

**201710-21500Title: Nitric Oxide Decreases Acute Kidney Injury and Stage 3
Chronic Kidney Disease after Cardiac Surgery.**

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CL, LB, LX and WMZ conceptualized the study. CL, LB, HD, SY, ZJ and LX revised and finalized the protocol. CL, HD, LH and MC collected patients' descriptive data and collected plasma and urine samples. LH and MC conducted standardized anesthesia. SY, WC, HW and QZ screened patients and conducted surgery. JS delivered NO to patients. ZJ and TC conducted cardiopulmonary bypass. RZ conducted postoperative intensive care to patients. ER and BY measured plasma NO consumption. EC and VSS measured urine biomarkers of kidney injury. ER and FN led the data analysis. CL, LB, ER, JVB, LX and WMZ led data interpretation. CL, LB and ER wrote the first report. JVB, LX and WMZ critically reviewed and revised the initial draft. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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At a Glance Commentary

Scientific Knowledge on the Subject:

Kidney damage after cardiac surgery requiring prolonged cardiopulmonary bypass is a common and serious complication. Over the past decades all attempts to decrease kidney injury after heart surgery failed. Promising animal studies showed that administration of nitric oxide decreased renal dysfunction during hemolysis by oxidation of plasma oxy-hemoglobin to met-hemoglobin.

What This Study Adds to the Field:

In a randomized clinical trial in China of 244 adults undergoing elective, multiple valve replacement surgery mostly due to rheumatic fever, administration of 80 parts-per-million of nitric oxide during and after prolonged cardiopulmonary bypass reduced the incidence of acute kidney injury and improved renal function at follow up 1 year after surgery. Nitric oxide gas is the first pharmacological intervention to show a reduction in the incidence of acute kidney injury and an improvement of long-term kidney function in cardiac-surgical patients after prolonged cardiopulmonary bypass. These results should be assessed in non-Chinese patients without rheumatic fever.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

ABSTRACT

Rationale: No medical intervention has been identified that decreases acute kidney injury and improves renal outcome at 1-year after cardiac surgery.

Objective: To determine whether administration of nitric oxide reduces the incidence of post-operative acute kidney injury and improves long-term kidney outcomes after multiple cardiac valve replacement requiring prolonged cardiopulmonary bypass.

Methods: 244 Patients undergoing elective, multiple valve replacement surgery mostly due to rheumatic fever were randomized to receive either nitric oxide (treatment) or nitrogen (control). Nitric oxide and nitrogen were administered via the gas exchanger during cardiopulmonary bypass and by inhalation for 24h post-operatively.

Measurements and Main Results: Primary outcome: Oxidation of ferrous plasma oxyhemoglobin to ferric methemoglobin was associated to a reduced post-operative acute kidney injury from 64% (control group) to 50% (nitric oxide) (RR, 95% CI; 0.78, 0.62–0.97; $P=0.014$). Secondary outcomes: At 90-days, transition to stage 3 chronic kidney disease was reduced from 33% in the controls to 21% in the treatment group (RR, 95%CI; 0.64, 0.41 – 0.99; $P=0.024$); and at 1-year, from 31% to 18% (RR, 95% CI; 0.59, 0.36 – 0.96; $P=0.017$). Nitric oxide treatment reduced the overall major adverse kidney events at 30-days (RR, 95% CI; 0.40, 0.18 – 0.92; $P=0.016$, 90-days (RR, 95% CI; 0.40, 0.17 – 0.92; $P=0.015$ and 1-year (RR, 95% CI; 0.47, 0.20–1.10; $P=0.041$).

Conclusions: In patients undergoing multiple valve replacement and prolonged cardiopulmonary bypass, administration of nitric oxide decreased the incidence of acute kidney injury, transition to stage 3 chronic kidney disease and major adverse kidney events at 30-days, 90-days, and 1-year.

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INTRODUCTION

Acute kidney injury (AKI) is a common and serious complication of cardiac surgical procedures that require prolonged (>90 minutes) cardiopulmonary bypass (CPB)^{1,2}. While the presence of AKI after CPB is associated with increased mortality, no medical interventions have yet been shown to be associated with improved long-term kidney function¹⁻⁷.

