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CASE SERIES

First clinical kidney transplantation series using brief bubble and direct surface oxygenation as alternative for membrane oxygenation during hypothermic machine perfusion

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Abstract

Background: Active oxygen during hypothermic machine perfusion has the potential to improve mitochondrial preservation and subsequently decrease the harmful effects of ischemia reperfusion injury. Brief bubble, and subsequent surface oxygenation are an alternative oxygenation technique for membrane-oxygenated kidneys during hypothermic machine perfusion (HMP).

Methods: Between March 20, 2022, and June 13, 2022, 5 kidney grafts originating from 3 donors after circulatory death were oxygenated by bubble and surface oxygenation during HMP.

Results: No adverse events related to this new oxygenation technique were observed. All five recipients experienced no dialysis-dependency after transplantation with excellent initial graft function at 3 months after transplantation.

Conclusions: For the first time in human, this new oxygenation technique was successfully applied to 5 HMP-kidneys, originating from donation after circulatory death. If confirmed on larger scale cohorts, this innovative oxygenation technique, as alternative oxygenation technique for membrane-oxygenated kidneys, has the potential to be widely implemented because its simplicity and efficacy, and reducing economic and ecological costs by eliminating the need for a membrane oxygenator and oxygen source during transport.

KEYWORDS

bubble and surface oxygenation, kidney, oxygenated hypothermic machine perfusion, preservation, transplantation

1 | INTRODUCTION

Hypothermic preservation by reducing cellular metabolism and oxygen demand remains the gold standard to minimize the detrimental effect of ischemia. Hypothermic machine perfusion (HMP) has been demonstrated to improve transplant outcome as compared with static cold storage.[1–3] Given the oxygen consumption by the perfused kidney, additional oxygenation could promote oxidative processes, restoring the adenosine triphosphate (ATP)

[Correction added on March 23, 2023 after first online publication. The article category and article title has been updated in this version.]

debt and decreasing the ischemic accumulation of mitochondrial succinate.[4–7] A recent multicenter randomized clinical trial from donation after circulatory death (DCD) applying either oxygenated or non-oxygenated HMP during the entire preservation period, failed to demonstrate an improvement in 12-month estimated glomerular filtration rate (eGFR), but the graft failure rate was significantly lower in the oxygenated group (3% vs. 10%).[8] However, these positive findings were not replicated in two matched-case studies and one RCT, where oxygenated HMP was performed as an end-ischemic preservation strategy for extended criteria donors of donation after brain death kidneys after a preceding period of static cold storage.[4,9–11]

In both above-mentioned RCTs, active oxygenation was realized by the integration of a membrane oxygenator and oxygen source into the perfusion device. Whilst membrane oxygenation is undoubtably effective at oxygen transference, it is likely to be well in excess of the kidney requirement for sustaining aerobic metabolism of a single kidney at 4°C during HMP.[12] In contrast to normothermic perfusion where metabolic demands mandate oxygen is needed during the entire period of perfusion, it is less clear whether oxygenation is required during the total duration of HMP. Bubble and intermittent surface oxygenation during HMP were proven to be as efficient as continuous membrane oxygenation to obtain mitochondrial protection and superior graft function as compared to standard HMP without active oxygenation in a pig kidney DCD autotransplant model.[13] Herein, we report the first 5 cases of this innovative oxygenation technique during HMP in human renal transplantation, including the working mechanisms, benefices, and future perspectives.

2 | MATERIAL AND METHODS

2.1 | Bubble and surface oxygenation applied to kidney perfusion

The LifePort Kidney Transporter® (Organ Recovery Systems, Diegem, Belgium) was used in combination with a disposable perfusion pack with optional oxygenation tubing (reference LKT200X, Organ Recovery Systems, Diegem, Belgium). The perfusion circuit and organ reservoir were primed with 1 L University of Wisconsin machine perfusion solution (PumpProtect®, Carnamedica, Poland). In contrast with membrane oxygenation where oxygen diffuses through a thin gas-permeable membrane (Figure 1), bubble and surface oxygenation were applied in this perfusion set up to raise the dissolved perfusate O_2 concentration. The clinical implementation (Figure 2) is based on four principles.[4] First, bubble oxygenation is directly proportional to the oxygen volume and inversely proportional to the bubble size. This results in a highly effective O_2 transfer (achieving perfusate Po_2 of at least 80–100 kPa (600–750 mm Hg) after 15 min of bubble oxygenation).[13] Secondly, the solubility of oxygen increases as

