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HTK-N as a new preservation solution for human kidney preservation: Results of a pilot randomized controlled clinical phase II trial in living donor transplantation

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Abstract

Background: HTK-N was developed based on the traditional HTK preservation solution, resulting in stronger protection against reactive oxygen species as well as better tolerance to hypothermia and ischemia. Aim of the present study was to compare HTK-N to HTK in clinical kidney transplantation demonstrating safety and non-inferiority.

Methods: We performed a randomized controlled single blinded clinical phase II trial in patients undergoing living donor kidney transplantation. After retroperitoneoscopic nephrectomy kidneys were either perfused and stored with classical HTK solution or the new HTK-N solution. Primary endpoint was the glomerular filtration rate (eGFR according to CKD EPI) 3 months after transplantation. Secondary endpoints included graft and patient survival beside others.

Results: The study included 42 patients, of which 22 were randomized in the HTK-N group and 20 in the HTK group. The primary end point showed a mean eGFR of 55.4 ± 14.0 ml/min/1.73 m^2 in the HTK group compared to a GFR of 57.2 ± 16.7 ml/min/m^2 in the HTK-N group ($P = .72$). Regarding secondary endpoints, there were no apparent differences. Posttransplant graft and patient survival was 100%.

Conclusion: This study is the first clinical application of HTK-N for kidney preservation and demonstrates non-inferiority compared to HTK in the setting of living donor kidney transplantation.

KEYWORDS
kidney transplantation, preservation solution, randomized controlled trial

1 | INTRODUCTION

Organ preservation remains one cornerstone for successful organ transplantation and defines the degree of ischemia-reperfusion injury (IRI) in the recipient. The complex cascade drives dominantly the damage of the allograft and influences short- and long-term outcomes.[1] Different preservation solutions protecting the allograft from IRI have been established in past decades and are since in clinical use. All of these have certain limitations.[2] One of the dominant solutions for solid organ preservation worldwide is HTK.

TABLE 1 Comparison of HTK-N and HTK Values are given in mM (mmol/L) unless stated otherwise

Preservation solution	HTK-N	HTK
Dosage form	Crystalloid solution with lyophilisate	Crystalloid solution
Content		
Sodium	16	15
Potassium	10	10
Magnesium	8	4
Calcium	.02	.015
Chloride	30.04	50
Histidine	124	198
N-Acetylhistidine	57	–
Mannitol	–	30
Sucrose	33	–
α-Ketoglutarate	2	1
Aspartate	5	–
Glycine	10	–
Alanine	5	–
Tryptophan	2	2
Arginine	3	–
Deferoxamine	.025	–
LK 614	.0075	–
pH	7.0	7.2
Osmolarity (mosmol/L)	305	310

During the almost four decades HTK solution has been in clinical use, additional knowledge about the mechanisms of cell and tissue injury during cold ischemia has been gained. Based on experimental findings, the traditional HTK solution was modified and named HTK-N: This solution is fortified with the amino acids glycine and alanine to inhibit the formation of the hypoxia-induced plasma membrane pore and was supplemented by the strong but poorly membrane-permeable iron chelator deferoxamine and the new, membrane-permeable iron chelator LK 614 to inhibit cold-induced cell injury.[3,4,13–18,5–12] Furthermore, recent studies have shown that the buffer histidine can have adverse effects on some cell types rich in "redox-active" iron.[19] Therefore, part of the histidine in the HTK solution was replaced by the superior derivative N-acetyl-histidine. The application of the vasodilator nitric oxide or L-arginine, the substrate of the endogenous nitric oxide-producing enzymes, proved to decrease microcirculatory disturbances.[20–23] Therefore, HTK-N has been supplemented with L-arginine. As mannitol is not impermeable to all cell types,[24] it has been replaced by sucrose. Moderate acidosis has been shown to protect against ischemic injury.[25,26] Therefore HTK-N has a slightly lower pH than the traditional HTK solution. Finally, aspartate has been added to allow the replenishment of intermediates of the tricarboxylic acid cycle and thus efficient energy production after reperfusion. A detailed comparison of HTK and HTK-N is given in Table 1.

The present study was designed to introduce HTK-N in clinical kidney transplantation and demonstrate in a first step the safety and non-inferiority of preservation compared to the standard solution HTK.

