Article published online: 2022-05-28

Effect of Ascorbic Acid on Cardiac Surgery-Associated Acute Kidney Injury Incidence

Anze Djordjevic[1] Stamenko Susak[2] Petra Kotnik[3] Mario Gorenjak[3] Zeljko Knez[3] Miha Antonic[1]

[1] Department of Cardiac Surgery, University Medical Centre Maribor, Maribor, Slovenia
[2] Department of Cardiovascular Surgery, Institute of Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Vojvodina, Serbia
[3] Faculty of Medicine, University of Maribor, Maribor, Slovenia

Address for correspondence Anze Djordjevic, MD, Department of Cardiac Surgery, University Medical Centre Maribor, Ljubljanska ulica 5 Maribor, Maribor 2000, Slovenia (e-mail: anze.djordjevic@gmail.com).

Thorac Cardiovasc Surg 2022;70:566–574.

Abstract

Objectives Acute kidney injury (AKI) is associated with higher perioperative mortality and morbidity. Oxidative stress has been proposed as a cause of postoperative AKI. Ascorbic acid (AA) supplementation was suggested as a novel and promising antioxidant. The aim of this study was to evaluate the capability of AA to reduce the incidence of postoperative AKI in cardiac surgery patients.

Methods A prospective randomized trial was conducted in patients scheduled for on-pump cardiac surgery. Subjects in the AA group received 2 g of AA intravenously during the induction of anesthesia, 2 g before aortic cross-clamp removal and 1 g every 8 hours for five postoperative days (the JERICA protocol). Postoperatively, the patients were monitored for AKI and other complications. Malondialdehyde levels were monitored in a subpopulation of 100 patients to evaluate the effect of AA on oxidative stress level.

Results The AA and control group consisted of 163 and 169 patients, respectively. The groups were well matched for baseline demographics and had similar intraoperative characteristics. The incidence of AKI in the AA and control group was 20.9 and 28.4%, respectively ($p = 0.127$). The estimated glomerular filtration rate did not differ between the study groups in the entire postoperative period. There was a trend toward higher malondialdehyde values with statistical significance on postoperative day 1 and lower in-hospital mortality in the AA group (0.6 vs. 4.1%, $p = 0.067$).

Conclusion Our results do not support the effectiveness of AA supplementation in reducing the incidence of postoperative AKI in on-pump cardiac surgery patients.

Clinical Registration Number This study was registered with the ISRCTN Registry under the trial registration number ISRCTN98572043.

Keywords
- acute kidney injury
- ascorbic acid
- cardiopulmonary bypass
- oxidative stress

received
September 18, 2021
accepted after revision
December 27, 2021
published online
May 28, 2022

DOI https://doi.org/10.1055/s-0042-1744262.
ISSN 0171-6425.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

Acute kidney injury (AKI) is represented by a rapid decrease in kidney function resulting in decreased ability to filter metabolic waste products, regulate acid–base balance, and manage fluid homeostasis[1] with an incidence up to 40%, depending on patients' comorbidities (endocarditis), type of surgery (complex multicomponent surgery, prolonged cardiopulmonary bypass [CPB] time), and diagnostic criteria.[2,3] The currently accepted definition by the Kidney Disease: Improving Global Outcomes (KDIGO) determines it as either an increase in serum creatinine of $\geq$26.5 µmol/L within 48 hours or more than a 50% baseline serum creatinine increase within 7 days.[4] Although the majority of patients developing postoperative AKI remain in a low-stage reversible kidney dysfunction, it still affects their outcome. It is associated with higher perioperative mortality, morbidity, prolonged in-hospital stay, readmission rate, costs, and increased risk of progression to chronic kidney disease (CKD).[5–9] Considerable efforts have been made to better understand the underlying pathophysiological patterns leading to AKI development with the goal of developing effective prophylactic and therapeutic options.[10,11] There are four main groups of mechanisms influencing AKI after cardiac surgery: (1) renal hypoperfusion, (2) inflammation and oxidative stress, (3) use of nephrotoxic substances, and (4) embolisms.[12,13] Malondialdehyde (MDA) has been often utilized as a valuable biomarker of oxidative stress. For more sensitive and accurate analysis of MDA in biological samples, the use of gas chromatography-mass spectrometry (GC–MS) with headspace extraction is proposed after derivatization with pentafluorophenyl hydrazine (PFPH). The complex is more volatile and stable to analyze with GC–MS. MDA levels do not peak at the end of the surgery, but continue to rise a few hours after surgery.[14] With the identification of reactive oxygen species (ROS) as a potential pathophysiological substrate, ascorbic acid (AA) has been proposed as a promising supplement that may reduce oxidative stress and subsequent incidence of postoperative AKI.[10] However, data on this topic are scarce and randomized prospective clinical studies are lacking.

The aim of this study was to evaluate the effect of perioperative AA supplementation on the incidence of postoperative AKI in patients after arrested-heart cardiopulmonary bypass surgery.

Materials and Methods

Study Sample

This bicentric randomized prospective study was conducted in patients who underwent open-heart surgery between December 2018 and May 2019 at the Department of Cardiac Surgery, University Medical Centre Maribor, Slovenia, and Department of Cardiovascular Surgery, Institute of Cardiovascular Diseases Vojvodina, Serbia. The study protocol was approved by the National Medical Ethics Committee of the Republic of Slovenia (0120–268/2018/4) and the Ethics Committee of the Institute of Cardiovascular Diseases Vojvodina (2378–1/14). It is in full accordance with the World Medical Association Declaration of Helsinki.