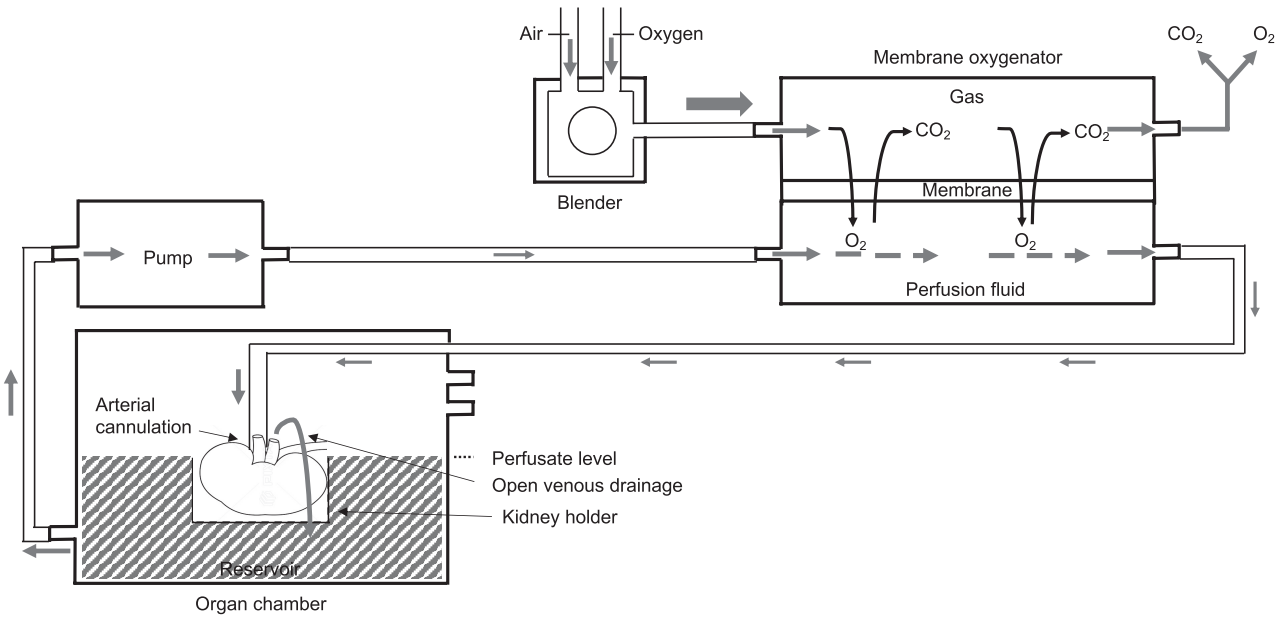

FIGURE 1 Basic principles of membrane oxygenation during kidney perfusion. Oxygen diffuses from the gas into the perfusion fluid, and carbon dioxide diffuse from the perfusion fluid into the gas for disposal. The oxygenated perfusion fluid is pumped into the kidney by arterial cannulation. Via open venous drainage, the perfusion fluid is collected into the reservoir of the organ chamber and again pumped towards the membrane oxygenator to reoxygenate the perfusion fluid.

