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Renal Normothermic Machine Perfusion: The Road Toward Clinical Implementation of a Promising Pretransplant Organ Assessment Tool

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C.M. and I.J. conceptualized the paper. T.L.H., B.O., and J.D.B. reviewed the literature. T.L.H., B.O., and J.D.B. drafted the manuscript. V.A.L., M.B.F.P., L.H.V., H.G.D.L., I.J., and C.M. performed the critical revision.

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ABBREVIATION LIST

AST, aspartate aminotransferase

ATP, adenosine triphosphate

COR, controlled oxygenated rewarming

DGF, delayed graft function

FMN, flavin mononucleotide

MAP, mean arterial pressure

NMP, normothermic machine perfusion

PNF, primary nonfunction

RBC, red blood cell

SCS, static cold storage

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ABSTRACT

The increased utilization of high-risk renal grafts for transplantation requires optimization of pretransplant organ assessment strategies. Current decision-making methods to accept an organ for transplantation lack overall predictive power and always contain an element of subjectivity. Normothermic machine perfusion (NMP) creates near-physiological conditions, which might facilitate a more objective assessment of organ quality prior to transplantation. NMP is rapidly gaining popularity, with various transplant centers developing their own NMP protocols and renal viability criteria. However, to date, no validated sets of on-pump viability markers exist nor are there unified NMP protocols. This review provides a critical overview of the fundamentals of current renal NMP protocols and proposes a framework to approach further development of ex vivo organ evaluation. We also comment on the potential logistical implications of routine clinical use of NMP, which is a more complex procedure compared to static cold storage or even hypothermic machine perfusion.

Supplemental Visual Abstract; http://links.lww.com/TP/C232

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INTRODUCTION

The shift towards the utilization of older organ donors with more comorbidities has stressed the importance of robust pretransplant organ viability assessment. Firstly, organs from expanded criteria donors or those donated after circulatory death are more susceptible to ischemia-reperfusion injury, resulting in a higher risk of delayed graft function (DGF), primary nonfunction (PNF), and graft failure.[1-3] Secondly, many kidneys offered for transplantation are ultimately not transplanted because there is doubt about their capacity to provide adequate short- and long-term function.[2,4-6] These organs in particular would benefit from reliable pretransplant organ viability and quality assessment because a significant number of kidneys that are currently discarded, would presumably provide a favorable risk-benefit ratio to a proportion of waitlisted individuals.[7,8] A plethora of nonperfusion-based pretransplant quality assessment tools exist, most often consisting of regression-derived prediction models that incorporate clinical donor and recipient variables. However, none of these models demonstrate adequate predictive power to guide clinical decision-making for individual donor kidneys.[9-11] Hence, the urgent need for more objective and accurate pretransplant kidney quality assessment tools remains.

Renal normothermic machine perfusion (NMP) provides a near-physiological organ preservation technique because it circulates a warm (35–37ºC) perfusion solution through the renal vasculature delivering oxygen and nutrients.[12] At normothermia, cellular metabolism can resume and replenish adenosine triphosphate (ATP) synthesis,[12] which makes it likely that assessment of renal functional capacities, as well as the severity of the renal injury, might be performed during NMP.[13,14] In addition, initial clinical experience shows that NMP has the potential to increase the number of kidney transplants by evaluating and transplanting kidneys that had initially been discarded for transplantation.[15,16]

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The potential of NMP reaches beyond its diagnostic applicability, also encompassing the possibilities to serve as a promising superior preservation strategy[17] and a platform for active organ reconditioning.[18-20] Indeed, NMP reduces cold ischemia time and may mitigate the detrimental impact of ischemia-reperfusion injury.[20,21] Preclinical and early clinical experience suggests that NMP might reduce DGF compared to static cold storage (SCS).[22-25] The first randomized clinical trial comparing NMP with SCS is currently being conducted by the Cambridge group in the UK (ISRCTN15821205).[26]

Hence, in recent years, considerable effort has been directed at the development of NMP as either an organ preservation, assessment, or repair platform. This effort has resulted in an increased heterogeneity among NMP protocols since transplant centers tend to mainly use their own developed procedure. Striving for more uniformity in protocols could enhance the progression towards standardized NMP viability criteria.

Although NMP as an ex vivo organ evaluation platform may seem intuitive and technologically within reach, the key question in this regard is what to assess while a kidney is on the pump.

In this review, we give an overview of the fundamentals of preclinical large animal and clinical NMP protocols used by several leading centers, because no single unified NMP protocol exists to date. We also provide a framework to consider when assessing kidney viability during NMP and discuss the logistical and economic impact that clinical implementation of NMP is likely to have on the renal transplant field.

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RENAL NMP PROTOCOLS

NMP uses extracorporeal membrane oxygenation and compound supplementation technology to provide the kidney with an oxygenated and nutrient-enriched perfusate throughout the NMP period. The different fundamental facets of NMP protocols can be roughly divided into the perfusate composition, arterial pressure delivered by the pump, oxygenation, temperature, and urine replacement (Figure 1).