The mechanisms leading to AKI are multifactorial and not fully elucidated. However, hemolysis has been shown to be closely associated with post-surgery AKI⁸⁻¹³. During hemolysis, hemoglobin (Hb) is released into the circulation in the form of oxyhemoglobin (Oxy-Hb). Plasma Oxy-Hb is filtered by the kidneys and facilitates development of AKI by intrarenal oxidative reactions¹⁴. Furthermore, plasma oxyhemoglobin depletes vascular nitric oxide (NO) via the dioxygenation reaction to form methemoglobin (Met-Hb)^{15,16}. Endogenous NO is a potent vasodilator which relaxes vascular smooth muscle, and NO depletion by plasma Oxy-Hb produces vasoconstriction, impairs tissue perfusion, and causes inflammation¹⁷⁻²². The administration of therapeutic levels of 80 parts-per-million (ppm) exogenous NO gas oxidizes plasma Oxy-Hb to Met-Hb. The oxidized iron (Met-Hb) species is unable to deplete plasma NO^{10,14,16, 23}. In a human model of blood transfusion, we found that breathing 80ppm of NO was safe and prevented depletion of plasma NO by circulating plasma Hb¹⁷. In an experimental canine model of free water-induced hemolysis, Minneci et al. showed that plasma hemoglobin oxidized by NO inhalation reduced serum creatinine and renal dysfunction¹⁹.

We hypothesized that administration of 80ppm NO during and for 24h after prolonged CPB would convert plasma Oxy-Hb to Met-Hb and prevent intrarenal oxidative reactions and NO scavenging by plasma Oxy-Hb, thereby preserving kidney function. We performed a randomized trial in cardiac surgery patients undergoing multiple valve replacements requiring prolonged CPB to test if NO could prevent AKI due to high levels of plasma Oxy-Hb caused by acute hemolysis. We followed patients for up to one year after surgery to assess survival and evaluate if patients who received NO benefited from improved renal function. Some of the results of these studies have been previously reported in the form of an abstract²⁴.

METHODS

Study design

This study was designed to determine whether NO administered during and after cardiac surgery requiring prolonged CPB reduces post-operative AKI. Patients were studied at 90 days and 1 year after surgery to assess the incidence of chronic kidney disease (CKD) and major adverse kidney events (MAKE)²⁵.

We performed a prospective, randomized controlled trial comparing treatment with NO versus nitrogen (N₂) in adult patients undergoing multiple valve cardiac surgery at the Departments of Anesthesiology and Cardiovascular Surgery of Xijing Hospital, Xian, China. The participants, the care-givers and investigators analyzing data and assessing the outcomes were blinded to group assignment. One perfusionist and ICU

physician were unblinded and prepared the appropriate test gas tanks and NO/N₂ meters, which were then covered and blinded to others.

Treatment gases, NO at 80ppm or N₂, were given via the CPB machine and following CPB via a mechanical ventilator. Treatment gases were commenced at the onset of CPB and lasted for 24h or less if patients were ready to be extubated early. Treatment gases were weaned off over a period of 2h.

Outcomes

The primary endpoint of this study was the incidence of AKI. AKI was defined as either an increase of serum creatinine by 50% within 7 days after surgery, or an increase of serum creatinine by 0.3 mg/dl within 2 days after surgery from pre-operative baseline levels of serum creatinine²⁵. Secondary outcomes included development of stage 3 CKD (eGFR<60 mL/min/1.73m²)²⁶⁻²⁷, loss of 25% of eGFR compared to baseline, and MAKE (defined as a composite outcome of loss of 25% of eGFR from baseline, end stage renal disease requiring a continuous renal replacement therapy and mortality)²⁵ at 30 days, 90 days, and 1 year following ICU admission. Together with the renal outcomes, other single organ dysfunction, self-care activities²⁸, and overall mortality were assessed. One-year follow-up visits after surgery and laboratory studies including plasma Hb, NO consumption, and urine biomarkers of kidney injury were completed in 2017.

Statistical Analysis

Continuous variables were expressed as mean±SD or median (IQR). Differences

between the two cohorts of patients were tested using a parametric unpaired Student's t-test or non-parametric Mann-Whitney *U*-test, as appropriate. Categorical variables were described as frequency (%). The relative risk (RR) and the median differences (NO group versus Control group), including 95% CIs, were used to describe the differences of perioperative characteristics, the occurrence of AKI and differences of intra-hospital, 30 day, 90 day and 1 year postoperative outcomes. We performed an intention-to-treat data analysis which includes patients who received hydroxyethyl starches in the cardiopulmonary bypass priming solution and a per-protocol data analysis excluding patients who received hydroxyethyl starches in the cardiopulmonary bypass priming solution.

