally, the necessity of expanding the donor pool to grafts of minor quality is underlined by Vogel et al. But simple cold storage is insufficient for preservation of “marginal donor grafts”.(15) According to Selzner et al. it will be possible to determine a larger donor pool if it turns out that normothermic ex vivo liver perfusion shows superior outcomes in preservation of marginal donor grafts compared to preservation by simple cold storage in trials on human liver transplantation.(97) Hence, more trials investigating NMP are required. For example, Selzner et al. recommended doing more research on whether it’s possible to reduce reperfusion injury, or even repair marginal grafts as well as to assess graft viability by using NMP for liver preservation.(97) Clinical trials involving more patients and including a long-term follow-up are also necessary for identifying whether NMP has the potential to decrease biliary complications and to increase patient and graft survival.(16,96)

Also important is an examination of the economic aspects of NMP because cost effectiveness could increase the acceptance of this preservation technique.(52,97)

An important role in the expansion of the donor pool is attributed to graft viability assessment before transplantation.(19) But so far, reliable and verified markers for the assessment of livers have not yet been found.(52,86) Probably, for assessing organ viability during machine perfusion, scores consisting of a combination of several markers will be established, but first need to be evaluated in trials with an adequate number of participants.(30)

Organ preservation at subzero temperatures is a further topic of interest. It includes organ freezing, vitrification and supercooling.(134) Of them, supercooling seems to be most promising.(134) It has the potential to prolong preservation times which could contribute to an increased utilization of donor organs.(135) It could increase the period in which a certain donor organ is available for transplantation and could thus also enable global distribution of donor organs.(136) Longer preservation times can be realized because at subzero temperatures the metabolism is slowed down even more than in hypothermic preservation, which lowers energy consumption and oxygen demand even further.(137) Main challenges of preservation at subzero temperatures are ice crystal formation, protein denaturation and osmotic effects during freezing and thawing.(135) The technique of supercooling is based on cooling an organ down to -6˚C using cryoprotectants which obviate extra- and intracellular freezing.(138)

Thus, it is crucial to find suitable non-toxic cryoprotective additives to preservation solutions.(134) In a trial on rat livers, Bruinsma et al. added polyethlyene glycol and 3–O–methyl glucose as cryoprotectans during SMP. Afterwards, the livers were cooled down to -6˚C.(138) After supercooling, the livers were again perfused at subnormothermia for preparing them for transplantation.(138) With this protocol preservation period could be prolonged significantly compared to simple cold storage. The post-transplant survival of supercooled livers was 100% after 72 hours and 58% after 96 hours of preservation. For comparison posttransplant survival was 0% after 72 hours of SCS.(136,138) Nevertheless, it has to be added that transplantation after long-term preservation by supercooling has not yet been investigated.(134)

De Vries et al. supercooled human livers for demonstrating the feasibility of their approach using a preservation protocol slightly modified to the one of Bruinsma et al. As cryoprotectants they used trehalose and polyethylene glycol as well as glycerol which was chosen instead of 3-O-methyl glucose. It is important to mention that the addition of cryoprotectants elevates the solution's viscosity which poses more shear stress on the endothelial lining. Furthermore, a significant difference between human and rat livers is the following: Since human livers are much bigger than rat livers, uniform distribution of the cryoprotectants is more complicated, but it is essential for preventing ice formation. For achieving such a uniform distribution they added HMP as intermediate step between SMP and supercooling.(139)

Using cryoprotectants is not the only investigated way for preventing freezing in supercooling. Takahashi et al. applied high pressures on the preserved rat livers by pressurizing the preservation solution for inhibiting freezing at subzero temperatures with different results depending on the pressure applied.(140,141)

Another possible way of supercooling is the so called supercooling refrigerator applying electrostatic fields on the donor organ for preserving it at subzero temperatures without freezing it. It has been developed by Monzen et al.(142)

A further important future aspect in liver preservation is the application of therapeutic interventions and agents during machine perfusion for expanding the donor pool as well as improving graft quality and posttransplant outcomes. Examples for that were given above. Furthermore, for enabling more sophisticated therapeutic interventions and methods of organ reconditioning Pinezich and

Vunjak-Novakovic regard it as crucial to enable much longer preservation times over several days instead of only several hours, as is the current status in ex vivo machine perfusion.(21)

A further future perspective is the modification of grafts by decellularizing them and afterwards repopulating them with specific cells during organ preservation.(143-145) This approach could be applied for achieving different goals. First, a repopulation of the graft could be done with specific cells derived from the recipient. This could damp down the intensity of the recipient's immune rejection and could thus enable to reduce the immunosuppressive therapy.(143) Perhaps this technique could also be used for an ex vivo reconditioning of a patient's own dysfunctional organs, which would supersede at least some allotransplantations.(143) And someday, bioartificial livers might be able to replace malfunctioning livers.(146,147)

3.5 Kidney Preservation

When clinical kidney transplantation was established in the early 1960s, effective methods of organ preservation were still missing. This, of course, was an unacceptable state since organ preservation is a key aspect of organ transplantation. Following Starzl further research was done which led to understanding and use of hypothermia for preservation.(6) Matsuno et al. describe that machine perfusion systems for the preservation of kidneys were first established in the late 1960s.(12,148) Subsequently kidneys were preserved by machine perfusion or SCS in Europe and the USA during the 1970s and there were also trials comparing both approaches.(12,23,24) This changed fundamentally due to the emergence of University of Wisconsin solution which enabled the preservation of kidneys by simple cold storage for up to 72 hours. Following the introduction of University of Wisconsin solution many transplantation centres switched to using simple cold storage instead of HMP.(12) Since the late 1980s this method has been used predominantly.(11)

As in liver preservation there is a donor organ shortage in kidney transplantation, necessitating an expansion of the donor pool.(149) Because of a lack of kidney grafts it is necessary to think of extending the donor pool to non-heart beating donors, elderly donors and hemodynamically unstable donors. For maintaining the function of marginal organs derived from these donor groups, machine perfusion might be superior to simple cold storage and thus became a topic of interest

again.(12) Moreover, of high importance in this context is the graft assessment before transplantation since it might support the expansion of the donor pool by improving the ability to identify whether kidney grafts derived from high risk donors are suitable for transplantation or not.(22) Based on such a graft assessment it might be possible to estimate the risk for posttransplant complications like delayed graft function and graft loss.(22) While the opportunities for graft assessment are very limited during SCS, machine perfusion allows for a better observation of parameters representing organ function and injury.(22)

Besides research on viability assessment and different machine perfusion strategies there is also research on therapeutic interventions during kidney preservation as will be explicated below.

3.5.1 Static Cold Storage in Kidney Preservation

As already mentioned above, SCS has been the predominantly used preservation technique in kidney preservation since the late 1980s.(11) This was mainly a result of the introduction of the University of Wisconsin solution for SCS.(12) In 1992 Ploeg et al demonstrated that using University of Wisconsin solution for kidney preservation has led to better results regarding graft function and graft survival compared to EuroCollins.(150) This further contributed to the widespread use of University of Wisconsin solution in organ preservation.(19)

An important disadvantage of SCS, especially in the context of expanding the donor pool to higher risk donors, is its lack of a reliable viability assessment of the graft before transplantation. The graft assessment during SCS is mainly limited to donor characteristics and circumstances and sometimes also a zero-time biopsy of the renal graft. But validated scoring systems for zero-time-biopsies are missing. Because of this, their predictive value for graft assessment is reduced.(22) The role of biopsies in viability assessment of renal grafts will be discussed further below.

3.5.2 Machine Perfusion in Kidney Preservation

In the past years, machine perfusion has been rediscovered as a method for kidney preservation, after it had been superseded by SCS in the late 1980s.(11,29) A lot of research is therefore currently being done on machine perfusion in kidney preservation. Current findings will be presented below, arranged in order of the chosen machine perfusion protocols.

