Repeatability and reproducibility of measurements obtained via two-dimensional speckle tracking echocardiography of the left atrium and time–left atrial area curve analysis in healthy dogs

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Objective—To evaluate left atrial phasic function in healthy dogs by means of 2-D speckle tracking echocardiography with time-left atrial area curve analysis and to assess repeatability and reproducibility of obtained measurements.

Animals—6 healthy Beagles.

Procedures—Each dog underwent echocardiography twice on different days (3 nonconsecutive examinations/d). Images were analyzed with offline software; area of the left atrium was automatically calculated in each frame throughout the cardiac cycle to derive time-left atrial area curves. Variables used to assess left atrial phasic function (total, passive, and active emptying area and emptying fractions and mean active and total emptying rates) were calculated. Agreement between variables measured via speckle tracking echocardiography and a manual tracing method was assessed with modified Bland-Altman analysis. Within-day and between-day coefficients of variation were determined.

Results—Mean ± SD total, passive, and active emptying fractions of the left atrium were 49.8 ± 3.5%, 27.7 ± 4.0%, and 30.5 ± 4.3%, respectively. Mean ± SD total and active emptying rates were 16.0 ± 2.5 cm²/s and 25.1 ± 4.9 cm²/s, respectively. Within-day and between-day coefficients of variation were < 20% (range, 0.41% to 16.4%) for all variables except mean active emptying rate (between-day coefficient of variation, 29.2%). Agreement between variables measured via speckle tracking echocardiography and the manual tracing method was good, and differences between methods were nonsignificant.

Conclusions and Clinical Relevance—Evaluation of left atrial phasic function via speckle tracking echocardiography was feasible; repeatability and reproducibility of measurements were adequate in healthy dogs. Studies are needed to determine clinical applicability in canine patients. (Am J Vet Res 2013;74:864–869)

Abbreviations

CI Confidence interval
CV Coefficient of variation
LAAmax Maximum area of the left atrium at ventricular end-systole
LAAmin Minimum area of the left atrium at ventricular end-diastole
LAAp Area of the left atrium at onset of the P wave on the ECG

The 3 phasic functions of the left atrium are important for left ventricular filling. These include a reservoir function (expansion associated with inflow of blood from the pulmonary veins during ventricular systole), a conduit function (passage of blood from the pulmonary veins to the left ventricle during ventricular diastole), and a booster pump function (augmentation of left ventricular filling during atrial contraction). In humans with cardiac diseases, a strong correlation between left atrial dysfunction and the severity of cardiac diseases or occurrence of cardiovascular events has been described.
In humans, the evaluation of left atrial phasic function via time-left atrial area curve analysis has been performed by use of the acoustic quantification method, in which the blood-tissue border is automatically determined and left atrial area can be automatically measured via software; however, it is somewhat cumbersome to obtain reliable results with this technique because it is difficult to exclude the presence of pulmonary veins and the left atrial appendage from a region of interest. 

A novel method has been developed for tracking the left atrial wall movement via 2-D speckle tracking echocardiography, and this technology can be used to automatically perform time-left atrial area or volume curve analysis. The principle of the 2-D speckle tracking method relies on the formation of speckles in echocardiographic images by reflection, scattering, and interference between the tissue and ultrasound beam. These speckles appear homogeneously distributed within the myocardium in 2-D echocardiographic images and can be tracked from frame to frame throughout the cardiac cycle. With this method, it is possible to exclude pulmonary veins and the left atrial appendage from a region of interest and to precisely perform time-left atrial area or volume analysis. In humans, time-left atrial area or volume analysis determined via 2-D speckle tracking echocardiography is useful for evaluation of left atrial phasic function in patients with various cardiac diseases.

To our knowledge, the assessment of left atrial phasic function in dogs via time-left atrial area curve analysis has not been reported. To determine whether differences in data reflect clinically important changes resulting from disease progression in a patient, it is necessary to evaluate the repeatability and reproducibility of variables measured with this modality. The purpose of the study reported here was to evaluate left atrial phasic function in healthy dogs via 2-D speckle tracking echocardiography with time-left atrial area curve analysis and to assess the repeatability and reproducibility of measurements obtained with this technique.

