Association of Indoxyl Sulfate with Heart Failure among Patients on Hemodialysis

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Abstract

Background and objectives Indoxyl sulfate, a protein-bound uremic toxin, may be associated with cardiovascular events and mortality in patients with CKD. This study aimed to investigate the relationship between indoxyl sulfate and heart failure in patients on hemodialysis.

Design, setting, participants, & measurements Patients on hemodialysis for >6 months were enrolled within 6 months. Patients with congestive heart failure, angina pectoris, acute myocardial infarction, cerebral infarction, or cerebral hemorrhage within 3 months before the study or those <18 years old were excluded. The primary end point was first heart failure event during follow-up.

Results In total, 258 patients (145 men) with a mean age of 57.0±14.6 years old were enrolled. Median plasma indoxyl sulfate level was used to categorize patients into two groups: the low-indoxyl sulfate group (indoxyl sulfate ≤32.35 μg/ml) and the high-indoxyl sulfate group (indoxyl sulfate >32.35 μg/ml). Then, patients were prospectively followed up for a median of 48.0 (interquartile range: 33.5–48.0) months. During follow-up, 68 patients experienced episodes of first heart failure. Kaplan–Meier analysis revealed the incidence of first heart failure event in the high–indoxyl sulfate group was significantly higher than in the low-indoxyl sulfate group (log rank $P<0.001$). Cox regression analysis showed indoxyl sulfate was significantly associated with first heart failure event (indoxyl sulfate as the continuous variable: hazard ratio, 1.02; 95% confidence interval [95% CI], 1.01 to 1.03; $P=0.001$; indoxyl sulfate as the dichotomous variable: hazard ratio, 3.49; 95% CI, 1.97 to 6.20; $P<0.001$). After adjustment for other confounding factors, the results remained significant (indoxyl sulfate as the continuous variable: hazard ratio, 1.04; 95% CI, 1.02 to 1.06; $P<0.001$; indoxyl sulfate as the dichotomous variable: hazard ratio, 5.31; 95% CI, 2.43 to 11.58; $P<0.001$).

Conclusions Plasma indoxyl sulfate was associated with first heart failure event in patients on hemodialysis. Whether indoxyl sulfate is only a biomarker or involved in the pathogenesis of heart failure in hemodialysis warrants additional study.

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Introduction

Cardiovascular disease (CVD) mortality is significantly higher in patients on hemodialysis than in the general population (1). Congestive heart failure is considered to be an important contributor to this elevated mortality. In addition to the traditional CVD risk factors, accumulation of uremic toxins, especially protein-bound solutes and middle molecules, that are difficult to remove from the body by the current dialysis procedures, may play an important role in the pathogenesis of CVD in the hemodialysis setting.

Indoxyl sulfate (IS), a protein-bound uremic toxin, is one of the organic anions synthesized in the liver from indole, which is produced from tryptophan by intestinal flora (2). CYP2E1 is the major isoform of P450 enzymes responsible for microsomal oxidation of indole to indoxyl (3). Because of the high protein-binding rate, clearance of IS using current dialysis techniques is limited relative to small water-soluble solutes, such as urea (4).

In animal experiments, IS has been shown to lead to progression of renal function deterioration, glomerular sclerosis, and tubulointerstitial fibrosis by enhancing the expression of TGF-β1 (5), tissue inhibitor of metalloproteinase 1 (6), plasminogen activator inhibitor 1 (7), intercellular adhesion molecule 1 (8), nuclear factor (erythroid-derived 2)–like 2 (9), intrarenal renin-angiotensin-aldosterone, and epithelial-to-mesenchymal transition-associated transcription factor Snail (10). In a clinical study, IS was shown to predict CKD progression (11). IS also has a detrimental effect on endothelial and vascular smooth muscle cell proliferation (12,13). However, the clinical evidence of the association of IS and heart failure is uncertain. We, therefore, conducted a prospective study to investigate the relationship between IS and heart failure in patients on hemodialysis.

Materials and Methods

Study Population

Patients who had been on hemodialysis treatment at least 6 months were enrolled from the Blood Purification Center, Zhongshan Hospital, Fudan University.

The enrollment was completed within 6 months from July to December of 2009. Patients with congestive heart failure, angina pectoris, acute myocardial infarction, cerebral infarction, and cerebral hemorrhage within 3 months before the study or those <18 years old were excluded from this study. The study adhered to the Declaration of Helsinki and was approved by the Ethical Committee, Zhongshan Hospital, Fudan University. All participants provided written informed consent.