Perfusate composition

The perfusate's composition is not only of critical importance to ensure optimal organ preservation, it is also likely to affect the interpretation of potential renal viability markers during NMP. Hence, the chosen perfusate composition might depend on the specific aim of the application of NMP (ie, preservation, viability assessment, or repair). Almost all NMP protocols described so far use red blood cell (RBC)-based perfusates to ensure adequate tissue oxygenation. Nevertheless, recent preclinical research suggests that synthetic oxygen carriers feature equivalent oxygen-carrying capacities compared to RBCs.[27,28] NMP perfusates are typically supplemented with different compounds to provide nutrients that aim to preserve renal cell viability. The Cambridge group use a perfusate based on Ringer's solution, which has a relatively low oncotic pressure (Table 1).[12] This perfusate has been used extensively in their preclinical and clinical work, with typically short (~1h) NMP durations in their most recent studies.[26] Their perfusate was initially developed to reperfuse kidneys after a long period of SCS with the aim of organ reconditioning and assessment 1-2 hours prior to transplantation. The Toronto group employ a perfusate based on Ringer's lactate and STEEN solution, which creates a physiological oncotic pressure and osmolarity.[24,29] This NMP solution was developed to evaluate NMP as a technique to preserve organs for a longer period and it has been successfully utilized in prolonged normothermic kidney perfusions of up to 16 hours in a porcine autotransplantation model.[17] Another perfusate that is based on human albumin and

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electrolytes within physiological ranges is used by the MePEP consortium (Groningen and Rotterdam in the Netherlands, Oxford in the UK, and Aarhus in Denmark). This perfusate has mainly been used in porcine studies where NMP is combined with the addition of mesenchymal stem cells to repair kidneys during ex vivo perfusion.[30,31] The Oxford group have published a discarded human kidney NMP study in which a somewhat similar perfusate based on 5% human albumin solution was used. This perfusate was designed for prolonged renal perfusions that lasted up to 24 hours.[32] Groups in Brussels, Cleveland, Essen, Sacramento, Sydney, and Rotterdam have also conducted renal normothermic perfusion experiments, based on the existing perfusates used by the Cambridge and Toronto groups.[33-38]

In order to maintain a stable near-physiological environment during perfusion, various compounds are typically infused (Table 1). Although the necessity of individual additives has remained largely unstudied, almost all centers seem to agree on supplementing their perfusate with a vasodilator and glucose. Insulin is also added by the Cambridge, Toronto, and MePEP groups in various concentrations to facilitate glucose absorption. It remains challenging to reliably appreciate the individual merits of existing renal NMP perfusates, due to the great diversity in formulations between centers. To date, no study has convincingly investigated the roles of individual perfusate components or even compared existing fluids side-by-side to establish which components could lead to an optimal NMP, as well as the best posttransplant outcome.

Arterial pressure provided by the pump

In the literature, there is no consensus about the optimal perfusion pressure during NMP, whether it should be applied in a pulsatile or nonpulsatile fashion, and whether a centrifugal or roller pump is best. Most groups use centrifugal pumps, which are considered to be less harmful to RBCs compared to roller pumps especially during prolonged perfusions.[39] Although most groups apply a continuous pressure during normothermic perfusion, there is some evidence that

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pulsatile pressure during NMP results in enhanced renal blood flow, creatinine clearance, sodium reabsorption, and lower tubular injury.[40] Reported mean arterial pressure (MAP) during renal normothermic perfusion range between 40 and 95 mmHg.[26,34,40-42] Preclinical studies conducted by Hosgood and colleagues found superior outcomes for higher MAPs (75 and 95 mmHg) compared to 55 mmHg in terms of renal function and endovascular injury during NMP and subsequent simulated reperfusion.[43,44] They now typically set their pressure at 75 mmHg. The Toronto group set their pump speed to a fixed rate that induces a MAP of 75 mmHg at the start of NMP with an observed drop in perfusion pressure to 65 mmHg over time.[25]

Oxygenation

Most experimental kidney NMP systems use a supraphysiological perfusate oxygen concentration of approximately 550-650 mmHg. Administered oxygen is typically balanced with a small percentage of carbon dioxide to create optimal acid-base homeostasis. However, hyperoxia can promote reactive oxygen species production, resulting in additional renal injury.[45,46] The Cambridge group investigated the effect of altered oxygenation during NMP (95% O_2 / 5% CO_2, pO_2 550 mmHg; 25% O_2 / 5% CO_2 / 70% N_2, pO_2 206 mmHg; 12% O_2 / 5% CO_2 / 83% N_2, pO_2 81 mmHg).[47] In their study, a reduction in oxygen concentration led to a decrease in oxygen kinetics (ie, oxygen delivery, extraction, and consumption) but did not significantly influence tubular function, creatinine clearance, urine output, or biomarkers of renal injury during simulated reperfusion after NMP. However, further work on altering oxygen concentration during NMP should also incorporate assessment of oxidative damage.

Oxygen consumption during NMP is often reported. The most commonly used equation was introduced by Stubenitsky et al in 2000 [(arterial pO_2 – venous pO_2) x perfusate flow rate/weight].[48] Over the past years, more complex formulas to express oxygen consumption have been derived based on insights into various aspects of ex vivo renal physiology.[27,47,49-53] Most of these formulas also take the oxygen bound to an oxygen carrier into account, which

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more adequately reflects the total oxygen content when an oxygen carrier is added to the perfusate.

