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ORIGINAL INVESTIGATION

# Echocardiography-based pressure–volume loop assessment in the evaluation for the effects of indoxyl sulfate on cardiovascular function

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## Abstract

**Background** Indoxyl sulfate (IS), a uremic toxin, has been reported to have hypertrophic effects on the heart. Previous studies, however, have shown no association between elevated IS levels and cardiovascular outcomes in hemodialysis patients. We hypothesized that, despite left ventricular (LV) hypertrophy, myocardial contractility and ventricular–arterial coupling would remain preserved, and that this would explain the reason for the absence of prognostic impact of IS.

**Methods** We evaluated the association of IS with LV structure, contractility, vascular function, and mechanical efficiency (ventricular–arterial coupling and stroke work/pressure volume area) in 154 patients on hemodialysis, using echocardiography-based pressure–volume loop assessment.

**Results** As expected, subjects in the high IS group (IS ≥ 33.8 μg/mL) had greater LV mass index and end-diastolic volume index compared to subjects in the low IS group (IS < 33.8 μg/mL). These differences remained significant after adjusting for age, sex, body mass index, diabetic nephropathy, duration of hemodialysis, and NT-proBNP levels, suggesting a potential role of elevated IS levels in LV remodeling. However, no differences in LV contractility (preload recruitable stroke work, peak power index, and systolic mitral annular velocity) and mechanical efficiency (ventricular–arterial coupling and stroke work/pressure volume area) were observed between the groups.

**Conclusions** Deleterious effects of IS on LV remodeling are not accompanied by impaired LV contractility or mechanical efficiency, which could contribute to the absence of cardiovascular prognostic impact observed in previous studies performed on hemodialysis patients.

**Keywords** Cardiac function · Efficiency · Hemodialysis · Remodeling · Uremic toxin

## Introduction

End-stage renal disease afflicts millions of people worldwide, and the prevalence of patients requiring hemodialysis has been reported to continuously increase over recent decades [1–3]. Cardiovascular diseases are common and substantially contribute to increased morbidity and mortality among patients on hemodialysis [4]. Indoxyl sulfate (IS) is a gut-derived uremic toxin that accumulates in the serum of patients with chronic kidney disease (CKD) [5]. Removal of this toxin by conventional dialysis is limited, and serum IS levels are markedly elevated in patients on dialysis [6]. A growing number of studies have reported detrimental effects of IS on cardiovascular system, especially left ventricular (LV) hypertrophy and myocardial fibrosis [7–10]. However, we and other authors have shown that elevated IS levels are

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not associated with cardiovascular events among patients on dialysis [11–13]. This observation was confirmed in a recent meta-analysis performed on a wider group of patients with CKD [14].

It is known that abnormality in LV contractility is a key determinant of cardiovascular outcomes in patients with LV hypertrophy [15]. We have recently demonstrated that impaired LV contractility and abnormal ventricular–arterial coupling (the interaction of ventricular contraction and arterial afterload) are associated with adverse outcomes in hemodialysis patients [16]. As such, we hypothesized that, despite LV hypertrophy, myocardial contractility and ventricular–arterial coupling would remain preserved, and that this would partially explain the reason for the absence of prognostic impact of IS in hemodialysis patients. The echocardiography-based pressure–volume loop assessment provides detailed information on cardiovascular function, including LV contractility, arterial afterload, preload, ventricular–arterial coupling, as well as myocardial energetics (Fig. 1) [16, 17]. Accordingly, the aim of the current study was to investigate the effects of IS on cardiovascular structure and function in patients receiving hemodialysis, using the echocardiography-based pressure–volume loop assessment.

## Methods

A total of 171 patients on hemodialysis were recruited from the dialysis unit of Hidaka Hospital. Some participants' data from this study has been previously published [11, 16, 17], but they were not related to the association between IS levels and cardiac structure/function. Echocardiographic examination was performed in all participants within 6 months of the study. All included subjects were hemodynamically stable and hemodialysis was performed 3 times per week (3–5 h/day). After excluding patients with left-sided valvular disease (> mild stenosis, > moderate regurgitation; $n = 10$) and poor image quality ($n = 7$), 154 subjects remained for final analysis. The study protocol was approved by the institutional medical ethics committee of the Hidaka Hospital, and written informed consent was obtained from all patients.

