Contrast Echocardiography in two-dimensional left ventricular measurements: comparison with 256-row multi-detector computed tomography as a reference standard in Beagles

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ABSTRACT

Unenhanced echocardiography (UE), commonly used in veterinary practice, is limited by left ventricular (LV) foreshortening and observer dependency. Contrast echocardiography (CE) was used to compare two-dimensional (2D) LV measurements made using UE and 256-row multidetector computed tomography (MDCT) as a reference standard. Seven healthy beagle dogs were evaluated in this study. Measurements obtained using CE, including LV wall thickness, internal diameter, and longitudinal and transverse length, were significantly greater than those obtained using UE. Measurements of LV internal dimension in diastole (LVIDd) and systole (LVIDs) were significantly larger with CE compared to UE. Regardless of the cardiac cycle, LV longitudinal (LVLd and LVLs) and transverse diameter (LVTDd and LVTDs) measurements were significantly different with CE and approximated values from MDCT. Among automatically calculated parameters, LV end-systolic volume and the relative wall thickness were significantly different between UE and CE. In CE, the correlation coefficients of 4 major parameters ($r = 0.87$ in LVIDd; 0.91 in LVIDs; 0.87 in LVLd; and 0.81 in LVLs) showed higher values compared to the UE ($r = 0.68$ in LVIDd, 0.71 in LVIDs, 0.69 in LVLd, and 0.35 in LVLs). Inter-observer agreement was highest for MDCT and higher for CE than UE. In conclusion, CE is more accurate and reproducible than UE in assessing 2D LV measurements and can overcome the limitations of UE including LV foreshortening and high observer dependency.

Keywords: Contrast echocardiography; left ventricular measurements; cardiac computed tomography; multidetector computed tomography; dogs

INTRODUCTION

Echocardiography is a common and standard method for evaluating cardiac function in dogs with various heart diseases [1,2]. Although studies have identified limitations of echocardiography when compared to multi-detector computed tomography (MDCT) and
cardiac magnetic resonance imaging (CMRI) in human medicine [3,4], echocardiography plays a major role in daily veterinary practice because it can be easily performed and does not require general anesthesia. Thus, the correlation between echocardiographic measurements and prognosis has been evaluated in dogs [5]. Although a variety of advanced echocardiographic techniques, including tissue Doppler imaging, and speckle tracking technique, have been developed and used in veterinary medicine recently [1], two-dimensional (2D) echocardiography for the evaluation of cardiac structures remains the standard for assessing the prognosis of the dogs with heart disease. This technique is useful in daily practice because it is minimally affected by heart rate, respiratory rate, observer skill, and the use of diuretics [5,6].

Despite availability and convenience, echocardiography has limitations including shortened left ventricular (LV) length, a poor acoustic window in patients with a pulmonary disease or obesity, and high dependence on observer skill [7,8]. Contrast echocardiography (CE), widely used in human medicine, has proved to be efficacious, and can overcome the disadvantages of echocardiography [3,9,10]. In veterinary medicine, CE has been used to study pulmonary transit time [11], cardiac function [12], and to identify congenital anomalies [13]. However, due to the cost, requirement for intravenous catheterization, and the length of the procedure, CE is underutilized in veterinary medicine [12]. Furthermore, similar to human medicine, most studies in veterinary medicine have focused on the LV function, rather than the 2D measurements, which are commonly used in clinical practice.

MDCT has been used in human medicine in clinical and research settings to evaluate coronary arterial disease and ventricular opacification [14]. Although CMRI is considered the gold standard, recent studies demonstrate that 256-row MDCT has the ability to accurately assess cardiac anatomy and LV measurements. The accurate assessment is based on the short acquisition time, high spatial resolution, and full cardiac coverage [14,15]. Due to the advantages of MDCT, such as multiplanar reconstruction, it is used for anatomic evaluation of the heart and pericardial structures as a reference standard [16,17].

The purpose of this study was to verify the feasibility of using CE to assess the 2D LV measurements. We also tested the hypothesis that CE can overcome the limitations of echocardiography, including LV foreshortening and high observer dependency. This is the first veterinary study to directly compare the accuracy and reproducibility of CE with that of unenhanced echocardiography (UE) and 256-row MDCT regarding 2D LV measurements.

**MATERIALS AND METHODS**

**Animals**

Seven healthy beagle dogs (5 males and 2 females; 8–9 kg) aged 30–38 months were used in this study. To assess health status, all dogs underwent a physical examination, routine blood analysis, radiography, abdominal ultrasound, and prior echocardiography. The care, maintenance, and study design followed the protocols approved by Institution Animal Care and Use Committee of Konkuk University (Approval #KU14136).