3.5.2.1 Hypothermic Machine Perfusion in Kidney Preservation

In HMP the temperature is set at 4-10˚C. The low temperature enables to diminish the deleterious effects of ischemia by reducing the metabolic rate of the cells and with that also the oxygen demand.(27) Nevertheless, as the cellular metabolism is only decreased, oxygenation during HMP for maintaining the oxygen supply seems to be beneficial.(27)

The residual metabolism during HMP may also facilitate graft assessment.(22) But for assessing graft function these hypothermic conditions with decreased metabolism are not ideal. Nevertheless, it might be possible to evaluate the severity of the graft's injuries.(22)

This is not the only reason why HMP could play an important role in the expansion of the donor pool. Due to a donor organ shortage the transplantation of ECD and donation after circulatory death kidneys has become an important topic of research in the past years. But such an expansion of the donor pool carries an increased risk of delayed graft function and poor graft survival.(151) In their meta-analysis Yarlagadda et al. showed an association between delayed graft function and acute rejection, long-term renal function and graft survival.(152) Furthermore, an increase of the duration of delayed graft function has been found to be associated with an increase of death-censored graft loss.(153) A key driver of this increased risk of delayed graft function is IRI. HMP is a preservation method aiming at diminishing IRI by providing an improved environment for the organ compared to SCS during transfer and storage of the organ since it enables a continuous perfusion of the kidney graft with a hypothermic preservation solution.(151)

An important topic in HMP is the choice of the perfusion pressure. Which perfusion pressure should be applied has not yet been finally clarified. According to de Beule et al. there is great agreement that a pressure of 25-30 mmHg is a good choice for the pulsatile perfusion of the renal artery in HMP. By applying such a low perfusion pressure, damage caused by perfusion at higher pressures can be prevented.(22)

Because of the higher level of experience and more data on HMP in kidney preservation compared to liver and pancreas preservation, this chapter is focused on showing the results of systematic reviews instead of results of single trials.

A systematic review of O'Callaghan et al. comparing HMP to SCS was published in 2013. The only significant difference they found were superior results regarding

delayed graft function in HMP.(154) But the use of different preservation solutions might limit the results of their review. In some trials Collins or Euro-Collins were used for SCS instead of University of Wisconsin solution, whereas a further review by O'Callaghan had shown an increased risk for delayed graft function in organs preserved in Collins and Euro-Collins solution compared to those preserved in University of Wisconsin solution.(154,155) This means that differences between results of HMP and SCS might have been reduced, if superior preservation solutions would have been used for SCS.(154) Apart from that, data on primary nonfunction, acute rejection, long term renal function and patient survival did not show any significant differences between the compared methods. According to O'Callaghan et al. this might be caused by a to small number of preserved and transplanted kidneys in the investigated trials.(154)

Another review was published by Martínez Arcos et al. Based on the results of twelve included studies they concluded that hypothermic pulsatile machine perfusion has the potential to moderately reduce delayed graft function compared to SCS.(156) For primary non-function no significant differences could be found between the different preservation methods. Nevertheless, Martínez Arcos et al. assumed that hypothermic pulsatile machine perfusion might have the potential to reduce the risk for primary non-function in DBD donor kidneys, but this has to be investigated in further trials.(156)

There is also a recent meta-analysis of Tingle et al., e-published in 2020 comparing the effects of HMP to SCS of kidney grafts of deceased donors.(151) Tingle et al. included 14 studies with delayed graft function as primary outcome. Delayed graft function was defined as the necessity for dialysis within the first week after transplantation.(151) In total 2138 patients were included in their meta-analysis. Of them 1068 received a kidney preserved by HMP and 1070 received a kidney preserved by SCS.(151) The results showed that the relative risk of delayed graft function was 0.77.(151) Thus, 10.35 kidney grafts have to be preserved by HMP to prevent one case of delayed graft function.(151) The reduction of relative risk was significant and even stronger when looking at DCD kidneys only. The risk ratio in DCD kidneys was 0.75. This equals 7.26 DCD kidney grafts that need to be preserved by HMP to prevent one case of delayed graft function.(151) In the DBD group risk reduction was 0.78. According to this 13.60 DBD kidneys need to be preserved by HMP to prevent one case of delayed

graft function.(151) Across the included trials, data on 1-year renal graft survival was not standardized enough for enabling a reliable analysis according to Tingle et al.(151) While two powerful studies showed a statistically significant improvement of 1-year renal graft survival in the HMP groups (151,157,158), other studies didn't (151,159-162) or didn't provide p-values, that are necessary for evaluating the significance.(151,163,164) Graft survival of more than one year was only investigated in three studies. Moers et al. and Zhong et al. found a significantly higher 3-year renal graft survival in the HMP group.(151,157,158) Kwiatkowski et al. were looking at an even longer period of time. They examined the 10-year graft survival. They did not find a significant difference between HMP and SCS which might be caused by a lower power of the study.(165) Furthermore, in patient survival no significant differences were found in the studies reporting on it.(151,157,158,161,162,165,166) Nonetheless, according to Tingle et al., their review shows the superiority of HMP to SCS.(151) Thus, Tingle et al. recommend the application of HMP in preservation of deceased donor kidneys, particularly in kidneys that are prone to delayed graft function.(151) Nevertheless, whether HMP has the potential to improve kidney and patient survival in the long run should be further investigated.

A further important aspect besides posttransplant outcomes are economic aspects of organ preservation. An estimation of the cost effectiveness of HMP compared to SCS in kidney preservation was made by Groen et al.(167) Such an estimation of cost effectiveness, of course, depends on the trial data that is taken as its basis.(168) Groen et al. based their calculation on Moers et al.'s international randomized controlled trial involving 672 recipients of kidney grafts. According to them, it turned out that HMP is a cheaper method compared to SCS for DBD and DCD kidneys.(157,167)

Even if HMP seems to be superior to SCS, there are still open questions. For example, it is unclear for how long HMP has to be applied for optimizing graft viability.(169) Another unanswered question is whether it is more favourable to use a flow-driven or pressure-driven perfusion system.(169) Wszola et al. published the results of their study on this topic including 50 transplanted kidneys. Significantly more kidneys perfused by the flow-driven perfusion machine showed renal interstitial fibrosis and tubular atrophy in the histological examination.(170) While delayed graft function was similar in both groups, the required sessions of

dialysis were significantly more for patients that had received a kidney preserved by a flow-driven perfusion system.(170) Furthermore, one year graft survival was non-significantly higher in patients with kidneys preserved by a pressure-driven perfusion system.(170) These results could be explained by a higher pulse stress on the blood vessels under flow-driven conditions.(170) Nevertheless, the power of this study is limited by the small number of patients.(170)

There are also disadvantages of HMP. One disadvantage might be that hypothermic preservation methods in general and with that also HMP causes temperature-related changes in the conformation of proteins which can result in an altered protein function.(2) For instance, it was shown by Thuillier and Hauet that hypothermia alters the cytoskeleton of human endothelial cells severely by altering the structure of microfilaments and microtubules.(171)

3.5.2.1.1 End-ischemic Hypothermic Machine Perfusion

Matos et al. conducted a trial on kidney preservation by applying a combination of SCS and HMP and compared the results to the outcomes of kidney grafts preserved by SCS only. 54 deceased donor kidneys were hypothermically perfused for at least 6 hours after SCS for 22 hours on average. This approach enabled a reduction of the rate of delayed graft function and its mean length. But this was only observed in organs of donors younger than 51 years. Moreover, differences in renal function after 6 month could not be observed between both groups.(172)

Another study on end-ischemic HMP in kidney preservation was conducted by Gallinat et al. They included 66 ECD kidneys. First they were preserved by SCS for 364 to 1567 minutes and afterwards they were connected to a perfusion machine until transplantation for applying HMP for 98 to 912 minutes. They compared the outcomes to ECD kidneys preserved by SCS only. Primary non-function was significantly lower in the machine perfusion group. On the other hand, a significant difference in one-year graft survival could not be observed. It was only non-significantly higher in the HMP group. According to Gallinat et al. the missing significance in one-year graft survival might be due to a too small number of included kidneys.(173) Gallinat et al. regard end-ischemic HMP as an alternative to HMP during the whole preservation period, if this method is not applicable due to legal or logistical issues.(173)

Ravaioli et al. published the results of a clinical trial concerning hypothermic oxygenated machine perfusion of ten livers and kidneys. The organs were derived from ECDs and the results were compared to results of matched organs preserved by SCS.(76) No case of primary non-function was observed. The proportion of kidneys with delayed graft function was non-significantly higher in kidneys preserved by SCS. 96.7% of kidneys preserved by SCS survived the first 30 days after transplantation compared to 100% in the hypothermic oxygenated machine perfusion group.(76) For Ravaioli et al. these results underline the safety of hypothermic oxygenated machine perfusion in preservation of organs derived from brain dead ECDs.(76)

