Feasibility and reproducibility of echocardiographic assessment of regional left atrial deformation and synchrony by tissue Doppler ultrasonographic imaging in healthy dogs

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Objective—To assess the feasibility and reproducibility of longitudinal tissue Doppler ultrasonographic imaging with regard to determination of velocity, strain, and strain rate (SR) of the left atrium (LA) and use those data to characterize LA synchrony (LAS) for a group of healthy dogs.

Animals—15 healthy dogs.

Procedures—For each dog, apical 4- and 2-chamber echocardiographic views were obtained. Peak velocity, strain, and SR and time to peak value during systole, early diastole, and late diastole were measured for each of the 4 LA walls. To characterize LAS, mean and SD maximal late diastolic time difference (LAD) among the 4 walls were calculated on the basis of time to peak for velocity, strain, and SR; for each, the 95% confidence interval (mean ± 2SD) was calculated. Within-day and between-day intraobserver variability was calculated.

Results—For all dogs, tissue velocity and SR had peak positive values during systole and 2 negative peaks during early and late diastole. Atrial strain had a peak positive value during systole, positive values during early diastole, and a negative peak value during late diastole. Reproducibility was acceptable for most variables. Diastolic strain and SR had the highest variability, but times to peak values were always reproducible. For velocity, strain, and SR, the 95% confidence interval for the maximal LAD was < 50 milliseconds and that for the SD of the LAD was < 23 milliseconds.

Conclusions and Clinical Relevance—Longitudinal tissue Doppler imaging of LA deformation was feasible in healthy dogs, and its application may be useful for understanding atrial pathophysiologic changes associated with various cardiac diseases in dogs. (Am J Vet Res 2014;75:59–66)

The LA plays an essential role in cardiovascular performance. This is accomplished through its actions as a blood reservoir during ventricular contraction, a conduit during early ventricular diastole, and a booster pump during late diastole. Left atrial dilation, being an indicator of the severity of volume overload and increased cardiac pressures, is one of the most important prognostic factors for humans, dogs, and cats with heart disease. Moreover, in both humans and horses, atrial electrical, structural, and functional changes are associated with the presence of atrial fibrillation, and atrial enlargement and contractile dysfunction are predictors for the development of the arrhythmia. Finally, the recognition of reduced LA appendage contractility, assessed by the reduction of flow velocity, is a predictor of thromboembolic risk in cats with cardiomyopathy.
The understanding of atrial function and recognition of its structural and functional changes are essential when evaluating a patient with heart disease. However, quantifying atrial performance with noninvasive techniques is difficult, mainly because the atria are geometrically and structurally complex. In particular, the obliquity of the IAS, irregular shape of the right atrium, and difficulty with fitting the atrial auricles in a geometric model make the morphological evaluation of these structures challenging.

Various echocardiographic methods have been developed to analyze atrial morphology and function in human and veterinary patients. Recommendations for chamber quantification and measurement of LA diameter and volume have been proposed for people, and reference values determined with both biplanar and tri-dimensional methods are routinely applied in human medicine. In dogs and cats, several echocardiographic techniques for measuring LA dimensions have been described, but in clinical practice, a single diameter measurement obtained in a short-axis view at the level of the heart base is typically used as the main criterion to identify LA dilation. However, LA shortening fraction and ejection fraction are not routinely used to identify mechanical dysfunction in small animals, and only a few studies have focused on these variables. Doppler echocardiography can be used to indirectly assess LA function, mainly by analyzing the late diastolic A and Ar waves from the mitral valve inflow and pulmonary veins flow, respectively. Left atrial mechanical and electromechanical function has been assessed by pulsed-wave and color-coded TDI in both clinically normal horses and in horses with atrial fibrillation (after conversion to sinus rhythm). In those studies, LA TDI was feasible and useful in understanding atrial pathophysiologic changes and myocardial remodeling.