Patients were treated three times per week (4 hours per session) with standard bicarbonate dialysate (Na^+: 138.0 mmol/L, HCO_3^-: 32.0 mmol/L, K^+: 2.0 mmol/L, Ca^{2+}: 1.2 5 mmol/L, Mg^{2+}: 0.5 mmol/L) by low-flux hemodialysis using 1.4-m^2 dialyzers with synthetic membranes (BLS514SD; Sorin Group Italia, Mirandola, Italy and Polyflux 14L; Gambro Dialysatoren GmbH, Hechigen, Germany). The blood flow was 200–280 ml/min, and dialysate flow was 500 ml/min. The water quality conformed to the Association for the Advancement of Medical Instrumentation standard and was examined every month. Dry weight was targeted in every patient to achieve an edema-free state. During the study, dry weight was reevaluated every month to guarantee a proper dry weight in every patient. In our center, all patients on hemodialysis were advised to have a high-protein diet (at least 1.2 g/kg per day with mainly animal protein).

Anthropometric Measurements, Blood Sampling, and Clinical Data Collection

Height and weight were measured while patients were barefoot and wearing light clothes only. Blood sampling was performed on a midweek nondialysis day (Thursday for patients scheduled with a Monday/Wednesday/Friday dialysis cycle and Friday for patients scheduled with a Tuesday/Thursday/Saturday dialysis cycle) from 8:00 to 10:00 a.m. Demographic and clinical data were collected, including age, sex, smoking history, underlying kidney disease, dialysis duration, comorbidity, and history of taking medicine. BP, average interdialytic weight gain, and ultrafiltration volume were obtained by averaging all predialysis BP, interdialytic weight gain, and ultrafiltration volume values, respectively, during 4 weeks (12 times in total) before this study. Urinary volume was measured, and residual renal function (RRF) was estimated by calculating GFR expressed as a mean of urea and creatinine clearance (milliliters per minute per 1.73 m^2) (14). Normalized protein nitrogen appearance rate (nPNA) was calculated according to the work by Depner and Daugirdas (15).

Biochemical Measurements

Serum albumin, prealbumin, hemoglobin, BUN, serum creatinine (SCr), uric acid (UA), calcium (Ca), phosphorus (P), lipids, homocysteine (Hcy), iron, ferritin, and transferrin were measured using standard methods in the clinical laboratory. The concentrations of high-sensitivity C-reactive protein (hsCRP) and β_2-microglobulin (β_2M) were determined using immunoturbidimetry assay, and concentration of intact parathyroid hormone (iPTH) was measured using electrochemiluminescence immunoassay.

The standard of IS potassium salt (99.8%) was purchased from Sigma-Aldrich (St. Louis, MO). The internal standard of hydrochlorothiazide (99.5%) was provided by the Shanghai Institute for Drug Control. Use of stable isotope-labeled IS as the internal standard may be one of the best approaches in terms of accuracy of the assay. However, it is not practical, because isotope-labeled IS is not commercially available in many places. The HPLC tandem mass spectrometry method modified according to Wang and Korfmacher (16) was used to detect IS concentration in plasma. Briefly, 100 μl plasma was pipetted into a 2-ml polypropylene tube. Then, 500 μl internal standard/protein precipitation solution (50 ng/ml hydrochlorothiazide in methanol) was added to precipitate the proteins. The contents were vortex mixed for 1 minute. After centrifugation at 12,000×g for 10 minutes, a 100-μl aliquot of clear supernatant was mixed with 100 μl water in a polypropylene tube and transferred to the autosampler. A volume of 5 μl was injected into liquid chromatography tandem mass spectrometry. The chromatographic separation was achieved on a Venusil XBP Phenyl column (100×2.1 mm, 5 μm; Bonna-Agela Technologies Inc., Wilmington, DE). Mobile phase A was 2 mmol/L ammonium acetate in 0.1% (vol/vol) formic acid. Mobile B was methanol. The mobile phase (A:B=30:70) was delivered at a flow rate of 0.3 ml/min. The temperatures of the column and the autosampler were maintained at 40°C and 4°C, respectively. Then, IS and the internal standard were eluted at 1.8 and 1.4 minutes, respectively. The chromatographic run time of each sample was 4 minutes. Mass spectrometric detection was performed on an API 3000 triple quadrupole instrument (Applied Biosystems, Toronto, ON, Canada) in multiple reaction monitoring mode. A TurboIonSpray ionization interface in negative ionization mode was used. Turbo spray voltage was set at −4200 V. Source temperature was maintained at 500°C. The compound parameters of collision energy, declustering potential, entrance potential, and collision exit potential were −25, −40, −10, and −10 V for IS and −30, −58, −10, and −6 V for hydrochlorothiazide. Quadrupoles 1 and 3 were maintained at unit resolution. Dwelling time was set at 200 ms for all of the analytes. Mass transitions m/z 212.1→81.1 for IS and m/z 296.2→268.8 for hydrochlorothiazide were used. Data processing was performed with the Analyst 1.4.1 software package (Applied Biosystems). The standard curves for IS were set at 0.125, 0.25, 0.5, 1, 5, 10, 50, and 100 μg/ml, with an average r value of 0.999 (n=6). The lower limit of quantitation was 0.125 μg/ml. Intra- and interassay mean biases were 3.3% and 4.2%, respectively.

