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EARLY EXPERIMENTS WITH HYPOTHERMIC OXYGENATED MACHINE PERFUSION OF KIDNEY GRAFTS FROM EXTENDED CRITERIA DONORS

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Objective: to evaluate the safety and efficacy of hypothermic oxygenated machine perfusion (HOPE) for kidney grafts obtained from expanded criteria donors (ECD). **Materials and methods.** From June 2018 to June 2021, 200 surgeries involving kidney transplants from deceased donors were performed at Botkin City Clinical Hospital. Of these, 123 were men (61.5%) and 77 were women (38.5%). The mean age was 47.62 ± 11.69 (20–73) years. In 102 cases, kidney grafts were procured from ECD. In 92 recipients (90.2%) of kidney transplants from an expanded criteria donor, static cold storage done according to the standard technique was used to preserve the organ; these patients constituted observation group 1. In 10 recipients (9.8%), hypothermic oxygenated perfusion was used in addition to static cold preservation; these patients formed observation group 2. **Results.** No 30-day mortality was recorded in both observation groups. The mean static cold storage time in group 1 patients was 612.33 ± 178.88 (133–1180) minutes. Overall incidence of delayed graft function was 26.5% (53/200). Incidence of delayed graft function was 19.3% (19/98) for organs from standard donors using static cold storage and 35.8% (33/92) for ECD organs. Twenty-five patients (12.5%) had postoperative complications. Postoperative complications with delayed graft function were diagnosed in 12 patients, which was 22.6% (12/53), with immediate function in 13 patients, which was 8.8% (13/147). Mean cold storage time in group 2 patients was 319.11 ± 110.24 (311–525) minutes. Mean HOPE time was 202.34 ± 21.48 (150–210) minutes. Delayed graft function was recorded in 1 group 2 patient (10%). No complications, including perfusion-related one, were recorded in this group. **Conclusion.** The unique technique used at Botkin City Clinical Hospital for HOPE in kidney transplant is safe. It provides a low risk of delayed graft function for ECD kidneys.

Keywords: kidney transplant, expanded criteria donors, hypothermic oxygenated machine perfusion.

INTRODUCTION

Long-term outcomes in kidney transplantation are constantly improving. At present, the 10-year survival of kidney transplants is over 80% [1]. The effectiveness of transplantation as a method of treatment of end-stage chronic organ failure has consistently increased the number of patients in waiting lists both worldwide and in Russia [2, 3]. The increasing shortage of donor organs prompts the search for new ways to tackle this problem. The most widespread practice throughout the world is the practice of expanding the criteria for organ donation [4]. It should be noted that long-term outcomes of solid organ transplantation from a standard donor and from an expanded criteria donor differ slightly. Specifically, the 1- and 5-year survival rates of a renal transplant from a standard donor are 92% and 70%, respectively, and from an expanded criteria donor, the figure is 80% and 44%, respectively [5]. This difference is explained by the poorer tolerability of cold ischemia of organs from expanded criteria donor. Oxygen consumption in tissues decreases significantly at 4–10 °C, but the corresponding metabolism is still observed. Additional oxygen can support mitochondrial adenosine triphosphate (ATP) synthesis and, in turn, restrain the damage process [6].

In preclinical trials, HOPE was found to reduce the incidence of cell damage and macrophage activation [7, Experiments suggest that this technology in clinical conditions should improve kidney transplant outcomes. Currently, there are several clinical studies evaluating the effectiveness of this technology in liver and kidney transplantation in the world.

MATERIALS AND METHODS

From June 2018 to June 2021, 200 surgeries involving kidney transplants from deceased donors were performed at Botkin City Clinical Hospital. Of these, 123 were men (61.5%) and 77 were women (38.5%). The mean age was 47.62 ± 11.69 (20–73) years. In 102 cases,

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kidney grafts were procured from ECD. In 92 recipients (90.2%) of kidney transplants from an expanded criteria donor, static cold storage done according to the standard technique was used to preserve the organ; these patients constituted observation group 1. In 10 recipients (9.8%), hypothermic oxygenated perfusion was used in addition to static cold preservation; these patients formed observation group 2.

