Clinical use of controlled oxygenated rewarming of kidney grafts prior to transplantation by ex vivo machine perfusion. A pilot study

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Abstract
Background: Sudden restoration of normothermic conditions upon reperfusion of cold-stored grafts has been suggested to entail a massive energy demand not yet met by the cells that still suffer from hypothermic torpor. An adapted and gentle rise of graft temperature by ex-vivo machine perfusion has, therefore, been proposed. This should now be tested in the clinical setting.

Methods: In a first clinical series, six ECD-kidneys were subjected to controlled oxygenated rewarming (COR) during short term pre-implantation machine perfusion. Matched kidneys that were conventionally kept on ice served as controls.

Results: Early allograft function after transplantation was significantly improved by COR. On post-operative day 7, clearance of creatinine was more than twofold higher after COR and fractional excretion of sodium in the normal range, while significantly elevated in control kidneys. Good correlations were seen between ulterior graft function and real-time parameters obtained during pre-transplant machine perfusion (Lactate: \( r^2 = .9 \); TIMP2: \( r^2 = .74 \)). Conventional denominators of graft viability like kidney donor risk index KDRI were far less predictive (\( r^2 = .26 \)).

Conclusion: It is concluded that COR can be safely applied to renal grafts and appears to be a valuable tool to predict and improve early renal function after transplantation.

Keywords
controlled rewarming, machine perfusion, rewarming injury, temperature paradox

Abbreviations: COR, controlled oxygenated rewarming; DGF, delayed graft function; ECD, enhanced criteria donor; FE Na, fractional excretion of sodium; KDRI, kidney donor risk index; TIMP2, tissue inhibitor of metalloproteinase 2.

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**1 | INTRODUCTION**

In face of the conflicting shortage of donor organs, retrieval of kidneys from extended criteria donors up to and including organs retrieved after cardiac arrest of the donor has become widely accepted as a clinical routine to expand the number of grafts available for kidney transplantation. Moreover, it is not unlikely that this proportion of ‘less than optimal’ kidney grafts will expand in the future as will the limits of acceptance criteria for the use of such grafts. Although this represents a valuable advance to enlarge the total number of organs available for transplantation, these grafts are often conflicted with a reduced functional reserve and, hence, less resilient against preservation and reperfusion injury.\(^1\)

Previous research indicates that structural integrity of the graft is most affected at the time of warm, oxygenated reperfusion, whereas only minor, reversible lesions actually manifest during vascular flush-out and cold-storage in modern preservation solution.\(^2\) Moreover, the abrupt shift in temperature upon warm reperfusion of the cold organ has been shown to constitute a long neglected, genuine trigger of significant cellular impairments for example in the respiratory control ratio leading to subsequent graft dysfunction after transplantation (temperature paradox).\(^2\)

A strategy to alleviate this temperature paradox upon warm reperfusion after cold preservation was recently investigated by our group, using a gentle and adapted rise in temperature from hypo- to normothermia during isolated machine perfusion of the graft prior to transplantation.\(^3\)

In 2020, the first clinical application of this technique had been reported, implementing controlled oxygenated rewarming up to normothermia with a cell-free perfusion solution. The procedure was easily carried out on an enhanced criteria donor kidney and was accompanied with good clinical outcome of the recipient.\(^4\)

Beyond that single case observation, we now report on a first clinical pilot series, intended to address safety and feasibility of ex vivo controlled rewarming prior to transplantation and to give a first measure of efficacy versus standard cold storage in clinical routine.

**2 | PATIENTS AND METHODS**

All data were prospectively collected in patients undergoing renal transplantation between July 2019 and January 2021.

Only donor organs were eligible for this study that fell into the expanded criteria donor (ECD) category: donor age of 60 years or older donor age between 50 and 59 plus two out of the following criteria: arterial hypertonus, terminal serum creatinine >1.5 mg/dl or deceased after cerebrovascular event. The perioperative immunosuppression was similar in both groups: Intraoperative induction with Basiliximab and methylprednisolone followed by tacrolimus, adjusted in accordance with blood levels and mycophenolate mofetil.

After arrival of the kidney at the hospital, six kidneys were subjected to controlled, oxygenated rewarming by ex vivo machine perfusion immediately prior to implantation.

