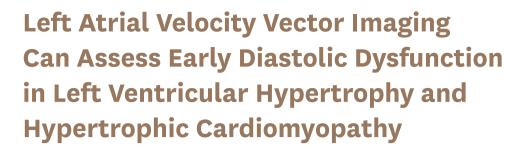
# Original Article





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# **ABSTRACT**

BACKGROUND: The function of left atrium (LA) is difficult to assess because of its ventricledependent, dynamic movement. The aim of this study was to assess LA function using velocity vector imaging (VVI) and compare LA function in patients with hypertrophic cardiomyopathy (HCMP) and left ventricular hypertrophy (LVH) with normal controls. METHODS: Fourteen patients with HCMP (72% male, mean age of 52.6 ± 9.8), 15 hypertensive patients with LVH (88% male, mean age of 54.0 ± 15.3), and 10 age-matched controls (83% male, mean age of  $50.0 \pm 4.6$ ) were prospectively studied. Echocardiographic images of the LA were analyzed with VVI, and strain rate (SR) was compared among the 3 groups. **RESULTS:** The e' velocity  $(7.7 \pm 1.1; 5.1 \pm 0.8; 4.5 \pm 1.3 \text{ cm/sec}, p = 0.013), E/e' <math>(6.8 \pm 1.6; 12.4)$  $\pm$  3.3; 14.7  $\pm$  4.2, p = 0.035), and late diastolic SR at mid LA (-1.65  $\pm$  0.51; -0.97  $\pm$  0.55; -0.82  $\pm$  0.32, p = 0.002) were significantly different among the groups (normal; LVH; HCMP, respectively). The e' velocity, E/e', and late diastolic SR at mid LA were significantly different between normal and LVH (p = 0.001; 0.022; 0.018), whereas LA size was similar between normal and LVH (p = 0.592). The mean late diastolic peak SR of mid LA was significantly correlated with indices of diastolic function (E/e', e', and LA size). **CONCLUSIONS:** The SR is a useful tool for detailed evaluation of LA function, especially early dysfunction of LA in groups with normal LA size.

Keywords: Left atrial function; Left ventricular hypertrophy; Hypertrophic cardiomyopathy

# INTRODUCTION

The left atrium (LA) functions not only as a reservoir and conduit to the left ventricle (LV), but also as an atrial pump. <sup>1)2)</sup> The hemodynamic importance of the LA for cardiac performance has been studied by using both invasive<sup>3)</sup> and noninvasive methods, such as M-mode, 2-dimensional, Doppler echocardiography, and acoustic quantification techniques. <sup>4)5)</sup>

https://e-jcvi.org 41

However, quantitative assessment of LA function using invasive methods has been clinically difficult. Although several noninvasive methods have been used to assess LA function, <sup>4)5)</sup> major limitations including single-plane assessment, dependence of altered left ventricular hemodynamics (dynamic movement as systolic distension, early diastolic contraction, and late diastolic atrial kick), and image quality have prevented clinical application.

Strain rate (SR) imaging has been proposed as a useful noninvasive echocardiographic technique to quantify regional myocardial function independent of cardiac rotational motion and tethering effects<sup>6)7)</sup> and has been introduced for the evaluation of regional left ventricular and right ventricular function.<sup>8)</sup> Traditionally, strain and SR have been derived from tissue Doppler imaging (TDI) and are Doppler angle dependent. An angle-independent method would allow the evaluation of regional function in all myocardial segments including LA. Velocity vector imaging (VVI) is a useful technique that is angle-independent and thus provides a more precise avenue to evaluate LA function.<sup>9)</sup>

The purpose of this study was to assess the feasibility of measuring LA function precisely in each systolic and diastolic phase using SR imaging and to compare LA function among patients with hypertrophic cardiomyopathy (HCMP) and left ventricular hypertrophy (LVH) with normal controls.

