Background: Indoxyl sulfate (IS), a uremic toxin, has cardiovascular as well as uremic toxicity. We evaluated the prognostic value of blood IS level for long-term outcome.

Methods and Results: This study followed 311 patients with coronary artery disease. Plasma IS level and estimated glomerular filtration rate (eGFR) were determined. The endpoint was a major adverse cardiac event (MACE). Median follow-up was 759 days. IS was significantly higher in patients with MACE than in those without (P<0.001). Patients were divided according to quartiles (Q) of plasma IS level (Q1, Q2, Q3, and Q4). On Kaplan-Meier analysis a significantly lower MACE-free rate was obtained for Q4 compared with the other quartiles (P<0.001). In patients with eGFR ≥90, 89–60, 59–30, 29–15, and <15 ml·min⁻¹·1.73 m⁻², the percentage of patients in Q4 was 0%, 13%, 29%, 100%, and 100%, respectively. In patients with eGFR 89–60 ml·min⁻¹·1.73 m⁻², there was no significant difference in MACE-free rate between Q4 and the other quartiles; in patients with eGFR 59–30 ml·min⁻¹·1.73 m⁻², a significantly lower MACE-free rate was obtained for Q4 compared with the other quartiles (P=0.832 and P=0.015, respectively).

Conclusions: Plasma IS level is a significant predictor of MACE, especially in patients with eGFR 59–30 ml·min⁻¹·1.73 m⁻².

Key Words: Cardio-renal syndrome; Chronic kidney disease; Uremic toxin
elective PCI between May 2009 and December 2011 at the Nagoya University Hospital. Patients with previous history of acute coronary syndrome within 6 months before screening for inclusion were excluded. Successful PCI was defined as final angiographic residual stenosis <30% on quantitative coronary angiography without flow-limiting dissection or occlusion of the large branch (>1 mm), and a resulting Thrombolysis in Myocardial Infarction grade of 3. Patients were also excluded if they had severe valvular heart disease diagnosed according to criteria in a previous report. The primary end-point of this study was a major adverse cardiac event (MACE) defined as death, non-fatal acute myocardial infarction, hospital admission for heart failure, or non-fatal stroke. The database was developed prospectively, and clinical data were analyzed retrospectively. All participants provide written informed consent. The institutional ethics committee approved the protocol for the current study and chart reviews.

All patients underwent laboratory measurements and echocardiography. In patients on chronic hemodialysis, blood samples were obtained from vein before hemodialysis on the hemodialysis day. For measurement of plasma IS, samples were filtered through a 0.20-µm membrane, then 10-µl aliquots were analyzed on reverse-phase high-performance liquid chromatography (Shiseido Capcell Pak MF Ph-1 SG80 MF 150 mm×4.6 mm; Shiseido, Tokyo, Japan). The mobile phase, 0.1 mol/L KH₂PO₄/tetrahydrofuran (95/5), was delivered at a flow rate of 1.0 ml/min at 35°C. Plasma concentration was measured on fluorescence detection (excitation, 295 nm; emission, 390 nm). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation modified with the Japanese coefficient (0.741×MDRD). Renal dysfunction was classified on the basis of eGFR: ≥90, 89–60, 59–30, 29–15, and <15 ml·min⁻¹·1.73 m⁻², respectively. Left ventricular ejection fraction was defined as left ventricular ejection fraction <50% or ratio of early transmitral flow velocity to early diastolic mitral annular velocity >1.5.

### Statistical Analysis

SAS version 6.10 (SAS Institute) was used for all statistical analysis. Variables with a non-normal distribution are described as median and interquartile range, and were analyzed using the Mann-Whitney test or Kruskal-Wallis test. Variables with a normal distribution are given as mean±SD, and differences were evaluated using the Student unpaired t-test. Categorical variables are presented as number (percentage), and comparisons across groups were performed using the chi-squared or Fisher’s exact test. Cumulative survival rates in each group were analyzed using the Kaplan-Meier method, and difference in survival rate between groups was estimated using the log-rank method. To determine significant predictors for MACE, multivariate Cox regression analysis, including established risk factors, was conducted. Statistical significance was defined as 2-tailed P<0.05.