Echocardiography

Transthoracic echocardiographic examinations were performed using a Philips echocardiographic machine (Philips IE33; Philips, Eindhoven, The Netherlands) with a 3.5-MHz multiphase array probe by a single experienced cardiologist on a midweek nondialysis day within 2 hours after blood sampling. Measurements of the left ventricular internal dimension, interventricular septal thickness, and posterior wall thickness were determined at end diastole in M-mode echocardiography according to the recommendations of the Penn Convention. Meanwhile, left ventricular ejection fraction (LVEF) was calculated from two-dimensional apical images according to Simpson's method. Left ventricular mass was calculated with the Devereux Equation (17), and left ventricular mass index (LVMI) was obtained by dividing left ventricular mass by height in meters raised to the power of 2.7. The height-based indexing was specifically chosen to minimize any potential distortion attributable to extracellular volume expansion.

End Point Evaluation

Heart failure was defined as dyspnea in addition to two of the following conditions: (*1*) raised jugular pressure, (*2*) bibasilar crackles, (*3*) pulmonary venous hypertension or interstitial edema on chest x-ray requiring hospitalization or extra ultrafiltration, or (*4*) LVEF≤40% (18,19). Follow-up was extended until July 31, 2013. Events, including heart failure, angina pectoris, acute myocardial infarction, arrhythmias, cerebral infarction, cerebral hemorrhage, and death (cardiac or noncardiac), were identified by a group of physicians who were blinded to the IS data. The primary end point was first heart failure event during follow-up. All patients were assessed every month for clinical events. If a patient was admitted to the hospital/emergency department or needed an unscheduled/urgent hemodialysis treatment, a record would be made by the doctor on duty and discussed on Friday afternoon of that week.

Statistical Analyses

All data were expressed as means±SDs, medians (interquartile ranges), or frequencies as appropriate. To compare two groups of normal data, independent samples *t* tests were used, whereas for skewed and categorical data, Mann–Whitney *U* and chi-squared tests were performed, respectively. The Kaplan–Meier method and Cox proportional hazard model were used to assess the association of IS and first heart failure event. To adjust for confounding risk factors for first heart failure event, we constructed model 1 (age, sex, and body mass index), model 2 (history of primary hypertension, coronary heart disease, and diabetes), model 3 (urinary volume, ultrafiltration volume, average interdialytic weight gain, RRF, and single-pool Kt/V), model 4 (albumin, prealbumin, BUN, SCr, UA, and nPNA), model 5 (LDL-cholesterol, HDL-cholesterol, apoprotein A (Apo-A), Apo-B, and Hcy), model 6 (P, Ca×P, iPTH, and 25-hydroxy vitamin D), model 7 (hsCRP and β_2M), model 8 (LVMI and LVEF), and model 9 (history of taking calcium channel blocker, angiotensin conversion enzyme inhibitor, angiotensin receptor blocker, β-blocker, α-blocker, aspirin, and statin). In model 10, hierarchical selection procedures were used. The inclusion criterion for model selection in a covariate set was predetermined as $P<0.10$ in univariate Cox proportional hazard models. The covariates with $P<0.10$ for predicting first heart failure event included age, systolic BP, history of primary hypertension, history of coronary heart disease, history of diabetes, prealbumin, 25-hydroxy vitamin D, LDL-cholesterol, Apo-B, LVMI, and LVEF. In model 10, the backward conditional method was used. IS entered the model either as a continuous variable or a dichotomous variable. A two-tailed *P* value <0.05 was considered statistically significant. All analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL).

Results

The patients' baseline characteristics are listed in Table 1. The cohort consisted of 258 patients on hemodialysis (145 men), with a mean age of 57.0±14.6 years old. Glomerular disease was the leading cause of ESRD, accounting for 44.2%. Among them, prevalence of primary hypertension, coronary heart disease, and diabetes was 30.6%, 6.6%, and 12.8%, respectively. The plasma IS level in these patients was 35.0±18.0 μg/ml. According to the median plasma IS level (32.35 μg/ml), patients were categorized into two groups: the low-IS group (IS≤32.35 μg/ml) and the high-IS group (IS>32.35 μg/ml). Compared with the low-IS group, patients in the high-IS group had higher height, nPNA, albumin, prealbumin, BUN, SCr, hsCRP, β_2M and Hcy. However, BMI, urinary volume, RRF, Apo-A, and proportion of taking β-blocker were significantly lower in the high-IS group. There were no significant differences in other characteristics.

Median follow-up time was 48.0 (interquartile range: 33.5–48.0) months. During follow-up, 68 patients had episodes of first heart failure, 24 patients had episodes of arrhythmias, 13 patients had episodes of angina pectoris, and eight patients had episodes of acute myocardial infarction. Nine patients were lost to follow-up owing to transfer to a different center; 11 patients received kidney transplantation, and 56 patients died, of which 25 deaths were classified as cardiac death and 13 deaths were classified as cerebral death. Other causes of death included infection (five deaths from severe pulmonary infection and one death from septicemia secondary to skin infection), sudden death (seven deaths), malignant tumor (four deaths), and asphyxia (one death).