Please refer to the online data supplement for the detailed study protocol.

RESULTS

Demographic data

Three hundred and twenty patients were screened and consented to participate in the trial. Sixty patients were excluded before randomization because their surgery was either cancelled (n=46), or the surgical plan was changed to single valve replacement (n=14). Thus, 260 patients were randomized either to receive NO or N₂. Sixteen patients dropped out from the study before surgery because, at the time of initiation of CPB, the gas treatment (NO or N₂) was not available (Table E1). Thus, 244 patients were included in the analysis. One-hundred and twenty-seven patients received N₂ gas (Control group, n=127) while 117 patients received NO (NO group, n=117)

(Figure1). Equally balanced patient and surgical characteristics of the two groups were assessed in Table1 and TableE2. Surgical procedures and indications were similar between the two groups (Table1 and TableE3). Elapsed surgical time and CPB duration was slightly higher in the NO group.

Primary Outcome

Significantly fewer patients in the NO group developed AKI within 7 days after surgery compared to the control group (intention-to-treat analysis: 50% vs 64%; RR, 95% CI; 0.78; 0.62–0.97; $P=0.014$; Table 2; per protocol analysis, excluding patients receiving hydroxyethyl starch: 50% vs 63%; RR, 95%CI; 0.78, 0.62–0.99; $P=0.022$; Table E4).

Plasma Biomarkers of Hemolysis

While plasma Hb levels increased at the end of CPB, there were no differences between the NO and Control groups. However, at 0h ($P=0.016$), 6h ($P<0.001$), and 24h ($P=<0.001$) post-ICU admission, plasma Hb was higher in the NO group (Figure2A).

With the exception of a single time-point (End of CPB, $P=0.034$), urine Hb levels did not differ between the treatment groups during the study period (Figure2B).

To determine whether administration of NO successfully oxidized circulating Oxy-Hb to Met-Hb, we measured NO consumption in plasma in a sample of 51 patients in the NO and 50 in the Control group. In the NO treatment group, plasma NO consumption was significantly lower as compared to patients treated with N₂ (end of CPB, $P=<0.001$; ICU admission, $P<0.001$; 6h, $P=0.012$) (Figure2C).

Urinary Biomarkers and Plasma Chemistry

To normalize for diverse urinary output rates, we examined the ratio of urinary KIM-1 (KIM-1_u) to urinary creatinine (crea_u). The value of this ratio was significantly higher at 0h ($P=0.003$) and 24h after ICU admission ($P=0.009$) in the NO group. Similarly, the ratio of urinary NGAL (NGAL_u) to crea_u (NGAL_u/crea_u) increased in both groups from the end of CPB to 24h after ICU admission. Urinary NGAL/crea_u increased significantly more starting at the end of CPB until 24h after ICU admission in the NO treatment group as compared with the N₂ group. The ratio of urinary NAG (NAG_u) to crea_u (NAG_u/crea_u) increased in both groups from baseline to ICU admission. Urinary NAG/crea_u ratio increased significantly more at ICU admission ($P=0.008$) in the NO treatment as compared with the N₂ group.

Urinary creatinine levels did not differ between the groups until 48h after ICU admission. Urinary creatinine levels and unadjusted levels of urinary biomarkers are reported in TableE5. Also, whole blood hemoglobin, white blood cell concentration, platelet concentration, and plasma bilirubin levels did not differ between the two treatment groups (TableE6).

Secondary Outcomes

Nitric oxide administration resulted in fewer patients transitioning to stage 3 CKD. Patients treated with NO also had a lower MAKE index at 30d, 90d and 1y compared to the control group (intention-to-treat analysis Table2; per-protocol analysis TableE4).

Twenty-four patients (21%) in the NO group and 22 (17%) in the control group had an eGFR lower than 60 mL/min/1.73m² at baseline, suggesting stage 3 CKD before surgery (Table1).

