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The Repeatability and Left Atrial Strain Analysis Obtained via Speckle Tracking Echocardiography in healthy Dogs☆

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Abstract *Introduction:* In left atrial (LA) strain—derived two-dimensional speckle tracking echocardiography, the reference intervals in healthy dogs can provide useful information to evaluate the LA function in dogs with heart disease.
Animals: Six laboratory beagles and 120 privately owned dogs without cardiac diseases were recruited.
Materials and methods: The LA strain and strain rate (SR) and echocardiographic indices were obtained in dogs who underwent standard echocardiography and off-line analysis for LA strain and SR measurement by speckle tracking echocardiography.
Results: The intra-observer within-day variations of strain variables showed adequate repeatability (coefficient of variation <20%). The mean values of strain were 25.37 for the LA reservoir function, 11.06 for the LA conduit function, and 14.17 for the LA booster-pump function; the strain was significantly correlated with the LA fractional volume change at each phasic function. The left atrial longitudinal strain

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during early ventricular diastole showed moderate correlation with the peak velocity of early diastolic transmitral flow (r = 0.5560) and ratio of peak velocity of early diastolic transmitral flow to peak velocity of late transmitral flow (r = 0.5515).

In multiple regression analysis, only age was significantly related to the strain/SR and volumetric change indices, indicating conduit function.

Conclusions: Left atrial speckle tracking echocardiographic analysis provided useful information to assess the LA function in healthy dogs. The influencing factors on strain and SR variables including the age, body weight, and heart rate should be considered in interpretation of these parameters in a clinical setting.

Abbreviation

A	peak velocity of late diastolic transmitral flow
BW	body weight
CV	coefficient of variation
E	peak velocity of early diastolic transmitral flow
LA	left atrium (atrial)
LA-FVC	left atrial fractional volume change
$LA\text{-}FVC_{active}$	left atrial fractional volume change during atrial contraction
$LA\text{-}FVC_{passive}$	left atrial fractional volume change during early ventricular diastole
LALS	left atrial longitudinal strain
$LALS_a$	left atrial longitudinal strain during atrial contraction
$LALS_e$	left atrial longitudinal strain during early ventricular diastole
$LALS_s$	left atrial longitudinal strain during ventricular systole
SR	strain rate
SR_a	second negative peak strain rate during atrial contraction
SR_e	first negative peak strain rate during early ventricular diastole
SR_s	positive peak strain rate during ventricular systole
STE	speckle tracking echocardiography
2D	two-dimensional

Introduction

The principle role of the left atrium (LA) is to modulate left ventricular filling and cardiovascular performance through three varying mechanical actions, namely, the change in the blood reservoir during ventricular systole, the phase of passive pulmonary venous return flow to the ventricle or conduit during early ventricular diastole, and the booster-pump function or active augmentation of ventricular filling during late diastole [1—3]. In evaluating patients with heart disease, it is important to consider the interaction among these atrial functions and the functional change [1,2]. The increased LA size and volume resulting in LA dysfunction is an important marker for the severity of valvular degenerative disease, cardiomyopathy, and other heart disease [2—9].

In humans, two-dimensional (2D) and three-dimensional echocardiography, cardiac computed tomography, and magnetic resonance imaging have been used to assess LA function [2,3]. In dogs, the assessment of LA function has been focused on calculation of the phasic size of the LA (area and volume) [6—14] and the percent fractional area change using 2D echocardiography [10—14]. In dogs, the severity of chronic mitral valvular heart disease was associated with worse prognoses in those with poorer LA systolic function [11]. However, most of the LA functional parameters are load dependent and influenced by hemodynamic changes related with mitral regurgitation [1]. In addition, LA function can be assessed by transmitral Doppler and pulmonary vein Doppler [15—18]. In addition, an alternative method, i.e., measurement of myocardial deformation through tissue Doppler—derived strain, has been developed [18—20]. However, Doppler-based methods have several limitations, such as angle dependence, tethering effect, and measurement of regional strain alone [1,2]. Two-dimensional speckle tracking echocardiography (2D-STE) based on tracking movement of the myocardial wall throughout the cardiac cycle provides a commonly available, highly reproducible, and angle independent tool for the quantification of LA function [1—3,20]. The 2D-STE allows evaluation of the atrial myocardial deformation expressed as strain and the rate of

deformation expressed as strain rate (SR) [20,21]. Although LA speckle tracking studies were feasible for the assessment of LA myocardial deformation in healthy dogs and in dogs with diseases [7,8,22,23], the data on repeatability and the reference intervals for LA longitudinal strain (LALS) and SR using different echocardiographic machines and offline analysis software applications can show different values [6,22,23]. Moreover, few reports indicated the reference values for conventional 2D echocardiographic parameters as well as other 2D-STE—derived variables including LA fractional volume change (LA-FVC) in dogs. The reference intervals for these variables are useful to estimate the LA function in a clinical setting. As several echocardiographic indices of cardiac function are considered to be influenced by non-pathological factors such as the patients' age, gender, and body size in both humans [24] and animals [9,14,25,26], these variables should be considered when providing normal values.