2 | METHODS

2.1 | Study design

We performed a randomized, controlled, single-center study (Eudra-CT Number 2013-005503-13; ISRCTN44414069). The study comprised two study arms (HTK-N vs. HTK) and was carried out as a single-blinded study. The study was approved by the local ethics committee (Ethics Committee University Duisburg-Essen, 16/02/2015, ref: 14-6075-AF) and followed the Declaration of Helsinki.

2.2 | Study population

The study population was selected from patients who underwent living donor kidney transplantation. Subjects of each gender were included in the study. All patients undergoing living donor kidney transplantation in our transplant center were considered for the trial as far as the inclusion criteria were given.

For the inclusion criteria of the kidney transplant recipients, the following requirements had to be met:

- Recipients undergoing their first kidney transplantation
- Living donor kidney transplantation
- Recipient's age ≥ 18 years
- Signed informed consent before randomization

Exclusion criteria for the recipients included participation in other clinical trials, panel reactive antibodies > 85%, ABO-incompatible kidney transplantation, and known hypersensitivity/anaphylaxis against iron chelators.

2.3 | Study procedures

An overview of the study is provided in the Study Flow Chart (Table S1). The study procedures and the timing of the procedures are summarized. Patients with signed informed consent who met all inclusion criteria, and did not meet any exclusion criteria were randomized. After randomization to one of the study arms, all procedures followed the study protocol strictly.

Specialized teams carried out kidney donation and transplantation as a standardized procedure at our transplant center: A retroperitoneoscopic approach was chosen in most donors. Further treatment of the organ was carried out at the back table. Immediately after explanation, the arterial vessel was cannulated, and the cold (approximately 4°C) perfusion solution (HTK-N or HTK, Dr. F. Köhler Chemie GmbH (Germany) according to randomization) was applied (details of

ingredients is given in Table 1, viscosity of both fluids is similar). The perfusion was carried out with 250–1000 ml of perfusion solution until clear venous effluent was observed. During the procedures, the kidney was kept on sterile ice and bathed in the particular perfusion solution. The following preparation of the kidney, including control of the vessels on leakage and removal of the fatty tissue, was carried out under sterile hypothermic conditions. After completing the preparation, the kidney was carefully packaged, bathed in the particular perfusion solution, and stored in sterile containers until the implantation procedure.

The allograft was transplanted, as suggested by standard guidelines,[27] into the ipsi- or contralateral Fossa iliaca, and anastomosis was performed to the respective iliac artery and iliac vein. Details of transplantation techniques can be read elsewhere. After anastomosis of the renal artery and vein of the donated organ to the iliac artery and vein of the recipient, the clamps were removed and the blood flow released. The particular perfusion solution contained in the organ (approximately 50 ml) was released into the recipient's blood. Both operations were carried out in one operating suite, sequentially. The perioperative care was similar in both groups, as well as the concept of immunosuppression. Preoperatively, calcineurin-inhibitors were applied (adjusted per the trough level of the drug). Induction therapy (Basiliximab 20 mg) in combination with intravenous corticosteroids (500 mg methylprednisolone) were applied intraoperatively. Postoperatively, calcineurin-inhibitors in combination with corticosteroids and mycophenolate mofetil were utilized. The second infusion of Basiliximab was applied on postoperative day 4.

After transplantation, all patients were observed daily for 1 week. Additional follow-up visits were carried out 1 and 3 months after transplantation at our outpatient clinic.

2.4 | Objectives and endpoints

The objective of this investigation was to demonstrate non-inferiority in the outcome of HTK-N against HTK in living donor kidney transplantation.

2.4.1 | Primary objectives

The primary objective was to demonstrate the non-inferiority of HTK-N versus HTK for living donor kidney perfusion concerning the renal function of the recipient, reflected by the glomerular filtration rate (GFR) at 3 months after transplantation. A lower value of less than 10 ml/min in the HKT-N group was considered as acceptable for non-inferiority.

The GFR is either calculated from serum Cystatin C with the following formula: Cystatin C Equation: GFR (ml/min*1.73 m^2) = 74.835 x Cystatin C (mg/l)$^{-1.333}$

Another way to calculate the GFR is the CKD EPI Equation as recommended for GFR values lower than 60 ml/min*1.73 m^2: CKD EPI Equation: eGFR (ml/min*1.73 m^2) = 141 x min (SCr/k,1)^a x max (SCr/k,1)$^{-1.209}$ x .993^{Age}x [1.018 if female] x [1.159 if black] SCr is

serum creatinine (mg/dL), k is .7 for females and .9 for males, a is -.329 for females and -.411 for males, min indicates the minimum of SCr/k or 1, and max indicates the maximum of SCr/k or 1.