**Anesthetic protocol**

The dogs were premedicated with 0.05 mg/kg glycopyrrolate (Mobinul, Myungmoon Pharmaceutical Co., Ltd, Korea) subcutaneously and anesthetized with a 6 mg/kg intravenous bolus of propofol (Provive, Myungmoon Pharmaceutical Co., Ltd.). Following endotracheal intubation, anesthesia was maintained with isoflurane and oxygen at 2.5%–3.5% in inspired fraction.
intubation, anaesthesia was maintained with isoflurane with 100% oxygen. Heart rate, respiratory rate, blood pressure, and concentration of end-tidal carbon dioxide were monitored using a patient monitor (VP-1000; VOTEM, Korea). Each dog was anesthetized twice, once for echocardiography and once for MDCT. There was a minimal 2-week interval between the procedures to minimize anesthesia complications.

**Unenhanced echocardiography**
UE was performed under general anesthesia for direct comparison with MDCT using a commercially available ultrasound machine (Prosound Alpha 6; ALOKA, Japan). Echocardiographic measurements were obtained following the recommendations for chamber quantification of American Society of Echocardiography [8] and listed in Table 1. The Teichholz formula is used to calculate LV volume. M-mode images were obtained to assess the left ventricular internal dimension in end-diastole (LVIDd) and end-systolic (LVIDs). Measurements of the interventricular septal thickness in end-diastole (IVSd) and end-systole (IVSs) and the left ventricular free wall thickness in end-diastole (LVFWd), and end-systole (LVFWs) were obtained (Fig. 1). The LV fractional shortening (FS), stroke volume (SV), ejection fraction (EF), end-diastolic volume (EDV), end-systolic volume (ESV), LV mass (LVM), and relative wall thickness (RWT) were automatically calculated based on the equations from a previous study [18]. LV length, defined as the length between the mitral annulus and the endocardial border at the LV apex, was measured at end-diastole (LVLd) and end-systole (LVLs) (Fig. 2). Transverse diameter of left ventricle in end-diastole (LVTDd) and end-systole (LVTDs) were obtained as the vertical bisector of LVLd and LVLs, respectively. Images from three consecutive cardiac cycles were stored by the observer (J. Kim) and reviewed in the DICOM file format by three authors (J. Kim, S. Kim, Y. Lee).

**Contrast echocardiography**
CE was performed immediately after UE in the same manner. Based on a prior study [12], a 22-gauge catheter with a 3-way stopcock (Easyfix 3-Way Stopcock; Sewoon Medical, Korea) was placed in the left cephalic vein. To prevent microbubble destruction, a low mechanical index (< 0.3) was used. 0.1 mL/kg Sulfur hexafluoride microbubbles (Sonovue; Bracco, Italy)

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**Table 1. List of the left ventricular measurements**

| Left ventricular measurements                      |
|----------------------------------------------------|
| Teichholz method on the right parasternal 4-chamber view |
| Interventricular septal thickness in end diastole    |
| Left ventricular internal dimension in end diastole  |
| Left ventricular posterior wall thickness in end diastole |
| Interventricular septal thickness in end systole     |
| Left ventricular internal dimension in end systole   |
| Left ventricular posterior wall thickness in end systole |

| 2-dimensional left ventricular measurement on the left apical view |
|------------------------------------------------------------------|
| Longitudinal diameter of the left ventricle in end-diastole      |
| Transverse diameter of the left ventricle in end-diastole        |
| Longitudinal diameter of the left ventricle in end-systole       |
| Transverse diameter of the left ventricle in end-diastole        |

| Automatically calculated left ventricular parameters             |
|------------------------------------------------------------------|
| Fractional shortening                                            |
| Stroke volume                                                    |
| Ejection fraction                                                |
| End-diastolic volume                                             |
| End-systolic volume                                              |
| LV mass                                                          |
| Relative wall thickness                                          |

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was injected intravenously followed by a 2 mL of saline flush. If the LV apex did not fill with a single contrast agent, an additional contrast agent (0.05 mL/kg) was injected again. To lengthen the LV longitudinal length, CE was performed at the extended intercostal window.
M-mode images and 3-consecutive cardiac cycle cine images, when the LV was homogeneously filled with microbubbles, were stored. The CE measurements were made in the same manner as the UE measurements. After the CE, each dog was monitored for a minimum of 2 hours to detect any side effects of the contrast agent.

**256-row multidetector computed tomography**

To obtain reference images of cardiac anatomy, a 256-row MDCT (Brilliance iCT; Philips Healthcare, Netherland) was performed. The dog was placed in sternal recumbency to minimize the motion artifact during respiration. A 50:50 mixture of saline and a non-ionic contrast medium (Iomelon 300; Bracco) was injected 2 mL/kg into the cephalic vein using a power injector (Philips Contrast Injector; Philips Healthcare) at a rate of 2 ml/sec. Image protocols were: 120 kVp, 150 mAs, 0.9 mm in slice thickness, spacing 0.45 mm. For synchronization of the cardiac cycles, retrospective electrocardiogram (ECG) gating technique was used to obtain end-diastolic (0% of the R-R interval; the largest left ventricle) and end-systolic images (35%–40% of the R-R interval; the smallest left ventricle).