The exclusion criteria were patients under the age of 18, off-pump surgery, hyperoxaluria or history of nephrolithiasis, known allergy to AA, critical preoperative state or emergency/salvage type of procedure (as defined by European System for Cardiac Operative Risk Evaluation [EuroSCORE] II), immunosuppressive or antioxidant therapy within 1 month before surgery, hypothermic circulatory arrest, preoperative CKD with an estimated glomerular filtration rate (eGFR) $<$30 mL/min/1.73 m^2, and death before completion of AA supplementation protocol (before 5th postoperative day). Once a patient was considered eligible for enrollment, he or she was informed about the study protocol and asked to sign a written informed consent. The enrolled patients were randomly assigned to either the AA group or control group. Patients in the AA group received 2 g of AA (100 mg/mL solution for injection in 5 mL ampoules, BB Pharma) intravenously before the procedure (during induction of anesthesia), then 2 g before the aortic cross-clamp was removed and 1 g three times a day for 5 days after the surgery (the Judiciously Extreme however Reasonably Increased vitamin C use in Adult cardiac surgery [JERICA] protocol) (►**Fig. 1**). This protocol was developed by our group specifically for the present study based on the results of our previous research.[10,15] A PRISMA flowchart is added as ►**Fig. 2**.

Basic demographic, laboratory and medical data were collected before the surgery. A surgical risk profile using the EuroSCORE II system was calculated for every patient. Baseline kidney function was assessed by determining the preoperative serum creatinine levels and the calculation of eGFR with the Modification of Diet in Renal Diseases equation.[16]

Surgery

All the patients received the same standard preoperative premedication (an antibiotic, in most cases 2 g of cefazolin, 40 mg of pantoprazole, and 7.5 mg of midazolam) and underwent the usual anesthesia protocol using standard medications for the induction and maintaining of anesthesia. Standard full sternotomy or upper left J-ministernotomy was performed in all the patients. The CPB circuit was primed with Ringer solution (1,300 mL), 250 mL of 20% mannitol, methylprednisolone sodium succinate (3 mg/kg), unfractionated heparin (7,500 units), and an antibiotic (in most cases 1 g of cefazolin). Standard unfractionated heparin (starting dose 300 units/kg) was used to target an activated clotting time between 400 and 600 seconds. A non-pulsatile flow between 2.2 and 2.4 L/m^2 of body surface was maintained by a heart-lung machine using a roller pump and a hollow-fiber polypropylene membrane oxygenator. The target mean arterial pressure was 60 mm Hg and the lowest accepted hematocrit level during CPB was 0.23.

A cold blood or crystalloid cardioplegia applied in antegrade or combined antegrade/retrograde fashion was used for cardiac protection. After separation from CPB, heparin was reversed with protamine sulfate.

Collected intraoperative data included CPB time, aortic cross-clamp time, and diuresis. Postoperative serum creatinine, white blood cell (WBC) count, and MDA were recorded

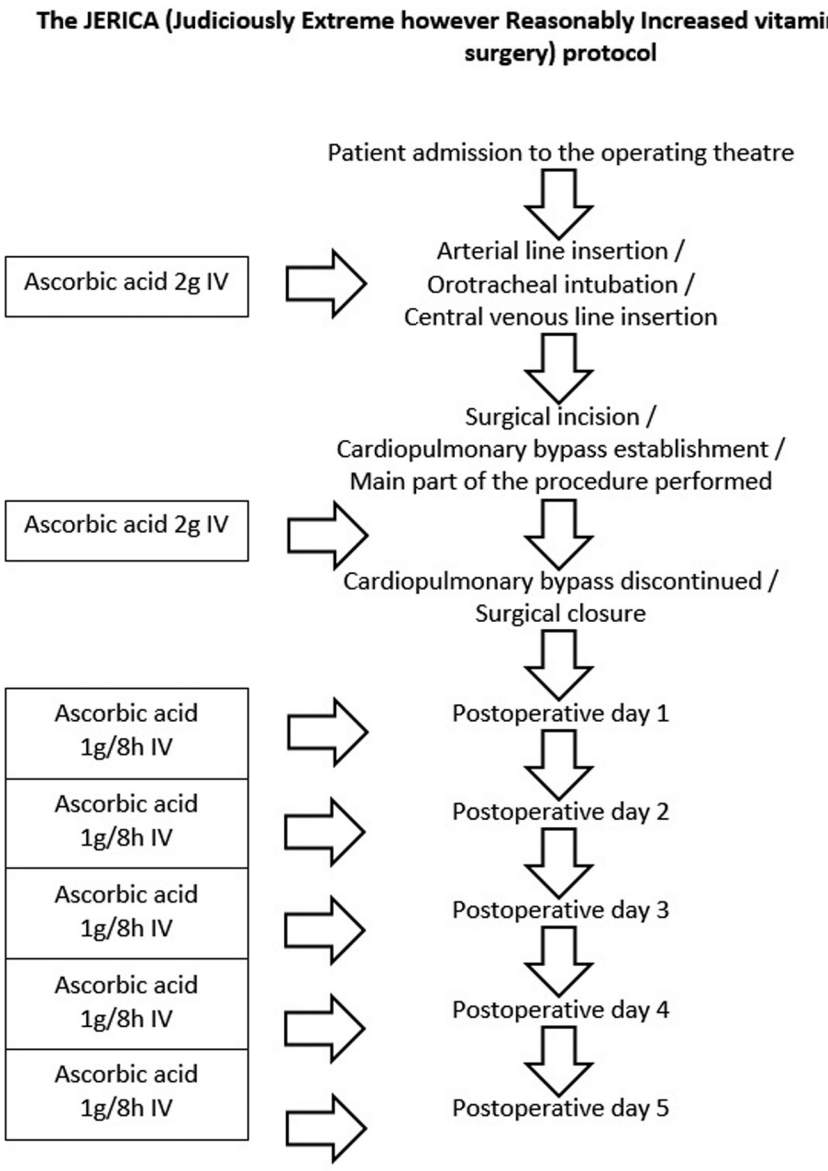

Fig. 1 The judiciously extreme, however, reasonably increased vitamin C use in adult cardiac surgery (JERICA) protocol.