The primary endpoint at 3 months after transplantation will consider the CKD EPI Equation. For the other equation, see secondary endpoints.

2.4.2 | Secondary objectives

As secondary objectives, early graft function of kidneys flushed with HTK-N solution compared to kidneys flushed with HTK solution was assessed by calculated GFR through the Cystatin C Equation on the postoperative day 7 and 1 and 3 months after transplantation. The GFR calculated through the CKD EPI GFR Equation will also be considered on postoperative day 7 after transplantation. Furthermore, conservative parameters like serum creatinine and serum urea on postoperative day 7 and 1 and 3 months after transplantation were used to assess renal function. Incidence of primary-non-function (PNF, defined as grafts that never gain function after transplantation) and delayed-graft-function (DGF, defined as the need for dialysis during the first post-transplant week) are low in living donor kidney transplantation. Accordingly, incidences of PNF and DGF in the study period were secondary objectives. Postoperative complications were graded by the classification of Clavien-Dindo.[28] Thus minor complications were defined as grades 1, 2 and 3a. Major complications were defined as grades 3b, 4 and 5. The occurrence of minor and major complications in the different groups was a secondary objective. Furthermore, graft survival and patient survival during the study period were secondary objectives. In addition, biopsy-proven rejections, biopsy-proven Calcineurin-Inhibitor (CNI)-toxicity, and dialysis requirement during the study period served as secondary objectives.

2.5 | Monitoring

All trial related procedures were monitored and controlled by the center for clinical trials and innovation Witten/Herdecke (ZKS-UW/H (Zentrum für klinische Studien der Universität Witten/Herdecke), according to ICH-GCP guidelines.

2.6 | Sample size calculation and statistical analysis

Definitions of inferiority or non-inferiority in renal function remain an issue of ongoing debate. Several approaches using calculated GFR after transplantation have been carried out. A difference in GFR of 5 ml/min to 10 ml/min was determined as clinically relevant differences in renal function (36). More than 50% of studies assessing differences in the renal function used differences of 10 ml/min in GFR as a clinically significant difference.[29] As the present study aimed at identifying non-inferiority of HTK-N compared with HTK, we adopted this definition of clinically relevant difference and set the non-inferiority margin

to 10 ml/min GFR at 3 months after transplantation. The mean calculated GFR after living-related kidney transplantation was reported 60 ml/min with a standard deviation of 15 ml/min (36). The sample size was calculated, considering a statistical power of 80% and a one-sided t-test with an alpha level of .05. The relevant difference is thus about two-third of the expected standard deviation. The calculation results in a sample size of 30 patients per group, or 60 patients in total. Moreover, we assumed a 10% dropout rate and a small number of patients lost to follow-up so that there was a 20% higher number to be included in the trial: 36 patients per group (meaning 72 in total) were required for this study. The primary endpoint was evaluated in the intention-to-treat (ITT) population with at least one measurement of renal function.

For all patients in the ITT population, donor and recipient characteristics, as well as procedural and postoperative data, were documented. Variables were analyzed and presented by appropriate descriptive statistics, for example, frequency (absolute and percentage), number of available and non-available values (non-missing or missing data), mean, standard deviation, median, quartiles, and range. The intention-to-treat population (and the per-protocol population) was used for this purpose.

2.7 | Changes in the conduct of the study

The study was terminated before the required sample size had been reached. The reason for this was the slow patient inclusion. The expected duration of the study had already been extended by 2 years.

The analysis was performed as planned, based on a lower than the optimal sample size.

3 | RESULTS

3.1 | Recruitment and follow up

The study included the first patient on June 16, 2015. Forty-two patients were included, therefore, 58% of the intended 72 patients. The last patient was included on March 18, 2019. The present analysis is thus based on 42 patients only, of whom 22 patients (52.4%) received a kidney perfused with HTK-N, and 20 patients (47.6%) received a kidney perfused with the standard HTK. There was no case of preterm termination or dropout; all patients completed the study as planned.

