

# Abnormal Tissue Doppler Images are Associated With Elevated Plasma Brain Natriuretic Peptide and Increased Oxidative Stress in Acute Kawasaki Disease

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**Background** The aims of this study were to evaluate myocardial mechanics using pulsed tissue Doppler imaging (TDI), and to determine the relationship between abnormal myocardial performance and plasma brain natriuretic peptide (BNP) levels and oxidative stress in acute Kawasaki disease (KD).

**Methods and Results** Consecutive TDI parameters, including peak systolic velocity (Sw) and early (Ew) and late diastolic excursion of the mitral annuli were obtained in 42 patients with KD (mean age: 2.4±0.4 years) in weeks 1, 2, and 3, and during convalescence. Plasma BNP level and urinary 8-isoprostane were also examined during the acute phase. These data were then compared with TDI profiles from 62 healthy children, plasma BNP levels in 38 controls with other febrile illnesses, and urinary 8-isoprostane levels in 13 healthy children. Ew in week 1 was significantly lower than in controls, subsequently normalizing in the convalescent stage. Plasma BNP level in acute KD patients was significantly higher (65±9 pg/ml) than in controls (13±2 pg/ml). Urinary 8-isoprostane level in acute KD patients was significantly higher as compared with control (596±37 vs 379±26 pg/ml Cr, p<0.05). There was a significant negative correlation between week 1 Sw and plasma BNP level (r=-0.55, p=0.0001). Change in Sw velocity in the BNP ≥51 group was significantly greater than in the BNP <51 group. There was a significant negative correlation between week 1 Sw and urinary 8-isoprostane level (r=-0.48, p=0.001).

**Conclusions** Latent abnormal tissue Doppler profiles, possibly reflecting long-axis systolic and diastolic dysfunction have been noted in KD patients. Abnormal myocardial mechanics may contribute to the increased plasma BNP level and enhanced oxidative stress may contribute to cardiac dysfunction in KD. (*Circ J* 2007; 71: 357–362)

**Key Words:** Brain natriuretic peptide; Kawasaki disease; Oxidative stress; Tissue Doppler echocardiography

**K**awasaki disease (KD) is a systemic vasculitis that primarily affects small and medium-sized arteries!<sup>1</sup> Transient myocardial dysfunction in children with acute KD has been reported<sup>2,3</sup> and moreover, myocardial inflammation, including myocarditis and microvascular damage in the myocardium, has been shown to cause aberrations in cardiac function<sup>4</sup> Several recent studies have discussed the usefulness of tissue Doppler imaging (TDI) to evaluate left ventricular (LV) function<sup>5–14</sup> Tissue Doppler measurement of myocardial Doppler velocity can be used to measure long-axis functions, which seem to be both more sensitive to minor disturbances in LV function and relatively preload-independent.<sup>11,13–14</sup> In the present study we used TDI to evaluate disturbance of myocardial function in acute KD patients.

The plasma brain natriuretic peptide (BNP) level is associated with cardiac function in adult patients with congestive heart failure!<sup>5,16</sup> Although elevation of BNP has been reported in acute KD patients,<sup>17</sup> no relation between BNP and cardiac function has been found.

Reports have suggested that increased systemic oxidative stress is associated with progression of cardiovascular disease, including ischemia–reperfusion injury, atherosclerosis, and heart failure!<sup>8–24</sup> The isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism!<sup>18,19</sup> The level of 8-isoprostane is used as a pathophysiological marker of lipid peroxidation. We previously reported elevation of urinary 8-isoprostane levels in acute KD patients;<sup>25</sup> and although the exact pathological role of increased oxidative stress in acute KD is uncertain, we believe that it is associated with oxidative injury to systemic vessels!<sup>25</sup> We hypothesize that this combined with disturbance of myocardial microcirculation may contribute to myocardial dysfunction in acute KD. Therefore, the purpose of the present study was to investigate the relationship between abnormal myocardial performance and plasma BNP levels, and to investigate the association between abnormal myocardial performance and enhanced oxidative stress.