Several studies in humans have focused on the assessment of atrial function with advanced echocardiographic techniques, such as pulsed TDI, color TDI, and speckle tracking echocardiography and through evaluation of strain and SR. Left ventricular diastolic function was assessed by pulsed-wave and color-coded TDI in both clinically normal horses and in horses with atrial fibrillation (after conversion to sinus rhythm). In those studies, LA TDI was feasible and useful in understanding atrial pathophysiologic changes and myocardial remodeling.

In dogs and cats, TDI and strain assessment techniques have been used to assess both left and right ventricular function in normal and pathological conditions to estimate intracardiac pressures and myocardial dysfunction, diagnose cardiomyopathies, and assess inter- and intraventricular synchrony. However, to the authors’ knowledge, studies focused on the evaluation of LA function by TDI and strain and SR assessment techniques are lacking in the field of small animal cardiology. The purpose of the study reported here was to assess the feasibility and reproducibility of longitudinal TDI with regard to determination of velocity, strain, and SR of the LA and use those data to characterize LAS for a group of healthy dogs.

Materials and Methods

Animals—Fifteen client-owned dogs were enrolled in the study. Inclusion criteria were absence of clinical signs and any other clinically relevant systemic disease (eg, clinically detectable endocrine disorders, gastrointestinal or respiratory tract diseases, or neurologic issues). The cardiovascular system was considered normal in each dog on the basis of results of a physical examination, including cardiac auscultation, 12-lead surface ECGs performed as previously described, noninvasive systolic and diastolic systemic arterial pressure measurement by use of an oscillometric method, and transthoracic echocardiography according to standard guidelines. Prior to study commencement, informed consent of owners was obtained and the Ethical Committee of the University of Bologna approved the protocol of the study.

The group of dogs included 3 Cocker Spaniels, 2 mixed breeds, 2 Golden Retrievers, 2 American Staffordshire Terriers, 1 Boxer, 1 Yorkshire Terrier, 1 Maltese, 1 Italian Spitz, 1 Miniature Schnauzer, and 1 Australian Shepherd Dog. Six dogs were male (3 castrated and 3 sexually intact), and 9 were female (3 spayed and 6 sexually intact). The age of dogs ranged from 3 to 11 years (mean ± SD age, 6.6 ± 2.5 years); the dogs' weights ranged from 3.3 to 34 kg (mean body weight, 17.8 ± 9.6 kg).

Study protocol—The study was conducted at the Veterinary Teaching Hospital of Bologna University. A complete transthoracic echocardiographic examination with continuous ECG monitoring according to standard guidelines was performed on dogs placed in lateral recumbency. All the examinations were done by the same experienced echocardiographer (MBT) using an ultrasound unit equipped with a multifrequency phased array probe.

The diameters of the LA and aortic root were measured from the right parasternal short-axis view as previously described, and an LA:aortic root diameter ratio < 1.6 was considered normal. Doppler echocardiography was performed to exclude any degree of valvular insufficiencies, intracardiac shunts, or malformations. Left ventricular diastolic function was assessed from the left apical 4-chamber view; peak velocities of the early diastolic (E) and late diastolic (A) waves, their ratio (E:A), isovolumic relaxation time of the left ventricle, and deceleration time of the E wave were recorded on 3 consecutive beats; and the mean value was calculated. Similarly, early diastolic wave (E'), late diastolic wave (A'), and their ratio (E':A' ratio) were measured on the lateral mitral valve annulus with spectral TDI.

TDI analysis—Tissue Doppler ultrasonographic imaging analysis was performed from the left apical 4- and 2-chamber views with each dog placed in lateral recumbency. On the basis of the American Society of Echocardiography nomenclature, in addition to the IAS, the LA free wall was arbitrarily divided in
3 additional segments (ie, the AW, LW, and PW of the LA). The IAS and LW were visualized in the 4-chamber echocardiographic view, and the PW and AW were visualized in the 2-chamber view (Figure 1).

Attention was paid to attain good alignment of the atrial walls and the ultrasound beam. The specific software available on the ultrasonographic machine was activated, and a video recording of at least 5 consecutive beats was acquired for subsequent offline analysis. The TDI color sector was adjusted to include only the LA. Color gain, filters, and velocity scale were adjusted to avoid aliasing artifacts and to maximize myocardial color saturation. A frame rate $\geq 100$ frames/s during TDI was mandatory to perform the examination. Only good-quality video recordings were stored for subsequent offline analysis.