Blood samples were obtained before starting each dialysis session after overnight fasting. Serum blood urea nitrogen, creatinine, hemoglobin, calcium, phosphate, albumin, hemoglobin, ferritin, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were assessed with routine automated laboratory procedures. We measured total IS levels using high-performance liquid chromatography (Fushimi Pharmaceutical Co., Ltd., Kagawa, Japan).

Echocardiographic examinations were performed by an experienced sonographer using a commercially available ultrasound system (Aplio XV, TOSHIBA Medical Systems, Japan), equipped with a 3 MHz transducer. All subjects were studied before dialysis session after one-day inter-dialytic interval. Blood pressure (BP) was assessed at brachial artery during echocardiography, and end-systolic pressure (ESP = 0.9 × systolic BP) was calculated as previously described [18]. The LV end-diastolic and end-systolic volumes (EDV and ESV), LV mass, and ejection fraction (EF) were determined according to the current guidelines [19]. Stroke volume (SV) was determined using the biplane method of discs because 19% of subjects displayed prominent sigmoid septum, which would affect LV outflow Doppler profile [17]. Left atrial (LA) volume was calculated by the biplane area-length method. LV and LA volumes were subsequently indexed according to the

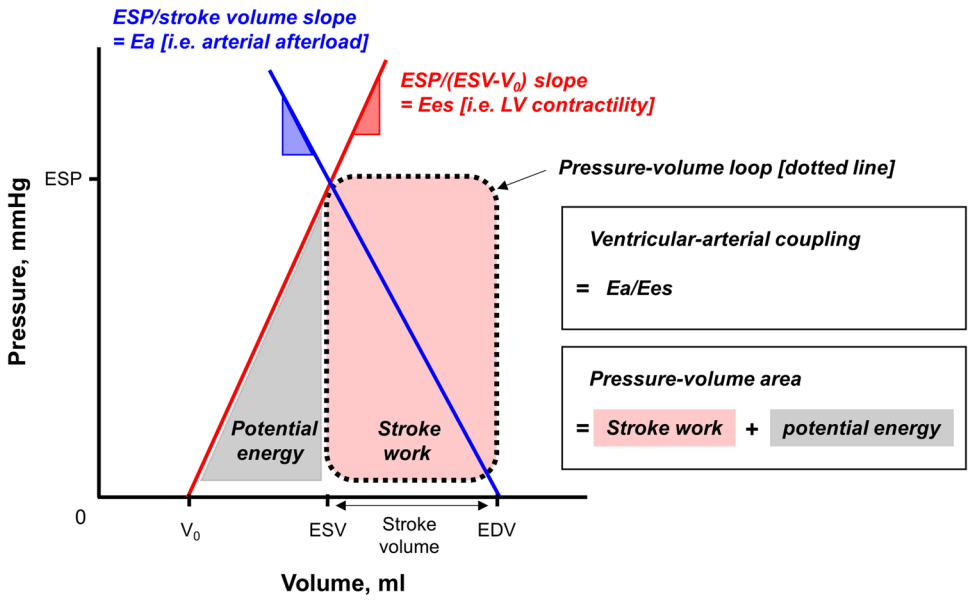


**Fig. 1** Pressure–volume loop relationship. *Ea* effective arterial elastance, *EDV* end-diastolic volume, *Ees* end-systolic elastance, *ESP* end-systolic pressure, *ESV* end-systolic volume; and $V_0$, left ventricular (LV) volume at LV pressure of 0 mmHg

body surface area. LV hypertrophy was defined based on sex-specific LV mass index [19]. The early filling (E-wave), the peak late diastolic (A-wave) velocities and deceleration time were assessed based on the trans-mitral flow. The peak systolic (s′), early diastolic (e′) and late diastolic (a′) mitral annular velocities were measured both at septal and lateral annulus, and averaged.

Pressure–volume loop represents relationships between LV pressure and volume in each cardiac cycle, and is determined by the balance among LV contractility (red line), arterial afterload (blue line), and preload (i.e., EDV) (Fig. 1). We evaluated parameters of pressure–volume relationship noninvasively using echocardiography. Effective arterial elastance (Ea) represents a measure of arterial afterload, which was calculated as 0.9 × systolic BP/SV. In other words, Ea is the slope of the ESP-SV line (blue line in Fig. 1). We also assessed other parameters of arterial afterload, including systemic vascular resistance (SVR, mean BP × 79.9/cardiac output) and total arterial compliance (SV/pulse pressure). End-systolic elastance (Ees) is the positive slope (red line in Fig. 1) reflecting load-independent LV contractility and passive chamber stiffness. The slope is determined by ESV, $V_0$, and ESP, and $V_0$ is a LV volume at a theoretical LV pressure of 0 mmHg. We estimated the Ees using the non-invasive single-beat technique [determined from BP, SV, and pre-ejection (PEP) and total systolic periods (SEP), determined on LV outflow Doppler, EF, and an estimated normalized ventricular elastance at arterial end-diastole]: [20].

