The Association Between Serum Glutathione Peroxidase-3 Concentration and Risk of Acute Kidney Injury After Cardiac Surgery: A Nested Case-Control Study

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Oxidative stress has an integral role in the pathophysiology of cardiac surgery-associated acute kidney injury (CSA-AKI). Glutathione peroxidase 3 (GPx3) is an important antioxidant enzyme in circulation and is mainly secreted by the kidney. This study aimed to evaluate the relation between GPx3 protein and CSA-AKI. This study is a nested case-control study in Zhongshan Hospital affiliated with Fudan University. We examined serum samples from 80 CSA-AKI patients and 80 age- and gender-matched non-AKI patients who underwent cardiac surgery. AKI was defined according to Kidney Disease: Improving Global Outcomes (KDIGO) 2012 criteria. We measured serum GPx3 concentration using the enzyme-linked immunosorbent assay. GPx3 ratio is the ratio of preoperative and 6 hours postoperative of GPx3 protein concentration. We applied dose-response relation analyses to odds ratio in different GPx3 ratio levels and integrated it into the logistic model to predict the risk of AKI. The receiver operating characteristic curve and area under the curve (AUC) was used to assess the prediction models. Postoperative serum GPx3 concentrations were significantly lower in the AKI group compared with the non-AKI group (1.78 ± 0.33 vs 2.03 ± 0.27, p <0.001). Malondialdehyde was higher in the AKI than in the non-AKI group (17.74 ± 8.65 vs 7.48 ± 4.59, p <0.001). The AKI risk increased in a dose-dependent manner, which was flat in the first half of the GPx3 ratio and then tended to be faster. The peaking odds ratio of CSA-AKI was 2.615 at the GPx3 ratio of 1.21 to 1.40. The AUC value to predict CSA-AKI only included the GPx3 ratio was 72.3%. After gradually integrating other covariates (body mass index, aortic crossclamp time, and cardiopulmonary bypass), the model showed an AUC of 82.6%. The serum GPx3 concentration was significantly lower in the CSA-AKI group. GPx3 ratio has a good predictive value for CSA-AKI, which may be a potential early diagnostic marker for AKI. **(Am J Cardiol 2023;209:29–35)**

Keywords: acute kidney injury, diagnostic marker, glutathione Peroxidase-3, cardiac surgery

Cardiac surgery-associated acute kidney injury (CSA-AKI) is the most common perioperative complication after cardiac surgery,[1–4] with an incidence of 20% to 70%.[5] CSA-AKI is independently associated with increased higher perioperative mortality, and short-term and long-term morbidity even for those patients with complete renal recovery.[2,6–8] Besides, CSA-AKI prolonged the length of stay in the intensive care unit and in the hospital, meanwhile, it also increased the cost of care.[9,10] Therefore, early identification of potential patients with CSA-AKI and early diagnosis of CSA-AKI remain a priority in the fields of nephrology and intensive care medicine. Glutathione peroxidase 3 (GPx3) is the only extracellular isoform secreted isozyme abundantly expressed in the kidneys and the thyroid.[11,12] GPx3 as a modulator of redox signaling accounts for the major anti-oxidative activity in the circulation.[13] Therefore, GPx3 protein concentration can be easily assayed in plasma, which makes it a useful prognostic and diagnostic biomarker. Oxidative stress is a crucial mechanism in the development of CSA-AKI.[2,14,15] In an animal model, it has been reported that persistent oxidative stress in renal tissues resulted in decreased GPx3 after renal ischemia-reperfusion injury (IRI).[16] In this study, we

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Funding: This article was supported by grants from Clinical Research Plan of Shanghai ShenKang Hospital Development Center (Shanghai, China), number SHDC2020CR2022B; Special Fund for Clinical Research of Zhongshan Hospital and Fudan University, 2018; Science and Technology Commission of Shanghai (Shanghai, China), 14DZ2260200.

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See page 34 for Declaration of Competing Interest.

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0002-9149/

https://doi.org/10.1016/j.amjcard.2023.08.141

sought to determine potential associations between circulating GPx3 protein concentration and the development of CSA-AKI.