**Materials and Methods**

**Dogs**—Six Beagles (2 males and 4 females; age, 1 year; body weight, 11.3 to 13.2 kg) that were part of a research colony owned by Hokkaido University were included in the study. All dogs were determined to be healthy, with no mitral regurgitation or congenital cardiac abnormalities, as determined on the basis of results of a complete physical examination, ECG, and standard echocardiographic examinations (including M-mode, pulsed-wave Doppler, and color flow Doppler imaging) performed prior to the start of the study to confirm normal heart anatomy and myocardial function. All procedures were approved by the Laboratory Animal Experimentation Committee, Graduate School of Veterinary Medicine, Hokkaido University.

![Figure 1](image-url)
Study design—Echocardiographic examinations were performed on 4 dogs over a 2-week period (ie, 3 dogs underwent the procedure on days 1 and 7, and the remaining 3 dogs underwent the procedure on days 8 and 14). On a given day, the 3 dogs were each examined 3 times; the 3 examinations of each dog were performed nonconsecutively. Each variable assessed for an individual dog consisted of the mean of measurements from 3 consecutive cardiac cycles during each examination.

2-D speckle tracking echocardiography—All echocardiographic examinations were performed by 1 of the authors (KN) using commercially available ultrasoundographic equipment equipped with a 3- to 7-MHz sector probe and offline software. All dogs were examined while manually restrained in left lateral recumbency. No sedation was used. An apical 4-chamber view was obtained with means of second harmonic grayscale imaging, with frequency, depth, and sector width adjusted for frame-rate optimization (154 frames/s). An ECG trace (lead II) was recorded simultaneously with echocardiographic imaging.

The echocardiographic images were analyzed with offline software by 1 observer (TO). A frame corresponding to the time of the peak of the R wave on the ECG was selected as indicating left ventricular end-diastole, and the endocardium of the left atrium was manually traced in that frame (Figure 1). The area of the left atrium was then automatically calculated by the software in each subsequent frame throughout the cardiac cycle to derive a time-left atrial area curve. Tracking quality was assessed visually. If the tracking quality was unsatisfactory (ie, the blood-tissue border was not tracked), manual tracing of the endocardium was repeated. The LAmax, LAAp, and LAmin for the left atrium were determined by the software. Variables used as indicators of left atrial phasic function were calculated as follows (Figure 2):

\[
\text{EAtotal} = \text{LAmax} - \text{LAmin}
\]

\[
\text{EApass} = \text{LAmax} - \text{LAAp}
\]

\[
\text{EAact} = \text{LAAp} - \text{LAmin}
\]

Total emptying fraction (%) = 100 × EAtotal/\text{LAmax}

Passive emptying fraction (%) = 100 × EApass/\text{LAmax}

Active emptying fraction (%) = 100 × EAact/\text{LAAp}

Mean total emptying rate = \text{EAtotal}/\text{time from LAmin to LAmax}

Mean active emptying rate = \text{EAact}/\text{time from LAAp to LAmin}

where EAtotal, EApass, and EAact represent the total, passive, and active emptying areas, respectively, of the left atrium. Total emptying fraction and mean total emptying rate were calculated as indicators of reservoir function, whereas passive emptying fraction was determined as an indicator of conduit function. Active emptying fraction and mean active emptying rate were calculated as indicators of booster pump function. Heart rate was calculated from the R-R interval on the ECG tracing during the same cardiac cycle used for echocardiographic measurements of the left atrium.

Manual tracing method—The same images of the left atrium analyzed automatically via offline software were analyzed via a manual tracing method.

![Figure 2](image)