3.2 | Donor, recipient, and perioperative characteristics

Demographic data of donors, recipients, and the perioperative characteristics are given in detail in Tables 2–4.

Donors underwent the standardized evaluation process and were essentially healthy average adults. The mean age was 52.5 ± 8.9 years with a BMI of 27.1 ± 4.0 kg/m^2.

Recipients were predominantly male. The mean age was 40.8 ± 14.2 years. Common indications for transplantation were glomeru-

TABLE 2 Living donor characteristics

	Unit	HTK N = 20	HTK-N N = 22	Total N = 42
Age	Years	52.2 / 52.5 SD 8.4 35 - 68	52.2 / 52 SD 9.5 26 - 66	52.2 / 52.5 SD 8.9 26 - 68
Male gender	N(%)	7 (35%)	12 (55%)	19 (45%)
Height	cm	171 / 167 SD 11 156 - 198	171 / 173 SD 11 142 - 192	171 / 176 SD 11 142 - 198
Weight	kg	80.5 / 83 SD 14.5 50 - 103	77.7 / 77 SD 13.8 54 - 105	79.0 / 80 SD 14.0 50 – 105
BMI	kg/m^2	27.6 / 27.6 SD 4.1 19.5 – 34.4	26.6 / 26.4 SD 3.8 21.0 – 31.8	27.1 / 27.1 SD 4.0 19.5 – 34.4
Serum urea	mg/dl	13.4 / 12.5 SD 2.3 8 - 19	15.5 / 15 SD 5.0 8 - 31	14.5 / 14 SD 4.2 8 - 31
Serum creatinine	mg/dl	1.0 / 1.0 SD .2 .7 - 1.2	.9 / .9 SD .2 .7 – 1.3	1.0 / 1.0 SD .2 .7 – 1.3

Continuous data are presented as mean / median, standard deviation (SD), range.

TABLE 3 Recipient characteristics

	Unit	HTK N = 20	HTK-N N = 22	Total N = 42
Age	Years	40.7 / 37 SD 15.4 21 - 67	40.9 / 39 SD 13.3 19 - 65	40.8 / 37 SD 14.2 19 – 67
Male gender	N(%)	13 (65%)	15 (68%)	28 (67%)
Afroamerican	N(%)	1 (5%)	1 (5%)	2 (5%)
Height	cm	175 / 173 SD 9 164 - 198	178 / 179 SD 12 159 - 198	177 / 176 SD 11 159 - 198
Weight	kg	80.3 / 81 SD 17.5 46 - 116	83.1 / 78 SD 22.0 45 - 133	81.8 / 80 SD 19.8 45 - 133
BMI	kg/m^2	26.3 / 27.3 SD 5.5 17.1 – 37.9	25.9 / 25.4 SD 4.9 16.7 – 34.6	26.1 / 25.5 SD 5.1 16.7 – 37.9
Serum urea	mg/dl	54 / 55 SD 22 14 - 104	65 / 60 SD 19 32 - 96	60 / 59 SD 21 14 - 104
Serum creatinine	mg/dl	7.7 / 7.9 SD 2.5 3.7 – 13.4	7.9 / 7.3 SD 2.9 3.7 – 13.5	7.8 / 7.7 SD 2.7 3.7 – 13.5
eGFR	ml/min/1,73 m^2	8.7 / 7.4 SD 4.8 3.4 – 22.8	8.3 / 7.1 SD 3.6 4.1 – 14.8	8.5 / 7.1 SD 4.2 3.4 – 22.8
Indication for Transplantation				
Diabetic nephropathy		1 (5%)	–	1 (2%)
Glomerulonephritis		3 (15%)	5 (23%)	8 (19%)
Nephrosclerosis (hypertension)		3 (15%)	1 (5%)	4 (10%)
Interstitial nephritis		2 (10%)	3 (14%)	5 (12%)
Cystic renal disease		2 (10%)	3 (14%)	5 (12%)
Chronic pyelonephritis		2 (10%)	1 (5%)	3 (7%)
Other indications		8 (40%)	11 (50%)	19 (45%)
Method of dialysis				
no dialysis		5 (25%)	10 (46%)	15 (36%)
continuous hemodialysis		–	–	–
intermittent hemodialysis		14 (70%)	9 (41%)	23 (55%)
peritoneal dialysis		1 (5%)	3 (14%)	4 (10%)
Length of past dialysis	days	698 / 462 SD 671 31 - 2372	813 / 592 SD 619 174 - 1989	751 / 527 SD 638 31 - 2372

Continuous data are presented as mean / median, standard deviation (SD), range.