(Received July 21, 2006; revised manuscript received November 15, 2006; accepted November 30, 2006)

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## Methods

Forty-two children with acute KD (boys/girls: 24/18, mean age:  $2.4 \pm 0.4$  years) were enrolled. All patients satisfied the diagnostic criteria; those with atypical KD were excluded. The children were admitted to hospital between April 2002 and July 2003. All patients were treated initially with a single dose of intravenous immune globulin (IVIG), 1–2 g/kg, in a single infusion over 12 h and were given aspirin orally within 7 days of onset. IVIG was begun  $5.0 \pm 0.7$  days (range 4–7 days) after onset. Twenty-eight children received a single dose of IVIG (total dose 1–2 g/kg), 8 required additional IVIG following the initial IVIG treatment (total dose 3–4 g/kg), and 6 underwent steroid pulse therapy (20–30 mg/kg of methylprednisolone after IVIG) because of IVIG-resistant KD. Mean plasma C-reactive protein (CRP) level before initial IVIG therapy was  $8.0 \pm 0.6$  mg/dl. Limited pericardial effusion and mitral valve regurgitation were observed in 7 and 6 patients, respectively. Gallop rhythms were detected in 4 patients and 2 patients underwent short-term dobutamine therapy because of transient cardiac hypokinesia. Transient coronary artery dilatation was observed in 2 patients after that treatment regimen and in 2 convalescent-phase patients.

### Echocardiography

Echocardiographic measurements were obtained with a commercial ultrasound system (Acuson SEQUOIA C256; Siemens, Mountain View, CA, USA). Complete 2-dimensional and conventional color flow imaging and Doppler echocardiography were performed initially. Heart rate (HR), LV shortening fraction (LVSF), LV end-systolic dimension (LVDs), LV end-diastolic dimension (LVDd), and mitral valve inflow Doppler pattern analysis of peak E-wave and A-wave velocities were calculated in the standard manner. End-systolic wall stress (ESWS) and HR-corrected velocity of circumferential fiber shortening (VCFc) were also calculated using established techniques.<sup>26</sup> After these echocardiographic evaluations, apical 4-chamber perspective tissue Doppler echocardiography was performed to measure longitudinal annular velocities at the lateral mitral wall. The first systolic (Sw), early diastolic (Ew), and late diastolic (Aw) tissue Doppler velocities were measured at the lateral mitral wall and averaged over 3 cycles in accordance with previous reports.<sup>5–14</sup> These echocardiographic evaluations were done in week 1 (before starting IVIG therapy), week 2 ( $\approx 7$  days after starting IVIG therapy), week 3 ( $\approx 14$  days after starting IVIG therapy), and in the convalescent stage (a mean of 5, 12, 19, and 36 days after onset). All patients agreed to initial echocardiographic evaluations before starting IVIG therapy in week 1. Changes from baseline (%) in Ew, Aw, and Sw were calculated as:  $\{( \text{the velocities at each phase} - \text{the velocities on week 1} ) / \text{the velocities on week 1} \} \times 100$ . The transmitral E/Ew ratio was calculated for each patient. Echocardiographic measurements in 62 age-matched healthy children without cardiac disease (mean age:  $2.4 \pm 2.0$  years) were also obtained as the control (TDI control group). All examinations were done by the same physician. Intraobserver variability was estimated as 4%.

### Measurement of Plasma BNP and Urinary 8-Isoprostane Concentrations

Blood samples were obtained to measure the plasma concentration of BNP. Acute phase concentrations were measured before IVIG therapy (mean  $5.0 \pm 0.7$  days after onset)

and during the convalescent phase (mean  $36 \pm 1$  days after onset), using an immunoradiometric assay from a commercially available kit (Shionogi Co Ltd, Osaka, Japan). Plasma BNP levels in 38 age-matched and CRP-matched (age:  $2.8 \pm 0.8$  years, CRP:  $9.2 \pm 0.8$  mg/dl) children with other acute febrile illnesses were measured as a control (BNP control group) for comparison with the acute KD group.