Temperature

Typically, the temperature during NMP is set at 37ºC, but this might be different, depending on the specific aim of ex vivo perfusion. Preclinical work has shown that, upon simulated organ reperfusion, tubular and renal function was better preserved when normothermic (37ºC), instead of subnormothermic (32ºC), perfusion preceded it.[54] Nevertheless, the subnormothermic perfusion system used by Brasile et al did not seem to cause relevant renal injury and showed superior posttransplant urine production and serum creatinine levels compared to nonperfused kidneys.[48] An important question is whether normothermia should be induced abruptly or gradually. It has been suggested that controlled oxygenated rewarming (COR) improves cellular homeostasis and mitigates rewarming injury in a porcine NMP model.[35,55] COR also enhances early posttransplant cortical microcirculation, thereby preventing the renal cells from being jeopardized by pumping against a high cortical resistance.[56] Gradual rewarming has typically been pursued up to 20ºC over a period of 90 minutes. As rewarming the perfusate gradually between 20ºC and 35ºC showed no additional protection, the upper limit to which the perfusate should be gradually rewarmed, starting from cold (0-7ºC) preservation, is suggested to be approximately 20ºC.[55] Ischemia-free kidney transplantation is another promising strategy to mitigate ischemic and hypothermia-induced injury. This logistically challenging method connects the kidney to the NMP device during organ procurement in such a way that ischemia associated with procurement, preservation, and implantation is avoided altogether.[57]

Urine replacement

Loss of circulating volume by urine production of the ex vivo perfused kidney should be replaced to maintain the circuit's circulating volume. The Toronto and Cambridge groups replaced this volume by adding Ringer's solution or Ringer's lactate to the perfusate.

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Alternatively, Weissenbacher et al showed that recirculating the urine is feasible and that it results in a significantly higher perfusate flow rate, as well as a revitalized metabolism determined by upregulated levels of ATP synthase, NADH dehydrogenase, and oligosaccharyltransferase.[58,59] Blum et al have applied urine recirculation in their experiments as they hypothesized that replacing proteinuric and hematuria urine with Ringer's lactate would lead to the gradual depletion of oncotic pressure and impair oxygen-carrying capacity.[34] Nevertheless, the Toronto group have performed stable perfusions for up to 16 hours without the use of urine recirculation.[60] Further preclinical investigation of urine recirculation during NMP is required to determine its full potential.

DIAGNOSTIC POTENTIAL

Even though the potential of NMP as a diagnostic platform has been recognized, the search for relevant and independently predictive viability markers has only just started and is likely to increase with wider clinical implementation of NMP. A detailed overview of potential biomarkers during NMP and their preclinical and clinical validation is displayed in Table 2. In the next paragraphs, we will summarize current knowledge on kidney viability assessment during NMP. For a more detailed review of the proposed framework, we refer to De Beule et al.[14]

Assessing nephron function and injury

Creatinine clearance and fractional sodium excretion are frequently reported as markers to assess nephron function.[36,47,50,61,62] It is not known whether these parameters during NMP are predictive for posttransplant function. Importantly, since perfusate composition and perfusion pressures will change hydrostatic and oncotic pressures, they influence filtration and ultimately production and composition of "urine". In addition, an ex vivo perfused kidney experiences no humoral influences, which are essential to maintain near-physiologic tubular function. As a result, typical renal functional markers such as those mentioned above, which are derived from

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our clinical in vivo reference frame, are unlikely to be useful for ex vivo organ viability assessment. Metabolic activity and oxygen consumption are high in tubular cells and therefore oxygen consumption has been proposed as a marker of kidney metabolic activity in animal models.[47,50,62,63] It is currently unclear how and if oxygen consumption indeed reflects viability as there is evidence that oxygen consumption in the kidney during NMP is dependent on the oxygen concentrations offered.[47] Injured and dying cells shed or leak cytosolic and mitochondrial content that could be used as injury markers in urine or perfusate and some of these are cell-specific; eg, kidney injury molecule-1 (KIM-1) originates from proximal tubular cells and neutrophil gelatinase-associated lipocalin (NGAL) originates from the thick ascending limb.[64-66] As distal tubular medullary segments (medullary thick ascending limbs and medullary collecting ducts) are more susceptible to ischemia compared to proximal tubular segments located in the outer medulla or the cortex, biomarker patterns might be informative on the location of the injury.[67] Additionally, there seems to be an inherent sensitivity of proximal tubular cells to warm ischemic injury whereas cold ischemia elicits distal tubular injury, with different patterns of response.[68] Non-cell-specific injury markers aspartate aminotransferase (AST) and lactate correlated with posttransplant renal graft function, measured by peak serum creatinine.[25] Flavin mononucleotide (FMN), a lesser-known biomarker, has also been shown to correlate with posttransplant renal graft function. In a pilot study, significantly higher levels of FMN during NMP were found in kidneys with DGF and PNF after transplantation.[69] The scarce data investigating perfusate or urine biomarkers during NMP originate from animal studies or small case series and none have been validated in large cohorts of kidney transplants.[25,62]

Assessing the vascular compartment

Endothelial damage is an important determinant of renal viability. Due to the limited regenerative capacity of endothelial cells, microvascular damage in the kidney has an adverse effect on long-term graft survival.[70] In vivo, endothelial damage and viability are reflected by