These patients were compared with six control subjects that were transplanted following standard procedures during the same time period in the same centre.

Control subjects were matched according to the kidney donor risk index (KDRI) and ischaemia time.

The procedure of ex vivo-controlled oxygenated rewarming as described herein had been approved for clinical application by the local Ethics Committee of the University Duisburg-Essen. The patients gave informed written consent to that procedure.

**2.1 | Controlled oxygenated rewarming**

During the last 2 h of the preservation period, kidneys are placed on a machine perfusion circuit and subjected to an end-ischaemic-controlled oxygenated rewarming (COR) protocol.

To that purpose, we used a CE certified (European Union certification) device, that allows for pulsatile perfusion of the kidney in a closed circuit (Kidney Assist®, Fa. Organ Assist, NL).\(^4\)

A mixture of 1 l Steen™ solution (XVIVO Perfusion, Göteborg, Schweden) and 1 l Ringer’s solution served as perfusate, after addition of 10 ml sodium bicarbonate 8.4%, 7 ml calcium gluconate 10%, 1 g ampicillin, and 10 mg of dexamethasone. Starting at 10°C, temperature is slowly elevated during ongoing perfusion to, 17°C, 30°C and 35°C after 30, 60, 75 and 90 min, respectively. Perfusion pressure is adapted in parallel from 30 mmHg up to 75 mmHg during the final steady state period at 35°C.\(^3\)

The oxygenator was continuously fed with 1 L/min of 100% oxygen resulting in adequate oxygen delivery to the graft during hypothermic perfusion as well as subsequent rewarming.\(^4\)\(^5\)

**2.2 |Endpoints**

The primary endpoint of the study was chosen to be the clearance of creatinine on day 7 after transplantation.

Further endpoints were fractional sodium excretion (FENa+), primary non-function, Delayed graft function
(DGF), hospital stay, rate of complications according to the Clavien-Dindo grading >3a and 3-month graft survival. DGF was defined as requirement for dialysis during the first week after transplantation. Moreover, correlations of selected parameters obtained during machine perfusion with ulterior graft function were calculated.

Tissue inhibitor of metalloproteinase 2 (TIMP2) was measured in the machine perfusate using a commercial ELISA kit (MyBioSource) according to the instructions of the manufacturer.

The kidney donor risk index (KDRI) was calculated for all grafts according to the formula published by Rao et al.

### 2.3 Statistics

Data are presented as mean ± standard deviation. Statistical comparison was done with the Mann-Whitney U test for nonparametric variables. Binary categorical parameters were analysed using Fisher’s exact test. Statistical analyses and calculation of Pearson correlation coefficient \( r \) were carried out using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. \( p \) values less than .05 was considered statistically significant.

### 3 RESULTS

#### 3.1 Donors and recipients

There were no significant differences of donor and recipient demographics or graft parameters (Table 1).

#### 3.2 Kidney function during COR

Perfusate concentrations of potassium showed an initial peak during the first 30 min of perfusion (5.8 ± 0.8 µmol/l) followed by a slow but steady decrease later on to 4.3 ± 0.4 µmol/L. Lactate levels were rather low during the initial hypothermic period (0.8 ± 0.2 mmol/L) and slightly rose upon transition to the metabolically active normothermic period. Mean lactate concentrations after 120 min of perfusion were 1.8 ± 0.4 mmol/L.

Perfusate flow averaged 507 ± 217 ml/min at the end of machine perfusion at 35°C with a venous partial oxygen pressure of 391 ± 56 mmHg.

