

# Prognostic Utility of Indoxyl Sulfate for Patients with Acute Coronary Syndrome

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**Aim:** We investigated whether indoxyl sulfate (IS), a protein-bound uremic toxin, predicts prognosis after acute coronary syndrome (ACS).

**Methods:** Serum IS level was determined prospectively in 98 patients who underwent successful primary percutaneous coronary intervention for ACS. Patients on hemodialysis were excluded. The endpoint of this study was six-month composite events including death, nonfatal myocardial infarction, heart failure requiring hospitalization, and adverse bleeding events.

**Results:** During the mean follow-up period of 168 days, composite events occurred in 13.3% of cases. Serum IS level was significantly higher in subjects who developed composite events than in those without events ( $0.14 \pm 0.11$  mg/dl vs.  $0.06 \pm 0.04$  mg/dl;  $p < 0.001$ ). After adjusting for confounding factors, a Cox proportional hazard analysis revealed that the IS level (hazard ratio (HR): 10.6; 95% confidence interval (CI): 1.63–69.3,  $p = 0.01$ ), hemoglobin level (HR: 0.61; 95% CI: 0.43–0.87;  $p < 0.01$ ), and left ventricular ejection fraction (LVEF) (HR: 0.95; 95% CI: 0.91–0.99;  $p = 0.03$ ) were independent predictive factors of composite events. Furthermore, IS level significantly conferred additional value to the combined established risks of LVEF and hemoglobin level for predicting the incidence of composite events (area under the curve: 0.82 vs. 0.88,  $p = 0.01$ ; net reclassification improvement: 0.67,  $p = 0.01$ ; and integrated discrimination improvement: 0.15,  $p < 0.01$ ).

**Conclusions:** The assessment of serum IS level has prognostic utility for the management of ACS.

**Key words:** Uremic toxin, Acute coronary syndrome, Cardiorenal syndrome

## Introduction

A positive correlation between the incidence and severity of renal disease (RD) and cardiovascular disease (CVD) has been identified<sup>1</sup>. Uremic toxins that accumulate with the progression of RD are associated with the underlying mechanism of atherogenesis<sup>2-4</sup>.

The natural metabolite indoxyl sulfate (IS) is a protein-bound uremic toxin that was recently shown to be a risk factor for CVD<sup>5,6</sup>. Vascular toxicity by IS generally contributes to the observed increase in oxidative stress with the subsequent elevation of the proinflammatory response<sup>7,8</sup>. Ito *et al.*<sup>9</sup> reported IS-enhanced leukocyte-endothelial interactions by the upregulation

of E-selectin via the JNK- and NF- $\kappa$ B-dependent pathways. IS has been also thought to induce cardiac fibrosis by the upregulation of TGF $\beta$ <sup>10,11</sup>. We previously reported that IS mediates the expression of monocyte chemoattractant protein-1 by the activation of aryl hydrocarbon receptor in human umbilical vein endothelial cells<sup>12</sup>. Furthermore, IS has been associated with the induction of endothelial senescence by modulating sirtuin 1 activity<sup>13</sup>. In clinical settings, the serum IS level is the therapeutic target for patients who develop endothelial dysfunction<sup>14,15</sup>. The IS level was also found to be a prognostic factor for patients with dilated cardiomyopathy, thus suggesting a robust relationship between IS level and cardiac function<sup>16</sup>. However, only

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a few studies have demonstrated the association between IS level and coronary artery disease<sup>17, 18</sup>. Given that RD is one of the prognostic factors of acute coronary syndrome (ACS), IS may play a pivotal role in the underlying mechanism. Therefore, we assessed whether serum IS is associated with the clinical outcome after ACS. Our results provide additional value to standard risk stratifications for patients with ACS.