Methods

This is a nested case-control study of hospitalized patients who underwent cardiac surgery in a third-class hospital in mainland China during the period of July 2021 to July 2022. These patients were evaluated at a high risk of CSA-AKI according to CSA-AKI risk score.[17] The inclusion criteria of participants were as follows: aged over 18 years, underwent elective cardiopulmonary bypass (CPB) cardiac surgery, and CSA-AKI risk score ≥4 (Supplementary Table 1). Patients were excluded if they had situations as follows: pregnant, chronic kidney disease stage 5, underwent renal transplantation, liver failure or hepatorenal syndrome, acute myocardial infarction within 1 week, participated in another clinical trial, or investigators considering that it is inappropriate to participate in this study. Informed consent was obtained from all participants.

The primary end points were AKI and moderate-to-severe AKI within 48 hours after cardiac surgery. AKI was defined according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guideline as follows: increase in serum creatinine by ≥0.3 mg/100 ml (≥26.5 μmol/L) within 48 hours. Severe AKI was defined as KDIGO stage 2 or 3.

GPx3 ratio is the ratio of preoperative and 6 hours postoperative of GPx3 protein concentration.

Demographic characteristics, including age, gender, height, weight, body mass index (BMI), co-morbidities (hypertension, diabetes mellitus, chronic kidney disease, and so on), intraoperative and postoperative data, were abstracted from history taking and the electronic medical record system. The biochemical data of the patients within 24 hours of admission were collected as baseline variables, including renal function, and other biochemical data. We also collected renal function 24 and 48 hours after surgery. The blood samples of GPx3 protein concentration and malondialdehyde (MDA) concentration were collected at 6 hours after surgery.

For each suberect, the serum sample was separated from the blood sample by centrifuging at 3,000 rotations/min for 15 minutes at 4°C, separated into 1-ml aliquots, and immediately stored at −80°C until analysis. GPx3 was measured using the commercial enzyme-linked immunosorbent assay, brought from AdipoGen Life Sciences, for quantitative determination of GPx3 protein concentration in human serum samples. A polyclonal antibody specific for GPx3 protein has been precoated onto the 96-well microtiter plate. GPx3 protein was detected with biotinylated polyclonal antibody and horseradish peroxidase-labeled streptavidin using 3,3′,5,5′-tetra methyl benzidine as horseradish peroxidase substrate. The intensity of the color reaction measured using the SpectraMax (Molecular Devices, Silicon Valley, California) at 450 nm is directly proportional to the protein concentration of GPx3 in the serum sample.

MDA concentration was determined using the MDA-TBA (thiobarbituric acid, TBA) method as described in the Lipid Peroxidation MDA assay kit brought from Beyotime Biotechnology. MDA levels were detected by microplate readers (BioTek Epoch 2，BioTek Instruments,Inc, Vermont) at 532 nm.

The statistical analysis was run in R 4.0.3 software. The data of normal distribution are described as mean ± SD, and the data of skewed distribution are described by the median and interquartile range and were compared by Student's *t* test and Wilcoxon test. Categorical variables were described by frequency and compared using Pearson's test. For dose-response relation statistics, a correlation model between GPx3 protein concentration and the incidence of CSA-AKI, and the likelihood ratio test was used to compare nonlinear variables. To assess the predictive ability of the GPx3 ratio for the primary end point, receiver operating characteristic curves, area under the curve (AUC), sensitivity, and specificity were calculated. The 95% confidence intervals (CIs) for the AUC and receiver operating characteristic were determined using boot-strap and the Clopper-Pearson method respectively. All statistical tests were two-tailed, and the statistical significance was set at $p < 0.05$.

Results

This study included 160 participants, the mean age was 65.33 ± 7.93 years, and 107 (66.9%) were male. In 80 patients with CSA-AKI, 73 (91.3%), 6 (7.5%), and 1 patients (1.3%) were in AKI stage 1, stage 2, and stage 3, respectively. Table 1 lists the major factors associated with CSA-AKI. Patients who had a higher BMI, prolonged CPB, and aortic crossclamp time (ACCT) were more likely to develop AKI. Although not statistically significant ($p = 0.071$), hypertension was more common in patients with CSA-AKI. Length of stay in the intensive care unit and the duration of mechanical ventilation in CSA-AKI patients was apparently longer than in non-AKI patients.