In this study, we aimed to determine the reference intervals of 2D-STE—based strain variables for estimating the LA function in healthy dogs and the relations of these parameters with 2D-STE—derived LA volumetric indices; in addition, we investigated the effect of the patients' age, heart rate, and body weight (BW) on STE indices.

Materials and methods

Animals

Six healthy beagles (2 males and 4 females, aged 1—4 years, with BW of 8.8—12 kg) from the experimental unit of the faculty of veterinary medicine, Hokkaido University, were recruited in the study for evaluation of the repeatability of 2D-STE indices. All dogs were deemed to be healthy based on routine physical examination including blood examination and standard echocardiography. Each dog underwent echocardiography on three different days at afternoon hours. On a given day, each dog was examined thrice by one of two experienced ultrasonographers (K.N. and T.M.) and thrice thereafter by another ultrasonographer. The order in which the ultrasonographer examined the dog was randomized. In each examination, an apical four-chamber cine loop containing three consecutive cardiac cycles was recorded for offline analysis. In each examination, 2D-STE indices were determined from the average of three cardiac cycles.

A sample of 120 privately owned dogs of varying age, breed, and BW, who were brought to Hokkaido University Veterinary Teaching Hospital between February 2014 and July 2018, were enrolled in the study to determine the reference intervals of 2D-STE indices. The owner's consent was obtained before the animal's recruitment in the study. All dogs were judged with normal cardiac function based on previous medical recordings, absence of any suspected sign of cardiac origin, absence of other abnormal structural cardiac defects, and normal physical and cardiovascular examination including electrocardiogram (ECG) and standard echocardiographic examination (including M-mode, pulsed-wave, and tissue-wave Doppler method) [15,16]. Exclusion criteria included the presence of abnormal heart sound, other concurrent cardiac abnormalities, and systemic diseases known to affect the cardiac structure and function; in addition, dogs undergoing treatment with cardioactive medication were excluded. Mild physiological tricuspid and pulmonic regurgitation from color flow Doppler was defined as physiological tricuspid or pulmonic valve regurgitation under condition of silent to auscultation and normal valve morphology [15]; dogs with mild physiological tricuspid and pulmonic regurgitation were considered as normal and not excluded from this finding. In this study, most of the dogs underwent echocardiogram as preanesthetic evaluation for scheduled magnetic resonance imaging, and the remaining, for purpose of medical health examination. Arterial blood pressure was indirectly measured by means of the oscillometric method [27].[c] Dogs were divided into three groups: Toy breed of BW $<$ 5 kg, small breed of BW 5—10 kg, and medium to large breed of BW $>$ 10 kg.

Echocardiographic study

Standard echocardiography In all privately owned dogs, standard echocardiography was fully performed by the same experienced ultrasonographer (K.N.) using an echocardiographic system[d] with a 3- to 6-MHz sector probe transducer array. All unsedated dogs were manually restrained on a table in the left and right lateral recumbency with simultaneous electrocardiographic recording (ECG trace recording lead II) during the examinations. The 2D-STE and standard echocardiographic indices were determined from the average of three consecutive cardiac cycles.

The left atrial-to-aortic ratio value was obtained at right-sided parasternal short axis view

[c] PetMAP graphic (Ramsey Medical Inc., Tampa, FL, USA).
[d] Artida; Toshiba Medical System Corp., Tochigi, Japan.

on the first frame after closure of the aortic valve [28]. With regard to 2D-guided M-mode echocardiographic method, the fractional shortening, left ventricular internal dimension at end diastole, left ventricular internal dimension at end systole, left ventricular free wall, and interventricular septal thickness in both systole and diastole were measured at right-sided parasternal short axis 2D view at the level of the chordae tendinae [29]. The left ventricular internal dimensions for both the end systole and diastole were normalized on the basis of BW using the following formulae [30]:

Normalized left ventricular internal diameter end systole = left ventricular internal diameter end systole (cm)/[BW (kg)]$^{0.315}$; normalized left ventricular internal diameter end diastole = left ventricular internal diameter end diastole (cm)/[BW (kg)]$^{0.294}$.

Based on the mitral flow velocity obtained at the left apical four-chamber view, the following measurements were obtained: peak velocity of early diastolic transmitral flow (E), peak velocity of late transmitral flow (A), and the ratio of E to A using pulsed-wave Doppler. For the mitral valve septal annular velocity derived from tissue Doppler—based imaging, the peak velocity of systolic, early diastolic, and late diastolic mitral annular motion were recorded. The corresponding ratio of peak velocity of early diastolic mitral annular motion to peak velocity of late diastolic mitral annular motion and the ratio of E to peak velocity of early diastolic mitral annular motion were obtained using tissue-wave Doppler imaging. The images used for strain analysis were individually acquired from the left apical four-chamber view with focusing on the LA by adjusting the depth and frame rate. The frame rate of the clips was between 78 and 288 frames per rate, and three consecutive cardiac cycles in sinus rhythm were digitally stored for subsequent offline analysis.