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an increase in vascular resistance related to a combination of disruption of the endothelial cell lining favoring thrombosis and the "no-reflow" phenomenon, which is the suboptimal restoration of perfusion after a period of ischaemia.[71,72] Tietjen et al showed the presence of RBC plugs in both cortex and medulla during 4 hours of NMP of discarded human kidneys, and these likely contribute to injury and the no-reflow phenomenon.[73] Little is known about the meaning of flow and resistance during NMP, although with increasing perfusion time, flow is usually seen to increase while resistance drops if perfusion pressures are maintained constant. Flow—not resistance—is one of the parameters of the kidney quality assessment score developed by the Cambridge group which combines macroscopic appearance, renal blood flow, and urine output during 1 hour of NMP performed at the end of SCS.[74] The use of this score has provided some proof that NMP kidney viability assessment can lead to transplantation of initially discarded kidneys.[15] It is, however, important to note that absolute values of renal blood flow and urine production will strongly depend on perfusion pressure settings as well as perfusate composition (additives and oncotic pressures). Therefore, scores such as the kidney quality assessment score might not necessarily be transferrable to other settings where different pumps, perfusate compositions, and additives are used. As healthy endothelial cells respond to vasoactive substances, the disappearance of such a vasoactive response could convey information about endothelial dysfunction and might be incorporated as a variable in viability testing. In a porcine model of NMP, Bath et al showed that kidneys, exposed to 2 hours of warm ischemia, did not elicit any vasodilating capacity when exposed to acetylcholine, suggesting irreversible injury of endothelial cells.[75] On the other hand, kidneys exposed to 16 hours of cold ischemia only showed a diminished response to acetylcholine.[75]

Assessing the immune (cell) compartment

In vivo, ischemia-reperfusion injury causes sterile inflammation, triggering activation of innate and adaptive immune systems, as well as leukocyte recruitment that is reinforced by cytokine

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and chemokine release.[76,77] Additionally, the endothelium and epithelial cells play a key immunological role in this postreperfusion inflammatory response.[71,78] Removal of circulating leukocytes from the NMP perfusate is thought to minimize inflammation compared to whole blood perfusion. However, it is interesting to note that whole blood perfusion showed lower AST levels compared to a red-cell-only-based perfusate in a model of pig liver NMP.[79] This illustrates that the ischemia-reperfusion cascade, its feedback loops, and its effects during ex vivo perfusion are insufficiently understood. Furthermore, despite the absence of circulating leukocytes during NMP, resident leukocytes are released. It is unclear what the implications of the presence and release of resident leukocytes are and whether the phenotype and behavior of these cells could be predictive of posttransplant outcomes.[36,80,81] The use of a leukocyte filter during 3 hours of ex vivo porcine lung NMP resulted in reduced T cell infiltration posttransplant compared to controls.[82] It has been established that inflammatory cytokines are released during kidney perfusion, although it is currently unclear which cytokines could be predictive of outcome. The use of a cytokine filter during 6 hours of pig kidney NMP reduced levels of interleukin-8 (neutrophil-attractant) and interleukin-6 (a proinflammatory cytokine) when compared to control.[42] Nevertheless, no difference in kidney function during NMP could be noted and these kidneys were not actually transplanted.

Long-term renal function

Currently, almost all NMP studies investigate biomarkers correlated with acute injury and short-term graft survival. Since most acute injury restores after transplantation; chronic renal damage will determine long-term graft survival. Predicting long-term posttransplant renal graft survival during NMP would make this technique particularly valuable as a pretransplant diagnostic tool. The main reason for late graft loss is the progression of renal fibrosis, which is mainly the result of the continuous alloimmune response to the donor graft despite immunosuppression.[83] This arises due to the deposition of unrecognized donor-specific

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antibodies or de novo antibodies on allograft capillary endothelial surfaces, which are produced after kidney transplantation and activate both coagulation and complement.[84] Thereby, pre-existing renal fibrosis in donor grafts is associated with diminished long-term graft survival, in which the severity of fibrosis correlates with the duration of graft survival.[85] Furthermore, long-term outcomes are also affected by the quality of the donor organ, which is mainly determined by the biological age of the donor.[83,86] The donor organ quality can also be affected by factors during donation and transplantation. It has been suggested that prolonged warm ischemia time and anastomosis time are associated with adverse long-term outcomes.[87,88] However, more clinical studies are needed to confirm this. Regarding recipient factors, increased (biological) age, recurrence of native kidney disease, anti-HLA immunization, ethnic background (African American), longer time on dialysis, and cardiovascular complications at the time of transplantation are associated with adverse long-term outcomes.[86] Since long-term graft survival and function are determined by multidimensional factors, an integrative approach may be required for pretransplant outcome prediction that combines viability measurements during NMP with donor, organ, and recipient characteristics, which all come with their own multifaceted complexity.