All clinical laboratory biochemicals are listed in Table 2. There was no significant difference in GPx3 protein concentration before surgery between AKI and non-AKI groups. The GPx3 protein concentration at 6 hours after surgery was significantly lower in the AKI group compared with the non-AKI group (1.78 ± 0.33 vs 2.03 ± 0.27, $p < 0.001$), and patients with AKI had higher postoperative serum creatinine levels (96.62 ± 34.74 vs 84.25 ± 19.36, $p = 0.006$). MDA was higher in the AKI than in the non-AKI group (17.74 ± 8.65 vs 7.48 ± 4.59, $p < 0.001$).

The dose-response relation between the GPx3 ratio and CSA-AKI was shown in Figure 1. It was figured out that the GPx3 ratio was positively correlated with the risk of AKI. The upward trend in AKI at GPx3 ratio ≥1.2 was faster than at GPx3 ratio <1.2 (odds ratio [OR] 2.8, 95% CI 1.430 to 5.536, $p < 0.0001$). Then we classified the GPx3 ratio into 6 levels, as listed in Table 3. Compared with the GPx3 ratio of 0.6 to 0.8, the risk continued to grow at a higher GPx3 ratio. The peaking OR of CSA-AKI was 2.615 (95% CI 1.003 to 6.822, $p = 0.0350$) at the GPx3 ratio of 1.21 to 1.40. The severe AKI showed a similar upward trend.

We used a logistic model based on GPx3 ratio to predict CSA-AKI (Figure 2). In the model with GPx3 ratio alone,

Table 1
Demographic characteristics

Variables	AKI (n = 80)	non-AKI (n = 80)	Total (n = 160)	
Demographic characteristics				
Age	65.71±7.69	64.96±8.19	65.33±7.93	0.560
Male gender, n (%)	55 (68.8)	52 (65.0)	107 (66.9)	0.737
BMI (kg/m^2)	24.12±3.12	22.43±2.96	23.27±3.15	0.001
Cardiac function (NYHA)				
I-II (n, %)	23 (28.8)	19 (23.8)	42 (26.3)	0.557
III-IV (n, %)	57(71.2)	61 (76.2)	118 (73.8)	0.557
Comorbidities				
Hypertension, n (%)	31 (38.8)	21 (26.3)	52 (32.5)	0.071
Diabetes mellitus, n (%)	6 (7.5)	5 (6.3)	11 (6.88)	0.755
Hyperlipidemia, n (%)	3 (3.8)	4 (5.0)	7 (4.3)	0.725
Hyperuricemia/gout, n (%)	8 (10.0)	5 (6.3)	13 (8.13)	0.399
CKD, n (%)	3 (3.8)	1 (1.3)	4 (2.5)	0.364
COPD, n (%)	1 (1.3)	0	1 (0.65)	0.494
MI, n (%)	4 (5.0)	3 (3.8)	7 (4.3)	0.782
Encephalorrhagia, n (%)	1 (1.3)	0	1 (0.6)	0.494
Cerebal infraction, n (%)	4 (5.0)	5 (6.3)	9 (5.6)	0.744
Life style				
Current or former smoker	21 (26.3)	23 (28.7)	44 (27.5)	0.860
Current or former drinker	13 (16.3)	13 (16.3)	26 (16.25)	0.542
Surgery style				
Valve, n (%)	58 (72.5)	62 (77.5)	120 (75.0)	0.200
Valve + CABG, n (%)	10 (12.5)	6 (7.5)	16 (10.0)	
Others, n (%)	12 (15.0)	12 (15.0)	24 (15.0)	
CPB	117.56 ±34.80	98.37 ± 33.12	107.35 ± 35.14	0.001
ACCT (Aortic occlusion time)	65.55±30.98	54.81 ± 27.04	59.84 ± 29.34	0.030
ECMO, n (%)	0	0	0	1.000
IABP, n (%)	1 (1.3)	1 (1.3)	2 (2.6)	1.000
Blood transfusion, n (%)	17 (21.3)	13 (16.3)	30 (18.8)	0.221
Duration of mechanical Ventilation, days	1 [0.83,1]	1 [0.5,1]	1 [0.7,1]	0.031
LOS in ICU	2 [1,3]	1 [1,2]	1 [1,3]	0.017
LOS after surgery	6 [4,9]	6 [5,9]	6 [5,9]	0.273