By 90 days, an eGFR below 60 mL/min/1.73m² was found in 24 patients (21%) in the NO group, while the number of patients with stage 3 CKD increased to 39 (33%) in the control group (RR, 95% CI; 0.64, 0.41 – 0.99; *P*=0.024); and by 1 year, 19 (18%) patients in the NO group had an eGFR below 60 mL/min/1.73m² compared with 34 (31%) in the control group (RR, 95% CI; 0.59, 0.36 – 0.96; *P*=0.017 (Table2). At 30d, only 3 patients in the NO treatment group (<3%) evidenced over 25% of eGFR loss from baseline value as compared to 11 patients in the control group (9%) (RR, 95% CI; 0.29, 0.08 – 1.00; *P*=0.025); at 90d, there were 2 patients (2%) in the NO treatment group versus 11 (9%) in control group (RR, 95% CI; 0.19, 0.04 – 0.84; *P*=0.014); and, at 1y, 1 patient in the NO treatment group (1%) versus 7 patients in the control group (6%) evidencing more than a 25% eGFR reduction (RR, 95% CI; 0.15, 0.02 – 1.20; *P*=0.037. There was a trend of a decreased mortality in the NO group intra-hospital and at one year after cardiac surgery. Taken together, these results show that the major adverse kidney events (MAKE) were markedly decreased in the NO group at 30d (RR, 95% CI; 0.40, 0.18 – 0.92; *P*=0.016), at 90d (RR, 95% CI; 0.40, 0.17 – 0.92; *P*=0.015), and at 1y (RR, 95% CI; 0.47, 0.20 – 1.10; *P*=0.041) (Table2). Per protocol-analysis is shown in the supplemental material (TableE4) and confirms renal-protective effects of NO on the above mentioned secondary outcomes.

No difference was found between the groups in other intra-hospital outcomes or in other long-term outcome variables (e.g., intrahospital and 1- year occurrence of other

organ injury, independence of activities in daily living, hospital readmission rate and mortality up to 1-year, Table2 and TableE4, TableE7, TableE8, and TableE9).

Safety of 80 ppm of NO delivery

Nitric oxide delivery levels were never reduced for safety concerns. Continuous measurement of NO₂ showed values always below 1 ppm in all patients during the entire 24h of NO treatment. Plasma MetHb significantly increased from baseline to the end of CPB in the NO group and was significantly higher at the end of CPB ($P<0.001$), 0h ($P<0.001$), 6h ($P<0.001$), and 24h after ICU admission ($P<0.001$) compared with the Control group. The highest value of MetHb measured in the NO group was 9.3%, and no patient exceeded 10% MetHb at any time (TableE6).

In the NO treatment group, no patient experienced post-operative hemorrhage requiring multiple blood transfusions or reoperation (TableE7). 76% of the patients in the control group and 68% in the NO treatment group required blood transfusion in the perioperative period (TableE2). There were no adverse events, complications, or other organ dysfunction associated with the use of NO (TableE7). All patients at 24h of treatment after CPB commenced were weaned off NO or Nitrogen over a period of 2h. If patients were extubated before 24h after CPB, the time of treatment gas weaning off was considered less than the intubation time. The total period of gas administration did not differ between the groups ($P=0.457$, TableE2). No patient required re-institution of gas treatment.

DISCUSSION

We investigated the effects on renal function of NO administration during and after multiple valve replacement heart surgery requiring over 90 minutes of CPB in a largely ethnic *Han* Chinese population with rheumatic heart disease. This phase IIb prospective, randomized controlled trial showed that NO reduced the incidence of AKI, transition to stage 3 CKD and MAKE index at 1 year after cardiac surgery whether hydroxyethyl starch was added to the priming solution of the CPB or not.

The beneficial clinical impact of using NO was associated with a 22% relative risk reduction in the rate of perioperative AKI (from 64% in the N₂ group, to 50% in the NO group). While not significantly different, intra-hospital RRT was initiated in 3% of the patients in the NO group versus 5% in the control N₂ group, and the mortality rate at 1-year was 3% and 6%, respectively. Favorable short-term effects of perioperative administration of NO translated into a 42% relative reduction of stage 3 CKD at 1 year (from 31% in the N₂ group, to 18% in the NO group). Overall, the rate of MAKE at 30d, 90d, and 1y was reduced in patients treated with NO.

At a biochemical level, plasma Hb concentration increased similarly at the end of CPB in the two treatment groups indicating extensive hemolysis. However, exposure to 80 ppm NO during and after CPB maintained lower levels of perioperative plasma NO consumption. Plasma NO consumption in the control N₂ group increased 10 fold when it was compared to levels before surgery. Taken together, these biochemical results suggest that administration of exogenous NO during hemolysis expedites the transition

of the highly unstable plasma Oxy-Hb to its reduced and inert form Met-Hb, which is unable to deplete NO from the vasculature.