and eGFR was calculated on admission to the intensive care unit (ICU) and once daily until the fifth postoperative day.

A standard postoperative ICU and ward care was provided to all the patients. All of them received vasopressor, inotropic, volume substitution, and diuretic therapy, as clinically required at the discretion of the intensive care physician. Generally, urine output >0.5 mL/kg/h, mean arterial pressure of 65 mm Hg, and central venous pressure of 10 to 15 mm Hg was targeted. Perioperative data collection included fluid intake (intravenous and oral), urine output, use of diuretics, and nephrotoxic antibiotics until the fifth postoperative day.

Malondialdehyde Detection

A subgroup of 100 patients (50 patients in each study group) was randomly selected for MDA detection. MDA was determined in serum samples. After centrifugation at 3,500 rpm (1,372 *g*) at 4°C for 10 minutes, the serum was transferred to cryovials and stored at −80°C until the samples were analyzed.

A sample of 1 mL serum was transferred into vial and 100 µL of 100 mg/L aceton-d6 (99.9 atom % D, CAS 666–52–4, Sigma-Aldrich), 20 µL MDA solution, and 150 µL 5% PFPH (≥98%, CAS 828–73–9, Sigma-Aldrich) in acetonitrile (≥99.9%, CAS 75–05–8, Fluka) were added. The pH was adjusted to pH 3 with 1 M HCl (37%, CAS 7647–01–0, Sigma-Aldrich). The derivatization was performed at 50°C for 10 minutes in a closed vial.

The GC–MS apparatus consisted of Shimadzu GC-2010 gas chromatograph and Shimadzu GC–MS 2010 QC Ultra mass selective detector. The volatile compound was separated on Phenomenex ZB-5MS column with helium as the carrier gas at a flow rate of 1 mL/min. Samples were injected in splitless

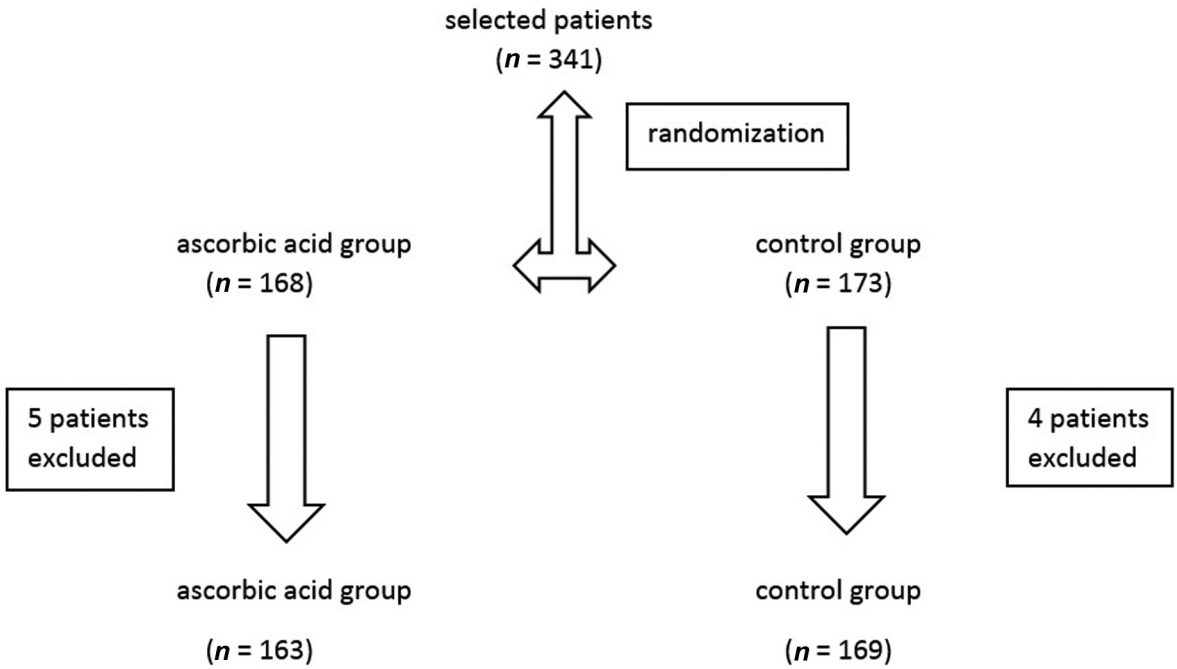

Fig. 2 A PRISMA flowchart.

mode at the injector temperature of 280°C. The separation was achieved using a temperature gradient: 35°C for 3 minutes, then increased by 10°C/min to a final temperature of 150°C and held for 2 minutes. The mass detector was operating in electron-impact mode (electron energy 70 mV) at 250°C. The full-scan mass spectra from 35 to 300 m/z was recorded for analyte identification. The compounds were quantified based on the calibration plots of MDA-PFPH.