### **METHODS**

## **Study population**

Between January 2006 and July 2010, we prospectively included patients and control group participants who were referred to our echocardiographic laboratories for risk stratification and chest pain from out-patient clinics. Fourteen patients with HCMP (72% male, mean age of 52.6  $\pm$  9.8), 15 hypertensive patients with LVH (88% male, mean age of 54.0  $\pm$  15.3), and 10 age-matched controls (83% male, mean age of 50.0  $\pm$  4.6) were prospectively and sequentially enrolled.

They were examined in a single center (Division of Cardiology, Severance Cardiovascular Hospital, Yonsei University College of Medicine). The rhythm of all patients was normal sinus rhythm. The diagnosis of HCMP was based on familial history, physical examination, electrocardiogram, and conventional echocardiographic demonstration of a non-dilated, asymmetric septal hypertrophic LV (septal thickness  $\geq$  15 mm) in the absence of other cardiac or systemic diseases capable of producing the magnitude of hypertrophy evident. Apical HCMP

and variant types were excluded. Patients with valvular heart disease, congenital heart disease, coronary artery disease, restrictive cardiomyopathy, chronic liver disease, or chronic renal disease were excluded.

## **Echocardiographic study**

Transthoracic echocardiographic studies were performed by standard techniques using a 2.5-MHz transducer interfaced with commercially available ultrasound systems (Sequoia C512; Acuson, Inc., Mountain View, CA, USA). Transthoracic studies were performed with the patients in the left lateral decubitus position, and images were obtained from parasternal and apical windows. The Doppler study included color, pulsed, and continuous-wave examinations. Two dimensional echocardiographic images of the LA and LV were obtained in the standard parasternal long-axis, and apical 2- and 4-chamber views with second harmonic imaging. LV ejection fraction were assessed by the modified Quinones method. LV hypertrophy was defined as abnormal LV mass index (LVMI), which was calculated using the following equations<sup>10)</sup>:

LV Mass = 0.8 (1.04 [LVID + PWTd + SWTd]<sup>3</sup> – [LVID]<sup>3</sup>) + 0.6 g LVMI = LVM/Body Surface Area LVMI (reference range, male)  $\leq$  115 g/m<sup>2</sup> LVMI (reference range, female)  $\leq$  95 g/m<sup>2</sup>

LA volumes were calculated from the parasternal long axis and apical 4-chamber view using the prolate ellipsoid model. <sup>10)</sup> For evaluation of diastolic function, sample volume (size 2 mm) of the pulsed wave Doppler was placed between the tips of the mitral leaflets in the apical 4 chamber view. Early (E) and late (A) transmitral flow velocities were obtained. Pulsed wave Doppler tissue imaging (DTI) was performed by activating DTI function in the same machine. Sample volume was located at the septal side of the mitral annulus. Early (e') and late (A') diastolic mitral annular velocities and the ratio of early diastolic transmitral flow velocities (E) to early diastolic mitral annulus velocities (E/e') were obtained. Studies were stored digitally and analyzed offline.

## **Measurements of SR imaging in LA**

To assess optimal LA function, the apical approach was obtained. The largest size of LA base and the longest LA length were captured to ensure alignment along the true long axis of the LA. <sup>10)</sup> Echocardiographic images of LA in apical 2- and 4-chamber views were analyzed by conventional manual tracing by software (Axius Velocity Vector Imaging; Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) (**Figure 1**). We compared the peak velocity, strain, and SR among the groups to assess left atrial dynamics. The offline software VVI provides

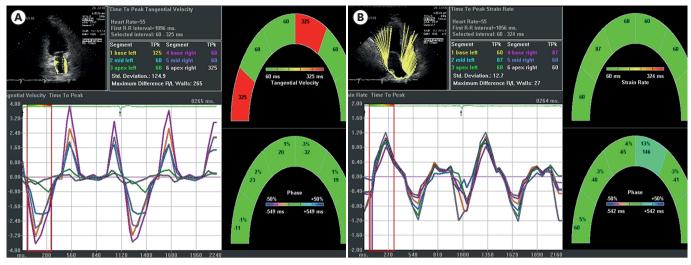


Figure 1. Velocity vector imaging in analysis of left atrium function. Graphic representation of the analyzed peak velocity (A) and peak strain rate (B).