Video recordings were successively reviewed in a dedicated software program to measure different LA TDI variables. For each LA wall segment, 4 ROIs were drawn to include the length and thickness of the entire wall, avoiding the surrounding lung parenchyma (Figure 1). The ROI was maintained in the same position by the motion compensation tool of the software. This tool prevents the displacement of the ROI during respiratory motion, but manual adjustment of the ROI was used for those frames severely affected by respiratory motion.

Velocity, strain, and SR profiles were obtained for each of the 4 LA wall segments. In total, 36 variables were measured; peak systolic, peak early diastolic, and peak late diastolic velocity, strain, and SR as well as time to each peak value were each measured on 3 consecutive heart cycles, and the mean for each variable was calculated. The same operator who performed the echocardiographic examinations did all of the TDI measurements.

**Intra-atrial synchrony**—The times to peak values for velocity, strain, and SR of each atrial wall were obtained with the aid of a digital timing caliper that was used to measure the interval between the onset of the P wave on the ECG tracing (atrial electrical activity) and the late diastolic peak on the TDI profile. Then the dispersion of LAS was calculated, on the basis of time to peak velocity, strain, and SR, as the mean maximal LAD and SD for timing among the 4 walls for the 3 analyzed cardiac cycles. The 95% confidence interval for overall LAD was calculated as mean LAD $\pm 2SD$ from the data for all 4 LA walls.

**Statistical analysis**—Data are presented as mean $\pm$ SD or median (range). Descriptive statistical analyses were performed with a commercial software program. Continuous data were tested for normality by use of a D’Agostino-Pearson test. Each TDI variable was compared among the 4 atrial walls by means of a 1-way ANOVA, followed by a Turkey post hoc test when necessary. Values of $P < 0.05$ were considered significant. This part of analysis was performed with a dedicated software program.

The intraobserver within-day measurement variability was determined from data generated by the same operator (MBT); this operator evaluated 4 dogs (animals already enrolled into the study) 2 times repeatedly during the same day. The intraobserver between-day variability was determined from data generated by 1 blinded observer (MBT); on 2 different days, this operator made 2 evaluations of the 4 dogs used for within-day variability. The variability was quantified as the CV by use of the equation $CV = (mean\ difference\ between\ measurements/mean\ of\ measurements)\times 100$ and expressed as a percentage. The degree of variability was arbitrarily defined as follows: $CV < 5\%$, very low variability; $5\%$ to $15\%$, low variability; $16\%$ to $25\%$, moderate variability; or $> 25\%$, high variability.

**Results**

**Left ventricular diastolic variables**—Left ventricular diastolic function was considered normal in all dogs. Velocities and time variables were within reference ranges.

![Figure 1](image-url)
In all dogs, the E':A' ratio was positive, but 2 dogs had an inverted E:A ratio on the transmitral flow. However, they were 7 and 11 years old, and this finding was considered a normal variant for old dogs, given that there were no alterations in the other diastolic variables. Mean ± SD of the recorded variables was as follows: E wave, 72.2 ± 14.8 cm/s; A wave, 61.4 ± 16.6 cm/s; isovolumic relaxation time of the left ventricle, 66.8 ± 8.9 milliseconds; deceleration time of the E wave, 113.4 ± 23.2 milliseconds; E:A ratio, 1.2 ± 0.3; E', 8.0 ± 2.0 cm/s; A', 6.5 ± 2.1 cm/s; and E':A' ratio, 1.3 ± 0.2.