TABLE 4 Procedural characteristics

	Unit	HTK N = 20	HTK-N N = 22
Immunosuppression before transplantation	yes	20 (100%)	20 (91%)
Cold ischemic time	min	150 / 142	131 / 125
		30	28
		117–227	77–225
Warm ischemic time	min	3 / 3	3 / 3
		1	1
		1–5	1–6
Anastomosis time	min	20 / 20	21 / 20
		5	5
		13–32	13–35
Kidney weight	g	171 / 175	184 / 188
		29	36
		116–228	118–239
Additional need for vasopressors after reperfusion	yes	1 (5%)	2 (9%)

Continuous data are presented as mean / median, standard deviation (SD), range.

lonephritis (19%), interstitial nephritis (12%), and cystic renal disease (12%). Most patients (55%) underwent intermittent hemodialysis before transplantation. Approximately one-third of patients (36%) were transplanted preemptively. The HTK-N group included numerically more preemptively transplanted patients ($n = 10$) compared to the HTK group ($n = 5$)($P = .021$). However, creatinine values of pretransplant dialyzed patients were worse in the HTK-N group (9.2 mg/dl vs. 8.2 mg/dl), resulting in comparable creatinine values at the time of transplantation in both groups.

The mean cold ischemic time was 140 ± 30.5 min. The mean anastomosis time was 20 ± 5 min.

3.3 | Primary endpoint

The GFR according to CKD EPI equation (creatinine-based) 3 months after transplantation was mean 55.4 ± 14.0 ml/min/1.73 m^2 in the HTK group compared to a GFR of 57.2 ± 16.7 ml/min/m^2 in the HTK-N group ($P = .72$). More details are given in Table 5.

The difference in GFR (HTK-N minus HTK) was 1.7 ml/min/1.73 m^2. The 95% confidence interval of this difference was -7.9 – 11.3 ml/min/1.73 m^2. The 90% confidence interval (used for one-sided assessment at .05 level) was $1.7 \pm 7.9 = -6.2 – 9.6$ ml/min/1.73 m^2. The pre-defined range of clinical equivalence was set at $\pm$ 10 ml/min/1.73 m^2. Since this was a one-sided hypothesis, GFR in the HTK-N group should not exceed -10 ml/min/1.73 m^2 with 95% certainty. The lower bound of the 90% CI of the difference is -6.2 ml/min/1.73 m^2, thus with 95% certainty, the true difference is
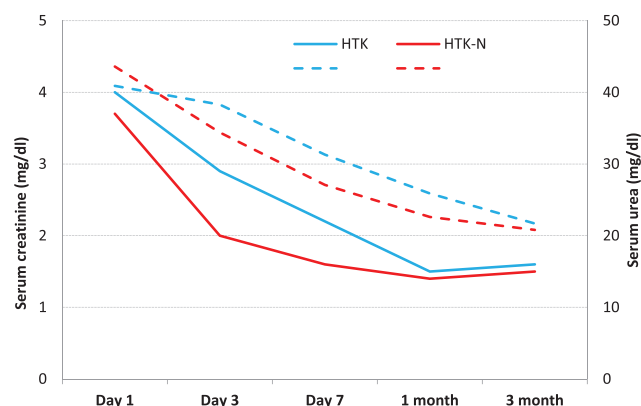

Serum creatinine (mg/dl) and serum urea (mg/dl) after transplantation (dotted line = serum urea, continual line = serum creatinine)

FIGURE 1 Serum creatinine (mg/dl) and serum urea (mg/dl) after transplantation (dotted line = serum urea, continual line = serum creatinine)

-6.2 ml/min/1.73 m^2 or higher (but not -10 ml/min/1.73 m^2). Thus HTK-N is non-inferior to HTK at .05 level, where the clinically relevant difference was set at 10 ml/min/1.73 m^2.

3.4 | Secondary endpoints

A delayed graft function (DGF) was observed in one recipient in the HTK group at postoperative day 3, which resolved in the further clinical course and was not observed anymore at postoperative day 7. None of the recipients developed DGF in the HTK-N group.