3.5.2.1.2 Hypothermic Machine Perfusion without Oxygenation

In a porcine model on nonoxygenated HMP Chatauret et al. were able to show that this preservation method has protective effects on the nitric oxide (NO) signalling pathway in the renal cortex.(174) After the kidneys had been rewarmed and oxygenated NO-dependent vasodilation of the renal arteries has been tested. It turned out that it was enhanced in kidneys preserved by nonoxygenated HMP. Furthermore, after transplanting the grafts an improved microcirculation in the renal cortex was observable in the machine perfusion grafts compared to grafts preserved by SCS.(174) Since NO-synthesis requires oxygen, oxygenated HMP might have even more beneficial effects, which should be investigated in further trials according to Chatauret et al.(174)

3.5.2.2 Subnormothermic Machine Perfusion

In SMP the chosen temperature is 20–25°C. At this temperature cold-induced injury can be obviated. At the same time metabolism is not as active as under normothermic conditions. Thereby, an oxygen carrier is not necessary for maintaining oxygen supply.(27) According to de Beule et al. there is no evidence on SMP in preservation of human kidneys yet.(22) Furthermore, viability assessment of kidneys preserved by SMP has not been investigated yet.(22)

3.5.2.3 Normothermic Machine Perfusion

In NMP the organ is preserved at 35-37˚C.(27) Since simple cold storage and also HMP are less complex and easier to handle while providing satisfying outcomes in organ preservation, NMP has not been of scientific interest for a long time.(169) But for the preservation of lower quality renal grafts it might be a promising method

and has therefore become a focus of research in organ preservation.(169) The application of NMP for preserving human kidneys only started a few years ago. Before that it has been investigated in studies on preservation of animal kidneys.(169)

According to Hosgood et al., the benefits of NMP are as follows: a metabolism close to the physiologic metabolism can be maintained or restored.(175) For example, a restoration of ATPe levels can be realized.(169) Furthermore, monitoring of the kidney and its metabolic activity is possible which enables a better and more reliable viability assessment.(175) Because of that NMP could contribute to expanding the donor pool. This is underlined by Hosgood et al.'s report that assessment under NMP enabled the successful transplantation of DCD kidneys which would otherwise have been discarded.(176) Hosgood et al. are convinced that ex vivo NMP of kidneys has the potential to enable an expansion of the donor pool. By that the number of transplanted kidneys could be raised. For the United Kingdom they assume a potential increase of about 10%.(177) Moreover, a further advantage of NMP is that injuries caused by hypothermia and ischemia can be prevented.(169) For maintaining the metabolism at normothermia, oxygenation is essential. Thus, an oxygen carrier in the perfusate is required.(22) For this purpose usually packed red blood cells are used. Synthetic haemoglobin-based oxygen carriers could be a good alternative. Compared to red packed blood cells they also offer advantages regarding logistics.(178)

Although NMP has become an important topic in organ preservation research, there are no finished randomized controlled trials supporting an application of NMP yet.(151) So far, most trials on NMP in kidney preservation followed protocols only including a short period of end-ischaemic NMP before transplantation. In these trials the application of NMP usually lasted for one hour.(22) Their results will be listed in the chapter end-ischemic NMP. NMP over a longer period of time has mainly been investigated in animal studies so far according to de Beule et al.(22)

It is still unknown for how long kidneys should be perfused normothermically and which composition of the perfusate would be best.(169) De Beule et al. outlined the relevance of the perfusate's composition in NMP. Although there are many different protocols, most perfusion solutions for NMP contain red blood cells, crystalloids, colloids, nutrients, a vasodilator and antibiotics.(22)

Economically it is still unclear whether potential benefits like a reduction of delayed graft function and an expansion of the donor pool can outweigh the higher costs caused by the high complexity of this method.(169)

3.5.2.3.1 End-ischemic Normothermic Machine Perfusion

Nicholson and Hosgood published the first study on ex vivo NMP for kidney preservation in 2013 and thereby showed its safety and feasibility. Directly before transplantation they perfused 18 ECD kidneys after SCS for about one hour. These kidneys showed a significantly lower rate of delayed graft function compared to 47 ECD kidneys preserved by SCS only. Significant differences in graft and patient survival after one year could not be found.(179)

Furthermore, there is an ongoing randomised controlled trial on pretransplant NMP for one hour after SCS compared to SCS only in preservation of DCD kidneys including 400 patients in the UK. Its ISRCTN registry number is 15821205.(180) According to the ISRCTN registry the results are intended to be published at the end of 2021.(181)

3.5.2.4 Combined Hypothermic and Normothermic Machine Perfusion

De Beule et al. mention that several perfusion methods can also be combined.(22) For kidney grafts considered untransplantable after HMP Kabagambe et al. suggest a subsequent NMP for a more reliable graft assessment.(182) Doing so could contribute to increasing donor organ utilization. In a trial Kabagambe et al. found all seven included previously discarded kidneys to be transplantable, but none of the seven organs was transplanted. Thus, posttransplant outcome could not be evaluated. For this purpose, a prospective clinical trial would be necessary. It could help finding out whether this combination might be favourable for assessing high-risk kidneys. In the study NMP was applied for three hours using a perfusate based on oxygenated packed red blood cells subsequent to HMP.(182)

3.5.2.5 Controlled Oxygenated Rewarming

When the transition from hypo- to normothermic conditions takes place too fast, dysfunction of mitochondria and proapoptotic signal transduction can be induced. The concept of controlled oxygenated rewarming could help averting such harmful fast temperature changes from hypo- to normothermia. It is based on slowly increasing the temperature of the perfusate and doing so contributes to preventing the development of cellular dysfunction.(183) Minor et al. were first to transplant a

human kidney graft gradually rewarmed from hypothermia to normothermia using a cell-free perfusate after several hours of SCS. The perfusate was oxygenated diluted Steen solution. Transplantation was carried out successfully and the patient's postoperative course was "overall event-free".(184) With this study, Minor et al. were able to show the feasibility and safety of controlled oxygenated rewarming in kidney preservation. This technique might contribute to a reduction of reperfusion injury and might become a method for graft reconditioning in ECD kidneys.(184)

Further knowledge on benefits of a gradual rewarming after SCS prior to reperfusion compared to sudden reperfusion without rewarming has been gained in animal models.(183,185)

3.5.3 Viability Assessment of Kidney Grafts

So far, decision making in kidney transplantation is often based on donor risk scores integrating several known risk factors for delayed graft function and graft loss. But these existing donor risk scores based on donor and recipient risk factors don't have enough predictive power which might be caused by the huge differences of individual recipients and donors and the variety of preservation methods.(186)

For determining and evaluating the viability of renal grafts before transplantation, it is of great importance to find more reliable measures.(149,169,187) Such biomarkers could help assessing the potential for recovery of the organ and predicting the posttransplant function. This might help increasing the donor pool to organs of higher risk donors which would counteract the donor organ shortage.(22) Determining whether a deceased donor kidney is transplantable or not is still quite subjective. In order to reduce the number of unnecessarily discarded kidneys, finding methods for a more objective viability assessment is crucial.(22) Preservation by machine perfusion allows for a good observation of several parameters representing organ function and injury.(22) Helpful for indicating graft injury might be intracellular material released by injured or dead cells detectable in the perfusate. Since different biomarkers originate from different cell types their concentrations in the perfusate might reveal important information on the pattern of graft injury.(22) For example, there are differences in the vulnerability for warm and cold ischemia between proximal and distal tubular cells. Cells of the distal tubulus seem to be more susceptible to cold ischemia whereas

cells of the proximal tubulus have been shown to be more susceptible to warm ischemia.(22,188)

A lot of potential biomarkers have been investigated so far, but a single biomarker that reliably predicts posttransplant outcome during machine perfusion has not been identified yet.(22,29) Due to the complexity of renal function it is more realistic to develop assessment scales composed of several biomarkers representing different aspects of organ function, injury and capability of regeneration than finding a single biomarker with a satisfying predictive value for posttransplant outcome at an individual level.(22)

The development towards a more biomarker-based decision making in organ preservation is in line with Naesens' and Anglicheau's opinion. They observe a shift of transplant medicine to a more individualized precision medicine, in which biomarkers play a very important role. Fundamental for this development, according to them, is advance made in the "omics".(189)

While NMP is expected to provide great opportunities in observing functional parameters as well as markers of organ injury for enabling reliable graft assessment(15,175), a viability assessment based on physiological parameters is not possible in HMP, because of the non-physiological hypothermic temperature and because of the used not blood-based perfusates.(29) Thus, in this setting only perfusion parameters and biomarkers indicating organ injury might be useful for assessing the preserved organ. This decreases the reliability as well as the predictive value of viability assessment in hypothermic conditions.(22,29) In order to compensate this disadvantage of HMP regarding graft assessment, a subsequent NMP after HMP could be applied, as mentioned above. This combination could provide a more reliable viability assessment of kidneys preserved by HMP.(182)

