Ischemia/reperfusion injury still remains a major impediment that negatively affects functional outcome after clinical kidney transplantation. The increasing use of grafts from expanded criteria donors (ECD), in order to compensate for the scarce availability of donor organs, has accentuated this problem because ECD kidneys exhibit a reduced resilience to preservation reperfusion injury. Therefore, normothermic ex vivo machine perfusion with erythrocyte-containing media has been proposed to restore circulation and enable functional restoration prior to transplantation.

However, experimental data indicate that the benefit of ex vivo normothermic perfusion decreases in proportion to the duration of preceding hypothermic storage. This phenomenon seems to be, at least in part, pertinent to a temperature paradox inherent to an abrupt rewarming of previously cold-adapted tissue and can be mitigated by controlled rewarming and normothermic perfusion with cell-free solution of a kidney prior to transplantation.

Cold preservation sensitizes organ grafts to exacerbation of tissue injury upon reperfusion. This reperfusion injury is not fully explained by the mere re-introduction of oxygen but rather is pertinent to the immediate rise in metabolic turnover associated with the abrupt restoration of normothermia. Here we report the first clinical case of gradual resumption of graft temperature upon ex vivo machine perfusion from hypothermia up to normothermic conditions using cell-free buffer as a perfusate. A kidney graft from an extended criteria donor was put on the machine after 12.5 hours of cold storage. During ex vivo perfusion, perfusion pressure and temperature were gradually elevated from 30 mm Hg and 8°C to 75 mm Hg and 35°C, respectively. Perfusate consisted of diluted Steen solution, oxygenated with 100% oxygen. Final flow rates at 35°C were 850 mL/min. The kidney was transplanted without complications and showed good immediate function. Serum creatinine fell from preoperative 720 µmol/L to 506 µmol/L during the first 24 hours after transplantation. Clearance after 1 week was 43.1 mL/min. Controlled oxygenated rewarming prior to transplantation can be performed up to normothermia without blood components or artificial oxygen carriers and may represent a promising tool to mitigate cold-induced reperfusion injury or to evaluate graft performance.

KEYWORDS
clinical research/practice, kidney transplantation/nephrology, organ perfusion and preservation, organ transplantation in general, tissue injury and repair

Abbreviations: DO₂, oxygen delivery; ECD, expanded criteria donor; pO₂, partial pressure of oxygen.
mitigated by gentle elevation of the perfusion temperature during oxygenated ex vivo machine perfusion from hypothermia. However, the hypothermic period precludes the inclusion of erythrocytes in the perfusate. Red blood cells lose their deformability and become fragile in the cold, eventually leading to impaired microcirculation and tissue oxygenation.

But then, recent experimental work has shown that, even at normothermia, no additional oxygen carrier was needed to achieve adequate tissue oxygenation during short-term renal perfusion, provided the perfusate partial pressure of oxygen (pO2) was maintained above 500 mm Hg.

Here we report the first clinical application of controlled oxygenated rewarming up to normothermia with cell-free perfusate in renal transplantation.

## 2 | PATIENT AND METHODS

A kidney from a 60-year-old female expanded criteria donor with a history of hypertension, who died of an acute brainstem bleeding, was retrieved after in situ flushing and cooling with histidine-tryptophan-ketoglutarate solution.

The Kidney Donor Risk Index was 1.43, indicating that the estimated risk of kidney graft failure from this donor was higher than 83% of all kidney donors recovered that year. Last serum creatinine was 61.9 µmol/L. The graft was packed on ice and preserved for a total of 12 hours 30 minutes until connection to the perfusion machine was effectuated in the operating room immediately prior to the preparation of the recipient.

Machine perfusion of the kidney was performed on a CE-certified Kidney assist® (Organ Assist, Groningen, The Netherlands) device that allowed for variable adjustment of perfusate temperature and perfusion pressure over time. Thus, our preclinically established perfusion protocol could be mimicked in a clinical setting.