Urinary 8-isoprostane levels were measured during the acute phase before IVIG therapy (mean  $5.0 \pm 0.7$  days after onset). Levels in 13 healthy children (mean age  $3.0 \pm 1.0$  years) were obtained as a control (8-isoprostane control group). The concentration of free 8-isoprostane was analyzed using a commercially available enzyme immunoassay kit (Cayman Chemical, MI, USA)<sup>27</sup> To eliminate contaminants, urine samples were purified before analysis with ODS gel (Silica Gel ODS-Q3, Fuji Gel, Tokyo, Japan) followed by a NH<sub>2</sub> Sep-Pak column (Sep-Pak, Vac NH<sub>2</sub>, Waters, MA, USA) as previously reported.<sup>28</sup> The detection limits of the assay were between 7.8 and 200 pg/ml. Microtiter assay plates were scanned with a computer-controlled adjustable wavelength microtiter plate reader. Results are expressed as pg/mg urinary creatinine (Cr).

The study protocol was approved by the ethical committees of the medical centers involved, and informed consent was given by all subjects.

### Statistical Analysis

Results are expressed as means  $\pm$  SEM. The statistical significance of differences between groups was determined by 1-way ANOVA followed by Tukey-Kramer's test. The unpaired T-test was used to analyze the difference between the control group and KD group in urinary 8-isoprostane levels. Differences in consecutive changes of echocardiographic parameters among each group were analyzed by repeated measures ANOVA. Pearson's correlation coefficient analysis was used to assess the association among echocardiographic parameters, plasma BNP and urinary 8-isoprostane level. All data analyses were performed with a commercially available statistical analysis software package (Statview 5.0, Abacus Concepts Inc, Calabasus, CA, USA).  $P < 0.05$  was considered significant.

## Results

### TDI in Acute KD Patients (Table 1)

HR was significantly lower after IVIG treatment. Blood pressure (BP), ESWS, VCFc, LVDd, LVDs, and LVSF did not significantly change. Ew velocity in week 1 was significantly lower than in the controls and subsequently normalized during the convalescent stage. Mitral inflow E/A and Ew/Aw at week 1 were both significantly lower than in controls and normalized in the convalescent phase. This finding was observed for Ew/Aw compared with E/A. E/Ew at week 1 was significantly higher as compared with control, but subsequently normalized during the convalescent phase. Sw, VCFc, ESWS, and LVSF showed no significant change at any stage. Sw velocity did not significantly correlate with LVSF at any stage ( $r = 0.1$ ,  $p = 0.19$ ). There were no significant differences between children with or without pericardial effusion, mitral valve regurgitation, or coronary artery lesion. There were no significant differences in TDI parameters between the responders to initial IVIG therapy ( $n = 28$ ) and non-responders ( $n = 14$ ).