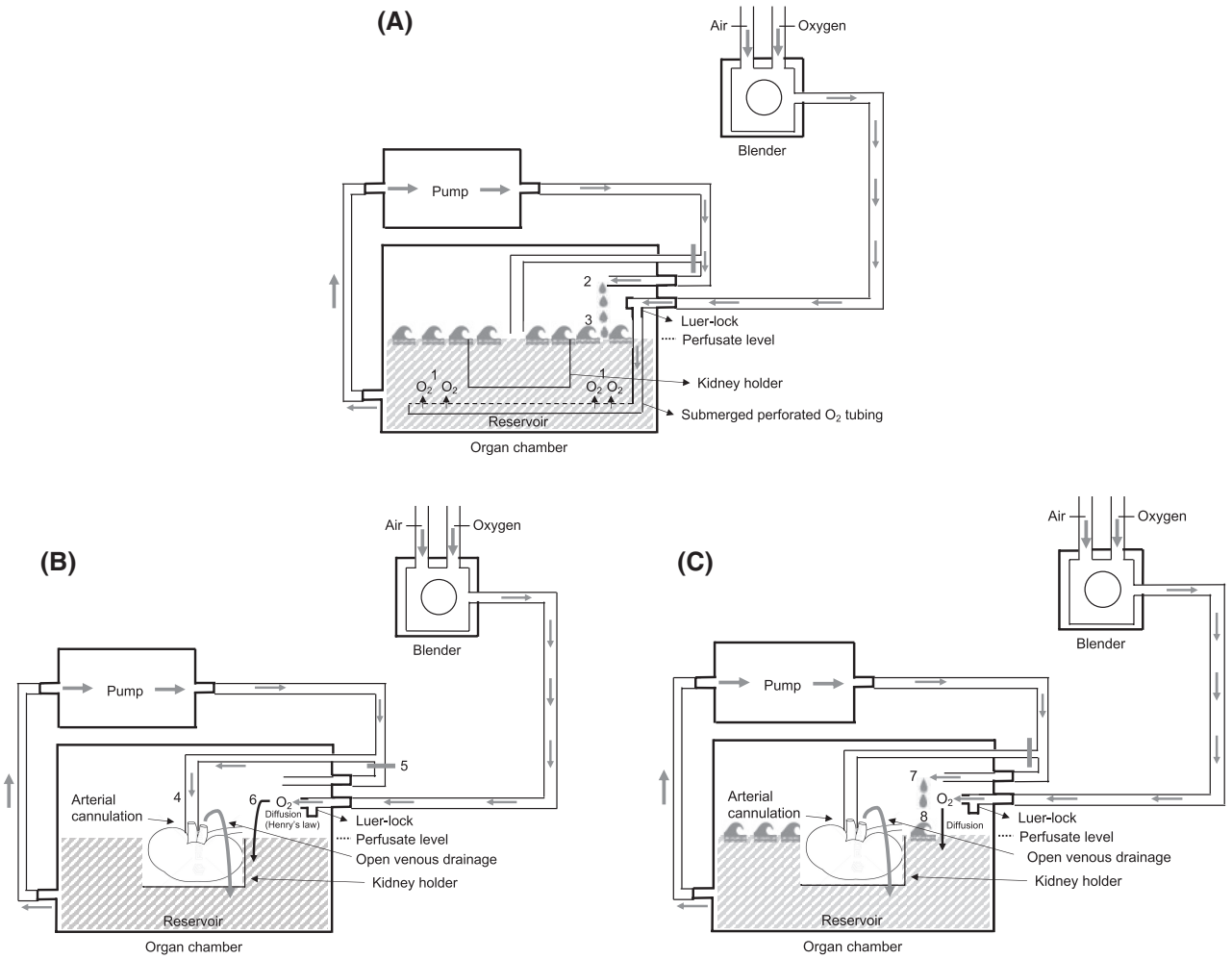

FIGURE 2 Basic principles of bubble and surface oxygenation during kidney perfusion. (A) Bubble oxygenation (90% carbogen (95% O_2–5% CO_2) was given at 500 ml/min) before connecting the kidney to the device via the submerged perforated O_2 tubing segment (1), and the perfusion fluid enters the reservoir by a separate wash line (2) and by creating waves the efficiency of this O_2 uploading process increases (3). (B) Surface oxygenation (90% carbogen (95% O_2–5% CO_2) was given at 200 ml/min) during kidney perfusion via arterial cannulation with a mean perfusion pressure of 30 mm Hg (4) (and closure of the wash line (5) and removing of the submerged tubing segment of the Luer-lock (6)). (C) the efficiency of surface oxygenation is enhanced during regularly scheduled wash cycles (7) resulting in breaking the perfusate's surface layer (8) and therefore increasing oxygen diffusion in the perfusate.

temperature decreases.[14,15] Thirdly, according to Henry's law, oxygen will slowly diffuse across the surface of the perfusate from the gaseous compartment on the top of it. The amount of O_2 diffusing into the perfusate will be proportional to the percent (=partial pressure) of O_2 above its surface. Fourthly, the efficiency of surface oxygenation is enhanced during the regularly scheduled wash cycles (every 10 min) during machine perfusion. According to the surgeon's preference, surface oxygenation during HMP can be performed in a continuous (from start until the end of HMP) versus intermittent (e.g., interrupted during organ transport) way (Video S1). In the context of a clinical trial (ClinicalTrials.gov identifier: NCT05430620), kidneys were randomized in one of the 2 following study groups: (1) continuous surface oxygenation (control group) and (2) intermittent surface oxygenation (interruption of active oxygenation during organ transport). The aim of this study is to evaluate the effect of oxygen interruption during organ transport of HMP-perfused kidneys on metabolic status at the end of the preservation period and on initial graft function.