In crude analysis by the Kaplan–Meier method, we found that incidence of first heart failure event in the high-IS group was significantly higher than that in the low-IS group (log-rank $P<0.001$) (Figure 1). It was well known that many conventional and unconventional risk factors were related to heart failure. Therefore, we constructed Cox proportional hazard models to adjust these confounding risk factors. First, a univariate Cox proportional hazard model was performed. The results showed that IS was significantly associated with first heart event (IS as continuous variable: hazard ratio [HR], 1.02; 95% confidence interval [95% CI], 1.01 to 1.03; $P=0.001$; IS as dichotomous variable: HR, 3.49; 95% CI, 1.97 to 6.20; $P<0.001$). Other significant variables included history of primary hypertension, history of coronary heart disease, 25-hydroxy vitamin D, LDL-cholesterol, Apo-B, LVMI, and LVEF. However, age, systolic BP, history of diabetes, and prealbumin were borderline significant (Table 2). Second, we constructed a series of models to adjust confounding risk factors (models 1–10). IS was still significant in models 1–9. In model 10, hierarchical selection procedures were used. The inclusion criterion for model selection in a covariate set was predetermined as $P<0.10$ in the univariate Cox proportional hazard model. The covariates with $P<0.10$ for predicting first heart failure event included age, systolic BP, history of primary hypertension, history of coronary heart disease, history of diabetes, prealbumin, 25-hydroxy vitamin D, LDL-cholesterol, Apo-B, LVMI, and LVEF. The results showed that IS, entered either as continuous (HR, 1.04; 95% CI, 1.02 to 1.06; $P<0.001$) or dichotomous (HR, 5.31; 95% CI, 2.43 to 11.58; $P<0.001$) variable, still remained significant after adjustment for the above confounding risk factors (Table 3).

Discussion

In this prospective cohort study, we found that the protein-bound uremic toxin IS could predict first heart failure event in patients on hemodialysis. The finding was independent of a series of conventional and unconventional risk factors, including age, sex, BMI, systolic BP, history of primary hypertension, history of coronary disease, history of diabetes,

Table 1. Baseline demographic, clinical, and biochemical characteristics of 258 patients on hemodialysis