WHEN TO START NMP

The optimal timing to start NMP largely depends on the aim of its clinical application, ie, preservation, viability assessment, or repair; all necessitating an individual tailored strategy (Figure 2). The potential of NMP to ameliorate renal preservation and serve as a repair platform, most likely demands prolonged NMP times (strategy 1). Using NMP as an assessment tool presumably has a wider range of options (strategy 2, 3 & 4). Today, a short period of NMP at the recipient center (strategy 3) has been most commonly reported.[26,89,90] This strategy provides the advantage to assess organ quality just prior to transplantation, also taking the effects of hypothermic-induced injury into account. Another possibility is to assess organs immediately

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after retrieval at the donor hospital (strategy 2). Applying NMP at the donor center avoids the complex logistics and safety issues associated with NMP during organ transport. Moreover, depending on a country's geographical area, expertise and experience of individual centers, the complexity of the various time-related strategies can be reduced by centralizing clinical renal NMP to larger hubs (strategy 4). This could also enhance the quality and allows better standardization of the procedure, as has been proposed for normothermic lung perfusion.[91]

LOGISTICAL & ECONOMIC IMPLICATIONS

NMP is technically complex, time-consuming, and entails a risk of technical failure, which would leave the organ exposed to ischemia at normothermic temperatures. Today, no truly "stand-alone" renal NMP devices are available, requiring any perfusion to be supervised at all times. Clinical implementation of NMP will result in structural changes to the current donation and transplantation logistics. This will most likely necessitate transplant centers to establish a specialized perfusion room or use existing operating theatres for the sterile conduct of NMP. In addition, dedicated staff, trained in organ perfusion, will need to join the surgical teams. Depending on when NMP is initiated in the donation and transplantation cascade (Figure 2), dedicated personnel may have to travel to the donor center or run organ perfusion hubs.

The actual costs associated with the clinical implementation of NMP remain largely unknown. Access to out-of-hours specialist expertise will be mandatory to ensure a proper course of action and this will likely be an important determinant of expenses.[92] Other major costs comprise the NMP disposables, perfusate components, equipment needed to obtain samples, analyses for viability assessment, facility fees, NMP training, and depreciation of the perfusion device. Future clinical trials comparing NMP with SCS or hypothermic machine perfusion should also incorporate cost-effectiveness studies. Moreover, clinical studies aimed at determining whether renal NMP can increase organ utilization rates would benefit from the inclusion of cost-effectiveness outcomes. Indeed, for liver transplantation, it has already been shown that NMP

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has the potential to salvage a substantial number of organs from discard.[89,93-96] In this regard, it has been suggested that the extent to which viability assessment of discarded livers alleviates the organ shortage outweighs the additional costs of NMP.[97] This could ultimately also be the case for NMP of deceased-donor kidneys.

PROSPECTS

Finding novel biomarkers and elucidating pathophysiological processes can eventually pave the way for meaningful pretransplant kidney assessment, followed by active initiation of regeneration by targeting the associated pathways ex vivo. Two promising innovative diagnostic approaches could help to accomplish this. One of these is multiomics analysis. The multidimensional biological complexity of assessing renal allograft function and predicting posttransplant outcome mandates an integrative approach with the implementation of multiomics data (for example, a combination of genomics, transcriptomics, proteomics, and metabolomics).[98] Such a multilayered omics approach has an unprecedented potential to find novel biomarkers by enabling hypothesis generation with fewer a priori assumptions and could be performed during NMP.[99] Once relevant biomarkers and pathways have been identified, analyses could be simplified to rapid point-of-care measurements, which will fit into the typically very short pretransplant assessment time window. In addition, multiomics-based understanding of ex vivo renal physiology could result in powerful models capable of mapping disease phenotypes and renal graft outcome.[98,100] Implementing such approaches during NMP could ultimately alleviate the organ shortage by simplifying as well as optimizing the decision-making process with regard to organ acceptance and discard. A second tool to advance pretransplant kidney viability assessment during NMP is through innovative imaging methods.[11,101-103] Near-infrared spectroscopy (NIRS), (functional) MRI ([f]MRI), positron-emission tomography (PET), contrast-enhanced ultrasound (CEUS), ultrafast ultrasound imaging (UUI), laser speckle imaging (LSI), and multiphoton microscopy (MPM) imaging all

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have the potential to unravel renal physiological processes during NMP in a noninvasive manner (Table 3). Additionally, these methods may provide more information about regional differences in the functional properties of the ex vivo perfused kidney. These techniques could be a valuable add-on to renal viability assessment during NMP in the near future.

CONCLUSION

An increasing number of centers are investigating renal NMP, either as a preservation tool, a viability assessment tool, or a repair platform. Great diversity exists among NMP protocols and interpretation of the read-outs during NMP. Moreover, to date, no validated (set of) ex vivo viability biomarkers have been identified. To establish effective preservation by NMP, as well as use NMP as an objective pretransplant organ assessment tool and eventually interpret NMP data on a standardized global basis, more uniformity in NMP protocols is of paramount importance. Best practice guidelines and consensus on protocols would likely progress the field. Future research should focus on identifying the ideal perfusate composition, perfusion duration and pressures, and the need for urine recirculation and specific additives, for each application area of renal NMP.

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Figure Legends

Figure 1. Schematic representation of a normothermic machine perfusion setup with the most commonly used components, as well as an indication of typical strategies to approach important aspects of the perfusion procedure.

Figure 2. Different applications of renal normothermic machine perfusion. (1): normothermic machine perfusion (NMP) for the entire preservation interval. (2): a short period of NMP at the donor hospital followed by cold preservation ([CP], either static cold storage or hypothermic machine perfusion) for transportation to the recipient center. (3): NMP at the recipient center only. (4): an intermittent period of NMP which could be executed in an organ hub or at the recipient center, after which kidneys are again preserved with CP. Reproduced from Jochmans et al[146] with permission from the publisher.