End Points

The primary end point was the postoperative occurrence of AKI within the first five postoperative days. AKI was defined according to the KDIGO criteria.[4]

The secondary end points were in-hospital mortality as well as eGFR and MDA levels in the postoperative period. In addition, pre- and postoperative WBC counts were compared between the groups.

The sample size estimation was based on the assumption that the incidence of AKI after open-heart surgery lies at approximately 25% and that the administration of AA would result in a 20% decrease of postoperative AKI. With a power of 80%, α error of 0.05, and β of 0.2, approximately 150 patients were required in each study group.

Statistical Analysis

Data analysis was performed using the IBM SPSS 25.0 software (IBM Inc., Armonk, New York, United States). Baseline characteristics are expressed as mean (SD) for normally distributed, as median [interquartile range] for nonnormally distributed continuous variables, and as frequency (percentage) for categorical variables. Differences between nominal variables were estimated using the Fisher's exact test. For determining the statistical differences of baseline or the highest peak quantitative variables between two groups, the independent samples t-test or Mann-Whitney U-test was used after the Kolmogorov–Smirnov test of normality. Each time point was first separately evaluated using logistic regression adjusted for sex, age, and baseline eGFR or MDA values. Additionally, repeated measure analysis was used for comparison of eGFR and MDA levels across time points and between groups. p-Values are presented for two-tailed tests and α level was set to 0.05. The trial complies with the Consolidated Standards of Reporting Trials Statement (CONSORT) checklist.

Results

A total of 332 patients were included in the trial. The AA group consisted of 163 patients and the control group included 169 patients. The groups were well matched for baseline demographics and comorbidities (►**Table 1**). Both groups had similar intraoperative characteristics with no significant differences in the type of surgery, CPB time, aortic cross-clamp time, diuresis, and fluid supplementation (►**Table 2**).

The data regarding the postoperative course are summarized in ►**Table 3**. There was no statistical significance between the two groups regarding the incidence of postoperative AKI (20.9 vs. 28.4%, $p = 0.127$). No significant differences were observed when comparing both groups regarding KDIGO stages ($p = 0.114$). eGFR was not found to be significantly higher in the AA group during the entire postoperative course (►**Fig. 3**). No significant difference was found between groups when comparing peak-to-peak values of eGFR ($p = 0.176$) or diuresis ($p = 0.978$). No significant differences

Table 1 Preoperative patient characteristics

	Ascorbic acid ($n = 163$)	Control ($n = 169$)	*p*-Value
Age (years)	67.61 (SD: 9.13)	65.70 (SD: 10.77)	0.129
Male sex	117 (71.8%)	118 (69.8%)	0.719
Body mass index (kg/m^2)	28.63 (SD: 4.24)	28.18 (SD: 4.37)	0.669
Diabetes mellitus	46 (28.2%)	38 (22.5%)	0.257
Chronic obstructive pulmonary disease	7 (4.3%)	7 (4.1%)	1.000
New York Heart Association			0.164
• class I	5 (3.1%)	2 (1.2%)	
• class II	91 (55.8%)	115 (68%)	
• class III	55 (33.7%)	52 (30.8%)	
• class IV	12 (7.4%)	0	
History of cerebrovascular insult	10 (6.1%)	9 (5.3%)	0.816
Peripheral arterial obstructive disease	40 (24.5%)	41 (24.3%)	1.000
History of acute coronary syndrome	46 (28.2%)	34 (20.1%)	0.096
History of cardiac surgery	2 (1.2%)	2 (1.2%)	1.000
EuroSCORE II	3.07 [2.8]	2.52 [1.9]	0.052
Preoperative creatinine (μmol/L)	87.54 (SD: 22.91)	90.72 (SD: 24.80)	0.325
Preoperative eGFR	79.82 (SD: 22.02)	74.38 (SD: 22.36)	0.066
Preoperative WBC count (10^9/L)	7.55 (SD: 5.04)	7.74 (SD: 2.46)	0.051
Preoperative MDA (μg/L)	23.39 (SD: 19.28)	17.86 (SD: 13.88)	0.174
Preoperative CKD staging			0.896
• stage 1	56 (34.4%)	52 (30.8%)	
• stage 2	72 (44.2%)	86 (50.9%)	
• stage 3	35 (21.5%)	31 (18.3%)	

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EuroSCORE, European System for Cardiac Operative Risk Evaluation; MDA, malondialdehyde; SD, standard deviation; WBC, white blood cell.

Table 2 Intraoperative data

	Ascorbic acid ($n = 163$)	Control ($n = 169$)	*p*-Value
Type of surgery			
• coronary surgery	92 (56.4%)	101 (59.8%)	0.503
• valvular surgery	85 (52.1%)	83 (49.1%)	0.661
1. Aortic valve	43 (50.6%)	42 (50.6%)	0.802
2. Mitral valve	32 (37.6%)	31 (37.3%)	0.889
3. Tricuspid valve	10 (11.8%)	10 (12%)	1.000
• Aortic surgery	12 (7.4%)	9 (5.3%)	0.505
• Miscellaneous (carotid, antiarrhythmic ...)	7 (4.3%)	17 (10.1%)	0.055
Cardiopulmonary bypass time (min)	84.62 (SD: 31.07)	89.55 (SD: 39.15)	0.597
Aortic cross-clamp time (min)	70.14 (SD: 26.55)	73.05 (SD: 29.97)	0.505
Diuresis (mL)	422.78 [305]	421.25 [305]	0.606
Crystalloid supplementation (mL)	2,179.26 (SD: 644.49)	2,246.08 (SD: 584.71)	0.507

Abbreviation: SD, standard deviation.

between the study groups were identified regarding peri- and postoperative fluid intake (►**Table 4**) or postoperative urine output (►**Table 5**).