**256-row multi-detector computed tomography analysis**

Three radiologists reviewed the post-contrast transverse series using a commercially available DICOM viewer (Osirix DICOM viewer; Pixmeo, Swiss). Using multiplanar reconstruction, CT images were reconstructed according to the echocardiographic images (Fig. 4). The values were measured three times by each observer and mean values were recorded.

**Statistical analysis**

Statistical analyses were done with commercially available software (SPSS 20.0®; IBM Corporation, USA). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to identify
normal distribution. The differences in measurements between UE and CE were compared with the paired t test, and the three modalities were compared using the repeated measures analysis of variance (ANOVA) with the Bonferroni post hoc correction. Correlations between LVIDd, LVIDs, LVLd, and LVLs were assessed by Pearson correlation test. Bland–Altman analyses were used to display the limits of agreement of LVIDd, LVIDs, LVLd, and LVLs among the three methods in dogs. Inter-observer reliability was assessed in LVIDd, LVIDs, LVLd, and LVLs using 2-way random ICCs. For all comparisons, the level of significance was set at $p < 0.05$.

RESULTS

The results of the repeated measured ANOVA and post hoc analysis by Bonferroni correction are shown in Table 2. In the measurement of wall thicknesses, decreases in wall thickness were observed in CE compared to UE, but there was no statistical difference among the three modalities. On the other hands, the LV internal dimensions in UE and CE were statistically different in both diastole and systole. LVIDd and LVIDs measured via CE did not show statistical difference when compared to MDCT values. The longitudinal and transverse dimensions of the left ventricle were significantly longer in CE than in UE. Although there was an increase in all values, LVTDD and LVTDS showed no statistical differences between UE and CE. Calculated parameters are summarized in Table 3. With the exception of FS and EF, all parameters were significantly different between UE and MDCT. In CE, only ESV and RWT were significantly different from the results based on UE and did not differ from MDCT measurements.
Echocardiographic parameters with CE showed statistical significance and strongly correlated to the MDCT. In CE, all of 4 major parameters showed statistical significance and higher correlation coefficients compared to the UE. The mean correlation efficient for MDCT was 0.87 ± 0.04 (r = 0.87 in LVIDd; 0.91 in LVIDs; 0.87 in LVLd; and 0.81 in LVLs) in CE and 0.61 ± 0.17 (r = 0.68 in LVIDd, 0.71 in LVIDs, 0.69 in LVLd, and 0.35 in LVLs) in UE.

The results of Bland–Altman analyses for the evaluation of mean differences between measured values obtained via echocardiography and MDCT were plotted (Fig. 5). ICCs in 4 echocardiographic parameters including LVIDd, LVIDs, LVLd, and LVLs were obtained. In the UE, the lower ICC (mean ± standard deviation [SD], 0.73 ± 0.06, 0.70 ± 0.05, 0.65 ± 0.09, and 0.63 ± 0.04, respectively) was obtained than CE (mean ± SD, 0.80 ± 0.03, 0.75 ± 0.05, 0.76 ± 0.02, and 0.73 ± 0.03, respectively). The best ICC were found in MDCT, which were 0.93 ± 0.01, 0.89 ± 0.03, 0.94 ± 0.02, and 0.92 ± 0.03, respectively.

**DISCUSSION**

This study demonstrates that CE is effective for 2D measurement of the left ventricle. Due to convenience, and the ability to provide real-time evaluation without general anesthesia, echocardiography is a standard technique in evaluating cardiac structure and function in patients.
Contrast echocardiography in 2D LV measurements

Fig. 5. Bland-Altman plot of 4 major parameters in diastole and systole. Comparison of the limits of agreement in UE, CE, and MDCT as a reference. The diagram showing the mean difference (solid lines) and the limits of agreement (dashed lines) between echocardiographic and computed tomographic measurements of LVIDd (A, B), LVIDs (C, D), LVLd (E, F), and LVLs (G, H). For all variables, the limits of agreement were narrowed significantly in CE compared to UE.

UE, unenhanced echocardiography; CE, contrast echocardiography; MDCT, multi-detector computed tomography; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; LVLd, left ventricular length in diastole; LVLs, left ventricular length in systole.
veterinary medicine [1,2,12,19]. However, several limitations, including LV foreshortening, reduce the reliability of echocardiography [20,21]. Although three-dimensional echocardiography has recently been developed to overcome the limitations of 2D cardiac evaluation [21], it is difficult to use in veterinary practice due to lack research, small size of cardiac structures, rapid heart rate, and mechanical requirements. Because general anesthesia is needed to obtain ideal images from state-of-art equipment such as CMRI and MDCT in dogs, CE is a more useful and promising method for evaluating the heart in dogs.