Speckle tracking analysis of the left atrium

The obtained echocardiographic cine loops were analyzed using 2D speckle tracking software (2D wall motion tracking software[e]) by one investigator (A.D.). The LALS and SR were analyzed through 2D-STE using the QRS complex as the initiation of calculation. A line was manually traced along the clearly visualized internal edge of the LA wall using the point and click approach, and the epicardial surface of the LA was automatically generated by the software, thus creating an optimal region of interest, with LA myocardial wall thickness divided into six segments with adjustable width to fit the entire LA myocardial wall throughout the cardiac cycle. After tracking, the software generated the longitudinal strain and SR curve for each atrial segment and the average of all six segmental values (global strain and SR) in each dog. Before processing, a cine loop preview was used to confirm the speckle pattern movement after generation of the LA endocardium, and adequate imaging quality (without dropout speckle pattern) was visually inspected by the operator before inclusion in the study. The direction of the LA endocardial and epicardial surface at the junction of the pulmonary veins and LA appendage requires manual adjustment due to artefacts from tracing the region of interest [22]. In addition, the heart rate was recorded at the STE offline analysis. The LALS and SR analyses for each phasic function were measured from the global curve at three different time points, as reported in our previous study [23]: minimum strain at negative peak during the ventricular end-diastolic phase, maximum strain at peak during the ventricular systolic phase, and strain before atrial contraction. The LALS for reservoir function was calculated at the time of ventricular systole ($LALS_s$), that for conduit function at the time of early ventricular diastole ($LALS_e$), and that for booster-pump function at the time of late ventricular diastole or atrial contraction ($LALS_a$) corresponding to the cardiac cycle, using the following equations, as reported in the previous study:

$LALS_s$ = maximum strain − minimum strain; $LALS_e$ = maximum strain − strain before atrial contraction; $LALS_a$ = strain before atrial contraction − minimum strain.

Similarly, positive peak strain rate during ventricular systole (SR_s), first negative peak strain rate during early ventricular diastole (SR_e), and second negative peak strain rate during atrial contraction (SR_a) were identified at the time of ventricular systole at positive peak, early ventricular diastole at first negative peak, and late diastole or atrial contraction at second negative peak, respectively.

For LA-FVC measurement, the software generated automatic construction of LA volume at three frames of the cardiac cycle: maximal LA volume, the frame before opening of the mitral valve starts; pre-atrial contraction LA volume, the frame before the P wave on the ECG; and minimal LA volume, the frame at the mitral valve closure. The total, passive, and active LA emptying FVC were calculated based on the following formulae defining each of three LA phasic functions.

[e] UltraExtend, V3.10; Toshiba Medical Systems Corp., Tochigi, Japan.

LA-FVC during ventricular systole = 100 × (maximal LA volume − minimal LA volume)/maximal LA volume

LA-FVC during early ventricular diastole = 100 × (maximal LA volume − pre-atrial contraction LA volume)/maximal LA volume.

LA-FVC during atrial contraction = 100 × (pre-atrial contraction LA volume − minimal LA volume)/pre-atrial contraction LA volume.

Statistical analysis

Statistical analyses were performed using computer software.[f] In all statistical tests, $p < 0.05$ was considered a significant value. Normal distribution of data was evaluated by means of Shapiro–Wilk test. For evaluation of repeatability of 2D-STE indices, the intraday, interday, and interobserver coefficients of variance (CVs) were determined using the following linear model:

$$\begin{aligned} Yijkl = {} & \mu + \text{observeri} + \text{dayj} + \text{dogk} \\ & + (\text{observerXdog})\text{ik} + (\text{dayXdog})\text{jk} \\ & + \varepsilon\text{ijkl} \end{aligned}$$

where Y_{ijkl} was the value measured for dog k on day j by observer i; μ, the general mean; observer$_i$, the differential effect (considered as fixed) of observer i; dog$_k$, the differential effect of dog k; (observer X dog)$_{ik}$, the interaction term between the observer and dog; (day X dog), the interaction term between the day and dog; and ε_{ijkl}, the model error. Standard deviation of the intraday variability was estimated as the residual standard deviation of the model; standard deviation of the interday variability, as the standard deviation of the differential effect of day; and standard deviation of the interobserver variability, as the standard deviation of the different effect of observer. The corresponding CVs were calculated by dividing each standard deviation by the group mean. The degree of repeatability was identified as follows: CV <5%, very high repeatability; 5–15%, high repeatability; 16–25%, moderate repeatability; or >25%, low repeatability [31].

Descriptive statistics (median, range, reference intervals, and 90% confidence intervals of the reference limits) were determined for the 2D-STE indices. The reference intervals were calculated using a robust method with Box–Cox transformation [34].[g] The 90% confidence intervals of the reference limits were calculated by bootstrapping method.

As the assumption of normal distribution was not verified for all data, Spearman's correlation analysis was performed to evaluate the relationship between the 2D-STE indices and the standard echocardiographic indices, BW, age, and heart rate. Multiple linear regression analysis with forward stepwise selection based on Akaike's information criterion was used to elucidate the relationship between the echocardiographic indices and BW, age, and heart rate. Assumptions of linearity, normality, homoscedasticity, and independence of the residuals were evaluated by inspection of the standardized residual plots and quantile plots.