| Characteristic             | Control (\( N = 6 \)) | COR (\( N = 6 \)) | \( p \)-value |
|----------------------------|-----------------------|-------------------|--------------|
| Donor                      |                       |                   |              |
| Age (years)                | 64.8 ± 10.2           | 56.7 ± 3.7        | .130         |
| Last Creatinine (mg/dl)    | 1.25 ± 0.71           | 1.17 ± 0.64       | .738         |
| KDRI                       | 1.567 ± 0.4008        | 1.358 ± 0.1514    | .217         |
| Preservation               |                       |                   |              |
| Total ischaemic time (hours)| 12.57 ± 6.54         | 11.83 ± 2.48      | .999         |
| WIT (min)                  | 26.0 ± 7.2            | 20.2 ± 4.5        | .095         |
| Recipient                  |                       |                   |              |
| Age (years)                | 59.3 ± 13.6           | 45.0 ± 14.6       | .1212        |
| Sex, (m/f)                 | 3(3)                  | 1(5)              | .5455        |
| Last Creatinine (mg/dl)    | 7.46 ± 2.72           | 7.90 ± 3.29       | .9372        |
| Before transplantation     |                       |                   |              |
| Disease recipient          | PCKD, 2 × RPGN        | DM nephropathy    |              |
|                           | 2 × IgA-Nephropathy   | Alport-syndrome   |              |
|                           | 1 × unknown           | IgA-nephropathy   |              |
|                           |                       | Conn-syndrome     |              |
|                           |                       | PSH IgA-nephritis |              |
| Duration of dialysis (years)| 4.7 ± 1.08           | 5.6 ± 1.43        | .6099        |
| Previous Transplants       | 0/6 (0%)              | 1/6 patients (17%)|              |

Note: Values are given as mean SD

Abbreviations: PCKD, polycystic kidney disease; PSH, Purpura Schönlein-Henoch; RPGN, rapidly progressive glomerulonephritis.
3.3 | Post-operative outcome

In the COR group, renal clearance on post-operative day 7 amounted to more than 3 times the values observed in the control group (cf. Figure 1). Of note, this difference remained significant up to the end of follow-up after 3 months (cf. Table 2).

As controlled oxygenated rewarming has experimentally been associated with an improved mitochondrial recovery from hypothermia, we were also interested in the tubular cell function. Tubular reabsorption of sodium accounts for the major part of renal energy expenditure. We thus had a look on the fractional excretion of sodium (FENa+) 1 week after transplantation.

It was seen that COR resulted in a significant improvement of tubular sodium reabsorption reaching normal values of FENa (1.8%) in comparison to 4.3% ($p = .026$).

Further outcome parameters are summarized in Table 2. There was one case of PNF in the control group (16.7%) but no case in the COR group (0%).

Delayed graft function also tended to be more frequent in the control group (33%) versus 0% in the treatment group, albeit the difference was not significant. No differences were seen with regard to 3-month survival, total hospital stay or complication rates.

3.4 | Correlation of perfusion data with outcome

Some of the parameters, obtained during ex vivo machine perfusion fairly well correlated with graft function after transplantation, reflected by the clearance of creatinine on post-operative day seven.

The best correlation ($r^2 = .90$) was seen for the terminal concentration of lactate in the machine perfusate (cf. Figure 1). Perfusion levels of TIMP2 did also well correlate with later graft function. The product of lactate and TIMP2 after 120 min of machine perfusion reached a correlation of $r^2 = .95$.

By contrast, neither cold ischaemia time nor the more sophisticated kidney donor risk index (KDRI) turned out as valuable predictors for renal clearance on post-operative day seven. Figure 2.

4 | DISCUSSION

Controlled oxygenated graft rewarming by ex vivo machine perfusion with acellular perfusate could be shown to be clinically feasible and safe. Moreover, pre-transplant, controlled rewarming of renal grafts from enhanced criteria donors resulted in a significant improvement in early graft function as evaluated by creatinine clearance and tubular reabsorption of sodium.

![Figure 1](image1.png) **Figure 1** Tukey box plot showing post-operative recovery of renal function in kidneys that underwent controlled oxygenated rewarming (COR) and in control kidneys. Glomerular filtration rate (GFR) was approximated by the clearance of creatinine; fractional excretion of sodium (FE Na) was used to evaluate the energy demanding reabsorption of sodium as function of the renal tubular cells. (*: $p < .05$ Mann–Whitney U-test)

| Outcome | Control ($N = 6$) | COR ($N = 6$) | p-value |
|---------|------------------|---------------|---------|
| Creatinine clearance POD7 (ml/min) | 27.0 ± 12.7 | 66.1 ± 19.1 | .004 |
| FE Na+ (%) | 4.7 ± 2.6 | 1.8 ± 0.8 | .026 |
| PNF | 1/6 (17%) | 0/6 (0%) | .999 |
| DGF | 2/6 (33%) | 0/6 (0%) | .333 |
| Hospital stay (days) | 16.6 ± 5.0 | 17.8 ± 6.6 | .924 |
| 3-month graft survival | 5/6 (83%) | 6/6 (100%) | .999 |
| 3-month GFR (ml/min) | 45 ± 19 | 70 ± 13 | .023 |
| Adverse events (≥Clavien-Dindo 3b) | 1/6 (17%) | 2/6 (33%) | .999 |

Note: Values are given as mean ± SD or numbers and percentage where appropriate.