Lastly, no adverse events occurred due to the administration of 80 ppm NO for 24h, and total blood Met-Hb levels remained below 10% in all patients throughout.

Postoperative AKI is a common and major complication after cardiac surgery with associated increases in short-term and long-term morbidity and mortality. In a study from Duke University, 54% of 4,217 adult patients undergoing coronary artery bypass grafting surgery developed AKI⁴. In the United States, and in Europe, patients undergoing valve replacement have a rate of AKI as high as 60-70%^{4,8-16} requiring renal replacement therapy (RRT) in up to 16% of patients⁸. In a chart-review of 146 cases of multiple valve replacement from December 2012 to June 2013, we found a similar incidence of AKI (68%) with a 6% incidence of RRT requirements after multiple valve surgery in a *Han* Chinese population affected by rheumatic heart disease at Xijing Hospital (Xi'an, China) (data not published). In prior epidemiological studies, even a minimal rise in serum creatinine showed a strong association with increased long-term complications and increased mortality in cardiac surgery patients²⁹⁻³¹. In attempts to alleviate the burden of postoperative AKI, several trials have tested medical interventions without any success^{3,5-7}. By following patients for up to 1 year after surgery, our trial shows that a reduction of the rate of post-operative AKI by NO therapy resulted in improved long-term kidney outcomes.

Our trial focuses on the prevention of renal injury caused by hemolysis when complex cardiac surgery requires prolonged CPB. Plasma Hb and heme, products of

hemolysis, are scavenged by haptoglobin and hemopexin respectively. However, during extensive hemolysis plasma Hb accumulates in the circulation^{20,22} causing vasoconstriction^{32,33} and impairs tissue perfusion by scavenging nitric oxide^{8,32,33} resulting in renal injury^{9,19, 34,35}. The levels of circulating plasma Hb have been shown to be associated with the rate and severity of post-surgery AKI⁹. In animal and human physiological exploratory studies, administration of therapeutic exogenous NO gas has been shown to oxidize plasma Oxy-Hb to Met-Hb preventing pulmonary^{17, 36} and systemic vasoconstriction^{33,36} and organ injury^{19,32,37}. We found that prolonged CPB causes hemolysis with an increased concentration of plasma Hb and increased plasma NO consumption compared to levels measured before surgery. In contrast, exposing plasma to NO gas in the CPB oxygenator and after surgery for an additional 24h by inhaling 80 ppm NO prevented the depletion of plasma NO, which was associated with a decrease in AKI rate and transition to stage 3 CKD at 90 days and 1 year after surgery.

Other than preventing vasoconstriction due to oxy-Hb, NO might have improved pulmonary perfusion in this study by its well-described selective pulmonary vasodilator properties, which might have increased cardiac output especially in patients with PAH^{37,38}. In order to account for an elevated baseline PAH, patients with pre-operative PAH were allocated equally to both groups during the randomization process. However, during surgery cardiac function was not measured by trans-esophageal echocardiography or pulmonary artery pressure monitoring with an indwelling pulmonary artery catheter, as these are not standardly monitored at Xijing Hospital.

In addition to its pulmonary vasodilator effects, others have suggested that gaseous NO administration has protective properties against systemic inflammation and reperfusion injury^{39,40}. In a pediatric cardiac surgery study by Checchia et al.³⁷, NO gas delivered via the CPB circuit resulted in lower troponin and natriuretic peptide levels, improved diuresis, and a better postoperative intensive care unit course. The authors suggested that in children, NO delivered by CPB decreased ischemia-reperfusion injury, thereby improving cardiac and renal function. In a recent follow-up study, James et al. randomized 198 children to either receive intra-operative 20ppm NO via the CPB bypass oxygenator, or have standard conduct of bypass. The authors showed that NO gas reduced the incidence of a low cardiac output syndrome post-operatively³⁸.

Despite the remarkable improvement of kidney function in both short- and long-term renal outcomes, we observed a transiently higher peak in urinary biomarkers of kidney injury in patients who received NO at the end of CPB and surgery compared to the control N₂ group (Table E4). There are three considerations that should be noted when interpreting the observed discrepancy in this study between plasma creatinine levels and urinary biomarkers. First, KIM-1 was higher in the NO group at only two time points- at ICU admission and at 24h, and NAG levels were higher only at ICU admission. KIM-1 and NAG are very sensitive markers of tubule stress and might not reflect the overall function of the nephrons (GFR) especially if NO has a vasodilatory effect maintaining GFR despite some mild tubular injury.