## Methods

### Study Population

A total of 98 consecutive patients who underwent successful primary percutaneous coronary intervention (PCI) for ACS at Toho University Omori Medical Center (Tokyo, Japan) were prospectively enrolled in this study. The inclusion criteria were patients with ACS, including ST-segment elevation-ACS (STE-ACS) and non-STE-ACS, who presented with typical chest pain for  $\geq 30$  minutes within 12 hours of admission. Electrocardiography was used to define STE-ACS as ST elevation  $\geq 2$  mm in two contiguous anterior–lateral leads or ST elevation  $\geq 2$  mm in inferior leads totaling  $\geq 8$  mm or a new left bundle-branch block with concordant ST elevation of 1 mm<sup>19</sup>. The exclusion criteria were (1) patients undergoing any dialysis, (2) death within 24 hours after admission, and (3) patients who were prescribed AST-120 at admission. This study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local ethics committee in the Toho University School of Medicine (Approval code: 26-292). Informed consent was obtained from all patients. All patients underwent urgent care and examinations for suspected ACS and were enrolled in the study immediately upon admission according to the discretion of the attending physicians. Blood sampling and electrocardiography were performed in the emergency department. When diagnosed with ACS, 100 mg aspirin and 150 mg clopidogrel or 20 mg prasugrel were administered to the patients before catheterization. The arterial approach route depended on the choice of interventional cardiologists. The removal of the arterial sheath was encouraged as soon as possible. Primary PCI was performed according to the current guidelines<sup>20</sup>, and the deployment of a coronary drug-eluting stent was performed in all cases. There was no case of thrombolysis during primary PCI. Procedural success was defined as a successful guide wire and balloon crossing with residual stenosis  $> 50\%$  and thrombolysis in myocardial infarction (MI) grade 3 flow after coronary stenting. Optimal therapy after primary PCI included the administration of beta blockers, strong statins, and angiotensin-converting enzyme inhibitor/angiotensin II recep-

tor blockers, as appropriate. Serum creatinine was measured upon admission. Other standard clinical parameters were measured within 24 hours after the procedure. The Modification of Diet in Renal Disease formula<sup>21</sup> was applied to calculate the estimated glomerular filtration rate (eGFR), and eGFR  $< 60$  ml/min/1.73 m<sup>2</sup> was defined as chronic kidney disease. Cardiovascular risk factors including diabetes, dyslipidemia, hypertension, and current smoking were defined in accordance with the accepted criteria<sup>22-24</sup>. Body mass index was calculated according to patient height and weight measured during hospitalization. Left ventricular ejection fraction (LVEF) was measured by echocardiography within 24 hours after admission<sup>25, 26</sup>. Killip class II was defined as pulmonary congestion with wet rales in the lower half of the lung fields or S3 gallop. Killip class III was accompanied by severe respiratory distress, with crackles over the lungs and orthopnea with desaturation ( $< 90\%$ ) before treatment. Killip class IV was defined as evidence of tissue hypoperfusion induced by heart failure after the correction of preload. Adverse bleeding events were defined as types 3 or 5 of the Bleeding Academic Research Consortium criteria<sup>27</sup>. For each patient, the global registry of acute coronary events (GRACE) risk score was calculated using eight specific variables collected

upon admission (<http://www.gracescore.org/web-site/webversion.aspx>)<sup>28</sup>.

### Study Endpoint

The endpoint of this study was the composite events at six months including death, nonfatal MI, heart failure requiring hospitalization, and adverse bleeding event.

### Analysis of Serum IS

Serum IS level was determined from arterial blood drawn immediately after PCI. Each serum sample (10  $\mu$ L) was analyzed by reversed-phase high-performance liquid chromatography (Capcell Pak MF Ph-1 SG80S5 4.6 mm I.D.  $\times$  150 mm; SHISEIDO CO., LTD., Tokyo, Japan). The mobile phase, namely, 0.1 M KH<sub>2</sub>PO<sub>4</sub>/tetrahydrofuran (95/5, V/V) (pH 6.5), was delivered at a flow rate of 1.0 mL/min at 37°C. Serum IS levels were determined by fluorescence detection (excitation, 295 nm; emission, 390 nm).