**Table 1** Conventional Echocardiographic and Tissue Doppler Parameters in Acute KD Patients

	KD				
	Control	Week 1	Week 2	Week 3	Convalescent phase
HR (beats/min)	102 (3)	136 (4)*	108 (3) <sup>†</sup>	107 (2) <sup>†</sup>	108 (2) <sup>†</sup>
Systolic BP (mmHg)	98 (1)	100 (1)	93 (2)	94 (1)	97 (1)
UCG					
LVDd (cm)	2.9 (0.1)	2.9 (0.1)	2.9 (0.1)	3.0 (0.1)	3.0 (0.1)
LVDs (cm)	1.9 (0.1)	1.8 (0.1)	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)
LVSF (%)	35 (0.5)	34 (0.8)	35 (0.6)	36 (0.5)	36 (0.6)
ESWS	54 (2)	55 (2)	52 (2)	54 (2)	56 (2)
VCFc	1.14 (0.02)	1.15 (0.02)	1.16 (0.02)	1.15 (0.01)	1.15 (0.1)
Mitral inflow E/A	2.0 (0.1)	1.4 (0.1)*	1.6 (0.1)*	1.6 (0.1)*	1.8 (0.1)
TDI					
Sw	9.7 (0.2)	9.7 (0.2)	9.0 (0.2)	9.3 (0.2)	9.5 (0.2)
Ew	20.3 (0.5)	16.2 (0.6)*	17.1 (0.7)*	18.9 (0.6) <sup>†</sup>	20.1 (0.6) <sup>†,‡</sup>
Aw	8.5 (0.3)	11.4 (0.6)*	8.2 (0.3) <sup>†</sup>	8.5 (0.3) <sup>†</sup>	8.5 (0.3) <sup>†</sup>
Ew/Aw	2.5 (0.1)	1.6 (0.1)*	2.2 (0.1) <sup>†</sup>	2.3 (0.1) <sup>†</sup>	2.5 (0.1) <sup>†</sup>
E/Ew	5.6 (0.2)	7.3 (0.2)*	6.3 (0.3)	5.7 (0.2)	5.4 (0.2)
Change of Sw (%)		0	-4 (3)	-1 (3)	2 (3)
Change of Ew (%)		0	6 (2)	18 (3)	27 (3) <sup>†,‡</sup>

KD, Kawasaki disease; HR, heart rate; BP, blood pressure; UCG, ultrasonic cardiography; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVSF, left ventricular shortening fraction; ESWS, end-systolic wall stress; VCFc, heart rate-corrected velocity of circumferential fiber shortening; Mitral inflow E/A, early transmitral flow velocity to late transmitral flow velocity ratio; TDI, tissue Doppler imaging; Sw, systolic tissue Doppler velocity; Ew, early diastolic tissue Doppler velocity; Aw, late diastolic tissue Doppler velocity; E/Ew, early transmitral flow velocity to early diastolic tissue Doppler velocity ratio.

\* $p < 0.05$  vs control, <sup>†</sup> $p < 0.05$  vs week 1, <sup>‡</sup> $p < 0.05$  vs week 2.

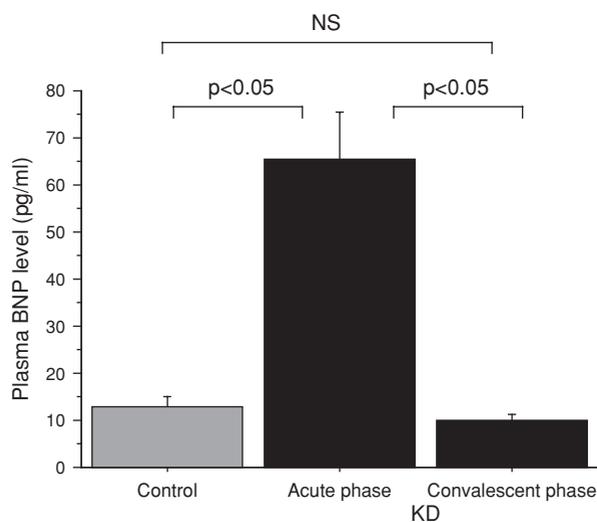


Fig 1. Plasma brain natriuretic peptide (BNP) level in patients with acute Kawasaki disease (KD) was significantly higher than in the control group.

#### Plasma BNP Level in Acute KD

Plasma BNP level in acute KD was significantly higher ( $65 \pm 9$  pg/ml) than in the control group ( $13 \pm 2$  pg/ml), but normalized in the convalescent phase ( $11 \pm 1$  pg/ml). No patient had a plasma BNP level above 50 pg/ml in the convalescent phase (Fig 1).