volumetric and regional functional information. Endocardial borders are automatically tracked throughout the cardiac cycle once a reliable endocardial tracing over one frame has been manually drawn. Ultrasound speckles in the image are tracked, a method commonly referred to as speckle tracking, and myocardial velocity is derived from the tracking as the ratio between frame-to-frame displacement and the time interval. These velocity vectors in the 2-dimensional plane are displayed throughout the cardiac cycle. From the same tracked contour of the endocardium, 2-dimensional strain and SR are obtained by comparing the displacement of the speckles in relation to one another along the endocardial contour throughout the cardiac cycle. The apical 2- and 4-chamber images used for conventional measurements of LA were analyzed with VVI by 2 investigators who were blinded to the conventional data. The endocardial border of the LA was traced manually and was automatically tracked by the software. The septal, lateral, anterior, and posterior walls of the LA were explored by positioning a region of interest in the basal, mid, and apical regions (total 12 points). Systolic, early diastolic, and late diastolic peak velocity; strain (S); and SR were measured as parameters of LA function among the 3 groups.

### Statistical analysis

Results are expressed as mean ± standard deviation, and the data were analyzed using SPSS (version 20.0; SPSS Inc., Chicago, IL, USA). To assess differences between the 3 groups, analysis of variance and *post hoc* analysis (Tukey's) were used, and to evaluate the correlation between each variable and LA function, Pearson's correlation coefficients were performed separately on clinical variables. A p-value < 0.05 was considered statistically significant.

## **RESULTS**

# Reproducibility and feasibility data and segmental variation of SR parameters

Inter- and intra-observer variabilities of the mean SR were evaluated in randomly selected controls (n = 10). For the assessment of inter- and intra-observer variabilities of SR variables, we repeated the entire processes from the initial 2-dimensional tissue Doppler image. The mean absolute differences and coefficients of variance (CV, %) of inter-observer variability for mean late diastolic SRs were  $0.38 \pm 0.16 \, \text{s}^{-1}$  at apex (CV: 7.5%),  $0.71 \pm 0.41 \, \text{s}^{-1}$  at mid (CV: 6.5%), and  $0.82 \pm 0.14 \, \text{s}^{-1}$  at base (CV: 7.2%).

# Clinical characteristics and baseline echocardiographic findings

The baseline systolic function, LA size, E velocity, A velocity and A' velocity were not different in the 3 groups. The e' velocity (normal; LVH; HCMP =  $7.7 \pm 1.1$ ;  $5.1 \pm 0.8$ ;  $4.5 \pm 1.3$  cm/sec, p = 0.013) and E/e' (normal; LVH; HCMP =  $6.8 \pm 1.6$ ;  $12.4 \pm 3.3$ ;  $14.7 \pm 4.2$ , p = 0.035) were significantly different between the groups (**Table 1**). Post-hoc analysis revealed significant difference of e' velocity and E/e' between normal and LVH groups (p = 0.001; 0.022; 0.018), but not between LVH and HCMP groups. LA size was similar between normal and LVH (p = 0.592; **Figure 2**).

# Comparison of late diastolic SR in 3 groups (1/sec)

The late diastolic SR in 12 segments of LA are listed in **Table 2**. The mean late diastolic SR showed a tendency for the absolute value to decrease from base to apex.

The mean late diastolic SRs were significantly different in mid and apical levels of LA between the 3 groups (mid, normal;