**Figure 2**—Schematic representation of the time-left atrial area curve (top) generated during a single cardiac cycle (represented as an ECG tracing; bottom) in a healthy dog. Measurements of left atrial area are indicated. From the onset of ventricular systole, atrial area progressively increases, reaching its maximal dimension at ventricular end-systole. After mitral valve opening, atrial area rapidly decreases during early ventricular diastole. During diastasis, left atrial area remains constant or is slightly increased. At the onset of atrial systole, atrial contraction begins which causes the atrial area to decrease to its minimal dimension. EAact = Active emptying area. EApass = Passive emptying area. EAtotal = Total emptying area. MVC = Mitral valve closure. MVO = Mitral valve opening.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Within-day</th>
<th>Between-day</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>112 ± 18</td>
<td>14.10 ± 12.50</td>
</tr>
<tr>
<td>LAmax (cm²)</td>
<td>6.73 ± 0.79</td>
<td>0.38 ± 5.72</td>
</tr>
<tr>
<td>LAAp (cm²)</td>
<td>4.87 ± 0.85</td>
<td>0.23 ± 4.77</td>
</tr>
<tr>
<td>LAmin (cm²)</td>
<td>3.38 ± 0.48</td>
<td>0.23 ± 6.88</td>
</tr>
<tr>
<td>Emptying area (cm²)</td>
<td>3.35 ± 0.31</td>
<td>0.29 ± 8.70</td>
</tr>
<tr>
<td>Passive</td>
<td>1.86 ± 0.33</td>
<td>0.26 ± 14.00</td>
</tr>
<tr>
<td>Active</td>
<td>1.49 ± 0.44</td>
<td>0.13 ± 9.25</td>
</tr>
<tr>
<td>Emptying fraction (%)</td>
<td>49.8 ± 3.5</td>
<td>2.65 ± 5.31</td>
</tr>
<tr>
<td>Passive</td>
<td>27.7 ± 4.0</td>
<td>2.76 ± 9.98</td>
</tr>
<tr>
<td>Active</td>
<td>30.5 ± 4.3</td>
<td>2.61 ± 8.57</td>
</tr>
<tr>
<td>Mean emptying rate (cm²/s)</td>
<td>16.0 ± 2.5</td>
<td>1.44 ± 8.97</td>
</tr>
<tr>
<td>Total</td>
<td>25.1 ± 4.9</td>
<td>2.71 ± 10.80</td>
</tr>
</tbody>
</table>

Heart rate was calculated from the R-R interval of an ECG tracing during the same cardiac cycle used for echocardiographic measurements of the left atrium.

**Table 1**—Mean values with within-day SDs and CVs for heart rate and left atrial variables determined via 2-D speckle tracking echocardiography in 6 healthy Beagles.
od. The endocardium of the left atrium was manually traced at 3 time points, and the resulting areas were calculated via automated calculation software provided with the ultrasonographic machine. The LAA-max was measured in a frame obtained just before mitral valve opening (left ventricular end-systole). LAAp was measured in a frame obtained at the onset of the P-wave on ECG, and LAAmin was measured in a frame obtained at mitral valve closure (left ventricular end-diastole). Total, passive, and active emptying areas and emptying fractions were calculated as previously described.

Statistical analysis—Normal distribution of the data was confirmed by means of a Shapiro-Wilk test. Results are expressed as mean ± SD. Statistical analyses were performed with computer software. The following linear model was used for variables in within-day and between-day variability analysis:

\[ Y_{ijk} = \mu + day_i + dog_j + (day \times dog)_j + \epsilon_{ijk} \]

where \( Y_{ijk} \) is the kth value measured for dog j on day i, \( \mu \) is the general mean, \( day_i \) is the differential effect of day i, \( dog_j \) is the differential effect of dog j, \( (day \times dog)_j \) is the interaction term between day and dog, and \( \epsilon_{ijk} \) is the model error. The SD of repeatability was estimated as the residual SD of the model and the SD of reproducibility as the SD of the differential effect of day. The corresponding CVs were determined by dividing each SD by the mean. In accordance with suggestions from reports of previous studies, clinical acceptability was defined as a CV ≤ 20%.

A Bland-Altman analysis with modifications for repeated measures was performed to assess agreement between measurements for variables determined automatically with speckle tracking echocardiography and those obtained by manual tracing; mean values for measurements obtained from the same images via the 2 methods were plotted against the difference (value determined via speckle tracking echocardiography minus the value determined by manual tracing). The mean difference (bias) as well as SD, 95% CI, and \( P \) values of the differences were calculated. For all statistical comparisons, values of \( P < 0.05 \) were accepted as significant.