$$\text{Ees} = \left[\text{Diastolic BP} - (E_{Nd(est)} \times \text{ESP})\right] / \left[E_{Nd(est)} \times \text{SV}\right]$$

Where

$$E_{Nd(est)} = 0.0275 - 0.165 \times \text{EF} + 0.3656 \times (\text{DBP/ESP}) + 0.515 \times E_{Nd(avg)}$$

And

$$E_{Nd(avg)} = 0.35695 - 7.2266 \times (\text{PEP/SEP}) + 74.249 \times (\text{PEP/SEP})^2 - 307.39 \times (\text{PEP/SEP})^3 + 684.54 \times (\text{PEP/SEP})^4 - 856.92 \times (\text{PEP/SEP})^5 + 571.95 \times (\text{PEP/SEP})^6 - 159.1 \times (\text{PEP/SEP})^7.$$

We also assessed preload recruitable stroke work (PRSW) and peak power index (PWRI) as a measure of LV contractility [18, 21]. Single-beat PRSW was determined from stroke woke/[EDV − k × EDV + (1 − k) × LV wall volume, where stroke work (SW) = SV × mean BP, LV wall volume = LV mass/1.05, and k was assumed to be 0.7. The PWRI was calculated as: (peak LV outflow velocity × LV outflow area × systolic BP)/EDV. The interaction between the heart and large arteries was assessed using the ratio of Ea to Ees (ventricular–arterial coupling) [22]. The ventricular–arterial coupling represents mechanical efficiency in transferring the blood from the heart to the arterial system, and elevated coupling (Ea/Ees > 1.2) indicates worse cardiac efficiency. Our group has recently reported that higher Ea/Ees ratio predicts adverse outcomes in patients on dialysis [16]. In the pressure–volume loop concept, SW is the area surrounded by the pressure–volume loop (pink area in Fig. 1), and equals to the actual external work performed by the heart. In contrast, potential energy is the area surrounded by the end-systolic pressure–volume relationship line, isovolumic relaxation phase of the pressure–volume loop, and volume axis (*x*-axis) (gray area in Fig. 1), and it reflects energy loss that does not participate in ejection of blood into the aorta. The pressure–volume area (PVA) is the sum of the SW and potential energy, and has been shown to correlate linearly with myocardial oxygen consumption [23, 24]. Thus, the ratio of SW to PVA represents LV mechano-energetic efficiency converting metabolic energy to external cardiac work. We estimated the SW (SV × mean BP) and the PVA as previously reported: [25] PVA = 0.5 x ESP x [SV + (EDV − $V_0$)] where $V_0$ = ESV − ESP/Ees. An experienced investigator who was unaware of the patients' characteristics analyzed all echocardiographic measurements (MO).

All continuous variables were presented as mean ± SD or median (interquartile range). Comparisons between the two groups were performed using Fisher's exact test, Chi-squared test, Student's *t* test (normally distributed variables), or Mann–Whitney *U* tests (skewed variables). Spearman's correlation was performed to analyze associations between IS levels and echocardiographic parameters. Multivariable linear regressions were used to determine independent associations of IS levels with echocardiographic parameters. Two-sided *p* values < 0.05 were accepted as statistically significant. All data were statistically analyzed using JMP 10.0.0 (SAS Institute, Cary, NC).

## Results

Overall, participants had markedly elevated IS levels [median IS, 33.8 (interquartile range 23.4–46.2) μg/mL] [6]. Clinical characteristics of study participants according to median IS values are shown in Table 1. Compared to subjects in the low IS group, subjects in the high IS group were younger and hypertensive, and had higher levels of serum creatinine, blood urea nitrogen, albumin, and NT-proBNP. No differences were found in sex, BMI, cause of end-stage renal disease, dialysis duration, pre-dialysis weight, dry weight, or ultrafiltration volume. Subjects in the high IS were more likely to receive angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers (ACEIs/ARBs), while other medication use was similar between the groups. No correlation was detected between IS levels and age, body mass index, or dialysis duration (all *p* values > 0.1).