Results

Intraday, interday, and interobserver CVs of strains and SRs derived from 2D-STE are presented in Table 1. On the basis of CVs, the intraday, interday, and interobserver repeatability of strains and SRs were high to moderate (CVs of <20%). The signal characteristics in 120 healthy dogs included in the study are presented in Table 3. The mean arterial blood pressure obtained from dogs was 106 ± 11.6 mmHg. Among the various breeds of dogs, Chihuahua was the most frequently presented breed (n = 18), followed by Beagle (n = 13), Miniature dachshund (n = 11), Toy poodle (n = 9), Miniature schnauzer (n = 8), Yorkshire terrier (n = 8), mixed breed (n = 8), Cavalier King Charles spaniel (n = 6), Pomeranian (n = 5), Golden retriever (n = 4), Labrador retriever

Table 1 Variability of strain and strain rate analysis obtained by 2D-STE from healthy beagle dogs (n = 6).

Strain and SR parameters	CV (%)		
	Intraday	Interday	Inter observer
$LALS_s$	7.1	14.4	2.6
$LALS_e$	9.9	13.3	5.5
$LALS_a$	9.5	18.4	3.6
SR_s	9.3	19.0	18.6
SR_e	8.8	12.1	17.4
SR_a	10.7	15.8	8.3

CV: coefficient of variation; $LALS_s/SR_s$: left atrial longitudinal strain during ventricular systole/positive peak strain rate during ventricular systole; $LALS_e/SR_e$: left atrial longitudinal strain during early ventricular diastole/first negative peak strain rate during early ventricular diastole; $LALS_a/SR_a$: left atrial longitudinal strain during atrial contraction/second negative peak strain rate during atrial contraction; 2D-STE: two-dimensional speckle tracking echocardiography.

[f] JMP Pro, 12.2.0; SAS Institute Inc., Cary, NC, USA.
[g] Reference Value Advisor (Microsoft, Redmond, WA, USA).

Table 2 Descriptive statistics for the LA function indices in conscious dogs (n = 120).

Parameters	Median	Min-max	Lower RI	90% CI	Upper RI	90% CI
$LALS_s$	25.37	16.36–41.56	17.61	16.79–18.42	37.33	35.47–39.35
$LALS_e$	11.06	4.35–22.69	4.25	3.81–4.80	20.93	19.49–22.35
$LALS_a$	14.17	4.77–27.48	8.00	7.27–8.77	22.48	21.17–23.85
SR_s	1.87	1.15–3.46	1.23	1.18–1.29	2.88	2.72–3.02
SR_e	−2.32	−1.07 to −4.39	−1.13	−1.11 to −1.24	−4.25	−3.96 to −4.55
SR_a	−2.71	−1.17 to −4.65	−1.42	−1.25 to −1.58	−4.09	−3.91 to −4.28
LA-FVC_{total} (%)	52.44	35.93–67.13	40.84	39.30–42.38	65.19	63.49–66.82
LA-$FVC_{passive}$ (%)	32.75	13.98–48.74	17.64	15.61–19.63	47.13	45.36–48.87
LA-FVC_{active} (%)	28.99	15.08–53.17	17.36	16.07–18.87	45.86	43.39–48.40

$LALS_s/SR_s$, LA-FVC_{total}: left atrial longitudinal strain during ventricular systole/positive peak strain rate during ventricular systole, left atrial fractional volume change during ventricular systole; $LALS_e/SR_e$, LA-$FVC_{passive}$: left atrial longitudinal strain during early ventricular diastole/first negative peak strain rate during early ventricular diastole, left atrial fractional volume change during early ventricular diastole; $LALS_a/SR_a$, LA-FVC_{active}: left atrial longitudinal strain during atrial contraction/second negative peak strain rate during atrial contraction, left atrial fractional volume change during atrial contraction; Min-max: minimum–maximum; RI: reference interval calculated using a robust method with a Box–Cox transformation; CI: confidence interval.

Table 3 Body weight–separated values of LA strain and SR in 120 dogs.