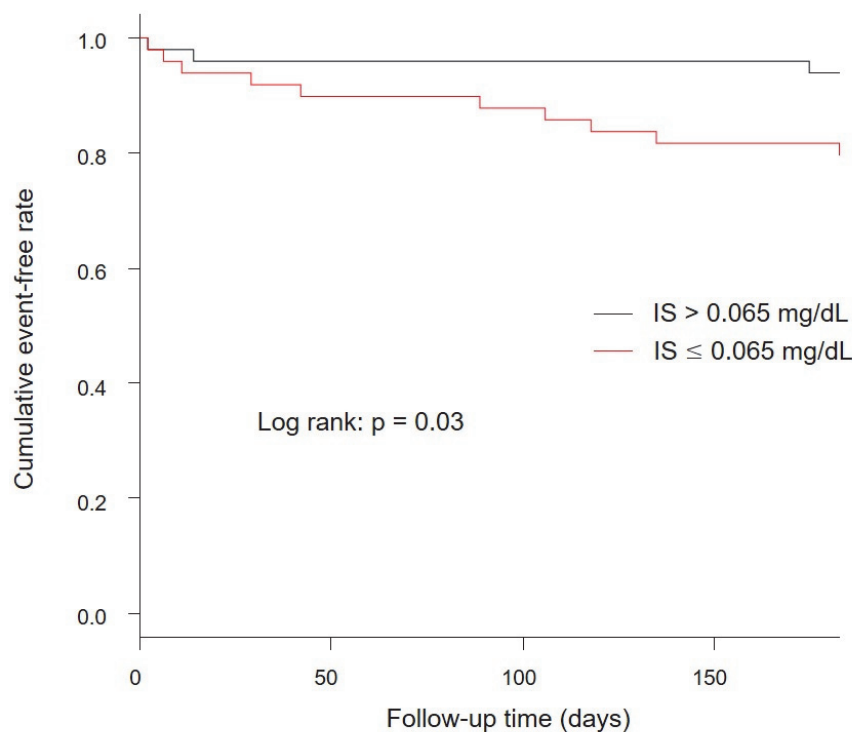
### Statistical Analysis

Data were analyzed using the Statistical Package for R (R Development Core Team, Vienna, Austria)<sup>29</sup>. The measured data were expressed as mean  $\pm$  standard deviation or median and interquartile range. The Kolmogorov–Smirnov test was applied to test for normal distribution. Continuous variables were compared using