Expanded criteria donors were defined according to the UNOS definition of 2003. [R.A. Metzger, F.L. Delmonico, S. Fenf et al. Expanded criteria donors for kidney transplantation. Am J of Transplantation. 2003; 3, suppl 4: 114–125] as a donor 50–59 years of age with at least two of the following conditions: cause of death from cerebrovascular accident, hypertension history, serum creatinine >133 μmol/L, or a donor ≥60 years of age had all, any two factors, any one factor or none of the factors presented above.

Protocol for HOPE in renal graft: renal graft perfusion was in the operating room with strict adherence to aseptic rules. The temperature of the preservative solution was measured using a non-contact Testo 805 thermometer, the target level was 4–8 °C.

The packaged kidney graft was removed from the shipping container. Punch biopsy of the kidney was performed. The graft was examined to assess its appearance and vascular anatomy. A soft silicone cannula previously attached to the arterial line was inserted into the mouth of the renal artery and fixed to the aortic site with 2–3 knotted sutures (Fig. 1).

If there were two or more renal arteries, each was cannulated using a Y-shaped adapter.

The cannula was connected to a circuit consisting of a roller pump of a stationary AIC apparatus, an oxygenator, and a pressure sensor built into the CPB pump (Fig. 2). The required volume circulation rate of the preservative depends on the hydrostatic pressure in the arterial line. The preservative delivery rate was gradually increased by the regulator until 40 mmHg target pressure was reached. The procedure was performed under constant monitoring by a surgeon. The tightness of the connection between the arterial cannula and the renal artery was constantly monitored, the renal graft temperature was determined every 15 minutes, the pH of the perfusate to assess oxygen saturation was determined every 30 minutes. Thawed refrigerants were replaced as needed. When the pressure in the system decreased, indirectly indicating a decreased resistance in the renal microcirculatory bed, the preservative delivery rate was adjusted to maintain 40 mmHg target pressure. Vascular resistance index was calculated as the ratio of pressure in the system to the volumetric blood flow rate.

Perfusion was completed when the recipient was admitted to the operating room. Before the kidney was incorporated into the recipient's bloodstream, another biopsy of the kidney from the primary punch biopsy site was performed. Intraoperative ultrasound examination with determination of the resistance index was performed before suturing the surgical wound. In the first week of the postoperative period, the following parameters were assessed daily: diuresis, urea, creatinine, K^+, concentration of blood calcineurin inhibitors, resistance index in ultrasound examination was estimated, based on which the decision on the need for renal replacement therapy was made. Postoperative complications were assessed.

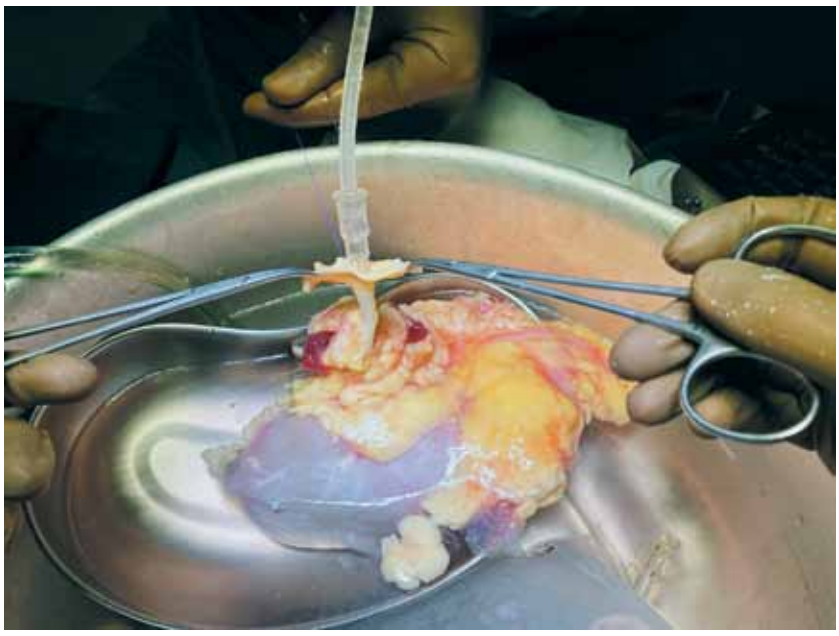

Fig. 1. Cannulation of kidney graft artery for oxygenated cold perfusion

RESULTS

No 30-day mortality was recorded in both observation groups. The mean static cold storage time in group 1 was 612.33 ± 178.88 (133–1180) minutes. The overall incidence of delayed renal graft function was 26.5% (53/200). When using an organ from a standard donor using static cold storage, the incidence of delayed graft function was 19.3% (19/98), and it was 35.8% (33/92) for ECD organs. Postoperative complications occurred in 25 patients (12.5%). Postoperative complications with delayed graft function were diagnosed in 12 patients, which was 22.6% (12/53), with immediate function in 13 patients, which was 8.8% (13/147).