The perfusion device provides a pressure-controlled pulsatile perfusion (60 beats per minute). A total of 2 L of perfusate is recirculated through the organ while venous outflow drains freely into the reservoir. Oxygenation of the solution is done via an interposed hollow-fiber oxygenator fed with medical grade oxygen to allow for arterial pO2 values above 500 mm Hg that have been shown to allow for sufficient oxygenation of the tissue during the rewarming process in the absence of oxygen carriers. Perfusion was started in the cold at 8°C at a pressure of 30 mm Hg. Temperature was then slowly elevated to 10°C, 17°C, 30°C, and 35°C after 30, 60, 75, and 90 minutes, respectively (Figure 1). Perfusion pressure was adapted accordingly to 40, 70, and 75 mm Hg after 60, 75, and 90 minutes. The last 30 minutes of perfusion was done at steady-state conditions of 35°C and 75 mm Hg. Perfusate consisted of 1 L Steen solution, diluted 1/1 with Ringers solution and supplemented with 16 mL 4% sodium bicarbonate, 7 mL 10% calcium gluconate, and 1 g ampicillin.

On the machine, renal perfusate flow increased progressively during ongoing perfusion in response to the elevation of the set value of the perfusion pressure that was adjusted to the raise in temperature (Figure 1). Final flow rates at 35°C were 850 mL/min.

Oxygen delivery (DO2) during normothermic perfusion was found to exceed the value of 391 µmol/min per kidney, reported as normal renal oxygen extraction rate in healthy humans. Moreover, venous oxygen partial pressures were always above ambient, also indicating that oxygen supply surpassed the demand (Table 1).

No cellular release of potassium was observed during ongoing perfusion with perfusate concentrations of 5.1 µmol/L after 30 minutes and 5.2 µmol/L at the end of the reconditioning after 120 minutes.

After ex vivo machine perfusion, the kidney was flushed with 500 mL of cold histidine-tryptophan-ketoglutarate solution and implanted into the recipient. Time for surgical anastomosis was 14 minutes.

The recipient was a 61-year-old man, weighing 79 kg and suffering from diabetic nephropathy. He was on hemodialysis for 3 years. HLA mismatch was 1-1-0, panel reactive antibody score 0%, and cross-match was negative.

The patient had been informed in detail about the intended treatment of the graft and written consent was obtained according to the advice of the local ethics committee.

A standard immunosuppressive regimen was applied with prednisolone (30 mg daily), tacrolimus to maintain trough levels of 6-10 ng/mL, and mycophenolic acid (720 mg 1-0-1). Immediate

**FIGURE 1** Time courses of renal perfusate flow (dashed line) and temperature (solid line) during ex vivo reconditioning perfusion prior to transplantation

**TABLE 1** Renal oxygen delivery (DO2) upon isolated machine perfusion at different temperatures and partial pressure of oxygen (pO2) in the venous line as well as the corresponding oxygen content in µmol/L

| Time (min) | 30  | 60  | 75  | 90  | 120 |
|------------|-----|-----|-----|-----|-----|
| Temperature (°C) | 10  | 17  | 30  | 35  | 35  |
| DO2 (µmol/min)    | 74.2| 107.1| 321.2 | 554.3 | 619.9 |
| Venous pO2 (kPa) | 73.7| 68.7| 59.4| 57.0| 62.2 |
| [venous O2] (µmol/L) | 1241 | 996 | 683 | 619 | 676 |

Perfusate oxygen content was determined using a temperature-compensated fiberoptic oxygen meter (Microx 4, PreSens precision sensing, Regensburg, Germany).
graft function was observed after transplantation of the kidney. Urine output amounted to >2.8 L during the first 24 hours after transplantation and remained between 2.2 and 3.9 L/d during the following week. Serum creatinine fell from preoperative 720 µmol/L to 506 µmol/L on postoperative day 1, and 178 µmol/L after 1 week. Creatinine clearance was measured 1 week after transplantation and was 43.1 mL/min.