**Table 1** Clinical characteristics

| Characteristics | Low IS ($n=76$) | High IS ($n=78$) | $p$ value |
|---|---|---|---|
| IS range (μg/ml) | < 33.8 | ≥ 33.8 | – |
| Median IS (μg/ml) | 23.4 (14.8–28.5) | 45.9 (38.8–57.6) | < 0.0001 |
| Age, years | 65 ± 11 | 62 ± 11 | 0.035 |
| Male gender | 53 (70%) | 62 (79%) | 0.20 |
| Body mass index (kg/m$^2$) | 23.1 ± 3.9 | 22.5 ± 3.3 | 0.38 |
| Hemodialysis data | | | |
| Cause of end-stage renal disease | | | |
| Diabetes | 31 (41%) | 27 (35%) | |
| Glomerulonephritis | 32 (42%) | 40 (51%) | |
| Cystic kidney disease | 6 (8%) | 4 (5%) | 0.42 |
| Hypertension | 2 (3%) | 0 (0%) | |
| Others | 5 (6%) | 7 (9%) | |
| Dialysis vintage, year | 5.3(2.3–12.5) | 6.1(3.4–13.5) | 0.22 |
| Pre-dialysis weight (kg) | 60.3 ± 12.6 | 61.7 ± 12.0 | 0.47 |
| Dry weight (kg) | 58.1 ± 12.1 | 59.1 ± 11.3 | 0.61 |
| Ultrafiltration volume (ml) | 2600 (1800–3550) | 3000 (2500–3625) | 0.07 |
| Comorbidities | | | |
| Hypertension | 61 (80%) | 72 (92%) | 0.035 |
| Diabetes | 34 (45%) | 28 (36%) | 0.32 |
| Dyslipidemia | 27 (36%) | 23 (29%) | 0.49 |
| Current smoker | 14 (18%) | 19 (24%) | 0.43 |
| Prior myocardial infarction | 2 (3%) | 5 (6%) | 0.44 |
| Laboratory data | | | |
| Creatinine (mg/dl) | 10.2 ± 2.8 | 12.1 ± 2.2 | < 0.0001 |
| Blood urea nitrogen (mg/dl) | 59 ± 14 | 66 ± 14 | 0.007 |
| Calcium (mg/dl) | 8.6 ± 0.8 | 8.6 ± 0.7 | 0.90 |
| Phosphate (mg/dl) | 5.1 ± 1.2 | 5.5 ± 1.2 | 0.06 |
| Albumin (g/dl) | 3.6 ± 0.3 | 3.8 ± 0.3 | 0.004 |
| Hemoglobin (g/dl) | 10.8 ± 1.1 | 10.8 ± 0.8 | 0.75 |
| Ferritin (ng/ml) | 40 (18–82) | 35 (20-60) | 0.52 |
| NT-proBNP (pg/ml) | 3035 (1338–5098) | 3940 (2363–7193) | 0.04 |
| Medications | | | |
| Beta blockers | 22 (29%) | 28 (36%) | 0.39 |
| Calcium channel blockers | 37 (49%) | 49 (63%) | 0.10 |
| ACEIs/ARBs | 38 (50%) | 52 (67%) | 0.049 |
| Statins | 16 (21%) | 16 (21%) | 1.00 |
| Phosphate binders | 59 (82%) | 64 (82%) | 1.00 |
| 25-Hydroxyvitamin D | 32 (44%) | 25 (32%) | 0.13 |

Values are mean ± SD, median (interquartile range), or $n$ (%)

*ACEI* indicates angiotensin-converting enzyme inhibitors, *ARB* angiotensin-receptor blockers; *IS* indoxyl sulfate, and *NT-proBNP* N-terminal pro-B-type natriuretic peptide

In contrast, serum IS levels were positively correlated with blood urea nitrogen ($r=0.20$, $p=0.01$), creatinine ($r=0.32$, $p<0.0001$), albumin ($r=0.20$, $p=0.01$), and NT-proBNP ($r=0.19$, $p=0.02$) levels.