The mean cold storage time in group 2 was 319.11 ± 110.24 (311–525) minutes. The mean hypothermic oxygenated perfusion time was 202.34 ± 21.48 (150–210) minutes. Mean renal graft temperature during perfusion ranged from 4.7 to 6.8 °C (Fig. 3).

The mean partial pressure of oxygen in the perfusate ranged from 323 to 574 mmHg (Fig. 4).

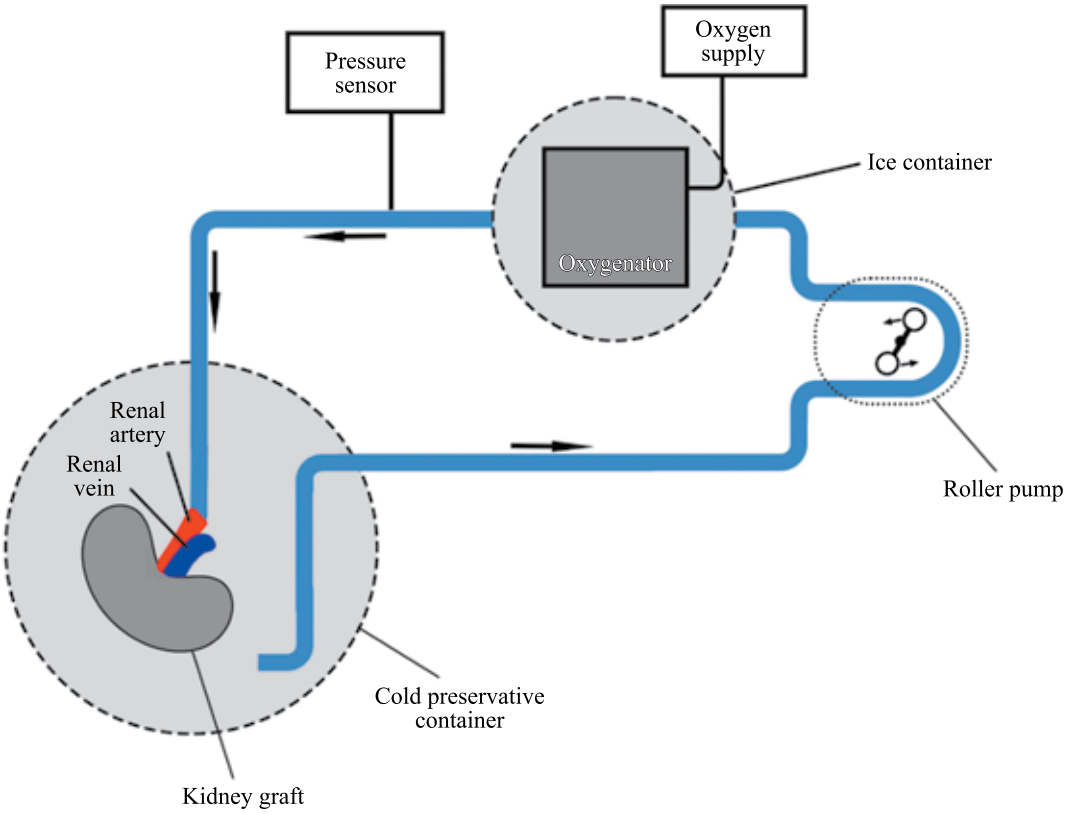

Fig. 2. Schematic layout of oxygenated cold perfusion of a kidney graft

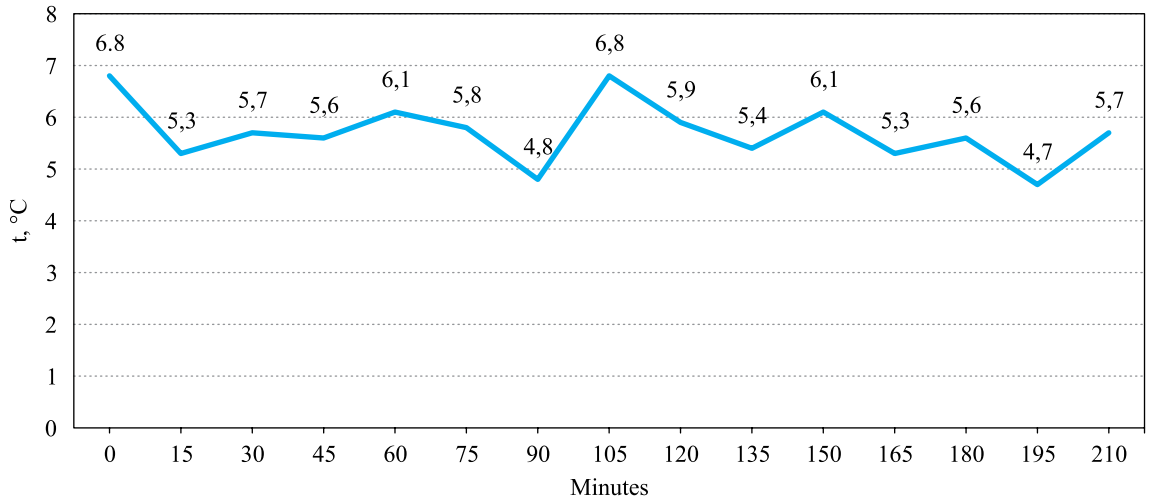

Fig. 3. Dynamics of average renal graft temperature during oxygenated cold perfusion

In one case, the vascular resistance index during perfusion increased by 0.02 (Table 1).

This group 2 patient (10%) had a delayed graft function. No complications, including perfusion-related complications, occurred in this group.

Electron microscopy of nephrobiopsy specimens from group 1 patients showed pronounced negative dynamics expressed in mitochondrial destruction (Fig. 5).

Electron microscopy of nephrobiopsy specimens from group 2 patients showed mitochondrial preservation after perfusion (Fig. 6).

DISCUSSION

Shortage of donor organs is a problem that limits the number of transplants performed. One solution is to expand the indications for donation, which leads to increased use of ECD renal transplants. According to our data, the use of ECD renal transplants significantly increases the likelihood of developing delayed function (35.8 vs 19.3%, p = 0.021). Development of delayed function, in turn, is associated with a significantly higher incidence of postoperative complications (22.6 vs 8.8%, p = 0.015), resulting in an increase in the average length of hospital stay and increased treatment costs. When evaluating our experience with the use of ECS organs, we obtained data on the effect of high donor body mass index (BMI) and cold ischemia time of the donor kidney on incidence of delayed function (Table 2).

In most cases, reducing the static cold storage time appears to be a difficult task. The patient has to come to the transplant center, undergo preoperative examination, in some cases dialysis is required. All this leads to