Between time point evaluation has shown that serum MDA concentrations tended to be higher in the AA group with statistical significance on POD 1 (►**Fig. 4**).

Table 3 Postoperative course

	Ascorbic acid ($n = 163$)	Control ($n = 169$)	p-Value
Incidence of postoperative AKI (any-stage)	34 (20.9%)	48 (28.4%)	0.127
KDIGO AKI staging			0.114
• Stage 1	28 (82.4%)	39 (81.2%)	
• Stage 2	3 (8.8%)	8 (16.7%)	
• Stage 3	3 (8.8%)	1 (2.1%)	
New onset RRT	3 (1.8%)	1 (0.6%)	0.367
Antibiotic use			
Cefazolin	146 (89.6%)	144 (85.2%)	0.296
Vancomycin	20 (12.3%)	24 (14.2%)	0.604
Aminoglycosides	2 (1.2%)	1 (0.6%)	0.617
Other	14 (8.6%)	11 (6.5%)	0.536
In-hospital mortality	1 (0.6%)	7 (4.1%)	0.067
WBC count 12 h post-surgery (10^9/L)	16.63 ± 7.97	16.76 ± 5.97	0.690
WBC count POD 1 (10^9/L)	13.99 (SD: 5.29)	14.84 (SD: 3.87)	0.347
WBC count POD 2 (10^9/L)	14.31 (SD: 4.95)	14.73 (SD: 3.96)	0.356
WBC count POD 3 (10^9/L)	11.34 (SD: 3.80)	11.03 (SD: 3.35)	0.301
WBC count POD 4 (10^9/L)	9.12 (SD: 2.83)	8.84 (SD: 2.71)	0.324
WBC count POD 5 (10^9/L)	9.00 (SD: 4.71)	8.62 (SD: 3.07)	0.474
	Ascorbic acid ($n = 50$)	Control ($n = 50$)	
MDA aortic cross-clamp removal (μg/L)	45.22 (SD: 44.83)	31.42 (SD: 28.05)	0.058
MDA 12 h post-surgery (μg/L)	31.87 (SD: 32.76)	40.90 (SD: 27.66)	0.625
MDA POD 1 (μg/L)	45.16 (SD: 35.28)	33.66 (SD: 26.00)	0.029
MDA POD 2 (μg/L)	51.10 (SD: 37.12)	26.38 (SD: 23.65)	0.095
MDA POD 3 (μg/L)	37.57 (SD: 33.52)	19.22 (SD: 13.69)	0.114

Abbreviations: AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; KDIGO, Kidney Disease: Improving Global Outcomes; MDA, malondialdehyde; NA, not applicable; OR, odds ratio; POD, postoperative day; RRT, renal replacement therapy; SD, standard deviation; WBC, white blood cell.

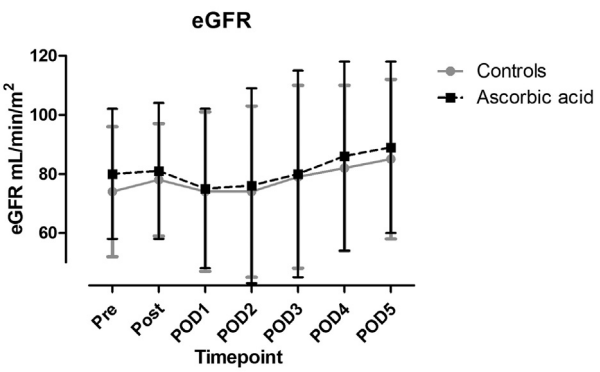

Fig. 3 Estimated glomerular filtration rate levels in the postoperative period. eGFR, estimated glomerular filtration rate; Pre, preoperative; Post, aortic cross-clamp removal; POD, postoperative day.

Additionally, using repeated measures analyses for both eGFR and MDA values, we did not observe any statistically significant differences of the measures between subjects. However, serum MDA levels were not proven to be significant predictors of postoperative AKI (►**Table 6**). There were no significant statistical differences between the groups regarding the preoperative and postoperative WBC count levels.

Nine patients (five in the AA group and four in the control group) died before the end of the study protocol and were therefore excluded from the study. From the included patients, one in-hospital death in the AA group and seven deaths in the control group show a trend toward lower in-hospital mortality (0.6 vs. 4.1%, $p = 0.067$). The cause of death in the patient who died in the AA group was sepsis. In the control group, three patients died of sepsis, two patients died of severe low cardiac output syndrome, one patient died of bowel ischemia due to a pre-existent (but undiagnosed) severe atherosclerosis of splanchnic arteries and one patient died of stroke with subsequent hemorrhagic transformation. Out of these eight patients, seven met the criteria for postoperative AKI (1/1 from the AA group and 6/7 from the control group). Postoperative new dialysis was necessary in three patients (1.8%) in the AA and in one patient (0.6%) in the control group ($p = 0.367$).