Table 2—Results of Bland-Altman analysis of agreement between measurements for left atrial variables determined automatically via speckle tracking echocardiography and by use of a manual tracing method for the same 6 dogs in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bias</th>
<th>SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAAm (cm³)</td>
<td>0.014</td>
<td>0.54</td>
<td>-0.16 to 0.20</td>
</tr>
<tr>
<td>LAAp (cm³)</td>
<td>-0.0019</td>
<td>0.44</td>
<td>-0.15 to 0.14</td>
</tr>
<tr>
<td>Emptying area (cm²)</td>
<td>0.0037</td>
<td>0.28</td>
<td>-0.093 to 0.010</td>
</tr>
<tr>
<td>Passive</td>
<td>0.0011</td>
<td>0.47</td>
<td>-0.12 to 0.17</td>
</tr>
<tr>
<td>Total</td>
<td>0.0056</td>
<td>0.26</td>
<td>-0.093 to 0.083</td>
</tr>
<tr>
<td>Emptying fraction (%)</td>
<td>0.11</td>
<td>4.92</td>
<td>-1.78 to 1.55</td>
</tr>
<tr>
<td>Passive</td>
<td>-0.049</td>
<td>6.01</td>
<td>-2.08 to 1.98</td>
</tr>
<tr>
<td>Total</td>
<td>-0.10</td>
<td>2.95</td>
<td>-1.10 to 0.89</td>
</tr>
</tbody>
</table>

The difference between methods was nonsignificant (\( P > 0.05 \)) for all variables.

Results

In the apical 4-chamber view, movement of the left atrial wall was tracked from frame to frame throughout the cardiac cycle with 2-D speckle tracking echocardiography. All of the obtained images from 108 cardiac cycles of 6 dogs were analyzed to derive time-left atrial area curves, and all required variables were determined for each curve (Figure 1). Subjective assessment of time-left atrial area curves revealed that left atrial area increased during left ventricular systole (reservoir phase), decreased passively during left ventricular early diastole (conduit phase), and remained relatively stable during left ventricular diastasis before decreasing actively during atrial contraction (booster pump phase).

Within-day and between-day CVs for variables obtained via speckle tracking echocardiography of the left atrium were summarized (Table 1). The CVs were < 20% (range, 0.41% to 16.4%) for all variables except the between-day CV for mean active emptying rate (29.2%). Overall, mean ± SD total emptying fraction was 49.8 ± 3.5%, passive emptying fraction was 27.7 ± 4.0%, and active emptying fraction was 30.5 ± 4.3%. The mean total emptying rate was 16.0 ± 2.5 cm²/s, and mean active emptying rate was 23.1 ± 4.9 cm²/s.

The dispersion of differences for values determined via speckle tracking echocardiography and the manual tracing method was evaluated by use of Bland-Altman analyses with modifications for repeated measures (Table 2). Agreement was good (ie, the differences were nonsignificant [\( P > 0.05 \) for all comparisons]) between the 2 methods for all variables.

Discussion

Analysis of results of the present study indicated that the use of 2-D speckle tracking echocardiography was feasible in dogs for evaluation of left atrial phasic functions (ie, reservoir, conduit, and booster pump). The repeatability and reproducibility of measurements obtained with this method were considered clinically acceptable for use in healthy dogs. All of the obtained images were of sufficient quality to allow analysis for derivation of time-left atrial area curves, and agreement between the speckle tracking echocardiography method and manual tracing was considered good for all variables. In addition, the within-day and between-day CVs for all variables measured via speckle tracking echocardiography were considered clinically acceptable (≤ 20%) except for the between-day CV for mean active emptying rate (29.2%).

Left atrial size increases in response to adverse hemodynamic conditions, such as increased left ventricular filling pressures and mitral regurgitation. Evaluation of left atrial size by determination of the left atrium-to-aortic root ratio is routinely performed via 2-D echocardiography, and this variable has been indicated as a valuable prognostic factor in dogs with chronic mitral valve disease. However, left atrial size does not always correspond to the severity of cardiac disease because an enlarged left atrium can be associated with normal or only slightly high left atrial pressure, therefore, evaluation of left atrial phasic function via speckle tracking echocardiography may
potentially be useful as a novel prognostic indicator in dogs with cardiac diseases.