ACCT = aortic crossclamp time; BMI = body mass index; CABG = coronary artery bypass grafting; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; CPB = cardiopulmonary bypass; ECMO = extracorporeal membrane oxygenation; IABP = intra-aortic balloon pump; ICU = intensive care unit; LOS = length of stay; MI = myocardial infraction; NYHA = New York Heart Association.

the AUC value was 72.3% (95% CI 64.4% to 80.2%). With the progressive enrollment of ACCT, CPB, the AUC value increased from 72.3% to 76.6% (95% CI 68.6% to 84.5%). With the progressive enrollment of BMI, the AUC value increased from 76.6% to 82.6% (95% CI 75.6% to 89.6%). Our data indicate that decreased GPx3 concentration predicts clinical CSA-AKI and the AUC of the GPx3 ratio after adjusting BMI, ACCT, and CPB reaches a better prediction ability than that of the GPx3 ratio alone.

Discussion

Both in human studies and in animal models, GPx3 protein concentration and its bioactivity have already been investigated in various diseases. The bioactivity of GPx3 was found to be inversely associated with critical illness.[18,19] There is a positive correlation between GPx3 bioactivity and its protein concentration in septic patients.[19] Lower plasma GPx3 levels were observed in patients and rat liver transplantation models with hepatocellular carcinoma recurrence.[20] GPx deficiency has previously been demonstrated in hemodialysis patients and those with CKD.[21,22] Jin et al[23] found that GPx3 deficiency promoted platelet-dependent thrombosis in vivo.

Previous studies have not demonstrated a consistent association between GPx3 and AKI after cardiac surgery. In this context, we are the first to deem that decreased plasma GPx3 is discovered in CSA-AKI patients, suggesting that GPx3 is a potential diagnostic marker for AKI and predicts CSA-AKI after cardiac surgery. GPx3 is primarily synthesized by the basolateral membrane of epithelial cells in the proximal renal tubule cells.[24−26] GPx3 is a member of the GPx family and plays a vital role in eliminating all forms of hydrogen peroxide and organic peroxides generated in the body[23] and catalyzing the conversion of glutathione to oxidized glutathione.[24,27]

In our report, we showed that in patients with CSA-AKI, GPx3 protein concentration at 6 hours after surgery was significantly lower than that in non-AKI patients. MDA was higher in patients with CSA-AKI. The potential regulatory mechanisms of GPx3 and its physiological and pathophysiological roles in CSA-AKI remain elusive. Mechanisms of