Table 4 Total fluid intake (intravenous + oral) in milliliters

	Ascorbic acid ($n = 163$)	Control ($n = 169$)	*p*-Value
Intraoperative	2,179 (SD: 645)	2,246 (SD: 585)	0.581
POD 1	3,470 (SD: 717)	3,259 (SD:752)	0.122
POD 2	4,066 (SD: 869)	3,963 (SD: 862)	0.525
POD 3	3,197 (SD: 833)	3,260 (SD: 776)	0.682
POD 4	2,842 (SD: 822)	2,857 (SD: 679)	0.931
POD 5	2,614 (SD: 856)	2,457 (SD: 997)	0.674

Abbreviations: POD, postoperative day; SD, standard deviation.

Table 5 Urine output in milliliters

	Ascorbic acid ($n = 163$)	Control ($n = 169$)	*p*-Value
Intraoperative	321 (SD: 197)	328 (SD: 170)	0.823
POD 1	1,389 (SD: 560)	1,526 (SD: 510)	0.160
POD 2	1,826 (SD: 702)	2,042 (SD: 746)	0.108
POD 3	2,347 (SD: 1005)	2,543 (SD: 1138)	0.343
POD 4	2,729 (SD: 1489)	2,445 (SD: 1090)	0.347
POD 5	2,372 (SD: 769)	2,659 (SD: 1214)	0.467

Abbreviations: POD, postoperative day; SD, standard deviation.

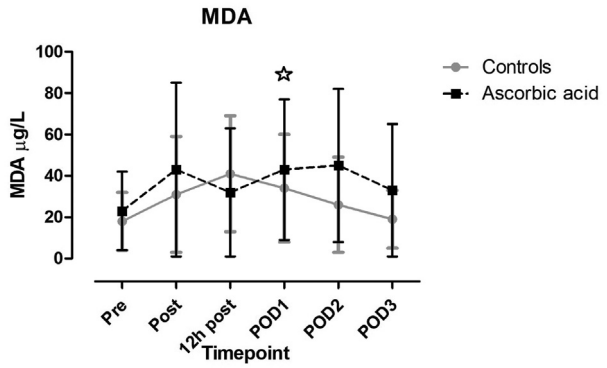

Fig. 4 Malondialdehyde levels in the postoperative period. MDA, malondialdehyde; Pre, preoperative; Post, aortic cross-clamp removal; POD, postoperative day.

Discussion

The exact pathophysiological mechanisms of postoperative AKI after cardiac surgery are complex and not yet fully understood. Oxidative stress generated by the unavoidable ischemia-reperfusion injury may further play a role in the development of postoperative AKI.[17] AA is a potent antioxidant that may be effective in protecting molecules such as DNA, lipids or proteins from oxidation by a variety of ROS.[18,19] However, reports have been published also on the pro-oxidative activity of the AA.[20]

In this trial, the beneficial effect of AA supplementation on the incidence of postoperative AKI in cardiac surgery patients could not be demonstrated. The groups were well matched in terms of preoperative characteristics, including preoperative renal function.

In both groups, the incidence of postoperative renal dysfunction was within the foreseen limits and in concordance with the previous reports.[2,3,10] As we have already hypothesized, it may be possible that in the studied population of rather young (mean age 67.6 and 65.7 years), low-risk patients (mean EuroSCORE II 3.07 and 2.52) with a favorable preoperative renal function, the harmful effect of a moderately short CPB (mean duration of 84 and 89 minutes) on the kidneys was not detrimental.

In 2017, our group conducted a randomized prospective trial on 100 elective on-pump coronary artery bypass grafting (CABG) patients and reported no significant reduction in the incidence of postoperative AKI in patients who perioperatively received AA (16 vs. 14%, $p = 0.779$).[10] Furthermore, the groups also did not differ in the postoperative C-reactive protein levels, indicating a clinically uncertain anti-

Table 6 Impact of malondialdehyde on postoperative acute kidney injury

	MDA pre-surgery	MDA post-surgery	MDA 12 h post-surgery	MDA POD 1	MDA POD 2	MDA POD 3	MDA high
p-Value	0.528	0.858	0.155	0.697	0.656	0.795	0.568

Abbreviations: MDA, malondialdehyde; POD, postoperative day.

inflammatory effect of AA. Similarly, even with a higher dosage of AA, no differences regarding WBC count could be detected in our present study. The results are also in accordance with the study of Hill et al, who reported no significant differences regarding postoperative inflammatory response as assessed by mean postoperative WBC levels ($p = 0.677$).[21]

In 2012, Jouybar et al[22] performed a similar, but smaller study investigating the effect of perioperative supplementation of AA in 40 elective on-pump CABG patients. No significant difference between the study groups regarding the postoperative inflammatory response as well as kidney function was identified. Besides the interleukin 6 and 8 levels, the groups also did not differ when comparing WBC counts.

The impact of the AA on postoperative AKI was also studied by Amini et al[23] in 272 elective off-pump CABG patients. AKI was seen in 14 patients (20.9%) in the AA group and in 10 patients (14.1%) in the control group with no significant difference in incidence, severity, duration, and day of occurrence of AKI among the groups. They also reported no difference regarding in-hospital mortality, whereas we observed a trend toward lower in-hospital mortality in the AA group (0.6 vs. 4.1%, $p = 0.067$).

Oxidative stress is an important factor in the pathophysiology of cardiovascular diseases. Since ROS are very difficult to directly detect and measure, MDA was chosen as an indirect oxidative stress marker. MDA levels did differ between the two groups on PODs 1 to 3. Surprisingly, MDA levels tended to be higher in the AA group indicating a potential pro-oxidative effect of AA in this patient population.