Profiles	Toy breed <5 kg (n = 57)	Small breed 5–10 kg (n = 40)	Medium to large breed >10 kg (n = 23)
Characteristics			
Age (years)	6 (1–16)	6 (1–13)	7 (1–12)
Sex (male/female)	28/29	21/19	10/13
Body weight (kg)	3.4 (1.2–4.8)	6.9 (5.1–10)	20.9 (10.4–60)
Heart rate (bpm)	118 (58–180)	106 (53–152)	107 (63–147)
Mean arterial blood pressure (mmHg)	106 (89–129)	101 (80–126)	110 (89–126)
Echocardiographic values			
Left atrial-to-aortic ratio	1.3 (1.1–1.6)	1.4 (1.0–1.6)	1.3 (1.1–1.5)
nLVIDd	1.3 (1.1–1.9)	1.4 (1.1–1.9)	1.4 (1.2–1.8)
nLVIDs	0.7 (0.4–1.1)	0.8 (0.6–1.3)	0.8 (0.5–1.2)
FS (%)	44.7 (28.1–62.2)	41.9 (27–58.8)	35.6 (28.7–50.9)
E (m/s)	60.7 (42–93.2)	72.2 (38.2–95.2)	72.7 (52–112.6)
A (m/s)	59.3 (25.7–96.2)	58.8 (31.3–100.3)	57.8 (39.9–87.9)
E:A	1.1 (0.6–1.9)	1.3 (0.6–2.2)	1.2 (0.9–1.9)
E' (m/s)	6.5 (3.0–12.2)	7.8 (4.2–12.0)	8.5 (5.3–12.6)
A' (m/s)	7.6 (5.2–12.1)	8.0 (3.7–12.9)	7.9 (5.1–11.2)
S' (m/s)	7.6 (5–11.9)	8.8 (5.7–12.9)	10.6 (7.4–17.1)
E:E′	9.2 (5.7–15.4)	8.8 (5.5–13.7)	8.9 (5.9–12.8)
2D-STE–derived parameters			
LA-FVC_{total} (%)	52.4 (35.9–66.8)	52.9 (42.3–63.4)	51.0 (41.9–67.1)
LA-$FVC_{passive}$ (%)	30.9 (13.9–47.7)	35.4 (16.9–47.9)	30.7 (15.7–48.7)
LA-FVC_{active} (%)	29.4 (15.1–51.2)	28.1 (15.6–40.2)	29.2 (16.3–53.2)
$LALS_s$	25.1 (16.4–37.9)	26.1 (19.7–41.6)	24.4 (17.6–34.8)
$LALS_e$	9.5 (4.7–19.5)	12.6 (4.3–22.1)	11.2 (5.3–22.7)
$LALS_a$	14.9 (7.5–27.5)	13.8 (8.6–19.0)	12.6 (4.8–23.2)
SR_s	1.9 (1.2–2.9)	1.9 (1.2–2.9)	1.9 (1.2–3.5)
SR_e	−2.4 (−1.1 to −4.1)	−2.5 (−1.1 to −4.4)	−2.2 (−1.3 to −4.1)
SR_a	−2.9 (−1.6 to −4.7)	−2.6 (−1.5 to −3.9)	−2.1 (−1.2 to −4.0)

nLVIDd: normalized left ventricular internal diameter end systole; nLVIDs: normalized left ventricular internal diameter end systole; FS: fractional shortening; E: peak velocity of early diastolic transmitral flow; A: peak velocity of late transmitral flow; E:A: ratio of E to A; E': peak velocity of early diastolic mitral annular motion as determined by pulse-wave Doppler; A': peak velocity of diastolic mitral annular motion as determined by pulse-wave Doppler; S': peak velocity of systolic mitral annular motion as determined by pulse-wave Doppler; E:E': ratio of E to E'; $LALS_s/SR_s$, LA-FVC_{total}: left atrial longitudinal strain during ventricular systole/positive peak strain rate during ventricular systole, left atrial fractional volume change during ventricular systole; $LALS_e/SR_e$, LA-$FVC_{passive}$: left atrial longitudinal strain during early ventricular diastole/first negative peak strain rate during early ventricular diastole, left atrial fractional volume change during early ventricular diastole; $LALS_a/SR_a$, LA-FVC_{active}: left atrial longitudinal strain during atrial contraction/second negative peak strain rate during atrial contraction, left atrial fractional volume change during atrial contraction; data are expressed as median (range); 2D-STE: two-dimensional speckle tracking echocardiography.