Table 1. Clinical characteristics and baseline echocardiographic findings

Characteristics	Normal group (n = 10)	LVH group (n = 15)	HCMP group (n = 14)	p-value
Age (years)	50.0 ± 4.6	54.0 ± 15.3	52.6 ± 9.8	0.060
Male (%)	83.3	87.5	72.2	0.120
Hypertension	0 (0)	15 (100)	5 (36)	0.020*
DM	0 (0)	3 (20)	2 (14)	0.235
SBP (mmHg)	$118.2 \pm 4.4$	$140.9 \pm 5.3$	$132.8 \pm 3.5$	0.812
DBP (mmHg)	$82.1 \pm 8.6$	$91.5 \pm 7.2$	$85.3 \pm 2.3$	0.698
LVEDD (mm)	$50.9 \pm 2.5$	$52.3 \pm 3.3$	$50.7 \pm 1.5$	0.396
LVESD (mm)	34.2 ± 3.9	$35.6 \pm 3.4$	33.1 ± 1.9	0.289
IVS end diastole (mm)	$9.8 \pm 1.1$	$12.3 \pm 2.3$	$15.7 \pm 2.8$	0.040*
PW end diastole (mm)	$10.8 \pm 0.9$	$13.0 \pm 1.5$	$15.8 \pm 2.1$	0.032*
EF (%)	$67.2 \pm 4.9$	$65.3 \pm 10.7$	$70.8 \pm 5.3$	0.210
RWT	$0.41 \pm 0.13$	$0.49 \pm 0.08$	$0.62 \pm 0.29$	0.024*
LV mass (g)	$194.4 \pm 39.1$	$269.7 \pm 25.5$	$353.6 \pm 22.8$	0.017*
LAVI (mL/m²)	$22.0 \pm 1.7$	$26.6 \pm 9.1$	$34.7 \pm 9.7$	0.008*
E (cm/sec)	$51.8 \pm 6.3$	$62.1 \pm 16.9$	$68.2 \pm 17.4$	0.672
A (cm/sec)	$58.1 \pm 9.4$	$78.0 \pm 16.6$	$69.7 \pm 18.6$	0.064
e' (cm/sec)	$7.7 \pm 1.1$	$5.1 \pm 0.8$	$4.5 \pm 1.3$	0.013*
a' (cm/sec)	9.2 ± 1.8	$9.1 \pm 1.5$	$7.6 \pm 1.8$	0.060
E/e'	$6.8 \pm 1.6$	$12.4 \pm 3.3$	$14.7 \pm 4.2$	0.035*

Values are presented as number (%) or mean ± standard deviation.

DBP: diastolic blood pressure, EF: ejection fraction, DM: diabetes mellitus, HCMP: hypertrophic cardiomyopathy, IVS: interventricular septum, LAVI: left atrial volume index, LV: left ventricle, LVEDD: left ventricular end diastolic dimension, LVESD: left ventricular end systolic dimension, LVH: left ventricular hypertrophy, PW: posterior wall, RWT: relative wall thickness, SBP: systolic blood pressure.

\*p < 0.05.

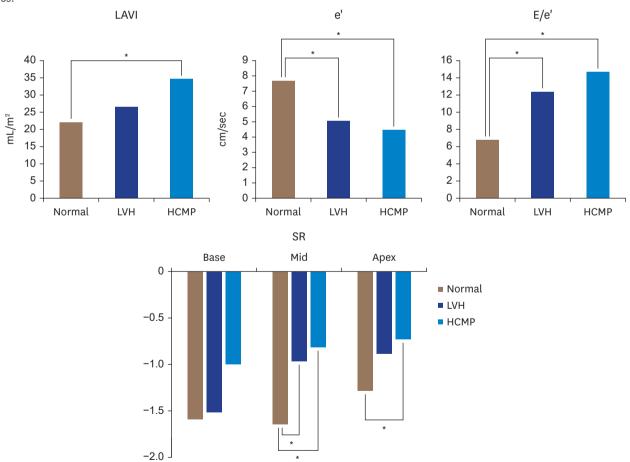


Figure 2. Comparison of diastolic functional index and mean late diastolic SR in 3 groups (1/sec). Post hoc analysis revealed significant difference of e', E/e', and mean late diastolic SR of mid LA between normal and LVH, but not between LVH and HCMP. LAVI did not show a significant difference between normal and LVH groups. HCMP: hypertrophic cardiomyopathy, LA: left atrium, LAVI: left atrial volume index, LVH: left ventricular hypertrophy, SR: strain rate.