Characteristic	All Patients (n=258)	Low-IS Group (IS≤32.35 μg/ml; n=129)	High-IS Group (IS>32.35 μg/ml; n=129)	P value
Age (yr)	57.0±14.6	58.0±13.3	56.0±15.9	0.28
Sex (men/women)	145/113	67/62	78/51	0.17
Height (m)	1.65±0.09	1.63±0.08	1.66±0.09	0.05
Weight (kg)	60.1±11.2	60.5±12.3	59.6±10.0	0.52
Body mass index (kg/m^2)	22.1±3.4	22.6±3.8	21.7±2.9	0.03
Interdialytic weight gain (%)	3.2±1.2	3.2±1.0	3.2±0.9	0.56
Ultrafiltration volume (ml)	2001±752	1981±802	2022±699	0.66
Systolic BP (mmHg)	136.6±17.8	135.9±18.3	137.3±17.3	0.54
Diastolic BP (mmHg)	82.2±10.5	81.7±10.7	82.7±10.4	0.44
Dialysis duration (mo)	43.2 (19.7–73.2)	42.0 (15.0–73.5)	44.0 (26.5–74.0)	0.12
Single-pool Kt/V	1.4±0.4	1.4±0.04	1.4±0.3	0.92
Urinary volume (ml/kg per 24 h)	0 (0–5.3)	1.8 (0–7.3)	0 (0–4.2)	0.03
Residual renal function (ml/min per 1.73 m^2)	0 (0–0.5)	0 (0–0.8)	0 (0–0.4)	0.02
Normalized protein nitrogen appearance rate (g/kg per day)	2.2±0.5	2.1±0.5	2.3±0.6	<0.01
Smoking history (%)	94 (36.4%)	44 (34.1%)	50 (38.8%)	0.44
Underlying kidney disease (%)				0.75
Glomerular disease	114 (44.2%)	56 (43.4%)	58 (45.0%)	
Diabetic nephropathy	23 (8.9%)	12 (9.3%)	11 (8.5%)	
Hypertensive nephropathy	23 (8.9%)	11 (8.5%)	12 (9.3%)	
Polycystic kidney disease	18 (7.0%)	12 (9.3%)	6 (4.7%)	
Others	35 (13.6%)	18 (14.0%)	17 (13.2%)	
Unknown	45 (17.4%)	20 (15.5%)	25 (19.4%)	
Comorbidity (%)				
Primary hypertension	79 (30.6%)	38 (29.5%)	41 (31.8%)	0.69
Coronary heart disease	17 (6.6%)	7 (5.4%)	10 (7.8%)	0.45
Diabetes	33 (12.8%)	18 (14.0%)	15 (11.6%)	0.58
Cerebral infarction	37 (14.3%)	21 (16.3%)	16 (12.4%)	0.37
Cerebral hemorrhage	8 (3.1%)	6 (4.7%)	2 (1.6%)	0.15
Medications (%)				
Calcium channel blocker	159 (61.6%)	84 (65.1%)	75 (58.1%)	0.25
Angiotensin conversion enzyme inhibitor	43 (16.7%)	18 (14.0%)	25 (19.4%)	0.24
Angiotensin receptor blocker	68 (26.4%)	38 (29.5%)	30 (23.3%)	0.29
β-Blocker	42 (16.3%)	27 (20.9%)	15 (11.6%)	0.04
α-Blocker	48 (18.6%)	26 (20.2%)	22 (17.1%)	0.52
Aspirin	53 (20.5%)	28 (21.7%)	25 (19.4%)	0.64
Statin	15 (5.8%)	8 (6.2%)	7 (5.4%)	0.79
25-hydroxy vitamin D	140 (54.3%)	68 (52.7%)	72 (55.8%)	0.62
Albumin (g/L)	39.2±3.8	38.7±3.6	39.8±3.8	0.02
Prealbumin (g/L)	0.33±0.08	0.32±0.08	0.34±0.08	0.03
Hemoglobin (g/L)	103.4±16.0	102.1±17.0	104.8±15.0	0.18
BUN (mg/dl)	142.4±32.1	137.1±30.5	147.7±33.0	<0.01
Serum creatinine (mg/dl)	11.5±3.0	10.6±2.9	12.5±2.8	<0.001
Uric acid (mg/dl)	7.3±1.5	7.2±1.5	7.4±1.5	0.37
Alkaline phosphatase (U/L)	72 (55–91)	73 (57–94)	69 (53–91)	0.41
25-Hydroxy vitamin D (ng/ml)	28.5±8.4	28.3±7.9	28.8±9.0	0.66
Ca (mg/dl)	8.88±0.81	8.87±0.79	8.90±0.84	0.78
P (mg/dl)	6.7±2.0	6.6±2.0	6.8±2.0	0.50
Ca×P (mg^2/dl^2)	59.7±19.1	58.9±19.2	60.5±19.0	0.50
Intact parathyroid hormone (pg/ml)	252.3 (129.7–561.4)	224.7 (114.0–518.9)	281.0 (136.9–607.7)	0.16
Unsaturated iron binding capacity (μg/dl)	139.4±43.8	140.8±43.7	138.1±44.0	0.49
Total iron binding capacity (μg/dl)	205.0±39.9	204.8±38.1	205.0±41.7	0.96
Iron (μg/dl)	65.2±32.2	63.8±31.1	66.6±33.2	0.51
Transferrin (g/L)	1.9±0.5	1.9±0.5	1.9±0.4	0.58
Ferritin (ng/ml)	130.0 (69.8–276.1)	126.4 (68.8–258.1)	133.7 (70.6–290.5)	0.81

Table 1. (Continued)

Characteristic	All Patients (n=258)	Low-IS Group (IS≤32.35 μg/ml; n=129)	High-IS Group (IS>32.35 μg/ml; n=129)	P value
High-sensitivity C-reactive protein (mg/L)	2.1 (0.7–6.1)	1.5 (0.6–5.2)	2.7 (0.7–6.3)	0.06
Triglyceride (mg/dl)	125.7 (94.9–169.2)	132.3 (98.2–182.5)	120.4 (93.4–166.6)	0.24
Total cholesterol (mg/dl)	169.8±43.9	170.4±45.2	169.2±42.9	0.84
HDL-cholesterol (mg/dl)	44.6±12.9	45.6±12.8	43.6±12.9	0.25
LDL-cholesterol (mg/dl)	95.0±35.5	91.4±34.8	98.3±35.9	0.15
Apoprotein-A (g/L)	1.2±0.3	1.3±0.3	1.2±0.2	0.06
Apoprotein-B (g/L)	0.8±0.2	0.8±0.2	0.8±0.2	0.99
Lipoprotein (a) (mg/L)	169 (116–288)	163 (120–281)	177 (111–291)	0.93
Homocysteine (μg/ml)	4.8 (3.8–6.2)	4.7 (3.5–5.7)	4.9 (4.0–6.2)	0.04
β_2-Microglobulin (mg/L)	35.8±11.0	34.3±11.8	37.3±10.0	0.04
Indoxyl sulfate (μg/ml)	35.0±18.0	21.3±7.4	48.5±14.7	<0.001
Left ventricular mass index (g/m$^{2.7}$)	58.2±20.5	59.2±19.5	57.3±21.3	0.51
Left ventricular ejection fraction (%)	66.6±7.1	66.3±7.4	66.8±6.8	0.64

Data are presented as means±SDs or medians (interquartile ranges) for continuous variables as appropriate and n (%) for categorical variables. Ca, calcium; P, phosphorus.