2.2 | Transplantation and immunosuppression

All oxygenated HMP ($HMPO_2$)-perfused kidneys were classically transplanted in the iliac fossa with vascular anastomosis to the iliac vessels and the ureter to the bladder according to the Lich-Gregoir procedure over

TABLE 1 Donor characteristics and blood results before procurement of 5 HMP-perfused kidneys with active oxygenation by surface oxygenation in a single center transplant program from march 2022 until June 2022

	Donor 1	Donor 2	Donor 3
Donor characteristics			
Type of deceased donor (DBD, DCD)	DCD	DCD	DCD
Category DCD, I to V	III	III	III
Age, years	28	44	40
Gender, male/female	Male	Male	Male
Weight, kg	80	100	91
Height, cm	180	180	185
Body mass index, kg/m^2	24.7	30.9	26.6
Blood group (A/B/AB/O)	B	A	A
Ethnicity	Caucasian	Caucasian	Caucasian
Diagnosis	Trauma capitis	Trauma capitis	Ventricular fibrillation
Arterial hypertension (yes/no)	No	No	No
Diabetes mellitus (yes/no)	No	No	No
Smoking (yes/no)	No	No	Yes
Alcohol abuse (yes/no)	Yes	No	Yes
Cardiac arrest (yes/no)	No	Yes	Yes
Hypotensive period (yes/no) (systolic pressure < 100 mm Hg)	No	No	No
Anuria or oliguria (yes/no) (<500 ml/24 h)	No	No	No
Vasopressors (yes/no)			
Dopamine	No	No	No
Dobutamine	No	No	No
(Nor)adrenaline	Yes	No	No
Sepsis (yes/no)	No	No	No
Antibiotics (yes/no)	No	Yes	Yes
Blood results			
Urea (mg/dl)	11	40	38
Last creatinine (mg/dl)	0.7	0.63	0.64
Peak creatinine (mg/dl)	0.7	1.35	1.31
CMV status (negative/positive)	Negative	Positive	Negative

Abbreviations: CMV, cytomegalo virus; DBD, donation after brain death; DCD, donation after circulatory death; HMP, hypothermic machine perfusion.

a double J stent. All recipients received our local immunosuppressive protocol (tacrolimus [1 mg/kg/day to maintain trough levels of 10–14 ng/ml], mycophenolate mofetil [500 mg twice daily] and methylprednisolone [16 mg daily, tapered with 4 mg per 2 weeks]). Induction therapy was added in high-immunized recipients (thymoglobulin 1.25 mg/kg/d) from day 1 until day 7 or plasmapheresis until one month after transplantation according to donor's compatibility and recipient's characteristics.

3 | RESULTS

3.1 | Donor and recipient details

The characteristics of the 3 DCD donors performed between March 20, 2022, and June 13, 2022, are illustrated in Table 1. Five of the 6 kidneys of these 3 donors were locally allocated by Eurotransplant and accepted by our own transplant team. The procurement data and recipient characteristics are summarized in Table 2.

TABLE 2 Recipient, procurement, and machine perfusion characteristics of 5 HMP-perfused kidneys with active oxygenation by surface oxygenation in a single center transplant program from march 2022 until June 2022