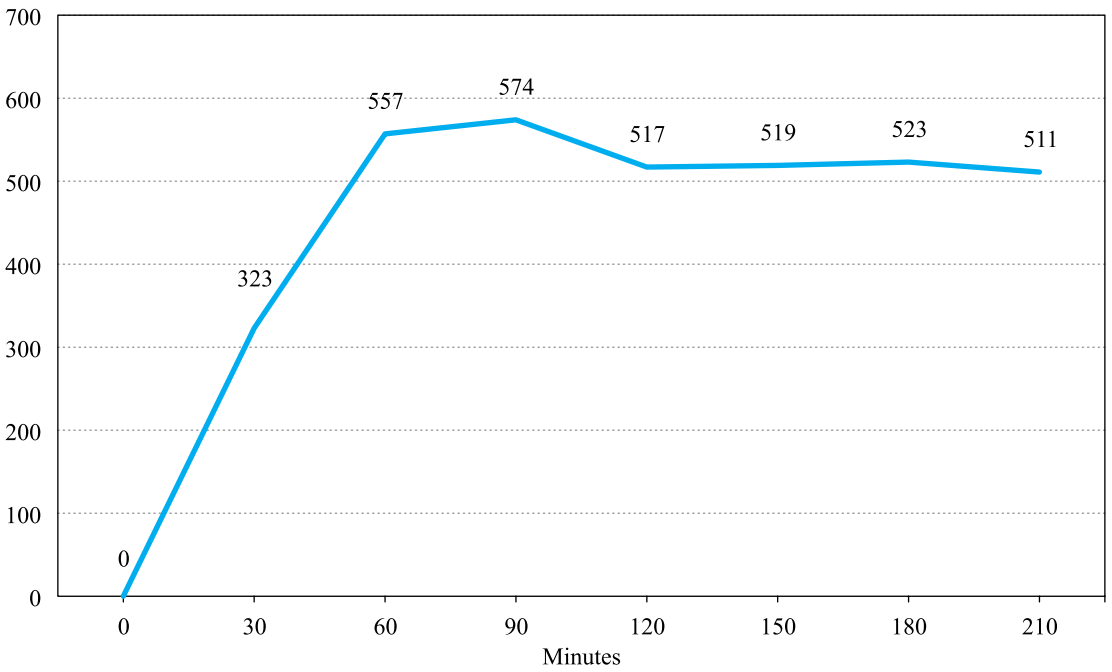

Fig. 4. Dynamics of mean partial pressure of oxygen in perfusate during oxygenated cold perfusion

Table 1

Indicators of oxygenated cold perfusion of the renal graft

S/N	Beginning-of-perfusion pressure	Beginning-of-perfusion volumetric blood flow rate	Beginning-of-perfusion vascular resistance	End-of-perfusion pressure	End-of-perfusion volumetric blood flow rate	End-of-perfusion vascular resistance
1	41	70	0.58	42	90	0.46
2	40	120	0.33	40	150	0.26
3	42	110	0.38	40	120	0.30
4	39	90	0.43	41	90	0.45
5	42	60	0.70	40	70	0.57
6	40	120	0.33	42	140	0.3
7	41	100	0.41	41	120	0.34
8	42	90	0.46	40	90	0.44
9	40	80	0.50	39	100	0.39
10	40	90	0.44	41	100	0.41

an average cold storage time of more than 10 hours at Botkin Hospital. Organs from standard donors tolerate the long cold ischemia period satisfactorily, ECD organs do so significantly worse. In our opinion, the solution to this problem is to replace static cold storage with HOPE. Delivery of oxygen dissolved in the preserving solution to the cells at a preserved low temperature constitutes the theoretical advantage of this technology. Oxygen supports aerobic metabolism in the cells, which slows down dramatically but does not stop completely in cold environments. Aerobic metabolism eliminates uncoupling of the mitochondrial respiratory chain with formation of reactive oxygen species, prevents the development of intracellular acidosis, and also maintains Na^+/K^+-ATPase activity at a normal level, which in turn reduces the risk of cell apoptosis.

Our proposed heart-lung machine for hypothermic oxygenated perfusion has all the qualities necessary for machine perfusion: it can control pressure and volumetric blood flow rate and oxygenate the preservation solution. An important problem we encountered in the development of the perfusion protocol is ensuring and maintaining the necessary temperature of the solution. The fact is that during perfusion the solution in the circuit is heated. So, on one hand, careful temperature control of the renal graft is required, and on the other, effective constant cooling of the solution is needed. We use a remote thermometer to dynamically measure the temperature of the kidney graft. For constant cooling of Custodiol, we use special sterile refrigerants, which are placed near the renal graft without touching it or mixing with the preservative, and we cover the oxygenator with ice as an element of the circuit with the largest area of contact with the solution. These measures allow to maintain the temperature of the graft within 4–8 °C throughout the perfusion period.

Addition of oxygen to the perfusion solution is the most important part of the protocol. Oxygen dissolves well in Custodiol; we managed to achieve an average partial pressure of oxygen in perfusate at 500 mmHg at a delivery rate of 4 liters per minute.