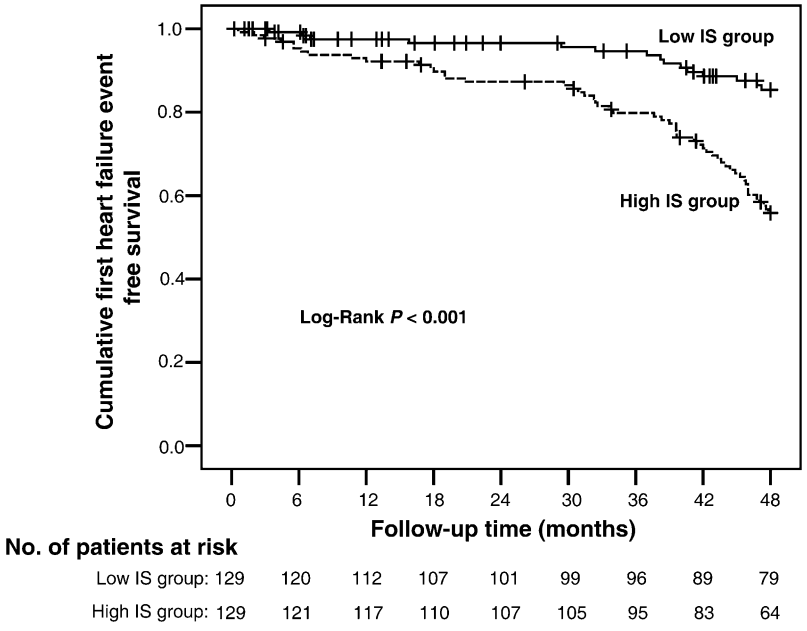

Figure 1. | Kaplan–Meier curves of first heart failure event during follow-up in patients on hemodialysis stratified by low- and high-indoxyl sulfate (IS) group.

urinary volume, ultrafiltration volume, average interdialytic weight gain, RRF, single-pool Kt/V, albumin, prealbumin, BUN, SCr, UA, nPNA, lipid profile, P, Ca×P, iPTH, 25-hydroxy vitamin D, hsCRP, β_2M, LVMI, LVEF, and history of taking cardioprotective agents.

There has been a growing mass of evidence that IS is involved in the progression of CKD by inducing oxidative stress or other pathways. IS-induced reactive oxygen species production through a pathway involving NADPH oxidase or NADPH-like oxidase (20,21). In addition, IS strongly decreased the level of the nonenzymatic antioxidant glutathione in endothelial cells (22,23). In patients on hemodialysis, IS was associated with level of pentosidine, a marker of carbonyl stress (24). Moreover, IS induced endoplasmic reticulum stress in tubular cells and inhibited cell proliferation through two downstream pathways of endoplasmic reticulum stress, namely CCAAT/enhancing-binding protein homologous protein and extracellular signal-regulated kinase-IL-6-p21 (25).

In recent years, more and more attention has been paid to the relationship between IS and CVD in CKD. Lin *et al.* (26)

Table 2. Univariate Cox proportional hazard model of first heart failure event during follow-up in patients on hemodialysis