Nonetheless, there is also evidence for a successful application of metabolic parameters in graft viability assessment during HMP as Guy et al. have shown. They reported that during HMP the metabolic activity of kidneys with delayed graft function deviated from kidneys showing immediate graft function. In the perfusate significant differences were observed for the levels of glucose, gluconate, inosine and leucin.(190)

Moreover, it has to be taken into account, that there is not much experience concerning NMP of kidneys yet. As a consequence, only little is known about

biomarkers in NMP.(22) In normothermically perfused porcine kidneys Kaths et al. found significant correlations between perfusates pH, base excess and HCO_3^- and posttransplant renal function. Lactate clearance was also correlated with renal function after transplantation.(191) Hosgood et al. assessed the viability of 56 declined human kidneys during NMP that was carried out for 1 hour. The kidney grafts were not transplanted afterwards. Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and endothelin-1 (ET-1) were investigated as potential biomarkers. Particularly, Hosgood et al. recommended neutrophil gelatinase-associated lipocalin, especially combined with functional perfusion parameters as an opportunity for improving kidney graft assessment prior to transplantation.(192)

Furthermore, Hosgood et al. developed an assessment score for kidneys preserved by ex vivo NMP for one hour. This score includes macroscopic appearance, renal blood flow and urine output. The lowest possible score is 1 and represents the least injured kidneys whereas the highest score is 5 and stands for serious graft injury.(177) The exact composition of Hosgood et al.'s score is as follows:(177)

	Score
macroscopic assessment	
grade I: excellent perfusion (global pink appearance)	1
grade II: moderate perfusion (patchy appearance)	2
grade III: poor perfusion (global mottled and purple/black appearance)	3
renal blood flow (ml per minuter per 100g)	
threshold ≥ 50	0
threshold < 50	1
total urine output	
threshold ≥ 43	0
threshold < 43	1

Table 1: ex vivo NMP assessment score after Hosgood et al.(177)

In a trial including 23 ECD kidneys, 5 higher risk standard criteria donor kidneys and 8 DCD kidneys this assessment score was applied prior to transplantation. All 36 included kidneys scored 1, 2 or 3 and were transplanted after normothermic ex vivo machine perfusion. Primary nonfunction was not observed. Delayed graft function occurred in 6% of kidneys scored 1, 0% of kindeys scored 2 and 38% of kidneys scored 3. 35 of the 36 grafts survived for one year.(177)

De Beule et al. mention that the application of such assessment scores for NMP might be limited to certain settings of perfusion and might not be conferrable to

other settings of NMP with different perfusion devices, perfusates or additives since these factors influence perfusion pressure and oncotic pressure which in turn affect renal blood flow and urine output.(22)

The following provides an overview of a selection of potential measures and methods for viability assessment in kidney preservation:

3.5.3.1 Histological Assessment

Histological assessment of the kidney is limited by its dependency on the examiner and by its inability to identify disorders, that are not visible in histological assessment like the inhibition of transport proteins. A further issue is the time lag: it takes time for injuries to become histologically visible.(193) Zero-time biopsies of the renal graft are sometimes used for viability assessment, especially in SCS. But validated scoring systems for zero-time-biopsies are missing. Because of this, their predictive value for graft assessment is reduced.(22) Similarly, the predictive value of such biopsies for posttransplant outcomes is regarded as debatable(194) and “at best moderate” according to Naesens.(195) Reese et al. mention examiner dependency and time pressure as aspects limiting the validity of biopsies for graft assessment.(196) Moreover, the results of an analysis conducted by Lentine et al. showed increased discard rates, especially in low risk renal grafts, when biopsies were used for graft assessment although biopsies don’t seem to improve the prediction of posttransplant outcomes significantly compared to clinical parameters like age or the clinical donor profile index. As discard rates might be unnecessarily high, when biopsies are used for graft assessment, Lentine et al. suggested to avoid dispensable biopsies for diminishing discard rates.(194)

3.5.3.2 Perfusion Parameters

In NMP, the predictive value of pump parameters like renal vascular resistance and flow for posttransplant renal function is largely unknown.(22)

In contrast, the role of renal vascular resistance in viability assessment during HMP has been investigated in several studies.(197-199) For example in Sung et al.’s trial, vascular resistance was used as part of viability assessment and discard rates were higher in ECD kidneys with higher vascular resistance.(200) The trend in renal vascular resistance during HMP was used for viability assessment by Bissolati et al. In their study on 65 kidney grafts this measure had a superior predictive value compared to pretransplant histological evaluation of renal

biopsies.(201) Moreover, Parikh et al. reported that in their large multicentre cohort study discarded kidneys tended to have worse pump parameters, of which renal vascular resistance is a part, compared to transplanted kidneys.(197) But experience on renal vascular resistance as predictor for graft function was regarded as uncertain by de Vries et al.(199) Therefore, several studies investigated its predictive value.(197-199) Jochmans et al.'s study involved 302 deceased donor kidneys. Renal vascular resistance at the end of perfusion turned out to be an independent risk factor for delayed graft function and one-year graft failure with only low predictive value. Thus, for graft assessment it should only be used in combination with other viability markers according to Jochmans et al.(198) De Vries et al. analysed the results of 440 DCD kidneys preserved by HMP. Renal vascular resistance in the beginning of perfusion turned out to be an independent risk factor for primary non-function and delayed graft function, also with a comparatively low predictive value. An association with patient or graft survival could not be found.(199) Parikh et al. reported an inverse association of elevated renal vascular resistance and estimated glomerular filtration rate (eGFR) at 6 month after transplantation.(197) As Jochmans et al., also Parikh et al. advocate integrating pump parameters into a combination of different viability markers, for example in the sense of a scoring system.(197,198) Using inflexible threshold values for pump parameters for assessing graft viability could otherwise increase the rate of renal grafts falsely considered untransplantable.(197) Correspondingly, Sonnenday et al. point out that pretransplant viability assessment should not only be based on perfusion parameters. They were able to successfully transplant renal grafts of acceptable donors, that had previously been discarded by other centres because of bad perfusion parameters.(202)

An integration of renal vascular resistance into a combination of different measures was proposed by Tai et al. In a study on 446 DCD kidneys preserved by HMP they investigated main predictors for posttransplant delayed graft function. Terminal resistance was identified as the most important one. This in addition to the cause of death, hypotension in the agonal phase and terminal serum creatinine showed a satisfying predictive value so that these measures might be valuable in viability assessment of DCD kidneys when integrated into a scoring system.(203)

It should also be noted that there may be differences in the predictive value of pump parameters between several categories of renal grafts. For example, Kataria et al. have the impression that vascular resistance and flow have a higher predictive value for DCD and marginal renal grafts compared to other renal grafts.(169)

3.5.3.3 Oxygen Consumption

In oxygenated HMP a significant correlation between oxygen consumption during preservation and glomerular filtration rate after preservation was found.(204) Thus, measuring oxygen consumption during preservation may help assessing kidney grafts during preservation.(22) But so far, many different ways of calculating oxygen consumption have been applied which hinders the comparability of trials on this topic.(22) Another obstacle for using oxygen consumption as a measure for viability assessment is the following: For calculating the oxygen consumption, haemoglobin concentration has to be included in the calculation. But besides haemoglobin also other oxygen carriers or acellular perfusion solutions are in use and it remains unclear how to calculate oxygen consumption in such a case.(22)

3.5.3.4 Perfusate's pH, Base Excess and HCO_3^-

In normothermically perfused porcine kidneys Kaths et al. found significant correlations between perfusates pH, base excess and HCO_3^- and posttransplant renal function.(191)

3.5.3.5 Lactate Clearance

Lactate clearance was correlated with renal function after transplantation in NMP of porcine kidneys.(191)

3.5.3.6 MicroRNA 21

Based on the observation that acute kidney injury elevates the renal expression of microRNA 21, Khalid et al. investigated its predictive value for posttransplant kidney function.They found a correlation between the microRNA 21 level in the perfusate at 60 minutes postperfusion and eGFR at 6 and 12 months after transplantation. Included in the trial were 11 ECD kidneys preserved by HMP. They concluded that the microRNA 21 expression might predict the early outcome after transplantation of kidneys and might therefore be a useful marker for viability assessment, at least in preservation by HMP.(187)