Table 2. Comparison of late diastolic strain rate in 3 groups (1/sec)

Groups/Locations	Anterior	Inferior	Lateral	Septum	Mean
Normal					
Base	$-1.58 \pm 0.68$	$-1.55 \pm 0.87$	$-1.25 \pm 0.92$	$-1.98 \pm 1.21$	$-1.59 \pm 0.70$
Mid	$-1.54 \pm 0.25$	$-1.66 \pm 0.60$	$-1.92 \pm 1.69$	$-1.47 \pm 0.77$	$-1.65 \pm 0.51$
Apex	$-1.11 \pm 0.47$	$-1.16 \pm 0.52$	$-1.38 \pm 0.96$	$-1.53 \pm 1.33$	$-1.29 \pm 0.50$
LVH					
Base	$-1.63 \pm 1.61$	$-2.47 \pm 2.06$	$-1.03 \pm 0.51$	$-0.94 \pm 0.61$	$-1.52 \pm 1.04$
Mid	$-1.06 \pm 0.82$	$-1.02 \pm 0.57$	$-0.99 \pm 0.65$	$-0.79 \pm 0.47$	$-0.97 \pm 0.55$
Apex	$-0.97 \pm 0.54$	$-0.93 \pm 0.43$	$-0.79 \pm 0.38$	$-0.87 \pm 0.46$	$-0.89 \pm 0.50$
HCMP					
Base	$-1.04 \pm 0.63$	$-0.88 \pm 0.76$	$-1.02 \pm 0.63$	$-1.21 \pm 0.70$	$-1.00 \pm 0.46$
Mid	$-0.95 \pm 0.64$	$-0.82 \pm 0.46$	$-0.76 \pm 0.56$	$-0.78 \pm 0.42$	$-0.82 \pm 0.32$
Apex	$-0.79 \pm 0.49$	$-0.66 \pm 0.40$	$-0.78 \pm 0.43$	$-0.64 \pm 0.34$	$-0.73 \pm 0.27$

HCMP: hypertrophic cardiomyopathy, LVH: left ventricular hypertrophy.

Table 3. Comparison of mean late diastolic strain rate in 3 groups (1/sec)

Locations	Normal	LVH	НСМР	p-value
Base	$-1.59 \pm 0.70$	$-1.52 \pm 1.04$	$-1.00 \pm 0.46$	0.122
Mid	$-1.65 \pm 0.51$	$-0.97 \pm 0.55$	$-0.82 \pm 0.32$	0.002*
Apex	$-1.29 \pm 0.50$	$-0.89 \pm 0.50$	$-0.73 \pm 0.27$	0.003*

HCMP: hypertrophic cardiomyopathy, LVH: left ventricular hypertrophy.  $^{\ast}p < 0.05.$ 

LVH; HCMP =  $-1.65 \pm 0.51$ ;  $-0.97 \pm 0.55$ ;  $-0.82 \pm 0.32$ , p=0.002; apex, normal; LVH; HCMP=  $-1.29 \pm 0.50$ ;  $-0.89 \pm 0.50$ ;  $-0.73 \pm 0.27$ , p = 0.003) (**Table 3, Figure 2**). *Post hoc* analysis revealed a significant difference between normal and LVH (p = 0.018) but not between LVH and HCMP (**Figure 2**).

The systolic and early diastolic SRs of LA were not significantly different between the 3 groups (not in table).

# Correlation between mean late diastolic SR and other variables

The mean late diastolic peak SRs of mid LA are significantly correlated with age (r = 0.434, p = 0.016), E/e' (r = 0.383, p = 0.037),

Table 4. Correlation between mean late diastolic strain rate and other variables

Variables		Base	Mid	Apex
Age	r	0.286	0.434	0.266
	p-value	0.126	0.016*	0.155
E/e′	r	0.149	0.383	0.348
	p-value	0.432	0.037*	0.059
E (cm/sec)	r	0.092	0.271	0.343
	p-value	0.630	0.147	0.064
A (cm/sec)	r	-0.401	-0.066	0.053
	p-value	0.380	0.745	0.791
e' (cm/sec)	r	-0.280	-0.453	-0.350
	p-value	0.134	0.012*	0.058
A' (cm/sec)	r	-1.181	-0.037	0.068
	p-value	0.366	0.853	0.735
LAVI (mL/m²)	r	0.334	0.407	0.354
	p-value	0.071	0.025*	0.055
EF (%)	r	-0.001	0.009	-0.118
	p-value	0.995	0.961	0.533

Values set in boldface denote statistical significance. EF: ejection fraction. LAVI: left atrial volume index.