Conventional Doppler ultrasonographic variables for transmitral blood flow, pulmonary venous blood flow, and mitral annular tissue velocities provide insight into the mechanics of the left atrium. Although these variables do not completely reflect left atrial phasic function and are also affected by left ventricular diastolic and systolic performance,¹⁵ some, including peak E wave (early diastolic transmitral flow wave) velocity, the ratio of E wave to A wave (late diastolic transmitral flow wave) velocities, and deceleration time of the E wave, were shown to be useful predictors of poor prognosis in dogs with chronic mitral valve disease and dilated cardiomyopathy.¹²,¹⁵ However, these Doppler-based techniques are affected by the angle between the wave source and receiver¹⁶ and translational movement of the heart.¹⁷ In fact, some variables determined with this technique in dogs are not adequately repeatable and reproducible.¹⁸,¹⁹

In human medicine, it is not known whether left atrial phasic function measurements have a better diagnostic or prognostic value, compared with the value for measurements of left atrial size and other results of conventional echocardiographic examination. However, in patients with hypertrophic cardiomyopathy, measures of left atrial phasic function are correlated with clinical signs of heart failure, independent of left atrial size or left ventricular systolic function.² Therefore, in our opinion, it is possible that evaluation of left atrial phasic function with speckle tracking echocardiography has the potential to provide additional information for the diagnosis of cardiac diseases and determination of prognosis in canine patients.

The good repeatability of measurements obtained with speckle tracking echocardiography in the present study may be partially attributed to the fact that the speckle tracking technique is independent of the angle between the wave source and receiver.²⁰ In humans, the variability of measurements of left atrial myocardial strain determined via speckle tracking echocardiography is adequate for routine clinical use.²¹ Also, in healthy dogs, the repeatability and reproducibility of measurements of systolic left ventricular torsion obtained by means of speckle tracking echocardiography are clinically acceptable.²² Measurements of systolic left ventricular torsion in dogs revealed within-day CVs between 9.1% and 16.4% and between-day CVs between 6.8% and 18.0%.²² In the present study, within-day and between-day CVs for most variables (range, 0.41% to 16.4%) were considered comparable to those findings. However, the between-day CV for mean active emptying rate of the left atrium was not clinically acceptable (29.2%). Measurements of cardiac function evaluated via cardiac catheterization are affected by changes in heart rate.²³ The variables in the present study may have been affected by this factor because the dogs were not sedated, and heart rates did not remain unchanged during the examination. Therefore, it is possible that the reproducibility of mean active emptying rate was negatively influenced by heart rate.

To our knowledge, the study reported here provides the first description of left atrial phasic function in dogs assessed with speckle tracking echocardiography and time-left atrial area curve analysis. Thus, there are no available reference intervals for areas of the left atrium during various functional phases or changes in left atrial area, emptying fractions, or emptying rates measured with any technique in healthy dogs. Among the variables assessed in the present study, the total, passive, and active left atrial emptying fractions may be more clinically valuable in assessment of left atrial phasic function than left atrial areas during functional phases (LAAmax, LAAp, and LAAmin) and differences between total, passive, and active emptying areas because the influences of body size are abolished in left atrial emptying fractions. The mean values of total, passive, and active emptying fractions (49.8%, 27.7%, and 30.5%, respectively) in the present study were fairly similar to previously reported values (47%, 31%, and 25%, respectively) determined via the acoustic quantification method in healthy adult humans.²⁴

The present study had several limitations. First, all of the dogs were healthy; therefore, caution has to be urged in extrapolating these data to groups of diseased dogs, especially those with high heart rates. Second, the apical 4-chamber view was obtained for analysis in our study, and other views were not evaluated. However, for the right parasternal short-axis view, it is impossible to exclude the presence of the left atrial appendage from a region of interest when manually tracing the outline of the left atrium. Also, for the right parasternal long-axis view, it is difficult to adjust the sector width of images for frame-rate optimization. Third, time-left atrial area curves, rather than time-left atrial volume curves, were obtained, although left atrial volumes can be calculated by the method²⁵ of Simpson et al.²⁵ by use of the same type of offline software²⁶ and would provide a more accurate estimate of left atrial size than do left atrial areas.²⁶ However, the left atrium is a complicated structure, and to our knowledge, the calculation of left atrial volumes in dogs by the method of Simpson et al.²⁵ has not been evaluated against a criterion-referenced technique such as MRI. Finally, no comparison was made between measurements obtained via speckle tracking ultrasonography and a criterion-referenced technique such as MRI, and further study in this area is needed.

Analysis of the results of the present study indicated that speckle tracking ultrasonography provided repeatable and reproducible quantitative measurements of most variables used to assess left atrial phasic function in healthy adult dogs. Investigations in dogs with heart disease are warranted to determine the clinical applicability of these variables.

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