Abbreviations: DGF, delayed graft function; FE Na+, fractional excretion of sodium on POD 7; GFR, glomerular filtration rate; PNF, primary non-function; POD7, post-operative day 7.
It is well known that kidneys from marginal donors and ECDs are prone to reduced graft function after transplantation and that delayed graft function (DGF) may not be the only transitory phenomenon but eventually correlated with poorer long-term graft survival. Although the rate of DGF observed in our control group was in the middle range reported for ECD kidneys, DGF did not occur in the kidneys that underwent controlled rewarming. However, as the limited number of patients in the present pilot study was not appropriate to reveal significances in binary parameters, we put our focus on the continuous functional parameter of glomerular filtration rate.

Previous experimental research from others and our group has given evidence for a temperature paradox phenomenon, meaning that abrupt warm perfusion of previously hypothermic tissue, although necessary for physiologic metabolism, may nonetheless be inflicted by mainly in mitochondrial alterations and functional deficits. It is thought, that normothermia precipitates an abundant demand in energy resources which is not adequately met by the still dys-homeostatic cellular microenvironment. Thus, abrupt rewarming can trigger mitochondrial injury, respiratory dysfunction and eventually lead to the induction of apoptosis and inflammatory reactions that negatively affect renal recovery after transplantation.

Much of the rewarming injury can be prevented using an adapted and controlled rise of the perfusion temperature from hypo- to normothermia, realized by during ex vivo machine perfusion as has been shown experimentally.

Our clinical data are in line with this; the technical procedure for controlled oxygenated rewarming turned out to be reliable in the hands of trained personnel. As previously documented experimental, erythrocytes are not essential for a short-term NMP provided that regulation of flow and oxygenation of the perfusion solution were adapted to the situation. All clinical acellular perfusions worked very well and oxygen delivery to the kidneys always exceeded oxygen consumption of the organ.

An important supplementary aspect of pre-transplant machine perfusion lies in the possible viability assessment of marginal grafts. In this study, the short normothermic perfusion provided several parameters that fairly well correlated with the ulterior glomerular filtration rate 1 week after transplantation.

TIMP-2, is a novel sensitive biomarker of early renal epithelial injury that prevents cells with a damaged DNA to perform mitosis (G1 arrest). It is upregulated upon stress situations and its concentration in the machine
perfusion can be used to approximate renal graft integrity, as instantaneous detection of TIMP-2 is possible by point of care detection systems.

Interestingly, functional parameters upon machine perfusion showed much superior predictive potential than conventional scores like KDRI or cold ischaemia time. After future validation in larger studies, a synoptic panel of functional machine parameters may thus improve the available accuracy in decision-making on graft acceptance or rejection eventually allowing for the expanded use of marginal renal transplants.

Owing to the pilot design of the study, several limitations are to be acknowledged.

First, the relatively small number of patients in the cohorts as well as some differences in the baseline characteristics with regard to ischaemia time and recipient age in favour of the COR group calls for a judicious interpretation of the results despite clear differences in functional post-transplant performance.

Second, our investigation only analysed early graft function after transplantation. This corresponded to the working hypothesis that controlled rewarming would alleviate initial metabolic disturbances upon reperfusion. If this improvement of early allograft function will translate into a reduction of chronic graft failure remains to be addressed.

In conclusion, the present report shows that COR is save and feasible and can easily be implemented into clinical practice. The apparent efficacy of the procedure to improve early recovery of cold stored renal grafts strongly encourages a confirmatory randomized controlled trial on a larger collective.

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AUTHOR CONTRIBUTIONS
Hristo Zlatev: performed research, analysed data, designed study, wrote paper. Charlotte von Horn: performed research. Moritz Kaths: performed research. Andreas Paul: performed research, revised paper. Thomas Minor: designed study, performed research, wrote paper.

CONFLICT OF INTEREST
All authors declare that they have no conflict of interest.

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