Table 2
Clinical biochemical indexes and CSA-AKI

Variables	AKI (n =80)	non-AKI (n = 80)	Total (n = 160)	P
GPx3 protein concentration (μg/ml)				
Before surgery	1.99 ±0.36	1.98 ± 0.29	1.99± 0.32	0.865
6h after surgery	1.78± 0.33	2.03± 0.27	1.90± 0.33	<0.001
MDA concentration (μmol/L)				
6h after surgery	17.74 ± 8.65	7.48 ± 4.59	12.74 ± 8.62	<0.001
Blood routine examination				
Hb (g/L)	128.01±15.29	133.28±18.27	130.63±17.00	0.055
RBC (10^{12}/L)	4.16±0.488	4.32±0.56	4.24±0.53	0.070
WBC (10^9/L)	6.57±1.14	5.63±1.547	5.76±1.51	0.308
Neutrophil(10^9/L)	56.98±7.38	56.97±8.66	56.98±7.97	0.993
Platelet (10^9/L)	167.13±51.59	183.32±70.08	175.2±61.80	0.106
Renal function				
BUN (mmol/L)				
Before surgery	8.69 ± 2.23	7.56 ±2.64	8.14 ± 2.21	0.186
24h after surgery	10.31 ± 2.73	7.86 ± 2.78	9.09 ± 2.61	<0.001
48h after surgery	12.57 ±3.48	10.29 ± 10.39	11.43± 7.80	0.080
SCr (μmol/L)				
Before surgery	96.62 ±34.74	84.25 ± 19.36	90.44 ± 28.71	0.006
24h after surgery	132.94 ± 39.21	88.23 ±20.85	110.72± 38.55	<0.001
48h after surgery	138.96 ± 55.87	85.04 ± 21.56	112.00 ± 50.13	<0.001
eGFR (ml/min/1.73m^2)				
Before surgery	70.97± 18.18	76.49± 16.94	73.66 ± 17.75	0.057
24h after surgery	48.00± 12.68	72.22 ± 16.78	59.85 ± 19.13	<0.001
48h after surgery	49.42 ± 20.10	75.10± 18.342	62.08 ± 23.11	<0.001
UA (μmol/L)				
Before surgery	411.81± 117.19	395.16±119.29	403.71± 118.11	0.390
24h after surgery	404.73± 97.26	324.01 ±105.72	364.37 ± 109.03	<0.001
48h after surgery	370.76 ± 101.69	270.04 ± 101.01	320.76 ± 112.93	<0.001
Liver function				
TB (μmol/L)	13.12±6.8	13.05±5.15	13.17±6.01	0.919
DB (μmol/L)	4.47±2.05	4.01±2.06	4.24±2.23	0.383
AST (U/L)	20.34±6.15	20.96±6.714	20.64±6.42	0.554
ALT (U/L)	17.86±10.314	17.88±11.862	17.87±11.063	0.991
Cardiac biomarkers				
BNP (pg/ml)	754[280,1754]	718[121,2033]	719[194,1819]	0.490
CK-MB (U/L)	11.86 ± 6.58	12.68 ± 8.51	12.26± 7.56	0.595
Other laboratory indexes				
Total protein (g/L)	63.44± 5.121	64.11±7.46	63.78± 6.37	0.514
Albumin (g/L)	39.91 ± 3.53	40.65 ± 3.203	40.28± 3.38	0.175
Globulin (g/L)	23.53 ± 4.008	23.45 ± 7.15	23.49 ± 5.75	0.933
FBG (mmol/L)	4.95 ± 0.937	5.05± 0.775	5.00± 0.86	0.525
GA (%)	12.30 ± 4.46	11.75± 3.68	12.02± 4.03	0.673
TC (mmol/L)	4.12 ± 0.98	4.22 ± 0.871	4.16 ± 0.927	0.512
TG (mmol/L)	1.43 ± 0.86	1.24 ± 0.742	1.34± 0.807	0.170
HDL (mmol/L)	1.27 ±0.842	1.28 ±0.58	1.27 ± 0.724	0.907
LDL (mmol/L)	2.29 ±0.793	2.43± 0.722	2.36 ± 0.761	0.260

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BNP = brain natriuretic peptide; BUN = blood urea nitrogen; CK-MB = creatine kinase MB isoenzyme; CSA-AKI = cardiac surgery associated acute kidney injury; DB = direct bilirubin; eGFR = estimated glomerular filtration rate; FBG = fasting blood-glucose; GA = glycated albumin; GPx3 = glutathione peroxidase-3; Hb = hemoglobin; HDL = high density lipoprotein; LDL = low density lipoprotein; RBC = red blood cell; SCr = serum creatinine; TB = total bilirubin; TC = total cholesterol; TG = triglycerides; UA = uric acid; WBC = white blood cell.