Variable	Unit of Increase	Hazard Ratio (95% Confidence Interval)	*P* value
Age	1 yr	1.02 (0.99 to 1.04)	0.06
Sex	Women versus men	0.82 (0.50 to 1.34)	0.43
Body mass index	1 kg/m^2	1.01 (0.94 to 1.09)	0.77
Systolic BP	1 mmHg	1.01 (0.99 to 1.03)	0.06
Diastolic BP	1 mmHg	1.01 (0.98 to 1.03)	0.68
Dialysis duration	1 mo	0.99 (0.99 to 1.01)	0.58
Average interdialytic weight gain	1%	1.02 (0.88 to 1.04)	0.68
Ultrafiltration volume	1 ml	1.01 (0.69 to 1.23)	0.46
Urinary volume	1 ml/kg per 24 h	0.96 (0.92 to 1.01)	0.11
Residual renal function	1 ml/min per 1.73 m^2	0.70 (0.46 to 1.08)	0.11
Single-pool Kt/V	1	0.59 (0.25 to 1.37)	0.22
Normalized protein nitrogen appearance rate	1 g/kg per d	0.98 (0.58 to 1.64)	0.93
Smoking history	Present versus absent	1.26 (0.78 to 2.04)	0.35
History of primary hypertension	Present versus absent	2.25 (1.39 to 3.64)	0.001
History of coronary heart disease	Present versus absent	5.14 (2.58 to 10.26)	<0.001
History of diabetes	Present versus absent	1.74 (0.93 to 3.24)	0.08
History of taking calcium channel blocker	Yes versus no	1.26 (0.76 to 2.09)	0.38
History of taking angiotensin conversion enzyme inhibitor	Yes versus no	0.98 (0.53 to 1.83)	0.95
History of taking angiotensin receptor blocker	Yes versus no	1.41 (0.86 to 2.32)	0.17
History of taking β-blocker	Yes versus no	0.84 (0.43 to 1.64)	0.61
History of taking α-blocker	Yes versus no	1.47 (0.84 to 2.57)	0.18
History of taking aspirin	Yes versus no	0.88 (0.48 to 1.61)	0.67
History of taking statin	Yes versus no	1.32 (0.53 to 3.28)	0.55
Albumin	1 g/L	0.99 (0.93 to 1.06)	0.84
Prealbumin	1 g/L	0.06 (0.01 to 1.27)	0.07
BUN	1 mmol/L	1.04 (0.99 to 1.08)	0.11
Serum creatinine	1 μmol/L	1.00 (0.99 to 1.01)	0.48
Uric acid	1 μmol/L	1.00 (0.99 to 1.01)	0.53
P	1 mmol/L	1.01 (0.69 to 1.46)	0.99
Ca×P	1 $mmol^2/L^2$	0.99 (0.99 to 1.01)	0.79
Intact parathyroid hormone	1 pg/ml	1.00 (1.00 to 1.01)	0.28
25-Hydroxy vitamin D	1 mmol/L	0.97 (0.96 to 0.99)	<0.01
LDL-cholesterol	1 mmol/L	0.68 (0.50 to 0.92)	0.01
HDL-cholesterol	1 mmol/L	0.88 (0.41 to 1.92)	0.75
Apoprotein-A	1 g/L	0.83 (0.29 to 2.37)	0.73
Apoprotein-B	1 g/L	0.28 (0.09 to 0.91)	0.03
Homocysteine	1 μmol/L	1.00 (0.99 to 1.01)	0.64
High-sensitivity C-reactive protein	1 mg/L	1.00 (0.99 to 1.02)	0.45
β_2-Microglobulin	1 mg/L	0.99 (0.98 to 1.02)	0.93
Indoxyl sulfate (continuous variable)	1 μg/ml	1.02 (1.01 to 1.03)	0.001
Indoxyl sulfate (dichotomous variable)	High versus low	3.49 (1.97 to 6.20)	<0.001
Left ventricular mass index ($g/m^{2.7}$)	1 $g/m^{2.7}$	1.03 (1.02 to 1.04)	<0.001
Left ventricular ejection fraction (%)	1%	0.95 (0.92 to 0.98)	0.001

indicated that IS was a valuable marker in predicting cardiovascular events in patients with advanced CKD. In patients on hemodialysis, Taki *et al.* (27) found that IS was significantly associated with the risk factors of atherosclerosis, pentosidine, and HDL-cholesterol. Yamamoto *et al.* (12) reported that IS promoted proliferation of vascular smooth muscle cell and aortic calcification in hypertensive rats. Later, Adijiang *et al.* (28) showed that IS induced aortic calcification by upregulating expression of osteoblast-specific proteins, such as core binding factor–α1, alkaline phosphatase, and osteopontin in hypertensive rats. The same results were also reported by Muteliefu *et al.* (29) in humans.

Lekawanvijit *et al.* (30) showed that IS stimulated cardiac fibroblast collagen synthesis and cardiac myocyte hypertrophy in neonatal Sprague–Dawley rats through activation of the p38 mitrogen-activated protein kinase, p42/44 mitrogen-activate protein kinase, and NF-κB pathways. Similarly, in Dahl salt-sensitive hypertensive rats, Yisireyili *et al.* (31) showed that IS aggravated cardiac fibrosis and cardiomyocyte hypertrophy with enhanced oxidative stress and reduced antioxidative defense. However, effects of IS on heart failure remained less clear. We, therefore, conducted this prospective cohort study to investigate the relationship of IS and heart failure in patients on hemodialysis.

Table 3. Multivariate Cox proportional hazard model of first heart failure event during follow-up in patients on hemodialysis (indoxyl sulfate entered as a continuous or dichotomous variable)

Model	Hazard Ratio	95% Confidence Interval	*P* value
Indoxyl sulfate (continuous variable)			
Unadjusted	1.02	1.01 to 1.03	0.001
Model 1	1.02	1.01 to 1.03	0.001
Model 2	1.02	1.01 to 1.04	<0.001
Model 3	1.02	1.01 to 1.03	0.003
Model 4	1.03	1.01 to 1.04	0.002
Model 5	1.03	1.01 to 1.04	0.001
Model 6	1.02	1.01 to 1.03	0.001
Model 7	1.02	1.01 to 1.04	0.001
Model 8	1.02	1.01 to 1.03	<0.01
Model 9	1.02	1.01 to 1.03	<0.001
Model 10	1.04	1.02 to 1.06	<0.001
Indoxyl sulfate (dichotomous variable)			
Unadjusted	3.49	1.97 to 6.20	<0.001
Model 1	3.66	2.05 to 6.56	<0.001
Model 2	3.38	1.90 to 6.02	<0.001
Model 3	4.14	2.07 to 8.26	<0.001
Model 4	4.36	2.13 to 8.90	0.001
Model 5	3.95	2.09 to 7.47	<0.001
Model 6	2.49	1.30 to 4.76	<0.01
Model 7	3.42	1.82 to 6.42	<0.001
Model 8	3.78	2.00 to 7.15	<0.001
Model 9	3.79	2.13 to 6.74	<0.001
Model 10	5.31	2.43 to 11.58	<0.001