	Recipient 1	Recipient 2	Recipient 3	Recipient 4	Recipient 5
Recipient characteristics					
Age, years	38	39	52	19	47
Gender, male/female	Female	Male	Male	Female	Female
Weight, kg	66	63	68	63	62
Height, cm	156	163	180	170	164
Body mass index, kg/m^2	27.1	23.7	21.0	21.8	23.1
Blood group (A, B, AB, O)	B	B	A	A	A
CMV status (negative/positive)	Negative	Negative	Positive	Positive	Positive
Primary nephropathy	Orofaciodigital syndrome type I with associated renal cysts	Glomerular disease	ADPKD	Glomerular disease	ADPKD
Residual urine output before transplantation, ml	0	0	1500	0	500
Rank of transplant (1, 2, 3)	2	3	1	1	1
Time on dialyses before transplantation, months	100	92	27	24	35
Peak PRA% pretransplant	96	86	0	0	71
HLA mismatch (0, 1, 2)					
HLA-A	1	1	1	2	0
HLA-B	2	1	1	1	1
HLA-C	1	1	1	1	0
Procurement data					
Kidney graft origin	Right kidney donor 1	Left kidney donor 1	Right kidney donor 2	Left kidney donor 2	Right kidney donor 3
Number of renal arteries, n	1	1	2	2	1
Damage (arterial, venous, parenchym or ureter) (yes/no)	No	No	No	No	No
Washout perfusion kidney (homogenous, patchy, blue)	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
Visual perfusion defect (yes/no)	No	No	No	No	No
Machine perfusion characteristics					
Study group: continuous versus intermittent surface oxygenation	Continuous surface O_2	Intermittent surface O_2	Continuous surface O_2	Continuous surface O_2	Intermittent surface O_2
Time of bubble oxygenation, min	58	48	59	73	65

(Continues)

TABLE 2 (Continued)

	Recipient 1	Recipient 2	Recipient 3	Recipient 4	Recipient 5
Duration of interruption surface oxygenation, min	NA	27	NA	NA	90
Total machine perfusion time, h:m	2:13	10:06	9:58	14:47	7:21
Renal flow at end HMP, ml/min	175	181	134	211	NA
Renal resistance at end HMP, mm Hg/ml/min	0.15	0.11	0.16	0.12	NA
Adverse event related to bubble and surface oxygenation (yes/no)	No	No	No	No	No

Abbreviations: CMV, cytomegalo virus; HMP, hypothermic machine perfusion; NA, not available.

3.2 | Bubble and surface oxygenation during HMP

Perfusion set up (included bubble oxygenation) was started once the surgeon considered the kidney technically feasible to connect on the HMP-device. Before connecting the kidney to the device, bubble oxygenation was realized. In the context of a (still ongoing) clinical trial, surface oxygenation was performed in a continuous way or intermittently (interruption of active oxygenation during organ transport) during HMP. Detailed machine perfusion characteristics are also illustrated in Table 2. No adverse event related to this new oxygenation technique was observed.

3.3 | Transplant data and clinical outcome

Transplant data and functional outcome of the 5 recipients are summarized in Table 3. On clamp release, all kidneys were well perfused in all areas and urine production was noted before the end of the transplant procedure. None of the HMP-perfused kidneys demonstrated delayed graft function nor an acute rejection episode during follow-up. Initial graft function of all recipients is illustrated in Figure 3. Recipient 1 received clotting factor VIII intravenously peritransplant for 24 h because of hemophilia type A and demonstrated slow initial graft function, but without the need for dialysis because of high initial urine output (see also Figure 3). Her postoperative course was complicated with a surgical drainage of a large retroperitoneal hematoma at day 7 and a symptomatic hospital-acquired influenza A infection at day 11 treated for 5 days with oseltamivir, 2 × 75 mg PO (Tamiflu®, Roche, Belgium) followed by hospital discharge at day 19. The postoperative course of recipient 2 was complicated with an asymptomatic urinary infection with E. Coli, treated for 6 days with oral antibiotics and double J stent removal afterwards.

4 | DISCUSSION

This is the first report of bubble and surface oxygenation, being applied in human renal transplantation. This oxygenation technique is easy and feasible and can be performed without apparent deleterious effect to the donor kidney or recipient.

Hollow fiber membrane oxygenators and oxygen cylinders were incorporated in the commercially available Kidney Assist Transporter (Organ Assist BV, Groningen, The Netherlands) and the VitaSmart (Bridge to Life, Northbrook, IL, USA).[4] The rationale to omit a membrane oxygenator during HMP is twofold. First, its oxygen transfer capacity is being significantly higher than what is needed for sustaining aerobic metabolism of a single kidney at ±4°C.[12,16] Secondly, current machine preservation solutions are still acellular and without oxygen carrier and oxygen must reach the organ tissue by diffusion.[4]