#### Correlation Between Plasma BNP Level and TDI Profile

There was a significant negative correlation between VCFc in week 1 and acute phase plasma BNP level ( $r = -0.37$ ,  $p = 0.018$ ). There was a significant negative correlation between LVSF in week 1 and acute phase plasma BNP level ( $r = -0.42$ ,  $p = 0.003$ ). There was a significant and stronger negative correlation between Sw velocity in week 1 and acute phase plasma BNP level than for VCFc and

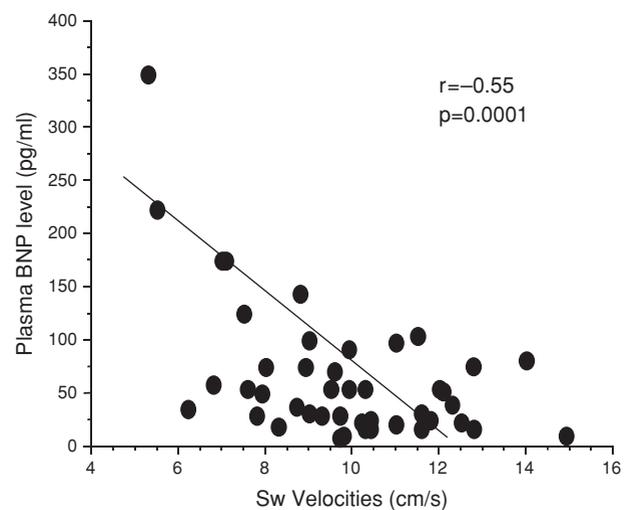


Fig 2. Correlation between plasma brain natriuretic peptide (BNP) level and week 1 systolic tissue Doppler velocity (Sw velocity) was a significant negative correlation.

LVSF ( $r = -0.55$ ,  $p = 0.001$ ) (Fig 2). We subdivided the KD group into 2 groups using median BNP (51 pg/ml): BNP  $\geq 51$  ( $n = 21$ ) and BNP  $< 51$  ( $n = 21$ ). The change in Sw velocity in the BNP  $\geq 51$  group was significantly greater than in the BNP  $< 51$  group ( $p < 0.05$ ) (Fig 3). Although the increase in LVSF in the BNP  $\geq 51$  group was also significantly greater than in the BNP  $< 50$  group ( $p < 0.05$ ) (Fig 4), Ew, Ew/Aw, E/Ew, and E/A did not significantly differ between groups.

#### Urinary 8-Isoprostane Level in Acute KD

The pre-IVIG therapy urinary 8-isoprostane level was significantly higher than in the control group ( $596 \pm 37$  vs  $379 \pm 26$  pg/ml Cr,  $p < 0.05$ )

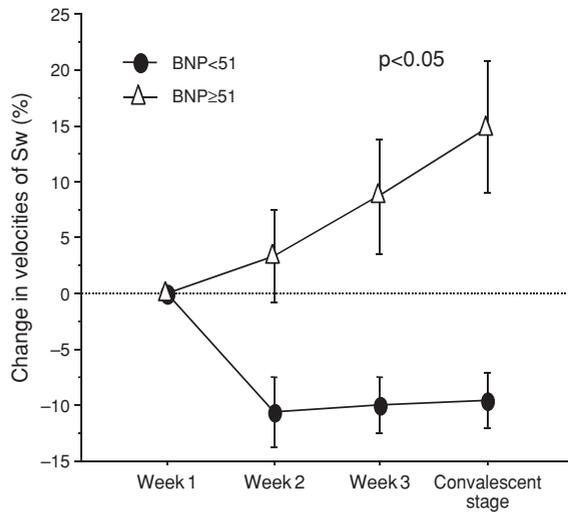


Fig 3. Time course of changes in the systolic tissue Doppler velocity (Sw velocity). Increases in Sw velocity in the brain natriuretic peptide (BNP)  $\geq 51$  pg/ml group were significantly greater than those in the BNP  $< 50$  pg/ml group.