Second, secretions of urinary biomarkers are triggered by a variety of renal and extra-renal stimuli. For example, NGAL is an iron-transporting protein and its release is

regulated by plasma iron levels. In the present study, plasma levels of Hb were higher in the NO group at ICU admission compared to the control N₂ group (Figure 2A). The increased plasma Hb, and possibly iron levels, in the NO group may have increased NGAL secretion. Third, renal biomarkers have their own nephron-protective properties. Therefore, it cannot be excluded that NO gas might directly increase secretion of these urinary biomarkers. This uncoupling phenomenon between the filtration of serum creatinine and secretion of urinary biomarkers is intriguing and might be a focus for future research elucidating the mechanisms regulating the secretion of urinary biomarkers and kidney repair during NO treatment. More recently, Friedrich et al. cast doubts on the clinical use of urine NGAL as a predictor of AKI and severity of renal injury⁴¹. Likewise, we were unable to determine benefits of using renal biomarkers to predict long-term kidney outcomes in this randomized trial.

In cardiac surgery, perioperative AKI is defined by rising serum creatinine levels in association with increased short-term and long-term renal complications and increased mortality^{1,2,4}. We herein demonstrate the safety and efficacy of one of the first medical interventions, namely NO gas, to prevent AKI and decrease long-term adverse kidney events, a common and serious complication of cardiac surgery requiring prolonged CPB.

Limitations: *First limitation: The trial selected “younger and healthier” patients undergoing valve replacement due to rheumatic heart disease as compared to the typical cardiac surgery patient profiles reported in the western literature.*

The present study predominantly selected a *Han* Chinese population of patients affected by rheumatic heart disease requiring multiple valve replacement. Whereas most cardiac surgery studies reported today are from Europe and North America, where degenerative valvular disease is the most common valve disease. It is important to recognize, however, that rheumatic heart disease remains a major cause of morbidity and premature death in the developing world. This high prevalence of rheumatic heart disease and the growing number of cardiac surgical procedures done in Asian countries make this study very unique^{42,43}. Rheumatic heart disease is estimated to be responsible for more than 500,000 deaths each year in Asia, with millions of patients waiting for definitive heart surgery⁴². The extraordinarily rapid economic development of many Asian countries, together with advances in surgical technologies and surgical skills, now gives thousands of these patients access to definite surgical treatment, rendering rheumatic heart surgery the primary reason for heart valve procedures in Asia. While the results of this study cannot be generalized to all races and pathologies requiring prolonged CPB for cardiac surgery, this study addresses one of the most common worldwide causes of heart disease and what will likely be the most common reason globally for prolonged CPB in cardiac surgery for the next decades.

Compared to available published literature on adults requiring cardiac surgery, the relatively young age of our patient population (mean age: 48 years old), the absence of pre-operative severe chronic kidney disease (eGFR<30 mL/min/1.73m²), and other cardio-vascular comorbidities (i.e., diabetes, obesity, atherosclerosis) should be considered to avoid generalization. Future studies will evaluate whether NO confers similar renoprotective properties in older patients and those with more co-morbidities.

Second limitation: The trial focused on prolonged (>90 minutes) CPB procedures.

Hemolysis is closely associated with the duration of CPB^{20,22}. While off pump procedures for coronary artery bypass graft and transcatheter valve replacement to avoid open-heart surgery have been adopted in recent years with a certain degree of success, most open-heart surgeries at major heart centers in the USA require more than 90 minutes of CPB time, as documented in recent literature^{1,44,45}.

Third limitation. This trial did not show a decreased mortality rate or cost-analysis benefits.

Our study was not powered to test whether NO exposure could reduce mortality, nevertheless, at 1 year, the rate of mortality in the NO treatment group was 3% compared to 6% in the control group ($P=0.088$, Table2). In addition, NO gas is expensive and to our knowledge, was used for the first time in China in this trial. However, promising and economically viable alternative NO delivery systems are emerging⁴⁶⁻⁴⁸.

Due to the current high cost of NO gas, the renal protective effects of NO gas need to be reproduced in phase III clinical trials before implementation in the clinical practice.

In conclusion, among Chinese patients requiring prolonged CPB for multiple heart valve replacement, 80 ppm NO gas exposure during and after prolonged CPB is safe, decreased the incidence of AKI, and reduced transition to stage 3 CKD at 90 days and 1 year.

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