Systolic, diastolic, and mean BPs, pulse pressure, and heart rate were similar between the groups (Table 2). As expected, subjects in the high IS group had a greater LV mass index than low IS group and nearly 70% of subjects in the group had LV hypertrophy (Fig. 2). Both EDV and ESV indices were larger in the high IS group (Fig. 2). While no differences were found in mitral inflow and annular velocities between the groups, LA volume index was larger in the high IS group. These differences remained significant after adjusting for either creatinine or blood urea nitrogen levels (all $p$ values < 0.05). Serum IS levels were directly correlated with LV mass index and EDV index ($r=0.25$, $p=0.002$ and

**Table 2** Cardiac structure and function

| Characteristics | Low IS ($n=76$) | High IS ($n=78$) | $p$ value |
|---|---|---|---|
| Hemodynamics | | | |
| Systolic BP (mm Hg) | 160 ± 27 | 167 ± 32 | 0.17 |
| Diastolic BP (mm Hg) | 74 ± 13 | 78 ± 17 | 0.07 |
| Mean BP (mm Hg) | 103 ± 16 | 108 ± 20 | 0.08 |
| Pulse pressure (mm Hg) | 86 ± 22 | 88 ± 25 | 0.61 |
| Heart rate (beats/min) | 72 ± 10 | 72 ± 10 | 0.96 |
| LV structure and morphology | | | |
| Septal wall thickness (mm) | 10 ± 2 | 10 ± 2 | 0.56 |
| Posterior wall thickness (mm) | 11 ± 2 | 11 ± 2 | 0.27 |
| LV mass index (g/m$^2$) | 116 ± 28 | 130 ± 28 | 0.003 |
| Relative wall thickness | 0.48 ± 0.09 | 0.46 ± 0.10 | 0.20 |
| LV hypertrophy (%) | 55 | 69 | 0.09 |
| End-diastolic volume index (ml/m$^2$) | 48 ± 12 | 58 ± 15 | < 0.0001 |
| End-systolic volume index (ml/m$^2$) | 16 ± 7 | 22 ± 11 | < 0.0001 |
| Arterial afterload | | | |
| Ea (mmHg/ml) | 2.99 ± 1.18 | 2.57 ± 0.70 | 0.008 |
| SVR (dyne/s*cm$^5$) | 2375 ± 788 | 2071 ± 547 | 0.006 |
| Arterial compliance (ml/mmHg) | 0.64 ± 0.25 | 0.78 ± 0.47 | 0.02 |
| LV contractility | | | |
| Ees (mmHg/ml) | 5.18 ± 2.70 | 4.05 ± 1.44 | 0.002 |
| PRSW (g/cm$^2$) | 71 ± 18 | 74 ± 19 | 0.41 |
| PWRI (mmHg/s) | 609 ± 181 | 557 ± 169 | 0.10 |
| s′ velocity (cm/s) | 8.5 ± 1.7 | 8.6 ± 1.9 | 0.80 |
| LV diastolic function | | | |
| E-wave (m/s) | 80 ± 23 | 82 ± 24 | 0.66 |
| A-wave (m/s) | 101 ± 23 | 103 ± 24 | 0.60 |
| Deceleration time (m/s) | 246 ± 64 | 247 ± 75 | 0.91 |
| e′ velocity (cm/s) | 6.7 ± 1.5 | 6.7 ± 1.6 | 0.90 |
| a′ velocity (cm/s) | 10.5 ± 2.0 | 10.5 ± 2.2 | 0.94 |
| E/e′ ratio | 12.4 ± 4.3 | 12.7 ± 4.4 | 0.71 |
| LA volume index (ml/m$^2$) | 36 ± 13 | 41 ± 14 | 0.03 |
| Integrated parameters | | | |
| Stroke volume index (ml/m$^2$) | 33 ± 8 | 37 ± 8 | 0.002 |
| Ejection fraction (%) | 69 ± 8 | 64 ± 10 | 0.005 |
| Cardiac index (l/m$^2$) | 2.3 ± 0.5 | 2.6 ± 0.6 | 0.0004 |
| Ea/Ees ratio | 0.65 ± 0.27 | 0.69 ± 0.27 | 0.39 |
| Stroke work (mmHg*ml) | 5403 ± 1776 | 6680 ± 2332 | 0.0002 |
| Pressure–volume area (mmHg*ml) | 10,084 ± 3649 | 12,419 ± 4374 | 0.0005 |
| Stroke work/pressure–volume area (%) | 54 ± 6 | 54 ± 7 | 0.92 |

Values are mean ± SD

*A* peak late diastolic mitral inflow velocity, *a'* late diastolic mitral annular velocity, *BP* indicates blood pressure, *E* peak early diastolic mitral inflow velocity; *e'* early diastolic mitral annular velocity; *Ea* effective arterial elastance, *Ees* end-systolic elastance, *LA* left atrial, *LV* left ventricular, *PRSW* preload recruitable stroke work, *PWRI* peak power index, *s'* systolic mitral annular velocity, *SVR* systemic vascular resistance; and other abbreviations as in Table 1

$r=0.30$, $p=0.0002$). These associations remained significant after adjusting for age, sex, body mass index, diabetic nephropathy, duration of hemodialysis, and NT-proBNP levels (both $p$ values $<0.05$), suggesting potential effects of IS on LV remodeling.