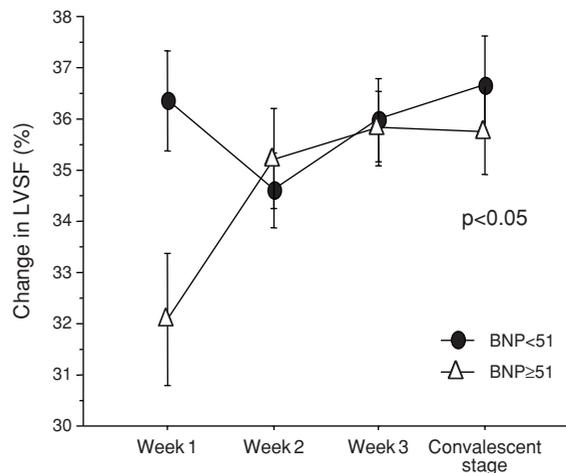


Fig 4. Time course of changes in left ventricular shortening fraction (LVSF). The increase in the LVSF of the brain natriuretic peptide (BNP)  $\geq 51$  pg/ml group was significantly greater than in the BNP  $< 50$  pg/ml group.

#### Correlation Between Urinary 8-Isoprostane Level and TDI Profile

LVSF in week 1 was significantly negatively correlated with urinary 8-isoprostane level ( $r = -0.34$ ,  $p = 0.02$ ). There was a significant negative correlation between Sw velocity at week 1 and urinary 8-isoprostane level ( $r = -0.48$ ,  $p = 0.001$ ) (Fig 5). VCFc showed no significant correlation with urinary 8-isoprostane level ( $r = -0.25$ ,  $p = 0.13$ ). E/Ew in week 1 showed a weak, but significant, positive correlation with urinary 8-isoprostane level ( $r = 0.31$ ,  $p = 0.04$ ).

#### Correlation Between Plasma BNP Level and Urinary 8-Isoprostane Level

The plasma BNP level was weakly but significantly positively correlated with the urinary 8-isoprostane level ( $r = 0.39$ ,  $p = 0.01$ ) (Fig 6).

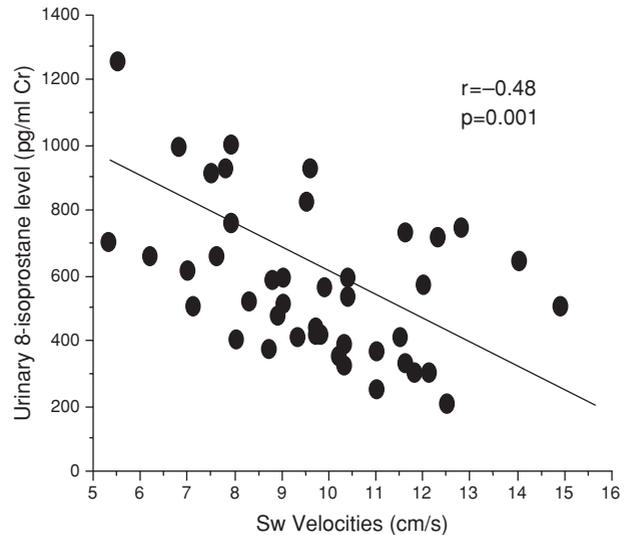


Fig 5. Correlation between systolic tissue Doppler velocity (Sw velocity) in week 1 and urinary 8-isoprostane level was a significant negative correlation. Cr, creatinine.