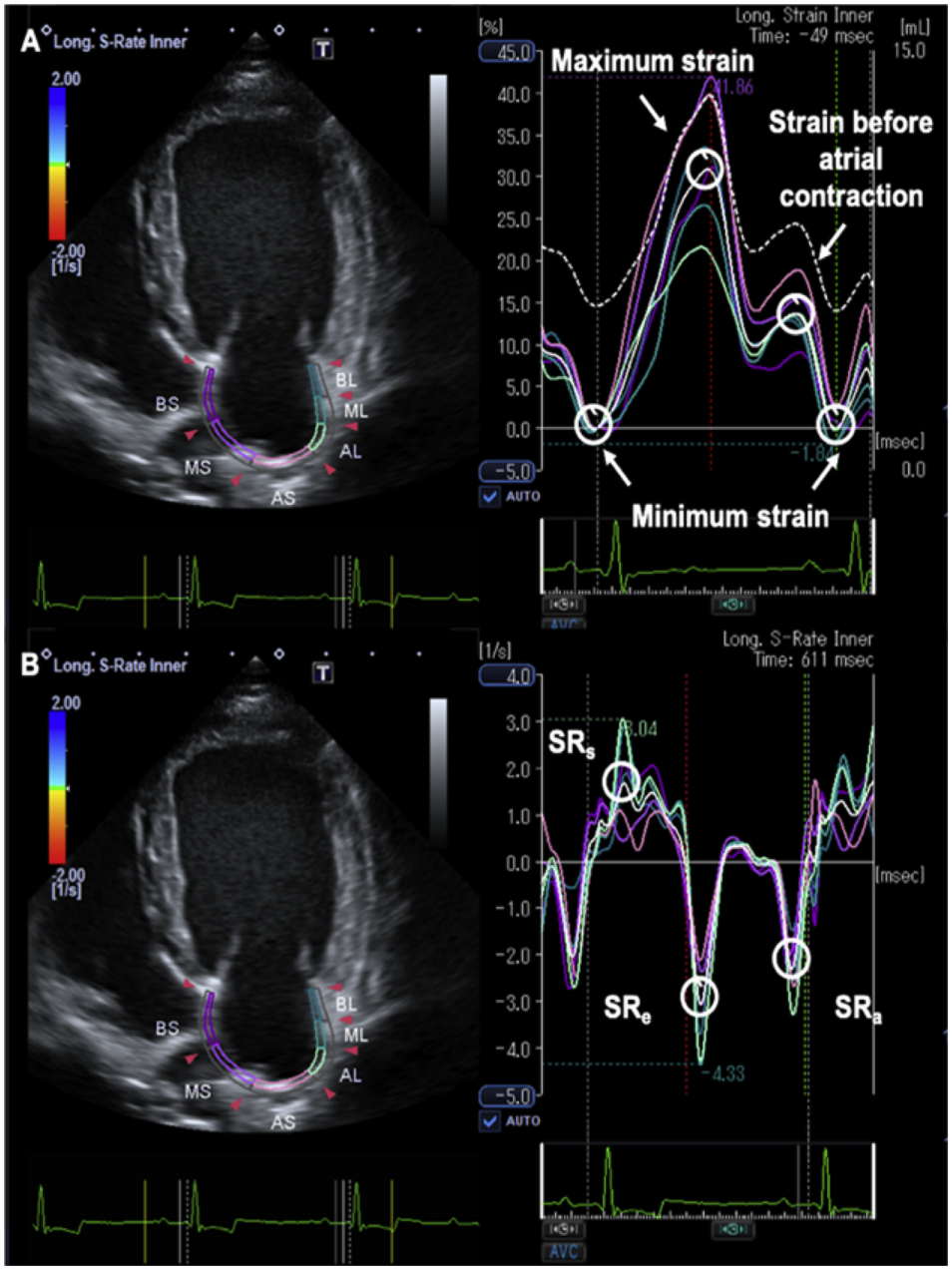

Figure 1 Longitudinal strain and strain rate images obtained from two-dimensional speckle tracking echocardiography. LA longitudinal strain and SR curves of healthy dogs obtained on the apical 4-chamber view. Strain and SR curves color-coded refer to the myocardial segment of LA. White lines represent the averaged global strain and SR (A) Minimum strain, maximum strain, and strain before atrial contraction were obtained from average global curves to calculate LALS during each LA phasic function; (B) SR_s was calculated by averaging first peak longitudinal strain rate values in all six segments of the LA. SR_e and SR_a were calculated by averaging the first and second negative peak longitudinal strain rate values in all six segments of the LA, respectively. SR_s, positive peak strain rate during ventricular systole; SR_e, first negative peak strain rate during early ventricular diastole; SR_a, second negative peak strain rate during atrial contraction; LA, left atrium; LALS, left atrial longitudinal strain.

(n = 3), Shiba (n = 3), Pug (n = 3), Papillon (n = 3), Maltese (n = 2), Welsh corgi (n = 2), Italian greyhound (n = 2), Bernese mountain dog (n = 2), American Cocker spaniel (n = 1), Standard schnauzer (n = 1), Border collie (n = 1), Jack Russell (n = 1), French bulldog (n = 1), Bichon frise (n = 1), Akita (n = 1), Pekingese (n = 1), Brussels griffon (n = 1), and Siberian husky (n = 1). The reference values of the longitudinal strain, SR profile, and LA-FVC using 2D-STE are displayed in Table 2. The data of LALS and SR based on the patients' BW separation are presented in Table 3.

Fifty-seven dogs were classified into the toy breed group, 40 dogs were classified into the small breed group, and 23 dogs were classified into the medium to large breed group.

In all dogs, the mean LA strain curve presented the first positive peak at ventricular systolic phase and decreased to a plateau at diastolic phase, followed by the second positive peak at preceding period of atrial contraction, and finally, the negative peak at postatrial contraction. In all dogs, the second positive peak was of less magnitude than the first positive peak (Fig. 1A). The SR profiles showed the first positive peak during ventricular systole (SR_s) and two negative peaks at early (SR_e) and late (SR_a) ventricular diastole (Fig. 1B). The SR_e curve was more negative than the SR_a, with the ratio of SR_e:$SR_a > 1$ in 47 dogs, whereas in 73 dogs, the SR_a was more negative than the SR_e, with the ratio of SR_e:$SR_a < 1$.