EF: ejection fraction, LAVI: left atrial volume index.

\*p < 0.05.

e' (r = -0.453, p = 0.012), and LA size (r = 0.407, p = 0.025) (**Table 4, Figure 3**).

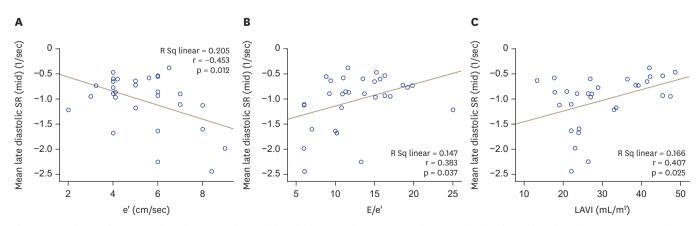


Figure 3. Correlation of mean late diastolic SR at mid LA and diastolic function. There was a significant correlation of mean late diastolic SR at mid LA and e', E/e', and LAVI.

LA: left atrium, LAVI: left atrial volume index, SR: strain rate.



# **DISCUSSION**

To our knowledge, this study is the first trial to show significant dynamic LA contractile function in HCMP and LVH with the automated speckle-tracking method using VVI. We demonstrated the difference of atrial contractile function among groups with HCMP, LVH, and normal controls with VVI. We also revealed significant correlation with current useful indices of diastolic function (e', E/e', and LA size) and early change of LA dysfunction.

Function of LA is important in correlation with symptoms, arrhythmic events, and prognosis. <sup>11)</sup> However it is difficult to assess because of its ventricle-dependent dynamic movement as systolic distension, early diastolic contraction, and late diastolic atrial kick. Strain and SR imaging has been proposed as a useful noninvasive echocardiographic technique to quantify regional myocardial function independent of cardiac rotational motion and tethering effect. <sup>6)</sup> It can detect those subtle changes in the myocardium that cannot be recognized by conventional echocardiographic parameters in 4 chambers. <sup>8)</sup> In assessment of LA function, strain and SR also showed useful results in many studies. <sup>12)13)</sup>

LA is mostly thought to be as a reservoir and conduit to the LV, but it also functions as an atrial pump. There are many studies assessing LA function in various diseases but few studies have been performed to show the contractile function of LA. Donal et al.<sup>14)</sup> represented the positive correlation between capacity of exercise in patients with congestive heart failure and end-diastolic pulsed wave Doppler tissue velocity at the mitral annulus. In our study, we enrolled relatively homogenous patients with mild diastolic dysfunction and normal systolic function. Although previous reports evaluating mitral flow Doppler and diastolic mitral annular motion suggested possible correlation with atrial dysfunction, mitral annular motion does not directly represent atrial function and it is heavily influenced by ventricular function. Thus, atrial SR and strain are suggested as strong indexes of regional myocardial function. <sup>10)15)</sup>

Another study in pediatric patients showed a significant difference in atrial function via SR with TDI between control and HCMP. They found differences in systolic, early and late diastolic SR of atrium, and late diastolic SR in mid LA. Further, they revealed significantly higher values in the control group as compared with HCMP (septum and lateral wall), which was similar to our results. In another study, Paraskevaidis et al. To analyzed longitudinal left atrial function with TDI and 2-dimensional strain in HCMP, LVH, and normal groups. They

showed longitudinal strain data of atrial contractile function using a different diagnostic tool (Echopac; GE Medical systems, Horten, Norway) and revealed significant differences of LA strain in all systolic, early diastolic, and late diastolic phases between those groups. Eshoo et al. 18) performed a similar study design with tissue Doppler, which showed reduced diastolic SR in HCMP. Zhang et al. 19) revealed that the active, less load-dependent booster pump functioned as well as a passive reservoir, that conduit functions of atria can be precisely evaluated by a tissue Doppler-based technique, and that TDI can provide additional information of atrial contractile function. The feasibility and reproducibility of TDI parameters, in particular peak velocity and peak SR of the active atrial contraction, have been demonstrated in a previous study. 20)