In a trial of 87 elective CABG patients, Safaei et al[24] reported lower MDA levels in the AA group but with no statistical significance compared with the control group. However, only elective non-diabetic CABG patients were included in the study. In addition, patients with aortic cross-clamp time of more than 120 minutes were excluded. Moreover, the blood samples were taken exclusively intraoperatively directly from the coronary sinus.

In contrast to our results, Dingchao[25] reported lower levels of serum MDA ($p < 0.05$) in patients who underwent heart surgery on CPB and perioperatively received AA. The study included 45 patients in the AA and 40 in the control group. A similar protocol for AA supplementation was implemented as in our present study. Neither any detailed data on the patient population characteristics is available, nor any data about the type of surgery and the length of the CPB is present. Moreover, a bubble oxygenator was used in all patients in the study, which is an important methodological difference to our study, where a state-of-the-art microporous polypropylene hollow-fiber membrane oxygenator with a biocompatible surface coating was implemented. It is generally known that bubble oxygenators trigger qualitatively distinct and more potent inflammatory response compared with membrane oxygenators, including cellular response with polymorphonuclear leukocytes and monocytes, as well as complement activation, both leading to increased generation of ROS.[26–28]

In 2001, Oktar et al[29] reported on significantly lower serum MDA levels in the AA group from the time of aortic declamping and until the first postoperative day. The test group received a one-time bolus of 4 g of AA, either just before the induction of anesthesia or added in the cardioplegic solution. The main limitation of this study is that it included only 48 patients, which were then divided into four study groups, each containing only 12 patients. Moreover, the MDA levels were analyzed with a less specific spectrophotometric method after thiobarbituric acid derivatization.

Kaźmierczak-Barańska et al[20] suggested that AA could act pro-oxidative in specific circumstances, mainly dependable on the availability of the iron ion. Iron reduced by ascorbate to Fe^{2+} easily reacts with oxygen, which in the Fenton reaction leads to the formation of ROS and HO2. In reaction with Fe^{2+}, they generate a highly reactive hydroxyl radical. Hypothetically, it may be possible that this potential pro-oxidative characteristic of AA could explain the tendency toward higher MDA levels in the AA group in our study. Due to the contradictory results of the antioxidant effect of the AA and its controversial protective effect on the postoperative function of kidneys further studies of its anti- and pro-oxidant characteristics are highly warranted to identify the optimal circumstances, dosing, timing, and patient population who may benefit most from its supplementation.

There are also some limitations of our study. Although it is a prospective and randomized study, it is a relatively short-term trial. Although creatinine-derived markers have been generally accepted as good renal function indicators, more sensitive and earlier biochemical markers of renal dysfunction exist, including neutrophil gelatinase-associated lipocalin, cystatin C, and Klotho.[30,31] However, the current definition of AKI still determines it as a specific increase in serum creatinine.[4] Also, MDA levels were measured and compared on a small subpopulation of only 50 patients in each study group.

Death was not included in the primary end point in this trial and it could be considered as a competing risk of the primary end point. However, out of eight in-hospital deaths, seven met the criteria of AKI (1/1 from the AA group and 6/7 from the control group). For this reason, we believe considering death as a competing risk factor, e.g., including it in a composite AKI, death end point would not substantially affect the results of this trial.

To the best of our knowledge, this is the largest randomized control study of the effect of perioperative AA supplementation on postoperative AKI. It is also the largest study specifically monitoring the perioperative changes of serum concentration of MDA as an indicator of oxidative stress in patients undergoing arrested-heart cardiopulmonary bypass surgery. Moreover, in this patient population, it is the largest study detecting the serum levels of MDA using a highly specific GC–MS method as an analytical method.

To conclude, our study did not show a significant reduction in postoperative AKI in cardiac surgery patients who perioperatively received AA. It demonstrated no improvement in eGFR levels during the postoperative period in the AA group. It did not show a beneficial impact of AA on

oxidative stress reduction, detectable via MDA measurements. In this patient population, oxidative stress was not found to have a significant impact on postoperative AKI.

Funding
The study was funded by the University Medical Centre Maribor within Internal Research Programs and by the Slovenian Research Agency (ARRS) within the Research Program P2-0046.

Conflict of Interest
None declared.