CSA-AKI include perioperative renal ischemia-reperfusion injury, oxidative stress,[28] inflammation, CPB-induced hemolysis, and pigment nephropathy.[29,30] They are interdependent and interrelated between IRI, oxidative stress, and inflammation.[29] Cardiac surgery also induces renal and systemic inflammation.[31] The exposure of blood to the CPB circuit, IRI, and oxidative damage all contribute to inflammation.[31] IRI disrupts the cellular redox balance and promotes the generation of reactive oxygen species (ROS) and superoxide in kidney.[16] ROS are derived from molecular oxygen and non-radicals like hydrogen peroxide. ROS induces inflammation by activating proinflammatory transcription factors[32] and proinflammatory signals such as Toll-like receptors and the nucleotide oligomerization domain (NOD)-like receptor thermal protein domain associated Protein 3 inflammasome.[33,34] Inflammation also

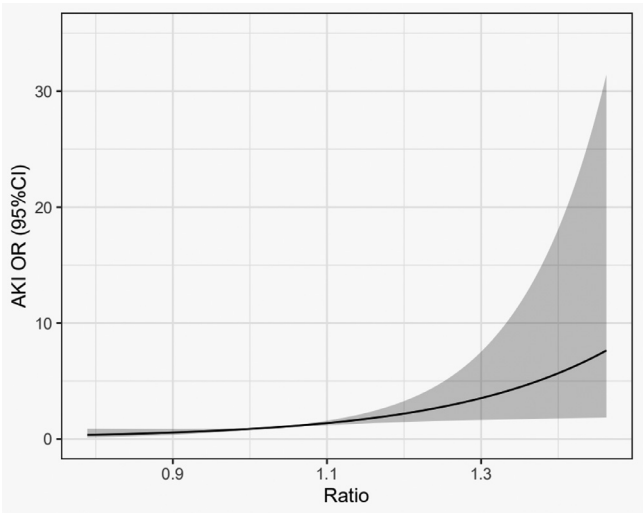

Figure 1. Dose-response relation of GPx3 ratio and CSA-AKI.

promotes ROS production.[35] As a major ROS scavenger in plasm, Gpx3 is heavily consumed during oxidative stress and inflammation. Previous studies indicated that the GPx3 protein or GPx3 activity was decreased in oxidative stress and inflammation. As previously mentioned, GPx3 is secreted by the proximal renal tubule cells (PTC).[26] When the proximal renal tubule was injured after cardiac surgery, the GPx3 protein concentration would be reduced. It has been reported that post-AKI rats had decreased renal GPx3 mRNA expression.[16] And CKD was related to lower GPx activity.[21,36]

Because of increase the excessive consumption and decrease the production of GPx3 in C patients with CSA-AKI during cardiac surgery, the GPx3 protein concentrations of CSA-AKI were lower than those without. We wonder what would happen, if we exogenously supplement Gpx3 and its related substances to patients with CSA-AKI. Ebselen is seleno-organic compound of GPx, which is able to ameliorate IRI of the kidney by decreasing oxidative and nitrosative stresses in rat renal IRI model.[37] Ebselen also has a renoprotective effect in a model of diabetic complications in the setting of enhanced oxidative stress.[38] It is a promising candidate for IRI induced AKI through selenium-containing nanomaterials to remedy Se imbalance and impede inflammatory responses in the kidney.[39] A potential use of Se supplementation has been proposed benefit patients with sepsis. Therapeutic role of recombinant GPx3 has not been used in renal diseases, whereas it has been explored in the hepatocellular carcinoma. GPx3 treatment at early phase was suggested as a new therapeutic

Table 3
Levels of GPx3 ratio and risk stratification for AKI

	n(160)	AKI			Severe AKI		
		n (%)	aOR (95% CI)	P	n (%)	aOR (95% CI)	P
GPx 3 ratio							
0.6-0.80	12	4 (33.3)	Ref		0		
0.81-1.00	54	16 (29.6)	0.870(0.295,2.559)	0.801	2 (3.7)	Ref	
1.01-1.20	63	35 (55.5)	2.167(0.713, 6.584)	0.158	1 (1.6)	0.684 (0.300, 1.561)	0.470
1.21-1.40	18	13 (72.7)	2.615 (1.003, 6.822)	0.035	1 (5.6)	1.130 (0.502, 2.545)	0.733
1.41-1.60	8	7 (87.5)	2.444 (1.082, 5.523)	0.017	1 (12.5)	1.322 (0.591, 2.959)	0.279
1.61-1.80	5	5 (100)	2.25 (1.084, 4.671)	0.012	2 (40.0)	1.891 (0.708, 5.049)	0.002