Model 1: adjusted for age, sex, and body mass index. Model 2: adjusted for history of primary hypertension, coronary heart disease, and diabetes. Model 3: adjusted for urinary volume, ultrafiltration volume, average interdialytic weight gain, residual renal function, and single-pool Kt/V. Model 4: adjusted for albumin, prealbumin, BUN, serum creatinine, uric acid, and normalized protein nitrogen appearance rate. Model 5: adjusted for LDL-cholesterol, HDL-cholesterol, apoprotein-A, apoprotein-B, and homocysteine. Model 6: adjusted for P, Ca×P, intact parathyroid hormone, and 25-hydroxy vitamin D. Model 7: adjusted for high-sensitivity C-reactive protein and β_2-microglobulin. Model 8: adjusted for left ventricular mass index and left ventricular ejection fraction. Model 9: adjusted for history of taking calcium channel blocker, angiotensin conversion enzyme inhibitor, angiotensin receptor blocker, β-blocker, α-blocker, aspirin, and statin. Model 10: adjusted for hierarchically selected covariates of age, systolic BP, history of primary hypertension, history of coronary heart disease, history of diabetes, prealbumin, 25-hydroxy vitamin D, LDL-cholesterol, apoprotein-B, left ventricular mass index, and left ventricular ejection fraction. In Model 10, the backward conditional method was used.

As we know, IS is derived from dietary protein. Therefore, dietary protein intake can influence plasma IS concentration, which was shown in our study in that nPNA, albumin, prealbumin, BUN, and SCr were significantly higher in the high-IS group. We also found that urinary volume and RRF were significantly lower in the high-IS group, supporting the idea that RRF had more effect on plasma IS level than hemodialysis treatment.

In the general population, many risk factors are associated with heart failure, such as age, sex, smoking history, disturbance of lipid metabolism, history of diabetes, history of primary hypertension, history of coronary heart disease, and so on. In patients on hemodialysis, the prevalence and incidence of heart failure are significantly higher than those in the general population. In addition to the above risk factors, uremic toxins, especially protein-bound and middle molecules, are believed to be the major contributors to heart failure. In Kaplan–Meier analysis, we found that incidence of first heart failure event in the high-IS group was significantly higher than that in the low-IS group. Because many risk factors were associated with heart failure and many factors were associated with IS, we constructed a series of Cox proportional hazard models to adjust these confounding risk factors. As we expected, the results were still significant.

In patients who underwent first-time coronary angiography for suspected coronary artery disease, Hsu *et al.* (32) found that serum IS was independently associated with the presence and severity of coronary artery disease. Additionally, in patients with both stable angina pectoris and successful percutaneous coronary intervention, Sato *et al.* (33) showed that higher plasma IS was closely associated with left ventricular diastolic dysfunction. The two studies support the idea that IS may be involved in the pathogenesis of heart failure.

Shimazu *et al.* (34) reported that serum IS was associated with cardiac dysfunction and predicted cardiac events (cardiac death or hospitalization for worsening of heart failure) in 76 patients with dilated cardiomyopathy and normal kidney function or mild to moderate CKD. However, the result was negative after adjustment for other risk factors. In a prospective cohort study including 139 patients with CKD, Barreto *et al.* (35) showed that higher serum IS was associated with increased overall and cardiovascular mortality. However, the predictive power of IS for cardiovascular mortality lost significance after adjustment for other risk factors. The relatively small sample size and different population may be the underlying causes. In a prospective cohort study in 521 United States patients on incident hemodialysis, Melamed *et al.* (36) found that elevated plasma IS was associated with all-cause mortality but not cardiovascular mortality. Different patient populations (61% white), underlying kidney diseases (diabetic nephropathy accounting for 47.8%), and comorbidities (primary hypertension in 97% and diabetes in 54.9%) may partially explain the differential results.

From the above studies, we can see that the associations of IS and cardiac events are uncertain. The results may be different in different populations, such as the general population, patients with early-stage CKD, and patients on hemodialysis. Also, the associations of IS and different cardiac events, such as angina pectoris, myocardial infarction, heart failure, and arrhythmias, are also different. Clinical studies with large sample size, longer follow-up time, and a specific cardiac event as the study purpose are needed in the future.

This study was observational, and causal relationships between IS and first heart failure event could not be obtained from this study. Using baseline data to predict prospectively

clinical events might be spurious. Data at regular intervals are needed in the future.

In conclusion, we showed that high plasma IS was associated with higher risk of first heart failure event in patients on hemodialysis. The finding persisted after adjustment for conventional and unconventional risk factors. Additional study is needed to elucidate the underlying mechanism.

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Disclosures

None.

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