The GFR calculated by the CKD EPI equation on postoperative day 7 and 1 month after transplantation was 41.8 (33.8–59.1)ml/min/1.73 m^2 and 56.7 (45.3–67.5)ml/min/1.73 m^2 in the HTK group and 50.8 (44.4–65.3)ml/min/1.73 m^2 and 61.0 (49.3–66.2)ml/min/1.73 m^2 in the HTK-N group, respectively.

The GFR calculated by the Cystatin C equation on postoperative day 7, 1 and 3 months after transplantation was 29.4 (20.5–39.4) ml/min/1.73 m^2, 39.0 (33.–46.3) ml/min/1.73 m^2 and 46.2 (37.4–50.4) ml/min/1.73 m^2 in the HTK group and 41.4 (31.1–54.6) ml/min/1.73 m^2, 42.4 (37.2–48.9) ml/min/1.73 m^2 and 46.5 (39.3–57.7) in the HTK-N group, respectively.

Serum creatinine and serum urea were measured at each visit after transplantation. There were no relevant differences between groups. More details of these data is given in Figure 1 and Table 5.

Complications were graded according to Clavien-Dindo. Complications of grades 1, 2, and 3a were classified as "minor" while complications of grades 3b, 4a, 4b, and 5 were classified as "major". Major complications were documented in six cases (30%) in the HTK group and three cases (13.6%) in the HTK-N group. None of these concerned the preservation solution.

Biopsy-proven rejections were documented in six cases (30%) in the HTK group and two cases (9.1%) in the HTK-N group. Further details are depicted in Table 6.

TABLE 5 GFR and primary endpoint

GFR according to CKD EPI equation (creatinine based) in ml/min/1.73 m^2		HTK N = 20	HTK-N N = 22	P-value (U-test)
7 days after transplantation	mean (SD)	45.5 (19.9)	52.7 (15.9)	.279
1 month transplantation	mean (SD)	55.3 (15.4)	59.3 (10.6)	.465
Primary endpoint : 3 months after transplantation	mean (SD)	55.4 (14.0)	57.2 (16.7)	.724
	95% CI	48.9–62.0	49.8–64.6	
	median	58.5	56.1	
	IQR	46.0 – 63.5	45.5–63.5	
	min-max	26.5–84.1	36.1–115.9	
Day of assessment 3 months after transplantation	median [IQR]	89 [83–93]	84 [79–92]	.986

TABLE 6 Biopsy findings, with visit of documentation

	Custodiol N = 20	Custodiol-N N = 22
Day 1	0	0
Day 3	N = 1 (Banff ND, day 3, mild)	N = 0
Day 7	N = 3 (Banff II, antibody, day ND, moderate) (Banff, borderline, T-cell, day 4; ND) (Banff IIB; T-cell, day 6; severe)	N = 1 (Banff IIB, T-cell, day 6, mild)
1 month	N = 2 (Banff ND, day 10, moderate) (Banff III, T-cell, day ND, moderate)	N = 1 (Banff IIA, T-cell, day 25, mild)
3 months	N = 1 (Banff ND, day 46, ND)	N = 0
At any time point	N = 7	N = 2

3.5 | Patient and graft outcome

Patient survival was assessed at each postoperative visit (since day 1) up to 3 months after transplantation. No patient died during that period.

Graft function was assessed at each postoperative visit (since day 1) up to 3 months after transplantation. There was no graft failure observed during that period in both groups. There was also no incidence of primary non-function (PNF), defined as grafts that never gained function after transplantation.

3.6 | Serious adverse events

Serious adverse events (SAEs) were similarly distributed between both randomized groups (Tables 2 and 3).

4 | DISCUSSION

The present study aimed to show the equivalence of the new solution HTK-N compared to the standard HTK solution in clinical kidney transplantation. The primary objective was allograft function, measured as glomerular filtration rate 3 months after transplantation. Filtration rate was measured as GFR estimated with the CKD EPI GFR equation, which is based on serum creatinine. The study showed that the observed values for GFR were similar in both groups: 55.4 for HTK and 57.2 for HTK-N.

The study design defined a GFR of 10 ml/min/1.73m^2 as worst acceptable value for comparability between groups. From a statistical point of view such difference between both groups can be excluded, therefore demonstrating non-inferiority between both preservation solutions.