References

1 Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 1996;334(22):1448–1460
2 Quan S, Pannu N, Wilson T, et al. Prognostic implications of adding urine output to serum creatinine measurements for staging of acute kidney injury after major surgery: a cohort study. Nephrol Dial Transplant 2016;31(12):2049–2056
3 Nadim MK, Forni LG, Bihorac A, et al. Cardiac and vascular surgery-associated acute kidney injury: The 20th International Consensus Conference of the ADQI (Acute Disease Quality Initiative) Group. J Am Heart Assoc 2018;7(11):e008834
4 Kellum JA, Lameire N. KDIGO Clinical Practice Guideline for acute kidney injury 2012. Kidney Int Suppl 2012;2:1–138
5 Thiele RH, Isbell JM, Rosner MH. AKI associated with cardiac surgery. Clin J Am Soc Nephrol 2015;10(03):500–514
6 Ortega-Loubon C, Fernández-Molina M, Carrascal-Hinojal Y, Fulquet-Carreras E. Cardiac surgery-associated acute kidney injury. Ann Card Anaesth 2016;19(04):687–698
7 Ozrazgat-Baslanti T, Thottakkara P, Huber M, et al. Acute and chronic kidney disease and cardiovascular mortality after major surgery. Ann Surg 2016;264(06):987–996
8 Antonic M. Preoperative renal dysfunction is a predictor of postoperative atrial fibrillation in coronary artery bypass patients. Acta Medico-Biotechnica 2019;12:29–38. http://actamedbio.mf.um.si/03_amb23_clanek_212-18.pdf
9 Grieshaber P, Möller S, Arneth B, et al. Predicting cardiac surgery-associated acute kidney injury using a combination of clinical risk scores and urinary biomarkers. Thorac Cardiovasc Surg 2020;68 (05):389–400
10 Antonic M. Effect of ascorbic acid on postoperative acute kidney injury in coronary artery bypass graft patients: a pilot study. Heart Surg Forum 2017;20(05):E214–E218
11 Luckraz H, Giri R, Wrigley B, et al. Reduction in acute kidney injury post cardiac surgery using balanced forced diuresis: a randomized, controlled trial. Eur J Cardiothorac Surg 2021;59(03):562–569
12 Wang Y, Bellomo R. Cardiac surgery-associated acute kidney injury: risk factors, pathophysiology and treatment. Nat Rev Nephrol 2017;13(11):697–711
13 Djordjević A, Šušak S, Velicki L, Antonič M Acute kidney injury after open-heart surgery procedures. Acta Clin Croat 2021;60 (01):120–126
14 Djordjević A, Kotnik P, Horvat D, Knez Ž, Antonič M Pharmacodynamics of malondialdehyde as indirect oxidative stress marker after arrested-heart cardiopulmonary bypass surgery. Biomed Pharmacother 2020;132:110877
15 Antonic M, Lipovec R, Gregorcic F, Juric P, Kosir G. Perioperative ascorbic acid supplementation does not reduce the incidence of postoperative atrial fibrillation in on-pump coronary artery bypass graft patients. J Cardiol 2017;69(01):98–102
16 Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth DModification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130(06): 461–470
17 Kramer RS, Herron CR, Groom RC, Brown JR. Acute kidney injury subsequent to cardiac surgery. J Extra Corpor Technol 2015;47 (01):16–28
18 Ozcan AV, Sacar M, Aybek H, et al. The effects of iloprost and vitamin C on kidney as a remote organ after ischemia/reperfusion of lower extremities. J Surg Res 2007;140(01):20–26
19 Dennis JM, Witting PK. Protective role for antioxidants in acute kidney disease. Nutrients 2017;9(07):718
20 Kaźmierczak-Barańska J, Boguszewska K, Adamus-Grabicka A, Karwowski BT. Two faces of vitamin C-antioxidative and pro-oxidative agent. Nutrients 2020;12(05):1501
21 Hill A, Borgs C, Fitzner C, Stoppe C. Perioperative vitamin C and E levels in cardiac surgery patients and their clinical significance. Nutrients 2019;11(09):2157
22 Jouybar R, Kabgani H, Kamalipour H, et al. The perioperative effect of ascorbic acid on inflammatory response in coronary artery bypass graft surgery; a randomized controlled trial coronary artery bypass graft surgery. Int Cardiovasc Res J 2012;6:13–17. https://applications.emro.who.int/imemrf/Int_Cardiovasc_Res_J/Int_Cardiovasc_Res_J_2012_6_1_13_17.pdf
23 Amini S, Robabi HN, Tashnizi MA, Vakili V. Selenium, vitamin C and N-acetylcysteine do not reduce the risk of acute kidney injury after off-pump CABG: a randomized clinical trial. Rev Bras Cir Cardiovasc 2018;33(02):129–134
24 Safaei N, Babaei H, Azarfarin R, Jodati AR, Yaghoubi A, Sheikhalizadeh MA. Comparative effect of grape seed extract (*Vitis vinifera*) and ascorbic acid in oxidative stress induced by on-pump coronary artery bypass surgery. Ann Card Anaesth 2017;20 (01):45–51
25 Dingchao H, Zhiduan Q, Liye H, Xiaodong F. The protective effects of high-dose ascorbic acid on myocardium against reperfusion injury during and after cardiopulmonary bypass. Thorac Cardiovasc Surg 1994;42(05):276–278
26 Nilsson L, Tydén H, Johansson O, et al. Bubble and membrane oxygenators–comparison of postoperative organ dysfunction with special reference to inflammatory activity. Scand J Thorac Cardiovasc Surg 1990;24(01):59–64
27 Cavarocchi NC, Pluth JR, Schaff HV, et al. Complement activation during cardiopulmonary bypass. Comparison of bubble and membrane oxygenators. J Thorac Cardiovasc Surg 1986;91(02): 252–258
28 van Oeveren W, Kazatchkine MD, Descamps-Latscha B, et al. Deleterious effects of cardiopulmonary bypass. A prospective study of bubble versus membrane oxygenation. J Thorac Cardiovasc Surg 1985;89(06):888–899
29 Oktar GL, Sinci V, Kalaycioglu S, et al. Biochemical and hemodynamic effects of ascorbic acid and alpha-tocopherol in coronary artery surgery. Scand J Clin Lab Invest 2001;61(08): 621–629
30 Kališnik JM, Hrovat E, Hrastovec A, et al. Creatinine, neutrophil gelatinase-associated lipocalin, and cystatin C in determining acute kidney injury after heart operations using cardiopulmonary bypass. Artif Organs 2017;41(05):481–489
31 Jerin A, Mosa OF, Kališnik JM, Žibert J, Skitek M. Serum Klotho as a marker for early diagnosis of acute kidney injury after cardiac surgery. J Med Biochem 2020;39(02):133–139