The LA phasic function estimated by LALS was significantly correlated with the parameters of left ventricle (LV) diastolic function and LA functional indices of conduit function. The $LALS_e$ represented significant moderate positive correlations with the E wave ($r = 0.5560$, $p < 0.001$) and the ratio of E to A ($r = 0.5515$, $p < 0.001$) and moderate negative correlation with the peak velocity of early diastolic mitral annular motion ($r = -0.5370$, $p < 0.001$). The $LALS_e$ showed significant weak negative correlation with the peak velocity of late diastolic mitral annular motion ($r = -0.2314$, $p < 0.05$) and A wave ($r = -0.2233$, $p < 0.05$). The correlation of the $LALS_s$, $LALS_e$, and $LALS_a$ was considered strong, strong, and moderate with the LA-FVC during ventricular systole ($r = 0.7628$, $p < 0.001$), LA-$FVC_{passive}$ ($r = 0.7465$, $p < 0.001$), and LA-FVC_{active} ($r = 0.5528$, $p < 0.001$), respectively. The $LALS_e$, SR_e, and LA-$FVC_{passive}$ were negatively correlated with the age ($r = -0.4585$, $p < 0.001$; $r = -0.4442$, $p < 0.001$; and $r = -0.4424$, $p < 0.001$, respectively), whereas the $LALS_a$ and LA-FVC_{active} were positively correlated with the age ($r = 0.2825$, $p < 0.05$ and $r = 0.4564$, $p < 0.001$, respectively). The $LALS_a$ showed negative correlation with the heart rate ($r = -0.2706$, $p < 0.05$), whereas the SR_s and SR_a showed positive correlation with the heart rate ($r = 0.1967$, $p < 0.05$ and $r = 0.3568$, $p < 0.001$, respectively). The SR_a and $LALS_a$ showed negative correlation with the BW ($r = -0.3709$, $p < 0.001$ and $r = -0.2272$, $p < 0.05$, respectively).

In multiple linear regression analysis, the relationships were significant, but generally weak. Only age was significantly associated with all the parameters for conduit function [standardized partial regression coefficient (β) $= -0.46$, $r^2 = 0.21$, $p < 0.001$ for $LALS_e$; $r^2 = 0.19$, $\beta = -0.46$, $p < 0.001$ for SR_e; and $r^2 = 0.19$, $\beta = -0.45$, $p < 0.001$ for LA-$FVC_{passive}$]. The age, BW, and heart rate were significantly related to the $LALS_a$ (age: $r^2 = 0.08$, $\beta = 0.25$, $p = 0.0025$; BW: $r^2 = 0.05$, $\beta = -0.25$, $p = 0.0039$; heart rate: $r^2 = 0.07$, $\beta = -0.31$, $p = 0.0004$). The heart rate and BW were significantly related to the SR_a (heart rate: $r^2 = 0.12$, $\beta = 0.31$, $p = 0.0003$; BW: $r^2 = 0.14$, $\beta = -0.31$, $p = 0.0003$). The age and heart rate were significantly associated with the LA-FVC_{active} (age: $r^2 = 0.21$, $\beta = 0.46$, $p < 0.001$; heart rate: $r^2 = 0.04$, $\beta = -0.21$, $p = 0.01$).

Discussion

The major findings of the study were as follows: (1) Assessment of the LA phasic function through the 2D-STE variables enabled LALS and SR analysis, which derived six different parameters ($LALS_s$, $LALS_e$, and $LALS_a$ and SR_s, SR_e, and SR_a) to estimate the three phasic functions of the LA including the reservoir, conduit, and booster-pump function, respectively) in healthy dogs; (2) The $LALS_e$ was moderately correlated with the parameters through LV diastolic Doppler (E wave, peak velocity of early diastolic mitral annular motion, and ratio of E to peak velocity of early diastolic mitral annular motion) and the corresponding FVC; and (3) All the LA functional indices indicating conduit function ($LALS_e$, SR_e, and LA-$FVC_{passive}$) were negatively related with the age, whereas those indicating booster-pump function ($LALS_a$ and LA-FVC_{active}) were positively correlated with the age.

Within-day intra observer variability for LALS was considered as a repeatable parameter with CV of <20% [32,33], although between-day intra-observer variability and interobserver variability of some SRs revealed relatively higher value than those in the strain variables. As reported in a previous study [22], the high variability of some SR variables may be software dependent due to automatic generation of the curves after tracing the region of interest. Therefore, the SR should be interpreted under careful consideration of the reliability. Moreover, the CVs relative to the LALS and SR obtained in our study were slightly higher than those (peak atrial longitudinal strain (PALS), peak atrial contraction strain (PACS) and contraction systolic index (CSI)) obtained in a previous study showing CVs <16% for all tested variables [6], which could be explained by the difference in the software and canine population in our study. However, the good repeatability of the STE might support the finding of lack of angle dependency,

which is an advantage over conventional techniques [1—3]. In humans, the LA strain parameter through offline analysis using a different software application was validated with high feasibility and good agreement, based on a previous report [35]. In addition, the repeatability and reproducibility of measurements of the LA function assessed with STE in healthy dogs are clinically relevant [6,13].

In our study, the strain for LA conduit parameters showed good correlation with the Doppler-based parameters used to describe left ventricular diastolic function and FVC estimated LA function, which highlights the potential clinical applicability of LA strain analysis using the new method. Among the strain variables, strain corresponding to the reservoir phase showed the strongest relationship with that corresponding to the conduit phase. The relationships of LA conduit function and Doppler-based parameters can be explained by the utility of mitral inflow as indirect parameters of the LV diastolic and LA functions based on occurrence initial filling of the LA and consequent relaxation of the left ventricle, resulting in high E wave as the blood enters the left ventricle and consequently a high passive LA emptying fraction [18,24,35]. Similarly, the strain parameters corresponding to each LA function were strongly related to the FVC used to describe each of the three LA phasic functions, indicating possible interaction of the LA myocardial deformation and volumetric change, particularly under conditions of cardiovascular disease states. However, for more detailed insight of the LA reservoir function and left ventricular hemodynamic measurement, other representative indices such as the pulmonary venous systolic flow, left ventricular end-diastolic pressure, left ventricular end-diastolic volume, left ventricular ejection fraction, or isovolumetric contraction time may be needed [35,36].