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Table 1. Perfusate constituents per research group

Components	Cambridge	Toronto	MePEP	Oxford
Red blood cells	1 unit[12]	125 ml[24]	170 ml[30] 288 ml[31]	1 unit[32]
Main component	200 – 400 ml[12] 500 ml[104] 1000 ml[47,54] Ringer's solution	200 ml Ringer's lactate + 150 ml STEEN solution[a,24]	250 ml of 50 g/l human albumin solution[30]	250 ml of 50 g/l human albumin solution[32]
		175 ml Ringer's lactate + 150 ml STEEN solution[a,29]	300 ml sodium chloride 0.9% + 100 ml of 200 g/l human albumin solution[31]	
Mannitol	20-25 ml, 10%[12,105] 5 g[47,54] 10 mg[104]	n/a	10 mg[30] 0.016 g[31]	10 ml, 10%[32]
Calcium gluconate 10%	n/a	1.8 ml[24]	3 ml[30] 4.8 ml[31]	10 ml[32]
Sodium bicarbonate 8.4%	12 ml[104,105] 10-40 ml[12]	8 ml[24]	5 ml[30] 8 ml[31]	5–15 ml[32]
Anticoagulants	2-4 ml heparin 1000 IU/ml[12]	1000 IU heparin[24,29]	n/a	n/a
Antibiotics	750 mg cefuroxime[104]	n/a	300 mg[30] 8 ml[31] Augmentin	750 mg cefuroxime[32]
	10 ml Augmentin 1.2 g[12]			
Other ingredients	2 ml (4 mg)[12] 2 ml (10 mg)[105] 3.3 mg/ml[47,54] 10 mg[104] dexamethasone	27 ml DRO filtered water[24,29]	6 ml glucose 5% + 5 IU insulin[30]	n/a
			9.6 ml glucose 5% + 8 IU insulin[31]	
Additives				
Vasodilator	0.5 mg epoprostenol	Verapamil (intraarterial)	1.25 mg[30] 2.5 mg[31] bolus	0.5 mg epoprostenol

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	Rate: 4 ml/h,[12] 5 ml/h[47,54]	Rate: 0.25 mg/h[24,29]	verapamil at the start of NMP Continuous infusion of verapamil during NMP (0.25 mg/h)[30,31]	Rate: 4 µg/h,[32] rate not described[59]
	25 mg sodium nitroprusside Rate: 25 ml/h (during the first hour)[104,105]			Verapamil, rate not described[59]
				Glyceryl trinitrate, rate not described[59]
Glucose	Glucose 5% Rate: 7 ml/h[12]	Amino acids and glucose (intravenous) 0,5 ml/h[24,29] Target glucose concentration: 5-15 mmol/l	Glucose 5% Added at a rate of 4ml/h if perfusate glucose levels became <4.5 mmol/l[31]	Bolus 2.5 ml of a lipid-free parenteral nutrition solution (total parenteral nutrition) if glucose dropped below 4 mmol/l
Other additives	Nutriflex infusion[12] was added together with the following: 100 units insulin 25 ml sodium bicarbonate 8.4% 5 ml multivitamins Rate: 20 ml/h	Insulin (intravenous) 5 IE/h[24,29]	60 mg[30] 1.6 ml[31] Augmentin was added every hour	n/a
			Calcium gluconate 10% Adding if perfusate ionic concentration was <1.1 mmol/l[31]	

DRO, double reverse osmosis; n/a, not applicable.

[a]Dextran 40, 5g/L; sodium chloride, 86 mmol/L; potassium chloride, 4.6 mmol/L; calcium chloride dihydrate, 1.5 mmol/L; sodium dihydrogen phosphate dihydrate, 1.2 mmol/L; sodium bicarbonate, 15 mmol/L; magnesium dichloride hexahydrate 1.2 mmol/L; D(+)-glucose monohydrate, 11 mmol/L; human serum albumin (200 g/l), 70 g/L; sodium hydroxide (1 M); sterile water.[106]

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Table 2. Potential biomarkers during NMP and their preclinical and clinical validation

Biochemical marker	Source	Site of measurement	Application during NMP	Validation *Preclinical and clinical*
NGAL	Cells of the thick ascending limb	Perfusate/blood and urine	**Cambridge**[15,26,42,44,61,62,107] **Toronto**[23,60,108] **Oxford**[32,59] **Other**[30,109]	**Preclinical** A higher level of urinary NGAL after NMP was associated with higher EVKP scores, reflecting more severe renal injury.[62] **Clinical** Some research suggests that NGAL is a promising biomarker to detect early posttransplant graft function.[110–114] However, NGAL measured during HMP failed to show an independent relationship with DGF, PNF, and 6-months overall graft loss and recipient mortality.[115] Moreover, 1 day posttransplant measured NGAL did not correlate with 1-year graft function.[116]
LDH	Parenchymal cells	Perfusate/blood	**Cambridge**[117,118] **Toronto**[17,23-25,49,60,108] **Other**[53,109,119]	**Preclinical** LDH measured during NMP did not correlate with posttransplant renal function in a porcine autotransplantation study.[25] **Clinical** In a DCD kidney transplantation study, LDH measured during HMP was associated with PNF.[120] However the diagnostic and predictive accuracy of PNF is relatively poor.[120,121]
AST	Parenchymal cells	Perfusate/blood	**Cambridge**[117,118,122,123] **Toronto**[17,23-25,29,49,60,108] **Other**[53,109,119]	**Preclinical** A positive correlation has been shown between AST levels during NMP and posttransplant peak serum creatinine in a porcine autotransplantation model.[25] Extended periods of warm ischemia are associated with higher AST levels during NMP.[118]
Lactate	Parenchymal cells	Perfusate/blood	**Toronto**[17,23-25,29,49,60,108] **Oxford**[59] **Other**[52,119]	**Preclinical** Increased warm ischemia times are associated with diminished renal lactate clearance during NMP.[17,25]