**Table 1.** Baseline characteristics

	Overall, <i>n</i> =98	No composite events, <i>n</i> =85	Composite events, <i>n</i> =13	<i>p</i> value
Age, years	65.1 ± 12.4	64.1 ± 12.4	71.4 ± 11.1	0.04
Male, %	77 (90.6)	66 (77.6)	11 (84.6)	0.72
BMI, kg/m <sup>2</sup>	24.0 ± 4.0	24.0 ± 4.22	24.5 ± 2.78	0.65
SBP, mmHg	129.5 ± 28.1	130.5 ± 28.1	123.0 ± 28.8	0.37
DBP, mmHg	75.9 ± 16.3	76.7 ± 15.9	70.5 ± 18.1	0.20
Heart rate, bpm	78.8 ± 17.0	78.9 ± 17.2	77.7 ± 16.4	0.81
GRACE risk score	121.8 ± 35.9	116.8 ± 32.8	154.5 ± 39.6	<0.001
Creatinine, mg/dL	0.90 ± 0.2	0.87 ± 0.22	1.06 ± 0.57	0.03
eGFR, mL/min/1.73 m <sup>2</sup>	67.2 ± 18.5	68.2 ± 18.2	60.6 ± 19.6	0.16
Hemoglobin, g/dL	13.2 ± 2.1	13.5 ± 1.9	11.1 ± 2.3	<0.001
HbA1c, %	6.38 ± 1.2	6.39 ± 1.3	6.30 ± 1.2	0.81
Total cholesterol, mg/dL	192.9 ± 49.2	197.1 ± 49.1	165.6 ± 42.4	0.03
LDL, mg/dL	123.8 ± 38.8	125.8 ± 39.1	105.3 ± 32.4	0.13
HDL, mg/dL	48.3 ± 12.5	48.6 ± 12.4	46.5 ± 13.1	0.56
Uric acid, mg/dL	5.81 ± 1.5	5.75 ± 1.4	6.17 ± 1.6	0.37
BNP, pg/mL	48.7 (18.2–127.8)	43.2 (17.7–101.1)	92.3 (41.7–324.0)	0.02
CK, mg/dL	135.5 (96.2–244.2)	133 (96.0–224)	157 (111–254)	0.92
CKMB, mg/dL	17.0 (10–33.5)	16.0 (10–28)	32.5 (15–64)	0.75
Troponin-I, ng/mL	0.29 (0.06–2.29)	0.28 (0.04–2.14)	0.69 (0.15–3.21)	0.74
IS, mg/dL	0.065 (0.03–0.10)	0.05 (0.02–0.09)	0.08 (0.07–0.16)	<0.001
LVEF, %	57.8 ± 15.7	60.3 ± 13.8	41.4 ± 17.9	<0.001
Hypertension, %	56 (57.1)	48 (57.6)	8 (61.5)	1.0
Dyslipidemia, %	37 (37.7)	36 (42.3)	1 (7.6)	0.01
Diabetes, %	22 (22.4)	18 (21.2)	4 (30.7)	0.48
Anemia, %	53 (54.0)	42 (49.4)	11 (84.6)	0.03
CKD, %	32 (32.6)	27 (31.7)	5 (38.4)	0.75
Current smoking, %	74 (75.5)	65 (76.4)	9 (69.2)	0.72
Prior stroke, %	6 (6.1)	3 (3.5)	3 (23.1)	0.02
STE-ACS, %	56 (57.1)	47 (55.2)	9 (69.2)	0.38
LAD culprit, %	50 (51.0)	42 (49.4)	8 (61.5)	0.55
Killip class ≥ II or greater, %	20 (20.4)	13 (15.2)	7 (53.8)	<0.01
Cardiac arrest, %	7 (7.1)	4 (4.7)	3 (23.0)	0.04
IABP, %	19 (19.3)	13 (15.2)	6 (46.1)	0.01
Medications before admission				
ACE-I, %	5 (5.1)	4 (4.7)	1 (7.6)	0.51
ARB, %	21 (21.4)	18 (21.1)	3 (23.0)	1.0
Beta blockers, %	14 (14.2)	12 (14.1)	2 (8.6)	1.0
Statins, %	17 (17.3)	15 (17.6)	2 (15.3)	1.0
Loop diuretics, %	5 (5.1)	1 (1.1)	4 (30.7)	<0.001
Anticoagulants, %	3 (3.0)	1 (1.1)	2 (15.3)	0.04
Medications after admission				
ACE-I, %	69 (70.4)	65 (76.4)	4 (30.7)	<0.01
ARB, %	16 (16.3)	14 (16.4)	2 (15.3)	1.0
Beta blockers, %	75 (76.5)	69 (81.1)	6 (46.2)	<0.01
Statins, %	67 (68.3)	63 (74.1)	4 (30.8)	0.002
Loop diuretics, %	18 (18.3)	13 (15.8)	5 (38.4)	0.08
Anticoagulants, %	13 (13.2)	11 (12.9)	2 (15.3)	1.0

Abbreviations: ACE-I-angiotensin converting enzyme inhibitor; ARB-angiotensin II receptor blocker; BMI-body mass index; BNP-brain natriuretic protein; CK-creatinine kinase; CKD-chronic kidney disease; CKMB-creatinine kinase MB; DBP-diastolic blood pressure; eGFR-estimated glomerular filtration ratio; GRACE-global registry of acute coronary events; HDL-high density lipoprotein; IS-indoxyl sulfate; LAD-left anterior descending artery; LDL-low density lipoprotein; LVEF-left ventricular ejection fraction; SBP-systolic blood pressure; STE-ACS-ST segment elevation-acute coronary syndrome.



**Fig. 1.** Patients with IS level  $>0.065$  mg/dL developed higher incidence of 6-month composite events after ACS.

Abbreviations: ACS-acute coronary syndrome; IS-indoxyl sulfate.

Student's *t*-test. In the case of nonlinearity, the data were logarithmically transformed using a base logarithm of two. Demographics, traditional risk factors, and clinical outcomes were compared using the Pearson  $\chi^2$  or Fisher's exact test, as appropriate, for categorical data and were expressed as percentages. Kaplan-Meier analysis was performed to calculate the unadjusted MACCE-free survival rate of patients categorized according to the median value of IS. Multivariate Cox proportional hazard analysis was performed after adjusting for confounding factors with *p* values of  $<0.1$  in the univariate analysis to identify the independent risk factors for six-month composite events. The discriminative ability of IS level with the endpoint of composite events was evaluated by the area under the curve (AUC). The incremental predictive ability of IS added to the risk model consisting of independent prognostic factors that were statistically significant in the multivariate analysis was also assessed by net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