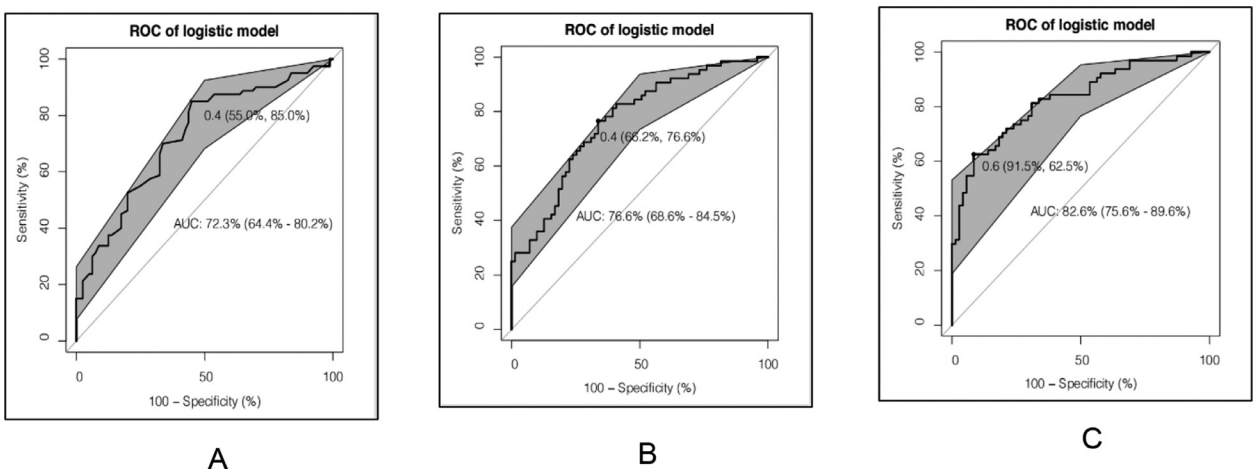

Figure 2. ROC curves for GPx3 as a predictor of CSA-AKI. (*A*) In model of GPx3 ratio alone, the AUC value was 72.3% (95% CI 64.4% to 80.2%). (*B*) With the progressive enrollment of ACCT, CPB, the AUC value increased from 72.3% to 76.6% (95% CI 68.6% to 84.5%). (*C*) With the progressive enrollment of BMI, the AUC value increased from 76.6% to 82.6% (95% CI 75.6% to 89.6%). ROC = Receiver operating characteristics.

strategy for patients with hepatocellular carcinoma after liver transplantation.[20]

However, our study possesses some limitations. This research is a single-center observational study design, and there may be some unmeasured confounding factors. Given the small sample size, we were unable to give a validation cohort. Additionally, we did not measure GPx3 bioactivity and thus could not know the relation between GPx3 bioactivity and CSA-AKI. The information on long-term kidney function and related prognosis was also not available.

In conclusion, the lower GPx3 protein concentration and higher GPx3 ratio are strongly associated with high risk of CSA-AKI. Gpx3 may serve as an early diagnostic marker for CSA-AKI. More further studies are needed to confirm the role of GPx3 in diagnoses and treatment for AKI.

Authors' Contributions

Zhouping Zou performed the analysis and drafted the manuscript, Teng Ren, Qi Zeng and Xiaoyan Wang helped to collect the data, Yang Li helped to guide the analysis, Xiaoqiang Ding conceived the idea, participated in manuscript writing and revision. Jiarui Xu and Ping Jia helped to revise the manuscript. All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors have no competing interests to declare.

Data Availability

Some or all data, models, or code generated or used during the study are available in a repository or online in accordance with funder data retention policies.

Ethical Approval

This study was approved by the Chinese Clinical Trial Registry (ChiCTR2000035568).

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2023.08.141.

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