3.5.3.7 Glutathione S-Transferase

According to a systematic review of Guzzi et al. on potential biomarkers in the perfusate of renal HMP, of all included potential biomarkers most data was found on glutathione S-transferase (GST). A majority of included studies reported a significant association between increased GST-levels and primary non-function. But there was no significant association between GST-levels and long-term outcome.(149) Thus, according to Guzzi et al. GST-levels might be a promising marker for primary non-function, but, however, only had a limited predictive value.(149)

3.5.3.8 Lactate Dehydrogenase

Guzzi et al. found only weak associations of lactate dehydrogenase (LDH) to primary non-function and delayed graft function when used as a biomarker for viability assessment in HMP, but, nonetheless, it may also be a promising biomarker.(149)

3.5.3.9 Creatinine Level

Shifts in the perfusate's creatinine level could potentially predict posttransplant outcome, but this remains unclear according to de Beule et al.(22)

3.5.3.10 Fatty Acid-Binding Protein

In a study on HMP Parikh et al. found levels of liver-type fatty acid–binding protein (L-FABP) to be inversely associated with eGFR at 6 month after transplantation. Nevertheless, the predictive value of this biomarker was regarded as low.(197)

3.5.3.11 Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is known as marker for acute kidney injury.(149) The baseline NGAL level in HMP perfusate was significantly associated with delayed graft function in only one out of three studies included in Guzzi et al.'s review on potential biomarkers for viability assessment.(149) Additionally, Parikh et al. found NGAL levels to be inversely associated with eGFR at 6 month after transplantation in a study on HMP. But they regarded its predictive value as low.(197)

Nonetheless, Hosgood et al. recommend neutrophil gelatinase-associated lipocalin, especially combined with functional perfusion parameters as an opportunity for improving kidney graft assessment prior to transplantation in NMP.(192)

3.5.3.12 Extracellular Histones

A potential biomarker for pretransplant graft assessment in HMP might be extracellular histones in the machine perfusate. Extracellular histones are cytotoxic and linked to cellular stress and cell death.(205) Van Smaalen et al. investigated the perfusates of 390 renal grafts derived from DCD donors. They focused on histone concentration and its association to graft function and graft survival. In machine perfusates of kidneys which developed a dysfunction after transplantation, the study showed significantly increased levels of extracellular histones. Extracellular histone level thus turned out to be a risk factor for delayed graft function and one-year graft failure.(205) Additional studies will be necessary to further investigate this association.(205)

3.5.3.13 N-acetyl-ß-D-glucosaminidase

The lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAG) has been shown to be a marker for tubular damage.(206) Moers et al. reported NAG to be a potential marker for delayed graft function.(207)

3.5.3.14 Magnet Resonance Imaging Using the Hyperpolarized Tracer [1-13C] Pyruvate

Magnet resonance imaging of the renal graft using the hyperpolarized tracer [1-^{13}C] pyruvate is a different way of assessing the metabolic state of kidney grafts and was investigated by Mariager et al. in a study on porcine kidneys. A potential advantag of this method is that it can be easily integrated into the perfusion system. Furthermore, the comparability between the metabolic states before and after transplantation is high if imaging of the transplanted organ is done prior to and after transplantation.(208)

3.5.3.15 Further Potential Biomarkers

Guzzi et al. published a systematic review on the capability of biomarkers in the perfusate of HMP for predicting outcomes in kidney transplantation.(149) In their review they also included the following biomarkers besides GST, LDH and NGAL: lactate, FABP, LPOPs and IL-18. For all of them only weak associations to delayed graft function and primary non-function could be found, if at all.(149) Guzzi et al. concluded that a single marker for reliably anticipating graft outcome during HMP is still missing. Combining different markers or identifying novel

markers might support graft assessment during HMP in the future and should therefore be evaluated in further trials.(149)

3.5.3.16 Rapid Sampling Microdialysis

Hamoui et al. used rapid sampling microdialysis in a new approach for continuously monitoring the metabolism of renal grafts during HMP. The information obtained from it might be used for graft viability assessment.(209) Rapid sampling microdialysis can be used for observing the function not only of kidneys but also of other organs. In another study it was also used for the assessment of pancreata.(210) Gowers et al. used their system for either monitoring two analytes in one organ – in their study lactate and glucose for observing the lactate/glucose ratio as marker for metabolic activities – or for monitoring one analyte – in their study lactate concentration – in two organs at the same time to compare their function.(210) Currently it is one objective of the researchers to integrate their system of rapid sampling microdialysis in a transportable device which can be used for monitoring the preserved organ also during the transport from the place of organ retrieval to the place of transplantation.(210) Bellini et al. identified rapid sampling microdialysis as a tool which could also enable a monitoring of the development of tissue ATP concentration and further metabolic measures during preservation. Rapid sampling microdialysis could thus also be used to observe the influence of temperature on metabolic measures.(17)

3.5.4 Therapeutic Interventions and Agents in Kidney Preservation

As already noted above, machine perfusion simplifies therapeutic interventions during organ preservation.(28) But there is also research on therapeutic interventions during SCS.(211) In the following, currently discussed approaches of therapeutic interventions during kidney preservation are specified.

3.5.4.1 Mesenchymal Stromal Cells

In a study on HMP of rat kidneys Gregorini et al. added mesenchymal stromal cells and extracellular vesicles of mesenchymal stromal cells to the perfusate. They observed a significantly diminished ischaemic damage in the kidneys perfused by this solution.(212) The application of mesenchymal stromal cells might be a topic of growing importance in the future of solid organ transplantation, since they convey anti-inflammatory effects and tissue-repair mechanisms and thus

have the potential to reduce organ injury.(213) Despite convincing results in first preclinical and clinical studies, a lot of further research is necessary according to Reinders et al.(213) An issue in this scientific field is the lack of comparability between studies on the application of mesenchymal stromal cells as was criticized by Issa.(214)

3.5.4.2 Gaseous Signalling Molecules

In finding therapeutic agents reducing IRI during kidney preservation, gaseous signalling molecules have become a topic of interest.(215) Lobb et al., for instance, found treating the kidney graft with hydrogen sulphide (H_2S) during preservation to be effective in reducing the effects of prolonged cold IRI.(216) H_2S has protective effects on mitochondria by decreasing the cellular metabolism. Thus, for reducing renal IRI and prolonging preservation times Dugbartey et al. proposed the addition of H_2S to preservation solutions or alternatively the induction of endogenous H_2S.(3)

Other evidence on gaseous signalling molecules comes from Sener et al., who added carbon monoxide releasing molecule 3 (CORM-3) to the preservation solution in SCS of renal grafts which reduced apoptosis as wells as tubular and glomerular necrosis. This might be due to stabilizing effects on the mitochondrial membrane.(211)

3.5.4.3 Thrombalexin

In a perfusion model Hamaoui et al. added thrombalexin, a cell binding thrombin inhibitor, to the perfusate during HMP before reperfusion with blood. This locally active antithrombotic intervention aims at reducing posttransplant microvascular thrombosis, that can occur due to IRI, without having the increased bleeding risks of systemically administered anticoagulant substances. In their study an improvement of postreperfusion parameters could be observed.(217)

3.5.4.4 Vasodilators

NO is a vasodilator produced by endothelial cells. A disruption of the blood flow results in a diminished NO production in endothelial cells which might result in vasoconstriction and might also be involved in activating platelets and leukocytes.(41) For antagonising this vasoconstriction, adding vasodilators to the perfusate could be helpful and has already been tried.(22,191) So far vasodilators as therapeutic agents in the perfusate have predominantly been tested in HMP

according to de Beule et al.(22) Polyak et al. investigated the effects of different vasodilators (verapamil, mannitol, trifluoperazine and prostaglandin E1) on ECD kidneys during HMP. While the application of verapamil, mannitol and trifluoperazine did not have any effect on early graft function and perfusion characteristics compared to the control group without any vasodilator, the addition of prostaglandin E1 reduced renal resistance, improved renal flow and diminished delayed graft function.(218,219) But one has to take into account that the effect of adding a vasoactive substance depends on the responsiveness of the endothelium.(22) It has been shown by Barth et al. that this responsiveness is reduced or even missing in damaged endothelial cells.(220)

3.5.4.5 Nanoparticles

Tietjen et al. are in favour of using NMP for molecular targeting of especially endothelial cells by applying nanoparticles. This could enable a target-oriented application of therapeutic agents. Nanoparticles added to the perfusate can easily reach the endothelial cells by circulating in the blood vessels.(221) Endothelial cells are of special interest since they are severely affected by IRI. Furthermore, they are first to have contact with the host's immune system. An intact endothelial lining might attenuate the host's immune response which seems to be advantageous in the long run. This underlines the necessity of protecting endothelial cells during preservation.(221)