In addition, our results indicated that LA function analyzed by speckle tracking was age dependent. The atrial strain and SR for conduit function were inversely related with increasing age. This may be due to the effect of aging process on impaired early left ventricular diastolic filling [24]. In contrast, the changes in strain corresponding to the atrial contraction phasic parameters were positively influenced by increasing age, which is most likely to be a compensatory mechanism for impaired left ventricular performance [24,35,37]. In this study, the strain/SR parameters corresponding to booster-pump function were relatively higher than those corresponding to conduit function. In contrast, our data indicated that volumetric value at conduit phase was relatively higher than that at booster-pump phase. Thus, the 2D-STE—derived LA strain parameters showed potential for use as sensitive indicators of the subclinical change in atrial function with aging; further studies are necessary to determine maintenance of the changes in volumetric indices with aging despite relative decrease in the values in LA strain. In addition, our results indicated a higher number of dogs with more negative SR_a curve than the SR_e, which extends an earlier observation regarding the adverse effect of aging on the LA conduit function [22,26]. Most dogs used in this study were in the older age group and represented the inversion of ratio of E to A on the pulse-wave Doppler interrogation of the mitral valve, without changes in the LA size and heart rate [18,22]. This trend may be considered as an important influencing factor on the age-associated change in cardiac function. However, to determine the effect of aging on LA function, a study including a large number of dogs of different age groups (young, middle, and older group) for subsequent analysis to detect the changes of LA function parameters is required [24,26,38—40].

Moreover, multiple linear regression analysis revealed that SR_a was associated with BW, in contrast to the absence of association between LA strain/SR and BW in a previous report [22]. Our study provides evidence of minor effect of BW on atrial SR, particularly booster-pump function, in dogs. The STE values in right ventricular strain in larger breed dogs was reported as lower than that in smaller breed dogs [41]. Therefore, we cannot exclude a possible effect of BW on the STE variables. The wide variation of SR in the study might lead to underestimation of the effect value of atrial SR_a. In our study, strain/SR parameters alone for booster-pump function were significantly associated with the BW and heart rate. These findings support the consideration of the effect of BW and heart rate on SR in interpretation of cardiac diseases [24,25].

Currently, the reference intervals in a large number of dogs of LALS and SR are lacking. In this study, we reported the central tendencies for estimating the mean of LALS and SR at each LA phasic function, including data by BW. The reported mean values of atrial strain and SR in the present study were also different when compared with those in other studies on healthy dogs [6,22]. In addition to tracking algorithm, the image quality, ultrasound system, and software used for analysis increase the differences in interpretation of longitudinal strain. The image of LA strain and SR in this study was generated by Artida and UltraExtend (Toshiba Medical System), compared

with MyLab and XStrain™ (Esaote) and iE33 and QLAB quantification software (Phillips) used in the previous studies. In humans, the longitudinal strain values of left ventricle using Artida were reported to be lower than those using Esaote [42,43]. However, this is the first report to provide the reference intervals of atrial deformation parameters through different software in healthy dogs as compared to previous literature. Although the reference intervals for LA strain and SR using different systems are needed, investigators should additionally consider variations due to different methodology as well as STE software for LA, instead of LV software, to provide suitable normal values for wider use in dogs with disease [20].

Our study has several limitations. First, repeatability data were obtained from a small number of healthy beagles which should be considered when interpreting LA strain and SR parameters using 2D-STE in dogs with LA dysfunction. Second, with regard to interpreting these parameters by different day and observer, data in our study indicated relatively high intra observer between-day and interobserver variation. Third, we did not present the results of this study in the form of body size—dependent allometric equations for LALS and SR variables due to the small effect of body size on the strain and SR variables. However, our study included a larger number of samples than other echocardiographic studies focused on normal LA deformation in animals [6,9,22] and provided better sample size for the recommended number of reference intervals in at least 120 reference subjects [44]. Fourth, we were unable to control the heart rate deviation because the respiratory cycle or stress factor in conscious dogs can lead to larger variability in this analysis. Fifth, the blood pressure was not measured in all dogs. Sixth, the present study lacked longitudinal follow-up of the dogs used to identify the reference intervals. Without long-term follow-up, it was not possible to examine the effect of the subclinical myocardial disease-free status of included dogs on the LA function. Accuracy of interpretation using LA strain analysis depends on the image quality and experience of tracking ability of the investigator [20] which should also be taken into account in the analysis.

Conclusion

The present study demonstrated that the LA phasic function indices of strain and SR were a feasible, reproducible measurement tool to assess the LA function in awake dogs without cardiac disease. However, the age, heart rate, and BW should be considered in the interpretation of these parameters in a clinical setting. The reference intervals of LA strain and SR enable future studies to investigate parameters to predict the cardiovascular outcome.

Conflicts of Interest Statement

The authors do not have any conflicts of interest to disclose.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvc.2019.01.006.

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