CE has been widely used in human medicine for early detection of ischemic heart disease and left ventricular opacification (LVO) [7,9]. In veterinary medicine, CE has focused on evaluating cardiac function, including LV volume and EF, and not 2D cardiac measurements [11,12,22]. Due to the large differences in the heart size and diseases between dogs and humans, previous studies have lacked clinical relevance in veterinary medicine. For example, not only is the size of the cardiac structure of most dogs is much smaller, many dogs have tachycardia, complicating the evaluation of cardiac function. The most common heart disease in dogs is acquired valvular heart disease; ischemic heart disease, frequently noted in humans, is rare in the dog [23]. In our opinion, research focused on LVO and 2D LV measurements is needed for the clinical application of CE in veterinary medicine.

In the present study, consistent with previous studies, UE underestimated most echocardiographic values compared to the MDCT [24-26]. Other values, including LV volume and 2D LV measurements of wall thickness and internal diameter, were underestimated compared to MDCT. In contrast to the previous study [24], the internal diameters of the LV were significantly underestimated regardless cardiac cycle. Since these values are used to predict volume overload and LV systolic function [5], the differences in measurements may have clinical significance. Although the differences are small, since 2D measurements are cubed to calculated LV volumes, it could be important clinically. As LV volumes including EDV and ESV are widely used as prognostic indicators in dogs with heart disease [5,6,27,28], CE expected to be used to accurately assess prognosis in dogs with heart disease.

One of major limitations of echocardiography is foreshortened LV longitudinal length. During an echocardiographic examination, only a few intercostal windows are available, and this issue is exacerbated in patients with lung disease and obesity [7]. Previous veterinary echocardiographic studies have not compared LV longitudinal length directly with MDCT, as a reference standard. In the present study, LVLd and LVLs were significantly underestimated in UE, and foreshortened more than 10 percentages compared to the MDCT. However, with CE the measurements were lengthened regardless of the cardiac cycle. It may due to primarily the sonographic contrast agent filled apex of the heart and LV trabeculations, clearly outlining the endocardial border [20]. With CE the observer can use an extended intercostal window that cannot be assessed in UE because of pericardial fat, ribs, and soft tissue causing an acoustic attenuation [12]. However, in CE, as the microbubbles fill the LV cavity, there is a contrast between myocardium and LV cavity even with an acoustic attenuation. This suggests CE will be useful and can be applied for patients with lung disease or obesity. On the other hands, inter-observer variability was reduced with CE. With UE, during cardiac contraction, the endocardial border can be confused due to the trabeculations and papillary muscle [7]. Although the inter-observer agreement with CE did not reach that with MDCT, CE improved inter-observer agreement regardless of the cardiac cycle.

Measurement of LV mass and RWT is used in human medicine to evaluate LV geometry, especially in patients with compensatory LV remodelling [29]. Classification of LV geometry
has been used as a prognostic indicator and to grade compensatory LV remodelling [29]. In veterinary medicine, LV mass has only been studied in cats with hypertrophic cardiomyopathy [30], and there is a lack of the previous study for the dog. In the present study, LV mass and RWT obtained by UE were overestimated, possibly due to the inability of the observer to clearly distinguish trabeculations and papillary muscles from LV endocardium. In contrast, CE did not overestimate these values when compared to values from MDCT. It suggested that CE may be useful in the evaluation of LV geometry in dogs.

The limitations of the present study include the small number of dogs and their lack of diversity in terms of breed, weight, age, and cardiac disease. Therefore, we could not estimate the effect of chest conformation, body condition score, and cardiac remodelling on measurements made with CE. In addition, although CE improves LV foreshortening, inter-observer agreement, and the underestimation of LV volume, the clinical significance of these improvements is unclear. Although the more accurate echocardiographic measurements were made with CE, it is unclear if this will influence drug prescribing, patient management, or improve prolong survival. To investigate the clinical relevance of CE, further studies with dogs of various breeds, with and without cardiac disease, and with long term follow up are needed. Another limitation is that microbubbles in the LV chamber can induce bias during measurement resulting in lengthening of the 2D LV measurements compared to the UE measurements. The difference in LV measurements may be exaggerated because of the volume of the contrast medium for MDCT scan.

In conclusion, this study demonstrated that CE is more accurate and reproducible than UE in assessing 2D LV measurements. CE overcomes the well-known limitations of UE, including LV foreshortening and high observer dependency. To the best of our knowledge, this is the first veterinary study to evaluate 2D LV measurements using UE and CE, statistically comparing these measurements with those from the 256-row MDCT as the reference standard.

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