3.5.4.6 Gene Therapies

Gene therapies during organ preservation for influencing the gene expression could also be important therapeutic interventions in the future.(126)

3.5.4.7 Inhibition of Matrix Metalloproteinases

Alterations in the expression of matrix metalloproteinases (MMPs) act a part in renal pathophysiology.(222) Moser et al. found an association between delayed graft function and significantly increased levels of the matrix metalloproteinases MMP-2 and MMP-9 in the perfusate.(223) A significant elevation in the expression of MMP-2 has already been shown to be associated with fibrotic changes in kidneys in the context of chronic active antibody-mediated rejection of kidney grafts.(224,225) MMP-9, which macrophages secrete, has the potential to trigger an epithelial-mesenchymal transition in tubular cells which plays a major role in the development of renal fibrosis.(226) Zhao et al. recommend to focus on inhibiting

MMP-9 by therapeutic interventions because of its role in the development of renal fibrosis.(224) Nevertheless it has to be stated that it is important to better understand the role of MMPs in renal pathophysiology, especially if therapies should be found which aim at inhibiting their profibrotic activities.(222) In an animal model Moser et al. added doxycycline, a non-specific MMP inhibitor, to the perfusate. This diminished preservation injury.(223) Another way of reducing MMP-activity is inhibiting the expression of MMP genes by adding gene-specific siRNA to the perfusate.(215,223)

3.5.5 Future Aspects in Kidney Preservation

The donor organ shortage in kidney transplantation necessitates an expansion of the donor pool.(149) Because of a lack of kidney grafts it is necessary to think of extending the donor pool to non-heart beating donors, elderly donors and hemodynamically unstable donors. For maintaining the function of marginal organs derived from these donor groups, machine perfusion could be superior to simple cold storage.(12) Finding suitable preservation protocols for expanding the donor pool, increasing organ utilization and improving posttransplant outcomes are very important future aspects in kidney preservation. Since many different preservation methods and perfusion protocols have been investigated in kidney preservation, the implementation of a registry could contribute to provide a better overview over these.(22) Also trials comparing different machine perfusion protocols will be necessary for improving kidney preservation.

Recently, based on the results of their review, Tingle et al. recommended the application of HMP in preservation of deceased donor kidneys, particularly in kidneys that are prone to delayed graft function.(151) Nevertheless, whether HMP has the potential to improve kidney and patient survival in the long run should be further investigated.

In any case, a better knowledge of kidney function during ex vivo perfusion is important not only for improving the evaluation of transplantability, but also for improving perfusion protocols and finding therapeutic agents that enable injured kidneys to be repaired.(22) Regarding therapeutical interventions, for example, the application of therapeutic agents carried by nanoparticles targeted to the place of action has the potential to find its way into organ preservation.(126) Further future approaches for therapeutic interventions include gene therapies and the application of mesenchymal stromal cells.(126,213) Also in the field of therapeutic

interventions, a lot of further research will be necessary before they can be adopted in clinical practice.

Regarding viability assessment of renal grafts, a single marker for reliably anticipating graft outcome during machine perfusion is still missing. Combining different markers or identifying novel markers might support graft assessment during machine perfusion in the future and should therefore be evaluated in further trials.(149)

Moreover, mammalian hibernation has become a topic of interest in kidney preservation, as in organ preservation in general, since adapting mechanisms of mammalian hibernation might contribute to improving the outcomes of organ preservation.(2,3,227) In mammalian hibernation long periods of hypothermia, called torpor, are usually interrupted by short episodes of normothermia.(227) The torpor goes in line with a decreased metabolic activity.(3) At the same time the organs of the hibernating animal don't get injured during torpor which is enabled by complex mechanisms that are not yet fully understood.(227) The torpor can be regarded as an equivalent to the hypothermic state in organ preservation while the transition to normothermia can be seen as an equivalent to rewarming and reperfusion in organ transplantation.(227) Better insights in the processes of mammalian hibernation could thus help developing strategies for reducing ischemia reperfusion injuries acquired during hypothermic preservation and rewarming of the preserved organ.(3,227) In addition to that, it may also enable remarkably longer preservation times.(227) But mammalian hibernation could not only be of interest in the field of hypothermic organ preservation but also for normothermic organ preservation as warm hibernators like lemurs show. An adaption of their hibernation mechanisms could be realized by inducing a warm torpor-like state in preserved organs as has been described by Hadj-Moussa and Storey.(228)

3.6 Pancreas Preservation

In comparison to transplantation of other solid organs, posttransplant graft failure and morbidity are highly significant in pancreas transplantation according to Branchereau et al.(229) Especially islet cells are very vulnerable to IRI and it is crucial to preserve and protect them well.(17,230) An improved pancreas preservation and a shorter cold ischemia time could contribute to an improvement of posttransplant results.(229,231)

2017 data on pancreas transplantation in the United States show that a high proportion of pancreata removed for transplantation is still discarded.(232) In particular that holds true for pancreata of donors aged 50 or older with a discard rate of 72.7%, whereas the discard rate of pancreata of donors aged 18 or younger was much lower (12.6%).(232) Moreover, the data shows a discard rate rising with the donor body mass index (BMI).(232) Furthermore, it is important that less than 3% of transplanted pancreata were grafts from donation after circulatory death donors.(232) Regarding the transplantation of marginal pancreas grafts the major limitations are development of a graft pancreatitis and of IRI.(233)

Expanding the donor pool in pancreas transplantation is not a new topic but has also been discussed in the past.(234-238) Using machine perfusion for pancreas preservation seems to have the potential for enabling an expansion of the donor pool.(14)

3.6.1 Static Cold Storage in Pancreas Preservation

Like in kidneys and livers, SCS still is the predominantly used method in pancreas preservation.(14) But this preservation technique causes damage in the graft which is increasing with time of SCS. Thus preservation time is an important limiting factor and should be as short as possible.(229)

SCS could be improved when an oxygen carrier is added to the preservation solution. M101 is such an oxygen carrier which can be added to preservation solutions for organ preservation under hypothermic conditions. This includes SCS as well as HMP. It might also have beneficial effects on graft quality not only in pancreas preservation. A decrease of necrosis and oxidative stress could, among other things, be responsible for these positive effects.(239)

Another potential method for improving the oxygen supply during SCS is a combination of SCS with oxygen persufflation. It aims at reducing ischemic injuries.(240) The effects of human pancreas preservation by such a combination before islet isolation were investigated in a study. The preservation by oxygen persufflation reduced inflammatory processes and increased metabolic gene expression. This in turn enhanced the function of β-cells. Moreover, the preservation time could be prolonged without any signs of negative effects.(240)

3.6.2 Machine Perfusion in Pancreas Preservation

After positive experience in kidney and liver preservation, the discussion about benefits of machine perfusion has also reached pancreas preservation and first trials on machine perfusion of the pancreas have already been performed.(14,17,231) An expansion of the donor pool to ECDs and DCD donors is also important for the future of pancreas transplantation. Currently pancreas grafts of DCD donors are not generally used because transplanting them could come along with an increased risk for complications.(241) However, SCS is not up to the increased vulnerability of pancreas grafts associated with the higher age and the higher rate of comorbidities of ECDs and the prolonged warm ischemia of DCD donors.(14) In contrast, the application of machine perfusion in pancreas preservation might support the expansion of the donor pool by enabling the transplantation of organs that would have been discarded in case of preservation by SCS. That applies to hypothermic as well as NMP since both might have beneficial effects on the preserved pancreas grafts.(14) But it isn't possible to simply apply the protocols used for perfusion of other organs in pancreas preservation as well. The physiological pancreas perfusion is characterized by low flow and pressure. Thus, the application of machine perfusion could injure the quite vulnerable endothelial lining of pancreatic blood vessels which would lead to thrombosis when the organ is reperfused.(14,231) Moreover, the pancreas is also susceptible to formation of oedema which poses a challenge to the application of HMP.(231)

So far there isn't much experience on machine perfusion in pancreas preservation. As a result, e.g. perfusion parameters and markers for viability assessment haven't been determined yet. Because of the missing experience further research on this topic is necessary.(14,17,242)

3.6.2.1 Hypothermic Machine Perfusion in Pancreas Preservation

Hamaoui et al. regard applying HMP in pancreas preservation as less complex than applying NMP.(231) Furthermore, it is also favourable that a failure of the perfusion system in HMP does not result in graft loss since the preservation can be continued as SCS. This is an important difference of HMP compared to NMP.(229)