Many researchers have actively studied the VVI technique for assessment of various cardiac functions. Yang et al.<sup>9)</sup> revealed a similar research design as our study using VVI comparing the control group with hypertensive patients with and without LVH. They identified a significant difference of early and late diastolic SR between the normal and LVH groups, and late diastolic SR revealed significant positive correlation with E/e'. Huang et al.<sup>21)</sup> reported RA function assessment in HCMP with longitudinal strain. Both reservoir and conduit SR were significantly lower than normal controls, and the booster function showed lower absolute values in HCMP patients.

Many studies have been performed on LA mechanics in LVH and HCMP using VVI, <sup>22)23)</sup> and studies discussing the LA contractile function with strain have focused on groups of atrial fibrillation or Fabry disease with VVI. <sup>24)25)</sup> Some remarkable studies revealed a strong correlation between active emptying of LA and LV diastolic function using 3D echocardiography<sup>26)</sup> or clinical output with cardiac magnetic resonance imaging. <sup>27)28)</sup>

However, some researchers showed that contractile function of LA was relatively preserved while reservoir and conduit function were significantly impaired among HCMP patients as measured by LA strain, <sup>22)23)29)</sup> results of patients with different ages, heart function, and analyzing systems. Kobayashi et al. <sup>29)</sup> calculated LA strain with LA wall length and presented impaired total and passive LA function in obstructive HCMP. Other studies showed preserved contractile function using XStrain software (Esaote S.p.A, Florence, Italy). <sup>22)23)</sup>

In the present study, we demonstrated the precise evaluation of contractile function of LA by means of SR imaging and showed the feasibility of mean late diastolic SR of mid LA in assessing LA function. Interestingly, mean late diastolic SR



of mid LA, E and E/e' showed a significant difference between normal and LVH but not LVH and LA size. Taken together, these results suggest that LA SR can detect the early change of LA remodeling before significant volume change develops. This point was also mentioned in recent studies using MR.<sup>27/28)</sup> Conclusively, it suggested that the more contractile mid LA is, the better LA functions.

SR is not significantly different in systolic and early diastolic phases, and in our data, SR of base is so diverse because of LV-dependent annular motion. Other peak velocity and strain are not significantly correlated with these variables of diastolic function.

VVI offers the advantages of simultaneous determination of global and regional functions<sup>30)</sup> and is another useful technique that can precisely assess LA function. This useful method needs further validation with invasive monitoring. Two limitations of this study are the relatively small sample size and the exclusion of severe heart failure, so a large-scale prospective and longitudinal study is needed to determine whether measurement with SR of LA function is accurate and objective in patients with not only mild diastolic dysfunction but also with more aggravated LA function.

In the present study, we demonstrated the precise evaluation of function of LA by means of SR imaging. VVI offers the advantages of simultaneous determination of global and regional function of LA and is another useful technique that can effectively assess atrial function and detect early LA dysfunction in groups with normal LA size.

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#### **Conflict of Interest**

The authors have no financial conflicts of interest.

#### **Author Contributions**

Conceptualization: Ha JW, Yoon SJ, Park S; Data curation: Yoon SJ, Seo HS, Shim CY, Ahn CM; Formal analysis: Yoon SJ, Seo HS, Shim CY, Ahn CM, Kim SA; Investigation: Yoon SJ, Choi EY, Shim CY, Ahn CM, Kim SA; Methodology: Yoon SJ, Park S, Choi EY; Supervision: Ha JW, Park S; Writing - original draft: Yoon SJ; Writing - review & editing: Ha JW, Shim CY.

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