To assess the effectiveness of perfusion in preserving cell mitochondria, we performed electron microscopy of nephrobiopsy specimens before and after HOPE. In all cases after completion of hypothermic machine perfusion, preserved mitochondria with cristae were detected

Table 2

Impact of expanded criteria donor risk factors on delayed renal graft function

Risk factor	Immediate function (n = 59)	Delayed function (n = 33)	p
Donor's age:			
55 to 65 years old	42	23	0.65
>65 years old	17	10	
Donor's gender:			
Male	27	16	0.73
Female	32	17	
Donor's BMI:			
<25	26	12	0.04
>25	33	21	
Recipient's BMI:			
<25	29	15	0.63
>25	30	18	
Donor's hospitalization time:			
<72 hours	44	22	0.29
>72 hours	15	11	
Cold ischemia time:			
<10 hours	26	12	0.03
>10 hours	33	21	

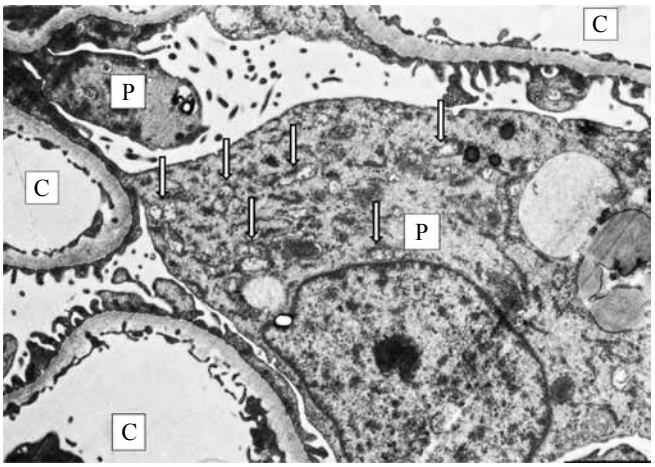

Fig. 5. Renal glomerulus fragment before HOPE. The podocyte (P) cytoplasm contains: small mitochondria with partially preserved cristae (arrows); short profiles of granular endoplasmic reticulum. C, capillaries. 9000× magnification

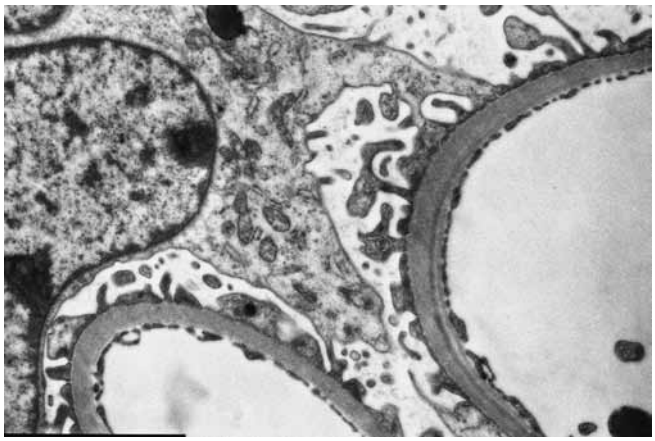

Fig. 6. Renal glomerulus fragment. The podocyte cytoplasm contains: small mitochondria with partially preserved cristae, short profiles of granular endoplasmic reticulum. 12000× magnification

in the mitochondria of the renal glomerulus, distal and proximal nephron fragments.

So, the developed technique makes it possible to maintain the energy balance of cells for a long time and prevent mitochondrial destruction, which inevitably occurs in the process of static cold storage.

The revealed morphological advantages of HOPE also correlate with clinical manifestations. Out of 10 patients who underwent kidney transplantation from a deceased ECD using the perfusion used, delayed graft function was observed in 1 patient (10%), which was significantly lower compared with Group 1 ($p = 0.035$). It was in this case that there was an increase in vascular resistance index from 0.43 to 0.45 during perfusion; in other cases, the index decreased on average by 0.07 ± 0.04 (0.02–0.13). This suggests that this parameter can be regarded as a predictor of delayed renal graft function.

The first experience of using our own HOPE protocol for an ECD renal graft showed its safety and efficiency. Further studies will clarify the optimal perfusion parameters, identify predictors of delayed organ function and primary nonfunction. Further reduction of the incidence of delayed function is possible through introduction of transport systems for hypothermic oxygenated perfusion.

CONCLUSION

The original technique used at Botkin Hospital for renal graft HOPE is safe. It is associated with a low risk of delayed renal graft function for ECD organs. Further studies will expand the indications for this technique.

The authors declare no conflict of interest.

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