The patient showed an overall event-free postoperative course and was discharged after 16 days with a serum creatinine of 143 µmol/L.

3 | DISCUSSION

Normothermic machine perfusion of kidney grafts has been recognized as a useful tool to improve pretransplant viability of the organ in line with the provision of functional data that might help to evaluate graft viability prior to engraftment.10

This is the first report of renal graft perfusion with cell-free buffer including a hypothermic starting period and subsequent controlled gradual rewarming up to normothermia prior to transplantation in a clinical setting. In doing so, all putative protective mechanisms of pretransplant ex vivo perfusion could be addressed.

Reoxygenation in absence of neutrophils and blood-derived inflammatory ligands reduces proinflammatory reactions during reperfusion7 and may allow for a better restitution of graft integrity prior to engraftment.

Pulsatile vascular perfusion stimulates endothelial mechanoreceptors that contribute to the restoration of an anti-inflammatory vascular phenotype prior to in vivo reperfusion.11,12

By virtue of starting the perfusion at hypothermic conditions, when tissue metabolism is still minimized, a rapid conversion of mitochondrial electron carriers from a reduced to oxidized stage can be obtained13 along with an improvement of cellular energy homeostasis. A unique feature of the presented perfusion protocol lies in the controlled gradual warming up of the graft.

Preclinical data suggest that reperfusion injury after cold preservation is minimized if reoxygenation takes place in the cold,14 while abrupt rewarming may elicit mitochondrial dysfunction and aggravation of cell injury.15 We and others have shown experimentally that this temperature paradox could be effectively mitigated by slowing down the rewarming process after hypothermic preservation.5,14,17

Controlled rewarming takes account of the hypothermic torpor of cellular metabolism that otherwise prevents immediate re-equilibration of energetic homeostasis upon abrupt normothermia (reviewed in [14]) and has shown protection superior to mere hypothermic machine perfusion in isolated kidneys.16 The duration of the machine perfusion and the timing of the rise in temperature have been adapted to the time usually available from arrival of the graft until readiness of the recipient. Further slowing down of the rewarming process did not produce superior results in preclinical experiments.19

However, an extension of the normothermic perfusion period beyond the intended 2 hours might be indicated, in case of irregularities with the patient preparation.

Such extension of acellular normothermic perfusion should be possible because experimental data in pig kidneys show stable renal parameters for several hours under conditions identical to those used in this clinical case (von Horn, unpublished observations).

The use of a cell-free perfusate facilitates the procedure and furthermore precludes the risk of residual cell material in red blood cell preparation or hemolysis during machine perfusion that could entail adverse effects to the graft.20

However, adequate DO2 must be provided instead by saturation of the perfusate with physically dissolved oxygen at supraphysiological concentrations.

We have intentionally chosen a temperature of 35°C so as to improve the ratio of physical oxygen solubility in the perfusate and oxygen consumption of the graft while still providing a near-normothermic milieu upon erythrocyte-free perfusion. In line with previous data from preclinical studies,7 it was found that oxygenation of the perfusate with pure oxygen was operative to prevent tissue hypoxia at any phase of the perfusion period.

The occurrence of multiple renal arteries might be considered an obstacle to machine perfusion, but this is not the case if the graft has been retrieved in standard technique with an aortic patch. Using a patch connector, available as a supplement to the perfusion disposible, all vessels included in the patch can be connected to the perfusion line. Distant pole arteries must be surgically anastomosed to the main artery prior to machine perfusion.

The present case was aimed at demonstrating clinical applicability of controlled oxygenated rewarming up to normothermia with a cell-free perfusate. Although immediate graft function had been conceivable in the present graft even without prior machine perfusion, the procedure could be shown as feasible and safe and may be useful to minimize reperfusion injury and to recondition marginal donor grafts prior to transplantation.

DISCLOSURE

The authors of the manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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