Atrial TDI profiles—All of the stored video recordings for TDI quantification were of good quality, and it was possible to obtain discrete curves for each atrial wall of each dog. For the 15 study dogs, a total of 180 curves of velocity, strain, and SR for each of the 4 atrial walls were analyzed (Figures 2 and 3). Both velocity and SR constantly had a positive wave during ventricular systole and 2 negative waves during early and late diastole, whereas the strain profile was characterized by a positive peak during ventricular systole, a rapid decrease in strain percentage during early diastole, and a negative deflection approaching zero during late diastole after the P wave. Findings for each variable and significant differences among the 4 LA walls were summarized (Table 1). Early diastolic velocities for the LW and AW were significantly (P < 0.05) higher than those for the IAS and the PW, whereas early diastolic SR was significantly (P < 0.05) higher for the LW than the values for the IAS and PW. Moreover, late diastolic velocity for the LW was significantly (P < 0.05) higher than values for the IAS and the other walls; also, late diastolic velocity for the AW was significantly (P < 0.05) higher than the value for the IAS. No significant differences in times to peak values were detected among the 4 LA walls.

Intra-atrial synchrony—Intra-atrial synchrony was assessed (Table 2). For characterization of IAS, the 95% confidence intervals of the maximal LAD and the SD of the LAD for velocity, strain, and SR were determined. In the healthy study dogs, the values of the 95% confidence interval for the maximal LAD for each variable were always < 30 milliseconds and the values of the 95% confidence interval for the SD of the LAD for each variable were always < 23 milliseconds. Moreover,
In this period, the LA acts as a reservoir chamber, and characterized. The LA strain profile reflects the normal physiologic changes of the chamber. During awake dogs, and reproducible curves were described and accepted CVs were recorded for the systolic variability. Moreover, the highest CVs were recorded for the strain measurements during early and late diastole, and acceptable CVs were recorded for the systolic variables. On the other hand, low and very low variability were observed for most times to peak values.

**Discussion**

To the authors’ knowledge, the study reported here is the first to test the applicability of TDI-derived longitudinal deformation imaging via a left apical view approach and to characterize intra-atrial synchrony of the LA in healthy dogs. On the basis of the study findings, the evaluation of velocity, strain, and SR derived by TDI for the LA was feasible and applicable in awake dogs, and reproducible curves were described and characterized. The LA strain profile reflects the normal physiologic changes of the chamber. During left ventricular systole, longitudinal elongation of LA walls occurs, with an increased strain percentage. During this period, the LA acts as a reservoir chamber, and the myofibers are stretched by the blood flow coming through the pulmonary veins with the mitral valve closed. When the mitral valve opens, during the early passive ventricular filling, the LA empties and its walls shorten, as proven by the reduction of strain value (conduit phase). After the P wave, the LA actively pumps the blood to complete the ventricular filling and a second negative wave coincides with the contraction of the atrial myofibers on the strain profile.3

The architecture of LA musculature is quite complex and has been incompletely studied in humans and other animals. The IAS, with its interatrial fascicle, offers a mechanical support for most of both right atrial and LA muscular fascicles. From the left portion of the IAS, a large fascicle originates, thereby creating the AW of the LA. This fascicle is attached to the atrioventricular fibrotic ring and has some pectinated fibers that embrace the left auricle and interweave with the intermediate pectinated muscles that originate from the interatrial fascicle. The atrioseptal fascicle creates part of the PW of the LA. The roof and the PW are created by at least 6 septopulmonary bundles that extend over the superior part of the LA. Some of these fibers cross in the middle of the LA roof and embrace the opening of pulmonary veins, creating a sphincter-like structure. This complex architecture results in extreme differences in the timing and strength of LA activation and contraction, giving rise to a gradient between the LA walls.48–51

In the present study, significant differences in TDI variables among the LA walls of healthy dogs were identified. The SR profile provided a lower peak value during early diastole for the IAS, compared with findings for the LW, and the LW had a higher SR, compared with that for the PW. The peak early diastolic velocities were higher for the LW and AW, compared with the value for the IAS, and the PW had lower values, compared with those for the LW. Finally, during late diastole, IAS peak velocity was lower than that of the LW or AW, and the highest peak late diastolic velocity was associated with the LW. Some gradients and differences between atrial walls have been also observed in echocardiographic studies in humans. This can be explained on the basis of the anatomic differences in the LA musculature. Interestingly, there were no significant differences in times to peak values among the atrial walls of the healthy dogs in the present study. In a
PW, and coronary sinus. Hence, the identification of preferential conduction pathways near the IAS, occurring through any of the LA walls because of the presence of atrial dysynchrony in some animals at risk for atrial fibrillation (ie, dogs with concomitant LA dilation, large-breed dogs, or predisposed breeds) or other supraventricular tachyarrhythmias could be of interest. As a matter of fact, in these patients, the arrhythmia could be perpetuated by a sick atrial substrate with preexisting local delayed conduction, and theoretically, ectopic atrial foci could even be localized. Further studies should be performed to elucidate whether LA TDI could be used as a noninvasive diagnostic and prognostic technique in that specific population of dogs.