3.6.2.1.1 Hypothermic Pulsatile Machine Perfusion

Branchereau et al. conducted a study on hypothermic pulsatile perfusion which was applied on 7 human pancreas grafts. For this purpose superior mesenteric artery and splenic artery were Y-anastomosed to the iliac bifurcation. The common iliac branch was then cannulated for machine perfusion. Additionally, there was a group of 2 pancreata that were perfused by hypothermic pulsatile perfusion in the head region while SCS was used for preserving the pancreatic tail.(229) Hypothermic pulsatile perfusion was carried out at a temperature of 4˚C using Perf-Gen® preservation solution. Branchereau et al. used a pressure controlled perfusion with a systolic pressure of 25 mmHg and a diastolic pressure of 10 mmHg.(229) As a control group 2 pancreata were only preserved by SCS using IGL-1 preservation solution. In all groups the preservation lasted for 24h.(229) All used pancreas grafts had been discarded before they were used for the trial.(229) While the perfused organs did not show any lesions after 12 hours, the grafts preserved by SCS showed signs of ischemic necrosis in the histological examination.(229) Hypothermic pulsatile perfusion for 24 hours did not cause oedema formation or necrosis of pancreas parenchyma or duodenal villi. Alterations of the immunostaining of insulin, glucagon and somatostatin were also not observable.(229) Not included in this study was the measurement of functional, dynamic and molecular markers. Since the grafts were not transplanted in the end, the study did not deliver any posttransplant results. Furthermore, IRI was not assessed since reperfusion was not done or at least imitated. Thus, further studies and randomized clinical trials including transplantation of the preserved grafts and posttransplant outcomes are necessary.(229)

An advantage of the method of pulsatile machine perfusion might be the stimulation of the expression of endothelial protective genes.(229)

3.6.2.1.2 End-Ischemic Dual Arterial Hypothermic Oxygenated Machine Perfusion

Leemkuil et al. were first to publish results on dual arterial hypothermic oxygenated machine perfusion of human pancreata in 2018.(230) They used hypothermic oxygenated machine perfusion for the preservation of 5 DBD and 5 DCD pancreata and compared the results to the results of 10 pancreata preserved by SCS.(230) Before hypothermic oxygenated machine perfusion was applied, the pancreata were stored by SCS during transport. Afterwards, for machine perfusion, the two main arteries of the pancreas, splenic artery and mesenteric

superior artery were separately perfused with a perfusion pressure of 25 mmHg for 6 hours. For perfusion they applied two centrifugal pumps. As perfusion solution University of Wisconsin Machine Perfusion Solution was used. The perfusion solution had a temperature of 4˚C to 7˚C and was oxygenated.(230) Oxygenated HMP is assumed to contribute to maintaining a residual metabolism with a distinctly decreased activity.(230) In line with this, during oxygenated HMP ATP concentrations were rising significantly in both, grafts derived from DBD and grafts derived from DCD donors. The DCD grafts started from a much lower ATP concentration, but reached the same level of ATP concentration as DBD grafts at the end of oxygenated HMP.(230) The increased ATP concentration observed in the HMP group may demonstrate a superior viability. Thus, oxygenated HMP may have beneficial effects, especially on DCD pancreata.(230) Furthermore, it was possible to perfuse the pancreata uniformly under hypothermic conditions without damaging the tissue.(230) An isolation of islets to evaluate their function was only performed in two pancreata and these islets were working properly.(230) A formation of oedema in the pancreatic tissue did not occur.(230) This might be an advantageous aspect in pancreas preservation, but it is still controversial, whether the formation of oedema in pancreatic tissue has negative or even beneficial effects on the outcome of islet isolation or not.(230,243-246) The feasibility of oxygenated HMP in pancreas preservation could be shown by Leemkuil et al. The results suggest that this technique might help expanding the donor pool to ECDs.(230) Nevertheless, it has to be considered that this trial ended with oxygenated HMP and the grafts were neither transplanted nor reperfused by NMP to imitate posttransplant conditions. Therefore, the effects of IRI couldn’t be evaluated, since reperfusion as a crucial part of it had not been investigated.(230) As a result further trails on this preservation technique are required.(230)

Hamaoui et al. developed a preclinical HMP protocol which they tested on 3 previously discarded human pancreas grafts.(231) After a long cold ischemia time of more than 24 hours the human pancreata underwent HMP for 5 hours at a pressure of < 20 mmHg using a modified University of Wisconsin solution. The pancreas grafts were perfused via splenic artery and superior mesenteric artery. Afterwards they were not transplanted. Instead, for imitating reperfusion, they were reperfused under normothermic conditions for 2 hours using an oxygenated perfusate based on Krebs-Henseleit buffer.(231) The decision for using a modified

University of Wisconsin solution supplemented by mannitol as perfusate for HMP was based on the following assumptions: on the one hand, mannitol as an osmotic agent could work against oedema formation. On the other hand, University of Wisconsin solution does not contain glucose which might be beneficial since glucose in the perfusion solution could unnecessarily stimulate pancreatic beta cells.(231) Hamaoui et al. measured the perfusion flow index during HMP and normothermic reperfusion and the oxygen consumption during normothermic reperfusion. Furthermore, the insulin secretion was tested during normothermic reperfusion by adding glucose to the perfusate 30 minutes after beginning of reperfusion and measuring the insulin and glucose levels in the effluent from portal vein at several points of time. Thereby, insulin secretion was stimulated in the human pancreata, but one human pancreas did not show endocrine function. In addition to that, amylase levels were determined as signs of cellular damage.(231) Furthermore, the grafts were examined regarding oedema formation and necrosis by histology.(231) While biopsies showed normal findings before HMP, necrotic areas were observable in the biopsies obtained after reperfusion.(231) In one pancreas a huge oedema was formed during normothermic reperfusion.(231) Of course, the results are strongly limited by the small number of preserved organs, but Hamaoui et al. were able to show that the use of HMP in pancreas preservation is feasible.(231) According to Hamaoui et al. further studies are required, especially for finding reliable markers for viability assessment.(231)

3.6.2.2 Normothermic Machine Perfusion in Pancreas Preservation

3.6.2.2.1 End-ischemic Normothermic Machine Perfusion

The first trial on NMP in preservation of the human pancreas was conducted by Barlow et al. and published in 2015.(233) 5 human pancreata were perfused of which 1 was excluded. The normothermic perfusion was performed for 1 to 2 hours using a perfusate at a temperature of 37˚ after a SCS of about 13 hours on average. They used a perfusate consisting of packed red blood cells, Gelofusine, mannitol, sodium bicarbonate, glucose and heparin. The perfusion system was pressure controlled with a pressure set at 50-55 mmHg. For oxygenation a gas mix of 95% oxygen and 5% carbon dioxide was used. The machine perfusion was performed via an iliac Y-graft.(233) The pancreas grafts were not transplanted. Thus, no posttransplant results were obtained.(233) Barlow et al. were able to show the feasibility of NMP in pancreas preservation but further research on this

topic is necessary, especially regarding viability assessment, clinical application and posttransplant results.(233) An important aspect is that machine perfusion and viability assessment should not further harm the pancreas graft in addition to the injuries occurring during organ procurement and SCS.(233) Injuries during NMP could be caused by active pancreatic enzymes in the perfusate which are not cleared from it and thus recirculate. Adding protease inhibitors to the perfusate might mitigate such harmful effects.(233)

Nassar et al. conducted a trial on NMP of 3 human pancreata from DBD donors. After an initial SCS for about 4 hours on average using histidine-tryptophane-ketoglutarate solution, the grafts were perfused under normothermic conditions for 6 or 12 hours via splenic artery, superior mesenteric artery and gastro-duodenal artery using packed red blood corpuscles and plasma in a 1:3 ratio as perfusate. They recommend a long lasting NMP for enabling the evaluation of organ viability.(242) The mean arterial blood pressure was about 60 mmHg and as perfusion rate Nassar et al. had chosen 55 ml/min/100 gm tissue.(242) As one of their main end points they had chosen the C-peptide level.(242) Additional major end points were the histology of pancreatic islets and the histology of the exocrine parenchyma of the pancreas.(242)

3.6.3 Markers for Viability Assessment of Pancreas Grafts

Before the preservation even begins, the assessment whether the pancreas of a potential donor could be used for pancreas transplantation is a first important step. In this context, Leemkuil et al. showed that an improved assessment of potential donors can considerably enhance the outcome of using donation after cardiac pancreata. DCD donors should not have any other important risk factors.(241) Supporting tools like the pancreas donor risk index can be used as part of the assessment.(241,247)