Arterial afterload was rather reduced in the high IS group compared to low IS group, with lower Ea and SVR and higher total arterial compliance. These differences remained significant after adjusting for the use of ACEIs/ARBs and calcium channel blockers (CCBs) (all $p<0.05$).

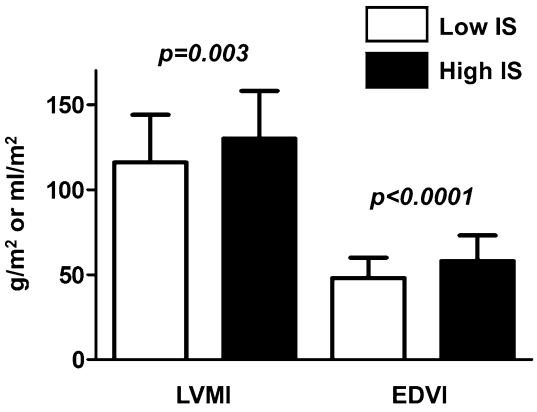


**Fig. 2** Left ventricular mass (LV) and size according to median indoxyl sulfate (IS) values. Compared to subjects in the low IS group, subjects in the high IS group display larger LV mass index, EDV index (EDVI), and left atrial volume index (LAVI). Abbreviations as in Fig. 1

While Ees was lower in the high IS group than in the low IS group, LV contractility as assessed by PRSW, PWRI, and s' velocity was similar between the groups (Fig. 3a, b). As compared to subjects in the low IS group, subjects with high IS displayed greater SV and cardiac indices, stroke work, and PVA. Elevated IS levels were positively correlated with cardiac index, stroke work and PVA ($r=0.28$, $p=0.0005$; $r=0.25$, $p=0.002$; and $r=0.24$, $p=0.003$), and these results remained significant after adjusting for age, sex, body mass index, diabetic nephropathy, duration of hemodialysis, and NT-proBNP levels (all $p$ values $<0.05$). Compared to subjects in the low IS group, LVEF was lower in the high IS group, but it was preserved (mean EF = 64%). In spite of greater LV remodeling and increased cardiac work in the high IS group, no differences in Ea/Ees ratio and LV mechanical efficiency (SW/PVA) were found between the groups (Fig. 3c, d).

## Discussion

This study investigated the association of serum IS levels with cardiac structure, ventricular and vascular function, coupling, and mechanical efficiency in patients receiving chronic hemodialysis using echocardiography-based pressure–volume loop assessment. Compared to subjects in the low IS group, subjects in the high IS group displayed greater LV remodeling. However, no reduction was found in LV contractility assessed using three measures of systolic function (PRSW, PWRI, and s' velocity), and arterial afterload was reduced in subjects with high IS group. In addition, elevated IS levels did not significantly affect LV mechanical efficiency, as assessed by ventricular–arterial coupling and SW/PVA. These data suggest that deleterious effects of IS on LV hypertrophy and dilation were not accompanied by impaired LV contractile function or LV mechanical efficiency, which could contribute to the absence of prognostic impact observed in previous studies performed on patients on dialysis [11–13].

Cardiovascular diseases in patients on dialysis have a complex and multifaceted pathophysiology, related to