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KIM-1	Proximal tubular cells	Perfusate/blood, urine, and tissue	**Cambridge**[62] **Oxford**[32,59]	**Preclinical** Urinary KIM-1 levels were not associated with perfusion parameters or renal function in the donor.[62] **Clinical** Posttransplant urinary KIM-1 is an independent predictor of graft loss.[124] Pretransplant tubular expression does not predict DGF but correlates with the degree of interstitial fibrosis in humans.[125,126] When measured in the perfusate during HMP, KIM-1 failed to show an independent relationship with DGF.[115]
L-FABP	Proximal tubular cells	Perfusate/blood and urine	**Cambridge**[26]	**Clinical** Higher urinary L-FABP concentrations are associated with slightly lower 6-month eGFR, only among recipients without delayed graft function.[114] Higher urinary L-FABP levels, obtained directly after transplantation, has been associated with significantly lower 2-year eGFR.[127]
FMN	Mitochondrial complex I	Perfusate/blood	**Cambridge**[69]	**Clinical** FMN levels during liver HMP were predictive of graft loss within 3 months after liver transplantation.[128] FMN levels during renal NMP were significantly higher in the kidneys that developed DGF and PNF.[69]
π-GST	Distal tubular cells	Perfusate/blood and urine	**n/a**	**Clinical** Elevated π-GST end-HMP levels have been independently associated with DGF.[121,129]
ET-1	Tubular cells	Perfusate/blood and urine	**Cambridge**[61,62,130]	**Preclinical** Increased urinary levels of ET-1 were associated with higher EVKP scores.[62] A period of warm ischemia is associated with higher urinary ET-1 levels during NMP.[61]
VWF	Endothelial cells	Perfusate/blood and tissue	**Cambridge**[123] **Other**[53]	**Preclinical** In a porcine study, there was no significant difference in secreted VWF between SCS, HMP, and NMP upon simulated reperfusion.[123]
VCAM-1/ ICAM-1	Endothelial cells	Perfusate/blood and tissue	**Other**[53,131]	**Clinical** VCAM-1 gene polymorphism has shown to be a risk factor for dialysis after transplantation.[132] ICAM1 genotype is an independent risk factor for

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				increased creatinine concentration after 12, 24, 36, 48 and 60 months of transplantation.[132]
TBARS	Lipid peroxidation	Perfusate/blood and tissue	**Other**[109,119]	**Clinical** MDA levels shortly after kidney transplantation were higher in DGF patients. Values at 7 days posttransplant represented an independent predictor of 1-year graft function.[133]
Protein carbonyls	Protein oxidation	Perfusate/blood and tissue	**Cambridge**[105,134]	**Preclinical** A significant correlation has been shown between protein carbonyls and creatinine clearance during NMP with extended warm ischemia times.[134]
8-isoprostane	Lipid peroxidation	Perfusate/blood	**Cambridge**[105,134]	**Preclinical** Plasma levels of 8-isoprostane, 60 minutes posttransplant, were significantly lower in the group of NMP compared to HMP in a porcine autotransplantation model.[105]
ATP content /ATP:ADP ratio	Parenchymal cells	Tissue	**Cambridge**[134,135] **Other**[119]	**Preclinical** In a study with discarded human livers, a significant increase in ATP content was observed after 3 hours of subnormothermic machine perfusion and a statistically nonsignificant increase in ATP:ADP ratio.[136]

ADP, adenosine diphosphate; AST, aspartate aminotransferase; ATP, adenosine triphosphate; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; ET-1, endotheline-1; EVKP, ex vivo normothermic kidney perfusion; FMN, flavin mononucleotide; π-GST, pi glutathione S-transferase; HMP, hypothermic machine perfusion; ICAM-1, intercellular adhesion molecule 1; KIM-1, kidney injury molecule-1; LDH, lactate dehydrogenase; L-FABP, liver-type fatty acid-binding protein; n/a, not applicable; NGAL, neutrophil gelatinase-associated lipocalin; NMP, normothermic machine perfusion; PNF, primary nonfunction; SCS, static cold storage; TBARS, thiobarbituric acid-reactive substances; VCAM-1, vascular adhesion molecule 1; VWF, Von Willebrand factor.