Already in 2003, Krieger et al. recommended not primarily focusing on donor characteristics like donor age or BMI in assessing whether a pancreas is suitable for transplantation, but also including qualitative assessment of the grafts in the decision making because this could contribute to an expansion of the donor pool.(236) The decision to discard a pancreas graft is often subjective according to Barlow et al. but it may be possible to objectify the decision making by establishing NMP which could enable a more objective evaluation of graft viability. This again might help lowering the discard rates. However, to make this possible, reliable

markers and measures for graft viability must first be found and determined(233), since they are still missing in pancreas preservation so far.(17) Markers proposed and used so far, include:

3.6.3.1 Endocrine and Exocrine Pancreatic Function

For viability assessment in NMP Hamaoui et al. suggest to consider endocrine and exocrine pancreatic function.(14) Concerning exocrine pancreatic function, Nassar et al. regard measuring amylase and lipase concentration as unsuitable for indicating organ viability or tissue damage. Instead they suggest measuring bicarbonate in duodenal secretions as possible marker for the exocrine function.(242)

Concerning the endocrine function, Nassar et al. state that the reliability of the insulin concentration as marker for organ viability is limited because an initial increase of the insulin concentration could be caused by cellular injury. Thus, a glucose stimulation test or a continuous increase of insulin concentration or C-peptide concentration might be required for a reliable evaluation of the endocrine function.(242)

Another way of assessing the endocrine function is the evaluation of the function of pancreatic islets. To do so, Branchereau et al. used an immunohistochemical evaluation via immunostaining for insulin, glucagon and somatostatin.(229)

3.6.3.2 Oedema Formation

Considering macroscopic oedema has been suggested for viability assessment of pancreas grafts in NMP.(14) As a marker for oedema Leemkuil et al. used the wet-to-dry weight ratio of the biopsy.(230)

Branchereau et al. don’t think that weighing the graft is a robust measure because of perfusion fluid in the duodenum. Instead, they favour macroscopic, histological and immunohistochemical examinations of the graft. In macroscopic examination they used a scale for evaluating the formation of oedema. Signs of oedema and necrosis were important aspects in the histological examination. (229)

3.6.3.3 Adenosine Triphosphate

ATP concentration or formation have been proposed as potential markers in hypothermic and NMP. (14,230)

3.6.3.4 Further Proposed Markers

For viability assessment in NMP Hamaoui et al. further suggested to consider aspects like signs for cellular apoptosis, inflammatory alterations of the endothelium and thrombosis, as well as the acid-base homeostasis and the oxygen consumption.(14) Branchereau et al. also proposed the perfusion resistance index as potential measure for graft assessment.(229)

3.6.4 Future Aspects in Pancreas Preservation

Hamaoui et al. defined several aspects of machine perfusion in pancreas preservation to be further investigated. These include the question whether an active oxygenation of the perfusate is beneficial.(14) Furthermore, ways for a reliable viability assessment need to be found. Important is that markers for viability assessment allow for a reliable estimation of the risk for a development of posttransplant complications including delayed graft function, thrombosis or graft pancreatitis. According to Hamaoui et al. a combination of markers might be superior to just relying on a single marker. Identifying potential benefits of therapeutic interventions applied during preservation is a further important aspect. Such interventions could help to improve posttransplant graft function.(14) In addition to that, the optimal timing of machine perfusion is still unclear. Should it be applied continuously from organ retrieval until reperfusion after transplantation? Should it be applied only in the end of the preservation process after SCS? Or should it be applied in an interrupted way? (14,229) Furthermore, the optimum perfusion pressure has not been determined yet. (229) Moreover, potential traumata caused by cannulation have not been investigated.(229) If answers on these open questions can be found, machine perfusion has the potential to enable an expansion of the donor pool to ECDs and DCD donors by diminishing preservation related injuries, by applying organ preconditioning for improving graft quality and by enabling pretransplant assessment of graft viability. Thereby, the number of wrongly discarded organs could be reduced.(231)

4 Discussion

SCS still is the predominantly used preservation method for liver, kidney and pancreas grafts. It will probably continue to have its place in organ preservation, either as a preservation method for low risk organs or as part of preservation in end-ischemic machine perfusion. However, a more widespread use of machine

perfusion is likely to become a reality in the future. This is suggested by the fact that, as mentioned above, there is some evidence of the advantages of machine perfusion, especially in the preservation of higher risk organs. Machine perfusion seems to have the advantage not only of attenuating IRI, especially in higher risk organs that are particularly prone to it, but also of enabling pretransplant viability assessment. Moreover, it seems to allow for longer preservation times and therapeutic interventions during preservation. Therefore, the use of machine perfusion seems to promise an expansion of the donor pool and better organ utilization.(52)

However, with regard to the current status of machine perfusion, the following aspects need to be considered: The rediscovery of machine perfusion in organ preservation has resulted in much research on this topic and in the meantime, multiple different approaches have been tried out. However, for many of these approaches, the studies have not yet gone beyond simply proving the safety and feasibility of the particular approach.(11,47,55,69,76,97,179) Though, many questions concerning machine perfusion remain unresolved, e.g. regarding the optimal setting of temperature, timing and duration of machine perfusion and the used perfusates.(17,169) To find answers on these questions, also randomized controlled trials comparing outcomes of different machine perfusion protocols are still missing.(73,89,151)

The variety of different preservation protocols regarding temperature, timing, oxygenation, perfusates, combination of different preservation methods and viability assessment leads on the one hand to experience with many different approaches of organ preservation, but on the other hand also to confusion, which makes comparisons difficult. In addition, there are also differences in outcome variables, which makes meta-analyses difficult to conduct. Furthermore, considerable differences regarding the description of methodology and nomenclature are observable, what impairs the comparability of different methods and their outcomes. This makes it even more complicated to carry out meta-analyses.(10) That’s why Karangwa et al. call for a standardized nomenclature in machine perfusion. Standardizing the description of methodology would finally enable objective comparisons between different trials and simplify meta-analyses.(10)

With regard to the evidence on machine perfusion, there are also obvious differences between the organs. In the field of machine perfusion of pancreas grafts there is by far the least experience. In liver preservation, on the other hand, there have been significantly more clinical trials on machine perfusion with many different preservation protocols. An important innovation in this field is ischemia-free NMP that has recently been introduced and may become an important method in liver preservation.(34) Moreover, the combination of hypothermic, rewarming and NMP, for instance, could also contribute to an expansion of the donor pool in liver transplantation as has recently been underlined by first study results.(111) In the field of machine perfusion of renal grafts, a lot of experience has already been gained, especially regarding HMP. Based on a recent meta-analysis, there is already a recommendation for the use of HMP in kidney preservation, especially for preserving kidneys with an increased risk of developing delayed graft function.(151) In addition to that, it has already been shown, that the application of HMP in kidney preservation is cost-effective.(167) But concerning NMP, an approach that has become an important topic in organ preservation research, there are no finished randomized controlled trials supporting its application in kidney preservation yet.(151)

Viability assessment of preserved grafts before transplantation is a further crucial topic in organ preservation. There are several different markers for viability assessment used and investigated in trials on machine perfusion in liver, kidney and pancreas transplantation. But, in general, the used markers often differed between the trials. Furthermore, meta-analyses can’t be done properly because the outcome variables often vary between the trials. As a result there is only very limited evidence for the validity of these markers so far.(30) Furthermore, single reliable markers have not yet been found.(22,29) Combinations of several markers may perhaps be established for assessing the viability of preserved grafts before transplantation.(14,30) Concerning viability assessment, NMP comes along with the best opportunities compared to other approaches of machine perfusion. That’s why some expect it to become the standard method in organ preservation.(29) But that is yet to be seen. Thus, a lot of further research on markers for viability assessment in liver, kidney and pancreas preservation is necessary.

Another key opportunity enabled by machine perfusion are therapeutic interventions for diminishing IRI and improving graft quality. Some of them have

been presented in this thesis. But concerning most of them, further research is also required.

Procedures that may lead to further improvements in organ preservation in the long run include supercooling of grafts or transferring them into a hibernation-like state for preserving them.(2,3,134,227) Another potential procedure could be the modification of grafts by first decellularizing and afterwards repopulating them with specific cells during organ preservation.(143-145)

In sum, it can be concluded that there is a trend towards more individualized approaches in organ preservation.(18) As a result, instead of a standard approach, different methods of organ preservation could be available in the future. In this case, the appropriate method would then be chosen according to the kind and quality of the organ and donor characteristics. This means, that choosing the right setting of organ preservation based on the individual case might be the most successful way in the future.(13,31)

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