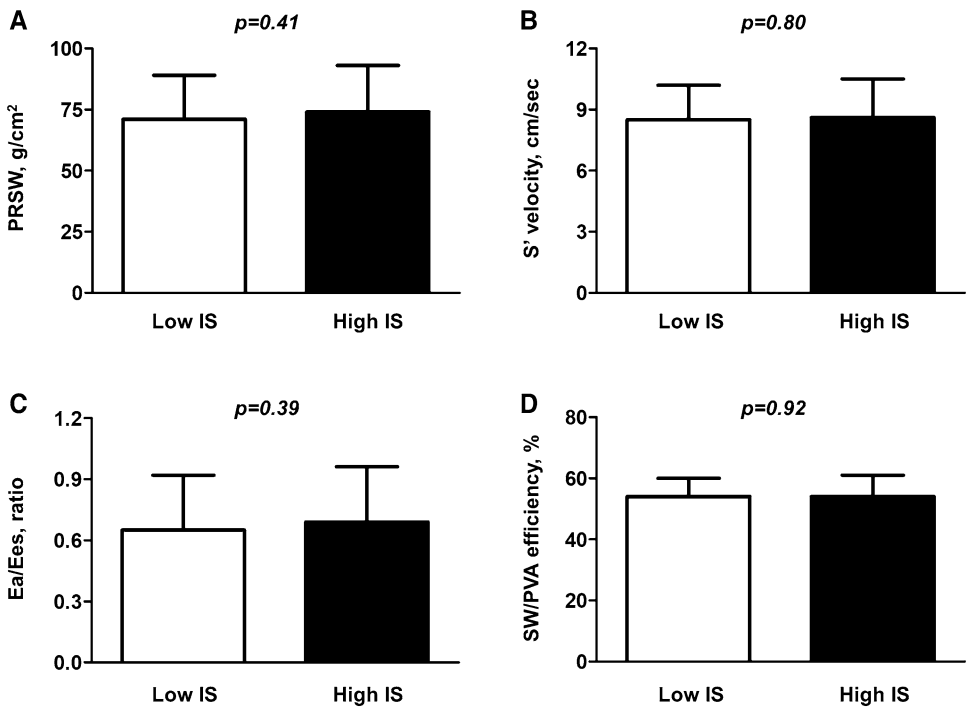


**Fig. 3** LV contractility and mechanical efficiency according to median IS values. LV myocardial contractility and mechanical efficiency are similar in low and high IS groups. **a** Preload recruitable stroke work (PRSW), (**b**) systolic mitral annular velocity (s′), (**c**) ventricular–arterial coupling (Ea/Ees ratio), and (**d**) stroke work/pressure volume area efficiency. Abbreviations as in Fig. 1

systolic and diastolic dysfunction, volume overload, arterial hypertension, coronary artery disease, insulin resistance, vascular calcification, and sympathetic nerve activation [26]. Beyond these traditional risk factors, growing evidence has shown that uremic toxins may be responsible for cardiovascular disease progression in patients with CKD [5]. Indoxyl sulfate is a gut-derived uremic toxin that has been extensively studied in recent years. It has been shown to have pro-hypertrophic, pro-fibrotic, and pro-inflammatory effects on cardiomyocytes and cardiac fibroblasts [7, 8]. Earlier studies found that elevated IS levels were associated with LV hypertrophy and worsening diastolic function [8–10], suggesting a potential role of elevated IS levels in adverse cardiovascular events occurrence through alterations in LV structure and function [5]. In a single center study performed on 258 patients on dialysis, elevated IS levels were associated with subsequent heart failure [27]. However, we and other authors have shown no relationship between IS levels and all-cause mortality or composite cardiovascular events in these patients [11–13]. Furthermore, a recent meta-analysis has confirmed that elevated IS levels were not associated with an increased rate of cardiovascular events in patients with CKD [14]. Nevertheless, little is known about the reasons of this discrepancy.

In the current study, echocardiography-based pressure–volume loop assessment might provide insights into the reasons for lack of association between IS and cardiovascular outcome in patients on hemodialysis. Consistent with previous experimental studies [28, 29], the current study found the association between elevated IS levels and LV remodeling. However, we showed for the first time that, using three independent measures of systolic function (PRSW, PWRI, and s' velocity), no reduction was found in LV contractility in the high IS group despite the LV remodeling. It is well recognized that depressed myocardial contractility could coexist in patients with hypertrophy despite of a normal LVEF, and that the presence of impaired myocardial contractility is associated with increased risk of cardiovascular events in patients with arterial hypertension or HF with preserved EF [15, 30]. Among patients receiving hemodialysis, depressed LV contractility was also shown to be an independent predictor of cardiovascular outcome [16, 31]. While the presence of LV hypertrophy or remodeling appears to lead to LV contractility decline, it may persist or even be enhanced in some patients, especially at an earlier stage of disease [15, 32, 33]. Thus, our data indicate that the lack of association between IS and cardiovascular mortality in patients on hemodialysis may be related to preservation of LV contractile function.