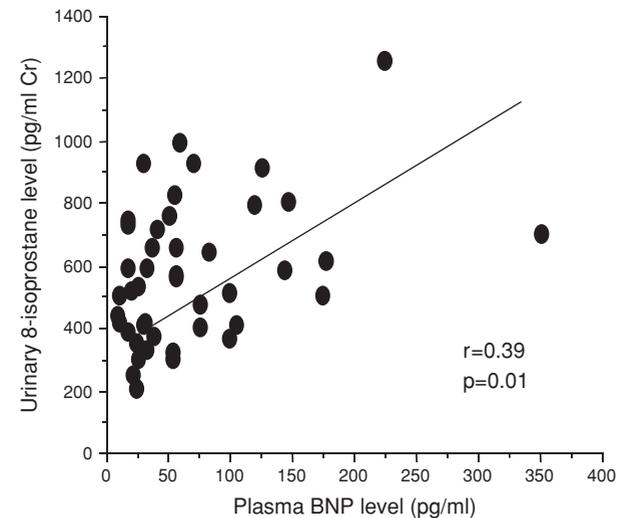


Fig 6. Correlation between plasma brain natriuretic peptide (BNP) level and urinary 8-isoprostane level was a significant positive correlation. Cr, creatinine.

## Discussion

#### Diastolic Function in Acute KD

The mean week-1 Ew velocity was significantly lower in the patients than in the controls, but normalized during the convalescent phase. It has been reported that decreased Ew velocity in conjunction with a decreased Em/Am ratio is strongly correlated with the isovolumic LV relaxation constant ( $\tau$ )<sup>29</sup> and reflects early diastolic recoil dysfunction.<sup>30,31</sup> Decreased preload index and increased ESWS in acute KD have been reported.<sup>3</sup> Although we also used ESWS as an after-load marker for the LV in this study, ESWS did not significantly change at any stage. HR was significantly higher in week 1 as compared with controls, but significantly decreased after week 2 because almost all patients become afebrile after IVIG or steroid pulse therapy. Although HR significantly changed between weeks 1 and 2, Ew

velocity increased by only 5%, with no significant increase between week 1 and week 2 and significant accentuation after week 3. This suggests that the change in Ew velocity in acute KD is relatively independent of both ESWS and HR. It has been reported that HR and BP do not have a significant effect on Ew velocity.<sup>10,32</sup> On the other hand, some studies report that increasing HR influences the diastolic parameters, especially Aw.<sup>33,34</sup> In contrast to the Ew velocity, the Aw velocity showed a significant reduction from week 1 to week 2, which resulted in a significantly increased Ew/Aw from week 1 to week 2. The influence of a change in HR on Ew/Aw needs to be taken into account.

#### *Systolic Function in KD*

It has been reported that Sw velocity sensitively reflects regional long-axis ventricular systolic function,<sup>6-9</sup> and we used Sw as a marker of longitudinal systolic function. We also used LVSF as an approximate indicator. In the present study, VCFc and LVSF did not significantly change at any phase. Newburger et al reported that both LVSF and VCFc were commonly suppressed within 10 days of onset,<sup>2</sup> contrary to the results of the present study. In their study, only 58% of patients received IVIG within 10 days of onset; in the present study, all patients received IVIG within 7 days of onset. This difference in initiating IVIG therapy may explain the differing results. Moran et al reported rapid improvement of abnormal mechanics after IVIG,<sup>3</sup> a finding consistent with ours. Takahashi suggested that relatively renewable structures such as intramyocardial microvessels explain the functional reversibility of abnormal myocardial mechanics in acute KD.<sup>4</sup>

#### *Plasma BNP Level in Acute KD*

In this study, significantly increased plasma BNP levels were observed in acute KD patients, but these quickly normalized in the convalescent stage, which is consistent with an earlier report.<sup>17</sup> The mechanism responsible for the increased plasma BNP level in acute KD is not yet known. Kawamura et al maintain that pro-inflammatory cytokines, such as interleukin-1 and TNF- $\alpha$ , cause myocarditis and stimulate secretion of BNP in acute KD.<sup>17</sup> They also reported no significant correlation between plasma BNP level and LV ejection fraction (LVEF) in acute KD. In contrast, in the present study, LVSF, VCFc, and Sw in week 1 significantly correlated with plasma BNP level. Moreover, the change in Sw velocity from baseline significantly differed between the BNP  $\geq 51$  pg/ml (high BNP) and BNP  $< 51$  pg/ml (low BNP) groups. These results suggest the Sw velocities obtained by TDI are more closely associated with increased plasma BNP level than with either LVSF or VCFc. Suppressed myocardial mechanics may partly contribute to BNP production from the ventricle in acute KD. Agricola et al assumed that TDI velocities are a more sensitive marker than the LVEF because TDI is relatively preload-independent and because of the architecture of myocardial fibers.<sup>35</sup>