Except for the electrical properties, the mechanical function of the LA was investigated in the present study as well. Increased intra-atrial pressure, atrial dilation, and atrial myocardial remodeling are known to be associated with valvular and myocardial diseases in humans and other animals. It has been observed that TDI-derived variables are more accurate for evaluation of LA function than traditional 2-D echocardiographic variables, and different behaviors of LA function are detected among patients with mitral valve insufficiency and mitral valve stenosis. A possible clinical application of LA TDI in veterinary medicine could be the analysis of atrial mechanical dysfunction in animals with concurrent left heart diseases with or without LA dilation; application of the technique could enable better and earlier assessment of the severity of disease, and possibly aid in stratifying the patients according to risk of decompensation or death.

The intraobserver within-day measurement variability was determined from data generated by the same operator, who made 2 evaluations of each of the 4 dogs, repeatedly during the same day, to test the variability of the quantification method. The intraobserver between-day variability was determined from data generated by the same observer blinded, who made 2 evaluations of the 4 dogs on different days. The variability was quantified as the CV by use of the equation CV = (mean difference between measurements/mean of measurements)×100 and expressed as a percentage. The degree of variability was arbitrarily defined as follows: CV < 5%, very low variability; 5% to 15%, low variability; 16% to 25%, moderate variability; or > 25%, high variability.

<table>
<thead>
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<th>Variable</th>
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<th>Between-day variability</th>
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difficult to obtain good TDI profiles, compared with profiles for the other wall segments, and might be the basis for the higher variability associated with the AW.

In general, the suboptimal reproducibility of LA TDI might affect the clinical applicability of this method, particularly in dogs. In the present study, we did not evaluate the influence of body weight and breed on the TDI variables. Similar to the ventricles, it is possible that atrial deformation properties are affected by body condition and specific breed characteristics. Increasing the number of dogs evaluated could allow creation of specific reference ranges according to weight class, possibly leading to a reduction in the SDs for some variables. On the other hand, very low and low variability were obtained for times to peak values, and time variables appear less dependent on heart rate. Thus, the analysis of intra-atrial synchrony appears to be much more promising as a clinically applicable technique for the study of LA dysfunction during irregular atrial rhythms.

In the present study, interobserver variability to determine the reproducibility of the method among echocardiographers was not investigated. It can be supposed that the variability among operators with different experience in the technique might be higher, compared with the intraobserver variability. Tissue Doppler imaging is angle dependent and is affected by artifacts such as reverberations, dropout zone, and low lateral resolution. Recently, the analysis of ventricular myocardial deformation has been improved with 2-D and 3-D speckle tracking, which overcame the disadvantages of TDI technique and improved the reproducibility of strain and SR measurements for ventricular chambers. In human medicine, the speckle tracking technique has been applied for the analysis of atrial deformation properties. Regardless of species, problems associated with the speckle tracking technique include the need for a high frame rate, very high-quality images, and use of dedicated software for analysis able to correctly track the thin atrial walls.

On the basis of the results of the present study, TDI-based longitudinal deformation properties and intra-atrial synchrony of the LA can be assessed in healthy dogs. In the future, this technique may be useful for the study of atrial dysfunction associated with heart diseases that cause LA dilation, such as chronic mitral valve disease or dilated cardiomyopathy, or may be used in the assessment of atrial electrical abnormalities in the presence of supraventricular arrhythmias or for the prediction of atrial fibrillation in dogs.

References