### Results

We investigated 311 consecutive patients. In the 29 patients with eGFR <30 ml·min⁻¹·1.73 m⁻², 21 (72%) were on chronic hemodialysis. The median follow-up was 759 days (range, 546–1,063 days). During follow-up, 30 patients (10%) experienced a MACE. The patient characteristics are listed in Table 1. Patients with MACE had significantly lower eGFR and significantly higher plasma IS (47±30 vs. 64±22 ml·min⁻¹·1.73 m⁻², P<0.006; and median, 1.5; IQR, 0.71–3.9 vs. 0.82, 0.49–1.3 µg/ml, P<0.001, respectively). To evaluate the relationship between plasma IS level and long-term outcome, patients were divided into 4 groups by quartiles (Q) of plasma IS (<0.50,
Plasma Indoxyl Sulfate Level Predicts MACE

Table 2. Predictors of MACE

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate Model 1</th>
<th>Multivariate Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.6 (0.76–3.2)</td>
<td>0.221</td>
<td>2.0 (0.94–4.2)</td>
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<tr>
<td>SBP</td>
<td>1.0 (0.98–1.0)</td>
<td>0.840</td>
<td>1.0 (0.98–1.0)</td>
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<tr>
<td>DBP</td>
<td>0.99 (0.96–1.0)</td>
<td>0.723</td>
<td>0.99 (0.94–1.0)</td>
</tr>
<tr>
<td>DM</td>
<td>2.2 (1.0–4.5)</td>
<td>0.037</td>
<td>1.8 (0.87–3.8)</td>
</tr>
<tr>
<td>MVD</td>
<td>2.7 (1.1–6.5)</td>
<td>0.031</td>
<td>2.3 (0.92–5.6)</td>
</tr>
<tr>
<td>eGFR (per stage progression)</td>
<td>3.9 (1.9–8.3)</td>
<td>&lt;0.001</td>
<td>2.8 (1.3–6.2)</td>
</tr>
<tr>
<td>High IS (Q4 vs. the others)</td>
<td>1.8 (1.3–2.5)</td>
<td>&lt;0.001</td>
<td>1.6 (1.1–2.2)</td>
</tr>
</tbody>
</table>
| No. MACE=30. CI, confidence interval; DBP, diastolic blood pressure; OR, odds ratio; SBP, systolic blood pressure. Other abbreviations as in Table 1.

0.50–0.84, 0.85–1.40, and >1.40 µg/ml for quartiles Q1, Q2, Q3, and Q4, respectively. Kaplan-Meier analysis showed that a significantly lower cumulative 3-year MACE-free rate was obtained for Q4 compared with Q1, Q2, and Q3 (97%, 91%, 95% and 78%, for Q1, Q2, Q3 and, Q4, respectively; log-rank test P<0.001; Figure 1). Diabetes mellitus, multi-vessel disease (MVD), left ventricular dysfunction, eGFR, and Q4 were significant predictors of MACE on univariate Cox proportional hazards analysis (hazard ratio [HR], 2.2; 95% confidence interval [CI]: 1.0–4.5, P=0.037; HR, 2.7; 95%CI: 1.1–6.5, P=0.031; HR, 3.9; 95% CI: 1.9–8.3, P<0.001; HR, 1.8; 95% CI: 1.3–2.5, P<0.001; HR, 9.2; 95% CI: 2.1–39.6, P=0.003, respectively; Table 2). Multivariate Cox proportional hazard analysis showed that left ventricular dysfunction and eGFR were significant and independent predictors of 3-year MACE (HR, 2.8; 95% CI: 1.3–6.2, P=0.010; and 1.6, 95% CI: 1.1–2.2,
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As eGFR declined, plasma IS tended to increase (P<0.001 for trend; Figure 2).

Figure 2. Plasma indoxyl sulfate (IS) as a function of estimated glomerular filtration rate (eGFR). As eGFR decreased, plasma IS increased (median, 0.41; IQR, 0.22–0.52 µg/ml; 0.72, 0.46–1.0 µg/ml; 1.0, 0.71–1.5 µg/ml; 2.4, 1.7–4.2 µg/ml; and 20.7, 8.3–32.4 µg/ml for patients with eGFR ≥90, 89–60, 59–30, 29–15, and <15 ml·min⁻¹·1.73 m⁻², respectively; P<0.001 for trend).

In patients with eGFR ≥90, 89–60, 59–30, 29–15, and <15 ml·min⁻¹·1.73 m⁻², the number of patients in Q4 was 0, 21 (13%), 28 (29%), 8 (100%), and 21 (100%), respectively. In patients with eGFR 89–60 ml·min⁻¹·1.73 m⁻², no significant difference in cumulative 3-year MACE-free rate was detected (P=0.007, respectively; Table 2, model 1). When high blood IS was included in the multivariate models, left ventricular dysfunction and high blood IS were significant and independent predictors of 3-year MACE (HR, 2.7; 95% CI: 1.2–6.1, P=0.013; and 2.6, 95% CI: 1.0–6.4, P=0.039, respectively; Table 2, model 2).