The duration of active oxygenation during HMP to obtain optimal mitochondrial protection is less clear. A pig kidney ex vivo preservation model was used to evaluate the effect of the interruption of surface oxygenation (mimicking organ transport in the Eurotransplant region) during HMP as compared to continuous surface and membrane oxygenation. Metabolic analysis (i.e., lactate, succinate, glutamate, ATP, ADP, AMP, NADH, NAD^+ and Flavin Mononucleotide [FMN]) on end-preservation cortical and medullar tissue biopsies demonstrated a similar mitochondrial preservation in all study groups (article in preparation). This was the rationale of the study design of

TABLE 3 Transplant, ischemia times and functional outcome characteristics of 5 HMP-perfused kidneys with active oxygenation by surface oxygenation in a single center transplant program from march 2022 until June 2022

	Recipient 1	Recipient 2	Recipient 3	Recipient 4	Recipient 5
Transplant data					
Manitol peroperative (yes/no)	Yes	Yes	Yes	Yes	Yes
Diuretics peroperative (yes/no)	Yes	Yes	Yes	Yes	Yes
Arterial or venous problems during graft implantation (yes/no)	No	No	No	No	No
Graft pink and well vascularized after reperfusion (yes/no)	Yes	Yes	Yes	Yes	Yes
Good turgor of kidney after reperfusion (yes/no)	Yes	Yes	Yes	Yes	Yes
Intra-operative urine production of kidney graft (yes/no)	Yes	Yes	Yes	Yes	Yes
Immunosuppressive therapy					
Tacrolimus	Yes	Yes	Yes	Yes	Yes
Mycophenolate mofetil	Yes	Yes	Yes	Yes	Yes
Methylprednisolone	Yes	Yes	Yes	Yes	Yes
Induction therapy	Yes	Yes	No	No	Yes
Thymoglobuline	Yes	No			Yes
Plasmapheresis	No	Yes			No
Ischemia times					
First warm ischemia time*, min	11	11	8	8	12
Anastomosis time**, min	40	30	58	33	23
Cold ischemia time, h:m	4:46	12:46	13:45	17:54	10:41
Clinical outcome					
Primary nonfunction (yes/no)	No	No	No	No	No
Delayed graft function*** (yes/no)	No	No	No	No	No
Acute rejection episode (yes/no)	No	No	No	No	No
Complications (yes/no)					
Wound infection	No	No	No	No	No
Cardiovascular	No	No	No	No	No
Urinary infection	No	Yes	No	No	No
Respiratory infection	Yes (hospital-acquired H. Influenza)	No	No	No	No
Needed surgical revision	Yes (retroperitoneal hematoma due to hemophilia A)	No	No	No	No
Respiratory failure	No	No	No	No	No
Transfusion need	Yes	No	No	No	No
Hospital stay after transplantation, days	19	12	9	10	8
Last serum creatinine (mg/dl)	1.15	1.29	1.49	2.37	1.07
Last eGFR (ml/min/1.73 m^3)	60	64	53	29	62
Last urinary protein/creatinine radio (g/g)	<0.105	<0.125	0.089	0.051	0.087

Note: Definitions: *Time from switch of ventilator until moment of start in situ cold perfusion of the organs after sternolaparotomy, **Time from kidney out ice water until moment of in vivo blood reperfusion, ***Defined as the need for dialysis in the first 7 days after transplantation and preceding the return of kidney function.

Abbreviations: eGFR, estimated glomerular filtration rate; HMP, hypothermic machine perfusion.