Due to a slower than expected inclusion rate (fewer patients were available than initially assumed), we terminated the study ahead of schedule. Though the number of included patient was 42 in total only, the study maintained enough power for a sufficient comparison of the primary end point between groups. The comparability of both groups is principally good, without significant imbalances. In detail, the HTK-N group included numerically more preemptively transplanted patients (n = 10) compared to the HTK group (n = 5) (P = .021). However, creatinine values of pretransplant dialyzed patients were worse in the HTK-N group (9.2 mg/dl vs. 8.2 mg/dl), resulting in comparable creatinine values at the time of transplantation in both groups. All patients and all allografts survived the study period, and the filtration rate was comparable over time.

Regarding the secondary endpoints, there were no apparent differences. However, the limited sample size limits the power of the study, hence, true differences are hard to detect in terms of the secondary end points. In terms of safety aspects, the number of SAEs was low and

evenly distributed in both groups. None of them was unexpected, and none of them was likely related to the study medication. All but one SAE could be resolved without sequelae.

Due to legislative limitations, it was impossible to perform this study after deceased kidney donations: In such a setting, all potential recipients in the EUROTRANSPLANT area would have to give informed consent to all study-related procedures, according to current laws. Hence, an RCT for deceased donor transplantation was not possible at present. Therefore, our study was carried out in the setting of living donor kidney transplantation. In living kidney transplantation, only organs of higher quality with short cold ischemia times are transplanted. It is clear that the real benefit of improved preservation is hard to detect. In our understanding, the present study fosters the applicability and eligibility to utilize the new solution HTK-N for clinical kidney preservation. This is underlined by several preclinical studies demonstrating advances of HTK-N: A porcine in vitro model of kidney reperfusion demonstrated higher renal blood flow as well as urine production and creatinine clearance if preserved by HTK-N.[30] In a pig model of kidney transplantation application of HTK-N resulted in less acute tubular injury, better creatinine clearance and less endothelial stress response.[31] The solution's true strength will presumably be detectable in the setting of deceased donor solid organ transplantation where allografts are more susceptible to IRI followed by allograft damages. We expect that not only non-inferiority but superiority compared to the classical preservation solutions will be detected. Therefore, it is of great interest to expand the utilization of HTK-N to the deceased donor setting.

The limitations of the present study are first of all the monocentric design and the limited sample size. Moreover, we had to conduct the story in the living donor setting so that extrapolation of the data to the deceased donor setting should be handled with care. In addition the current study compares HTK-N only with HTK and not with other state-of the art preservation solutions, thus, not allowing comparison with other solutions. We included all recipients undergoing living donor kidney transplantation, resulting in a rather heterogeneous recipient cohort. However, recipients were comparable in both groups. Lastly, our primary endpoint (GFR 3 months posttransplant) is afflicted with some downsides. It does not provide insights of the very early renal functional recovery, and might be influenced by several adverse events in the early weeks after transplantation like rejections or infections. However, this endpoint resembles often a first period of stable kidney function, without impact of pre-transplant creatinine values and/or perioperative multi-factorial trauma to the kidney. Additionally, inferior preservation of kidney allografts might result in inferior GFRs already 3 months after transplantation.

In conclusion, this randomized controlled trial of clinical kidney preservation with the new solution HTK-N showed non-inferiority to preservation with HTK. These data represent the first clinical application of HTK-N in kidney preservation and demonstrate safety for this new preservation solution. Further application in clinical practice will guide more results to assess the hypothetical and preclinically shown advantages of this solution.

AUTHOR CONTRIBUTIONS

Dieter P. Hoyer: ABDEF. Tamas Benkö: DF. Anja Gallinat: DF. R. Lefering: BCDE. M. Kaths: BDF. Andreas Kribben: BD Johannes Korth: BDF. Ursula Rauen: ABD Juergen W. Treckmann: ADF. Andreas Paul: ABDF.

A: Study design. **B**: Data analysis and interpretation. **C**: Drafting of article. **D**: Critical revision of article. **E**: Statistics. **F**: Data collection.

CONFLICT OF INTEREST

U.R. is one of the inventors of Custodiol-N. She is stated as one of the inventors in the patent on this preservation solution, but the patent is held by Dr. F. Köhler Chemie. The other authors have no conflict of interest to disclose.

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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