## Results

### Baseline Characteristics

The mean follow-up period was 168 days, and

composite events developed in 13.3% of cases (death = 7, nonfatal MI = 1, heart failure requiring hospitalization = 3, and adverse bleeding event = 4) (Table 1). Patients in the composite events group were older. There was no significant difference in the baseline prevalence of traditional coronary risk factors including hypertension, diabetes, dyslipidemia, gender, and smoking status between the two groups. However, patients in the composite event group were more likely to have anemia, history of stroke, and lower LVEF, and they had higher serum creatinine and brain natriuretic protein levels and lower hemoglobin levels. The composite events group had a higher incidence of Killip class II or higher. The patients in the composite events group were more likely to have cardiac arrest upon admission; therefore, they were more frequently subjected to intra-aortic balloon pumping. They were also more often treated with loop diuretics and anticoagulants prior to admission.

### IS as an Independent Predictor of Six-Month Clinical Outcome

The IS level was significantly higher for patients who developed composite events. On the basis of a Kaplan-Meier analysis, higher IS was associated with higher incidence of composite events when patients

**Table 2.** Cox proportional hazard analysis for composite events

	Univariate analysis		Multivariate analysis	
	HR, 95% CI	<i>p</i> value	HR, 95% CI	<i>p</i> value
log BNP	2.22, 0.94–5.57	0.06	0.76, 0.30–1.93	0.56
log CKMB	2.48, 0.86–7.14	0.08	1.78, 0.31–10.0	0.51
LVEF	0.92, 0.89–0.96	<0.0001	0.95, 0.91–0.99	0.03
Age	1.05, 1.00–1.10	0.04	1.02, 0.95–1.09	0.51
Anemia	5.24, 1.16–23.6	0.03	0.86, 0.07–9.45	0.90
Cardiac arrest	5.48, 1.50–20.0	0.01	1.62, 0.06–38.0	0.76
Creatinine	3.65, 1.19–11.1	0.02	1.26, 0.21–7.40	0.79
Dyslipidemia	0.12, 0.01–0.95	0.04	0.36, 0.04–3.18	0.35
Hemoglobin	0.59, 0.45–0.77	<0.001	0.61, 0.43–0.87	<0.01
log IS	27.6, 3.44–221.5	<0.01	10.6, 1.63–69.3	0.01
Killip class ≥ II or greater	5.66, 1.9–16.8	<0.01	1.44, 0.31–6.61	0.63
Prior stroke	6.21, 1.70–22.7	<0.01	0.41, 0.02–7.08	0.54
Total cholesterol	0.98, 0.97–0.99	0.02	1.00, 0.98–1.02	0.61

Abbreviations: BNP-brain natriuretic protein; CKMB-creatinine kinase MB; CI-confidence interval; HR-hazard ratio; IS-indoxyl sulfate; LVEF-left ventricular ejection fraction.

**Table 3.** Reclassification analysis by addition of IS to established risk stratification

	AUC	<i>p</i> value	NRI	<i>p</i> value	IDI	<i>p</i> value
LVEF + Hb	0.82	Ref.	–	Ref.	–	Ref.
(LVEF + Hb) + log IS	0.88	0.01	0.67	0.01	0.15	<0.01

Abbreviations: AUC-area under the curve; Hb-hemoglobin; IDI-integrated discrimination improvement; IS-indoxyl sulfate; LVEF-left ventricular ejection fraction; NRI-net reclassification improvement.

were divided according to the median value of IS (0.065 mg/dl) (**Fig. 1**). The Cox proportional hazard analysis conducted after adjustment for other confounding factors (LVEF, age, cardiac arrest at admission, creatinine, hemoglobin, Killip class of II or higher, prior stroke, total cholesterol, brain natriuretic protein level at admission, creatinine kinase MB level at admission, anemia, and dyslipidemia) found that IS (hazard ratio (HR): 10.6; 95% confidence interval (CI): 1.63–69.3;  $p=0.01$ ), hemoglobin (HR: 0.61; 95% CI: 0.43–0.87;  $p<0.01$ ), and LVEF (HR: 0.95; 95% CI: 0.91–0.99;  $p=0.03$ ) were associated with the incidence of composite events (**Table 2**).