#### *Oxidative Stress in Acute KD*

Urinary 8-isoprostane levels were significantly higher as compared with controls, suggesting that enhanced systemic oxidative stress may contribute to acute KD pathogenesis. Further study is necessary to clarify the mechanism responsible for this.

Week 1 LVSF and Sw velocity were significantly negatively correlated with urinary 8-isoprostane level. Wolfman et al reported a significant negative correlation between 8-

isoprostane level and LVEF in patients with chronic heart failure;<sup>36</sup> and Mobert et al<sup>37</sup> reported that 8-isoprostane induced a concentration-dependent decrease in coronary flow; alterations in coronary flow were associated with a parallel reduction of LV pressure and dP/dt (max). 8-isoprostane is a potent vasoconstrictor;<sup>38-40</sup> an effect that may contribute to enhanced peripheral vascular tone and have a negative influence on coronary arterial and intracardiac microvascular blood flow, thus leading to depressed cardiac functional capacity in acute KD patients. Interestingly, we observed a significant correlation between plasma BNP level and urinary 8-isoprostane level. Nonaka-Sarukawa et al reported a significant correlation between urinary F2-isoprostane concentration and plasma BNP level in patients with congestive heart failure.<sup>23</sup> The mechanisms responsible for these correlations are not yet known. In the present study, both BNP and 8-isoprostane levels correlated with week 1 Sw velocity. Therefore, we assume that ventricular dysfunction associated with enhanced oxidative stress resulting in suppression of cardiac capacity may contribute to increased production of plasma BNP from the ventricle.

#### *Study Limitations*

First, high-dose IVIG therapy for acute KD might affect the TDI profile because it has high osmolality<sup>41</sup> and is associated with volume overload. Second, because urinary 8-isoprostane may be influenced by many factors, it is difficult and premature to conclude that this substance has a clear effect on oxidative stress. Third, although an increased urinary 8-isoprostane level may reflect systemic oxidative stress, it is difficult to evaluate its local production and effects in the heart. Fourth, the influence of the administration of aspirin, a cyclooxygenase inhibitor, cannot be excluded, because small amounts of 8-isoprostane can be formed by human platelets and monocytes through a cyclooxygenase-dependent mechanism. Fifth, the 8-isoprostane levels in KD were only compared with healthy subjects; its levels in febrile conditions other than KD have not been revealed. Sixth, the number of patients enrolled in this study was quite low. Finally, the observation period in this study was very short; further long-term observation is necessary.

## **Conclusion**

Latent abnormal tissue Doppler profiles, possibly reflecting long-axis systolic and diastolic dysfunction, have been noted in acute KD patients. Increased plasma BNP level is associated with abnormal tissue Doppler profiles, suggesting that abnormal myocardial mechanics may partly contribute to the increased plasma BNP level observed in acute KD. Increased urinary 8-isoprostane level indicates the presence of enhanced oxidative stress in acute KD and the urinary 8-isoprostane level correlates with transient myocardial dysfunction, suggesting that enhanced oxidative stress may contribute to cardiac dysfunction in acute KD.

#### *Acknowledgments*

We thank F Sawa, MD, K Katsumori, MD, S Shirakawa, MD, K Takeuchi, MD, Y Uemura, MD, Y Shimoda, MD, Y Haga, MD and the nursing staff at Tokyo Rinkai Hospital for their assistance.

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