We performed a sub-analysis to examine the association of plasma IS and eGFR with 3-year MACE. As eGFR declined, plasma IS tended to increase (P<0.001 for trend; Figure 2). In patients with eGFR ≥90, 89–60, 59–30, 29–15, and <15 ml·min⁻¹·1.73 m⁻², the number of patients in Q4 was 0, 21 (13%), 28 (29%), 8 (100%), and 21 (100%), respectively. In patients with eGFR 89–60 ml·min⁻¹·1.73 m⁻², no significant difference in cumulative 3-year MACE-free rate was detected.
between Q4 and the other quartiles (95% vs. 94%, log-rank test P=0.832; Figure 3A). In contrast, in patients with eGFR 59–30 ml·min⁻¹·1.73 m², significantly lower cumulative 3-year MACE-free rate was obtained for Q4 than the other quartiles (79% vs. 96%, log-rank P=0.015; Figure 3B).

Discussion

The main findings are as follows: (1) increased plasma IS was significantly associated with poor long-term outcome; (2) this effect was independent of diabetes mellitus, MVD, and left ventricular dysfunction; (3) a decline in eGFR was significantly associated with an increase in plasma IS; and (4) in patients with eGFR 59–30 ml·min⁻¹·1.73 m², increased plasma IS was significantly associated with poor clinical outcome.

The concept of CRS has been established recently. IS is a uremic toxin with an accelerating effect on the progression of CKD. The association of IS with endothelial dysfunction, vascular smooth muscle cell proliferation, and increased risk of atherosclerosis in humans has been reported both in vivo and vitro. These findings suggest that IS is a key metabolite in CRS. Therefore, we evaluated whether plasma IS level could predict future CVD. Increased plasma IS was a significant and independent predictor for MACE. This suggests that IS plays a role in CRS, but, because the mechanism of CRS is a complex process involving other CKD-related or CKD-specific risk factors, further investigation is warranted.

In accordance with other studies, renal impairment as reflected by eGFR was an independent predictor for MACE, when the model included established risk factors other than blood IS level. When blood IS level was included in the multivariate model, however, the prognostic value of eGFR was canceled. We noted a high correlation of eGFR with plasma IS. We think that this might be the major reason why eGFR was not associated with MACE when blood IS level was included in the multivariate model. Healthy kidneys excrete IS from the circulation into urine from the renal tubules via organic anion transporters 1 and 3. IS accumulates when blood IS level did not predict future CVD. Considering these findings, we speculate that a sequential process of renal tubular dysfunction might be involved in IS excretion. We also suggest that high-protein diet might affect blood IS level. In patients with eGFR 89–60 ml·min⁻¹·1.73 m², a persistent increase might occur in blood IS, due to further progression of renal dysfunction and/or dietary pattern, which is associated with future risk. In the present study, all patients with eGFR <30 ml·min⁻¹·1.73 m² had increased blood IS. Therefore, we could not determine the prognostic value of blood IS level in patients with eGFR <30 ml·min⁻¹·1.73 m².

Severe renal dysfunction might not produce a tolerable range of blood IS, regardless of dietary pattern. Further investigations are needed to clarify this point.

To prevent progression of both CVD and CKD, reduction of blood IS level is a feasible and useful treatment goal. IS, however, cannot be easily removed via hemodialysis, because of its high binding affinity for albumin. We speculate that, in patients on chronic hemodialysis, blood IS level might be associated with MACE. Only 21 patients on chronic hemodialysis, however, were included in the present study, thus, we could not clarify this issue. AST-120 (Kreminz; Kureha Corporation, Tokyo, Japan), an oral charcoal, is an approved drug widely used in Japan, Korea, Taiwan, and the Philippines for treating pre-dialysis patients to reduce circulating IS and delay CKD progression. The present findings might contribute to clarify which patients should be treated with IS reduction therapy. To clarify the role of IS reduction therapy, including AST-120, in CKD patients, large-scale trials are warranted.

The limitations of the present study should be discussed. First, this study consisted of patients from a single center. Second, plasma IS level was examined at only 1 point. Furthermore, we did not collect data on nutritional state such as food intake or serum albumin level. Given that IS is a uremic toxin and binds to albumin, it is possible that the present results were affected by these conditions. Finally, the number of patients with severe renal dysfunction was relatively small. Therefore, it is possible that the statistical power of the study was not sufficient to detect the prognostic value of blood IS level in this patient type. For these reasons, the present findings should be interpreted with caution.

Conclusions

Plasma IS is a useful clinical marker to predict future CVD, especially in patients with eGFR 59–30 ml·min⁻¹·1.73 m².

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The authors have no competing interests to declare.

Disclosures

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References