### The Incremental Prognostic Value of IS in Addition to Established Risk Factors

According to the results from a multivariate analysis to clarify the prognostic factors, we compared the risk prediction value between IS and the established risk factors (hemoglobin and LVEF). We found that IS was significantly able to increase the AUC when added to the combination of hemoglobin and LVEF (0.82 vs. 0.88,  $p=0.01$ ) (**Table 3**). Furthermore, the inclu-

sion of IS led to the better categorization and discrimination of risk stratification than the inclusion of hemoglobin and LVEF results alone (NRI: 0.67;  $p=0.01$ ; IDI: 0.15;  $p<0.01$ ).

## Discussion

The main finding of this study is that serum IS level was an independent predictor of composite events (death, nonfatal MI, heart failure requiring hospitalization, and adverse bleeding event) within six months for patients with ACS who underwent successful primary PCI.

The relationship between CVD and RD is now known as cardiorenal syndrome, in which the functional deterioration of the two organs is highly interconnected<sup>30, 31</sup>. Notably, IS is one of the uremic toxins that were reported to contribute to the development of CVD in patients with RD<sup>32, 33</sup>. Particularly, investigations of the relationship between IS and coronary artery disease have been reported. Hsu *et al.*<sup>18</sup> demonstrated that the serum IS level was significantly higher for patients with coronary artery disease and was asso-



ciated with the atherosclerotic severity of the coronary artery. Others have also revealed a positive association between IS and an unstable lipid profile of coronary plaque<sup>17</sup>). We demonstrated that IS level at admission, along with the typical risk factors of LVEF and hemoglobin level, was an independent predictor of composite events in ACS.

In our sub-analysis, IS was significantly correlated with baseline renal function such as creatinine levels ( $r=0.40$ ,  $p<0.0001$ ) and eGFR ( $r=-0.29$ ,  $p=0.003$ ). However, creatinine levels were not associated with composite events even after we built the multivariate model excluding IS. This interesting difference may be because of the cytotoxicity of IS by enhancing oxidative stress and inflammatory response in cardiovascular systems during ACS. In an animal model of MI, lowering the IS level resulted in a reduction in the expression of TGF- $\beta$ 1 in the myocardium and kidney, thus suggesting the involvement of IS in the process of tissue fibrosis<sup>11</sup>). Fujii *et al.*<sup>34</sup>) also reported that a higher IS level after MI is associated with the upregulation of kidney injury biomarkers and the induction of cardiac hypertrophy via the enhancement of oxidative stress.

Our study may provide the basis for further investigations aimed at determining whether the pharmacologic modulation of IS is beneficial after ACS. Studies have shown that AST-120, which is an oral charcoal sorbent of uremic toxins, is an effective treatment for cardiovascular disorders<sup>15, 35</sup>). Yu *et al.*<sup>36</sup>) reported that AST-120 improved the endothelial dysfunction assessed by flow-mediated endothelium-dependent vasodilatation for patients with RD. Ideally, lowering IS by using AST-120 is practical for reducing oxidative stress and inflammation, which in turn may prevent tissue damage and endothelial dysfunction with the subsequent improvement of hemodynamic stability, even after ACS.

Finally, we conducted reclassification analysis to assess the incremental effect of IS on risk prediction by using standard risk factors. We found that 67% of patients were recategorized when IS was considered in conjunction with the established model based on LVEF and hemoglobin. Notably, AUC and IDI were also significantly improved by the addition of IS to these risks. Our findings indicate that the measurement of the IS level upon admission may improve care by informing risk stratification for patients with ACS.

### Study Limitations

This study has several limitations. First, this study was performed at a single center. Second, the assessment of baseline renal function was partially limited by the absence of information on proteinuria. Third,

because of the small sample size and the nature of the study design, the statistical power was limited. For example, although there was a tendency for IS to improve risk prediction by using the GRACE risk score (NRI: 0.27;  $p=0.34$ ; IDI: 0.12;  $p=0.03$ ), it did not reach statistical significance. Fourth, the small sample size may have prevented the emergence of a correlation between IS and other cardiac risk factors.

### Conclusions

Serum IS is a prognostic factor for patients with ACS who underwent successful primary PCI. Our findings may improve current risk stratification during ACS.

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### Conflict of Interest Statement

This research was supported in part by the Grants-in-Aid (15K09103 and 16K01433 to T.I.) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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