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Table 3. Examples of innovative imaging techniques that could be applied during renal NMP

Imaging technique	Modalities
Near-infrared spectroscopy (NIRS)	NIRS is a diffuse optical technique that uses the near-infrared region of the electromagnetic spectrum to measure oxy-, deoxy-, and total hemoglobin oxygen saturation in the microcirculation.[137]
MRI	MRI is a class of static and functional imaging methods developed to demonstrate regional, time-varying changes in physiological processes. Promising functional MRI methods are based on blood oxygenation level-dependent (BOLD) contrast and arterial spin labelling (ASL) perfusion contrast.[138] BOLD MRI is dependent on the paramagnetic features of deoxyhemoglobin to indicate perfusate oxygenation levels; ASL MRI magnetically labels perfusate flowing into the ex vivo kidney, allowing the assessment of renal perfusion without a contrast agent.[139]
Positron-emission tomography (PET)	PET is a medical imaging modality that uses radioisotope-labelled substances that emit positrons and act as molecular probes to display and measure biochemical processes.[140]
Contrast-enhanced ultrasound (CEUS)	CEUS uses microbubbles as a non-nephrotoxic contrast-agent and offers high-resolution mapping of the microvasculature of the kidney.[141]
Ultrafast ultrasound imaging (UUI)	UUI is based on the unfocused transmission of plane waves.[142] It can be used to display and quantify tissue stiffness, perfusate motion, and contrast dynamics with high frame rates.[143]
Laser speckle imaging (LSI)	LSI applies an infrared illumination to a surface, which records changes in laser speckle contrast. This technique provides an excellent spatial and temporal resolution for assessing the renal cortical perfusate flow.[139]
Multiphoton microscopy (MPM) imaging	MPM imaging is a technique that uses multiphoton excitation fluorescence microscopy for optical sectioning of renal tissue.[139] Dynamic processes, the interplay between segments of the nephron, and multiple renal functions can be quantitively visualized at near real-time speed and with submicron resolution (for instance, glomerular filtration rate, microvascular function, apoptosis, proximal tubule endocytosis, and protein expression can be measured).[139,144,145]

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Figure 1

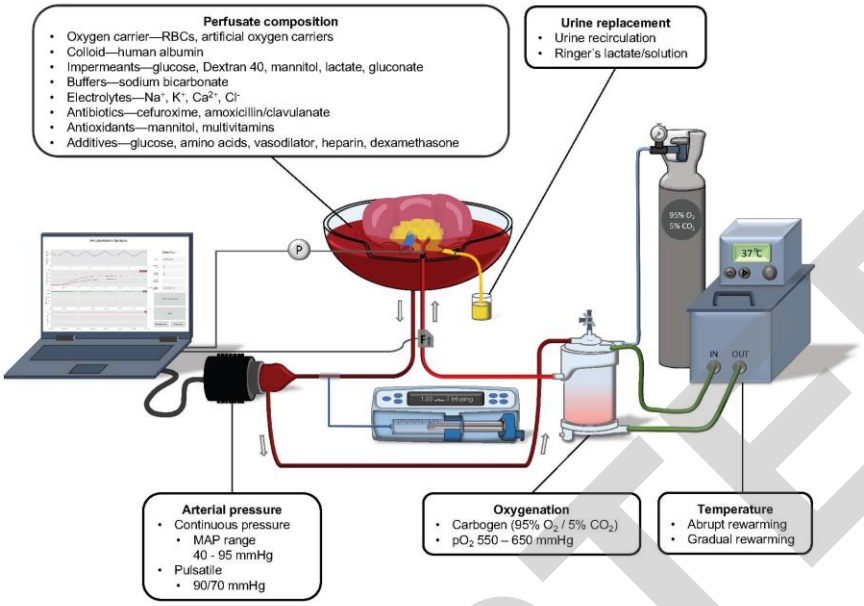

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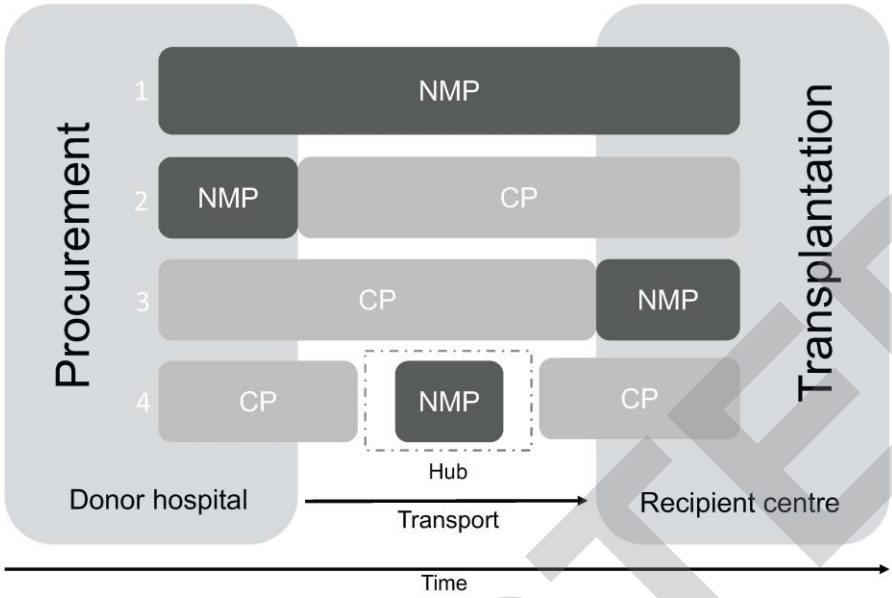
Figure 2



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