Unlike the other contractile parameters, Ees was lower in the high IS group. Ees is not a pure measure of contractility and influenced by chamber geometry and passive stiffness [15]. In hypertensive heart diseases, patients with heart failure and preserved EF, and dialysis patients, Ees increases with increasing vascular afterload (i.e., Ea) in order to maintain ventricular–arterial coupling. This increased Ees, however goes together with impaired contractile performance. Therefore, there is a well-known dissociation between contractility and systolic LV elastance (i.e., high Ees but low contractility). Conversely, in patients with high IS, decreased Ees could be an adequate response to decreased Ea. Further study is required to determine the mechanisms of the dissociation between Ees and other contractile parameters in hemodialysis patients who have high IS levels.

Experimental studies have reported that IS induces vascular smooth muscle cell proliferation and oxidative stress in endothelial cells [34, 35]. Barreto et al. [6] has demonstrated positive correlations of IS levels with pulse wave velocity and aortic calcification in a wide range of CKD patients (CKD stage 2–5D), though the observed correlations might be confounded by the progression of CKD. In contrast to these data, we observed reduced arterial afterload in the high IS group. Compared to subjects in the low IS group, those in the high IS group were more likely to be treated with vasodilators (ACEIs/ARBs 50 vs. 67%, $p=0.049$ and CCBs, 49 vs. 63%, $p=0.10$), which might influence the results. Although the differences in arterial afterload between the groups remained significant after adjusting for the use of ACEIs/ARBs and CCBs, we cannot exclude the possibility that the difference in vasodilator use may relate in part to the decreased arterial afterload. The cross sectional design of our study does not allow to asses causal relationship between IS and arterial afterload. Further study is needed to determine this relationship in hemodialysis patients.

Using the pressure–volume loop approach, we also demonstrated that ventricular–arterial coupling (Ea/Ees ratio) and SW/PVA efficiency were similar between the low and high IS groups. This suggests that IS had no or little effect on LV mechanical efficiency in patients on dialysis. The ventricular–arterial coupling, the interplay of the heart with the arterial system, is a key determinant of cardiac performance, and elevated coupling is known to be associated with deleterious cardiovascular outcomes [36]. We have recently reported that elevated Ea/Ees is associated with increased risk of adverse outcomes in patients on hemodialysis [16]. No difference in the prognosis between high and low IS groups could be explained by similar Ea/Ees ratio. Similar SW/PVA efficiency between groups shown in the current study further attributes a similar prognosis. A recent study has demonstrated that LV systolic function preservation is one of the predictors to maintain normal cardiac efficiency [37]. We speculate that restoration of ventricular–arterial coupling and SW/PVA efficiency may be related, at least in part, to the absence of the relationship between elevated IS levels and adverse cardiovascular outcomes in patients on hemodialysis.

Our study has several important limitations. This study is a single-center observational study and as such could have inherent flaws related to selection and referral bias. We cannot assess causality, since this is a cross-sectional study. Blood samples were drawn before starting dialysis sessions. Serum IS levels could be slightly altered by blood sample timing. We did not measure neurohumoral factors and cannot evaluate their effects on the association between IS and the heart. Loading conditions might affect some echocardiographic variables. Although SV was determined by using the biplane method of disks to avoid an overestimation due to a sigmoid septum, it might lead to underestimation of SV. Three-dimensional transthoracic echocardiography may overcome this limitation. The difference in vasodilator use between the groups might influence the results of arterial afterload. We cannot exclude the possibility that elevated IS levels are associated with increased risk of cardiovascular events or mortality based on these data. However, this is the first study investigating the possible reasons for the lack of the association between elevated IS and cardiovascular events in patients on hemodialysis using echocardiography-based pressure–volume loop assessment.

## Conclusions

The echocardiography-based pressure–volume loop assessment demonstrates no significant effect of elevated IS levels on LV myocardial contractility or mechanical efficiency in hemodialysis patients. These data suggest that deleterious effects of IS on LV remodeling are not accompanied by impaired LV contractile function or mechanical efficiency, contributing to the absence of cardiovascular prognostic impact observed in previous studies performed on patients on dialysis.

### Compliance with ethical standards

**Conflict of interest** Dr. Obokata received research funding from Kureha Corporation, Tokyo, Japan. The sponsor was not involved in the study design, data collection, analysis and interpretation, and preparation of manuscript. Dr. Negishi was supported by an award from the Select Foundation, which has no role in the preparation of this manuscript. Drs. Kurosawa, Ishida, Ito, Ogawa, Ando, and Kurabayashi declare that they have no conflict of interest.

**Ethical approval** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions.

**Informed consent** Informed consent was obtained from all patients for being included in the study.

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