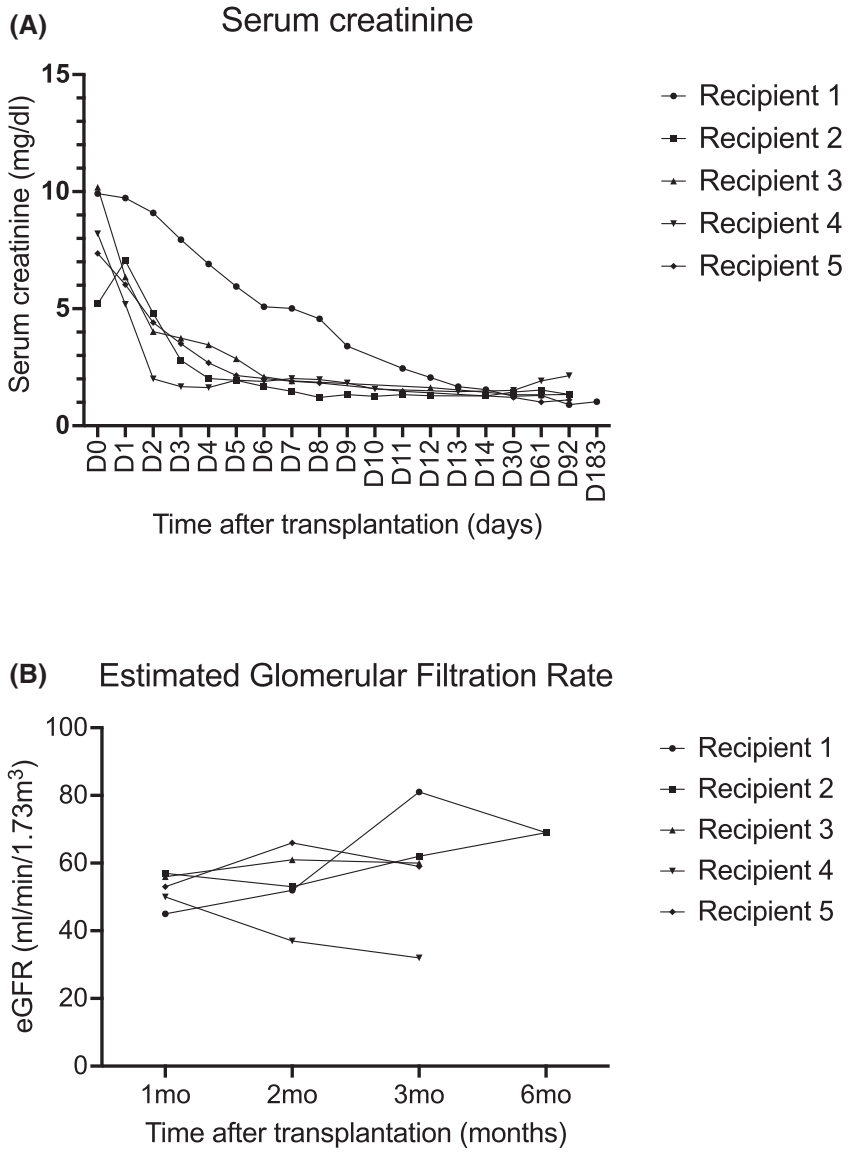

FIGURE 3 Evolution of serum creatinine (A) and estimated glomerular filtration rate (eGFR) (B) after transplantation of 5 HMP-perfused kidneys with active oxygenation by surface oxygenation in 5 recipients in a single center transplant program from march 2022 until June 2022.

our ongoing prospective single center trial using bubble and surface oxygenation for DCD kidneys (ClinicalTrials.gov identifier: NCT05430620).

The advantages of brief bubble and subsequent surface oxygenation are the following: (1) a simple and effective oxygenation method, and (2) might omit the need for a membrane oxygenator (±400–500€) and oxygen source (±100–150€) during organ transport (by simply using the existing oxygen connection sources available in donor and recipient center and therefore also allowing airplane transport) which results in a significant reduction of economic and ecological costs.

This new oxygenation technique can logistically easy be applied in transplant programs already using the LifePort Kidney Transporter® (Organ Recovery Systems). In addition, this technique is also easy to integrate in existing kidney-HMP devices (transportable and non-transportable) and merits exploration to other organs (e.g., pancreas and liver). However, the clinical evidence to use active oxygenation during HMP, independent of the oxygenation technique, is, at this moment, relatively limited and needs to be confirmed by future clinical trials.

In summary, bubble and surface oxygenation is a straightforward new oxygenation technique as alternative for membrane oxygenation and herein described for the first time in human renal transplantation.

AUTHOR CONTRIBUTION

Concept/data collection and analysis/interpretation/drafting article: Darius Tom. *Critical revision of article*: Devresse Arnaud. *Critical revision of article*: Buemi Antoine. *Critical revision of article*: Kanaan Nada. *Critical revision of article*: De Meyer Martine. *Concept/critical revision of article*: Mourad Michel.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The Saint-Luc Hospital-faculty Ethical Committee approved in June 2021 a clinical trial to explore this new oxygenation technique (ClinicalTrials.gov identifier: NCT05430620).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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