Assessment of left atrial function in feline hypertrophic cardiomyopathy by using two-dimensional speckle tracking echocardiography

CURRENT STATUS: UNDER REVIEW

Arisara Kiatsilapanan
Department of Veterinary Medicine Faculty of Veterinary Science Chulalongkorn University

Sirilak Surachetpong
Chulalongkorn University

Corresponding Author
sirilakd27@gmail.com

DOI: 10.21203/rs.3.rs-17226/v1

SUBJECT AREAS
Small Animal Medicine

KEYWORDS
Cats, Heart, Left atrium, Strain
Abstract
Background: Left atrial (LA) function plays an important role in diastolic dysfunction in cats with hypertrophic cardiomyopathy (HCM). Two-dimensional speckle tracking echocardiography (2D-STE) is a novel technique for assessing LA function. This study aimed to evaluate changes in the LA function in HCM cats compared to normal cats, using 2D-STE.

Results: Seventeen client-owned cats affected with HCM and twenty healthy control cats. Conventional echocardiographic and 2D-STE variables were measured and compared between groups (the normal and HCM groups). The difference between two groups was compared by using the independent Student’s t-test. A p-value less than 0.05 was considered significant. Variability from the 2D-STE variables tests displayed good reproducibility (coefficient of variation ≤15%). The mean value of peak atrial longitudinal strain (PALS) in the HCM group (13.16 ± 8.64) was lower in the control group (28.54 ±10.31) (p < 0.001). PALS was lowest at the LA roof region. The atrial longitudinal strain of septal and lateral regions was significantly lower in the HCM group than the normal group. PALS correlated with the percentage of the LA fractional shortening (LA-FS) (r=0.538, p =0.001), the percentage of the LA ejection fraction (LA-EF) (r =0.797, p <0.001), and the LA fractional area change (FAC) (r =0.746, p <0.001).

Conclusions: PALS can be used to evaluate changes in the LA function in HCM cats. It is a reproducible method for assessing the LA function in cats affected with HCM. Keywords: cats; heart; left atrium; strain

Background
Hypertrophic cardiomyopathy (HCM) is one of the most common myocardial diseases. The prevalence of HCM in cats was approximately 10-15% in cats and increased with age [1, 2]. Hypertrophic cardiomyopathy is characterized by a ≥ 6 mm thickness in diastole of left ventricular wall, as assessed by echocardiography [3]. Diastolic dysfunction can occur secondary to an increased left ventricular wall thickness. Some HCM cats may develop congestive heart failure or arterial thromboembolism [4]. The left atrium (LA) plays an important role in cardiac performance through its three phasic functions: reservoir, conduit, and booster pump function [5–9]. First is the reservoir phase, in which the left...
atrium obtains blood from the pulmonary venous flow during the left ventricular systole. Second is the conduit phase, when the left atrium passively transfers blood into the left ventricle during early diastole. The last phase is the booster pump that represents LA contraction during the late diastole [5–9]. Assessment of LA function can be used to indicate the chronicity, severity and progression of the disease [10].

Several methods have been used in the assessment of LA function in humans and dogs, such as echocardiography, magnetic resonance imaging and computed tomography [11, 12]. Echocardiographic techniques used for evaluating LA function in humans, dogs, and cats include phasic volume changes and tissue Doppler imaging, but these techniques have limitations, including load-dependence, angle-dependence, and the tethering effect [3, 8, 10, 12, 13].

Two-dimensional speckle tracking echocardiography (2D-STE) is a novel echocardiographic technique that can be used to assess LA function, by tracking acoustic speckle patterns of the LA wall, and to analyse the myocardial motion [7, 8, 14]. Two-dimensional speckle tracking echocardiography is feasible, reproducible, and more sensitive than conventional echocardiography for assessing LA function [7, 8, 14].

Two-dimensional speckle tracking echocardiography has been utilized to assess LA function in humans and dogs [5, 6, 9, 14, 15]. Few studies using 2D-STE to assess left ventricular function in HCM cats have been published [16–20]. To our knowledge, there are no studies focusing on the assessment of LA function by 2D-STE in feline HCM. We hypothesized that changes in LA function of HCM cats can be detected using 2D-STE. This study aimed to evaluate changes in LA function in HCM cats and compare them to healthy control cats, using 2D-STE.

Methods

**Animals**

The study population consisted of 20 healthy cats as controls and 17 client-owned cats affected with HCM. All cats presented at Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand during August 2018 – June 2019. The study protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science,
Chulalongkorn University, Thailand (Animal Use Protocol No.1831073). The sample size for each group (at least 17 cats per group) was calculated by the statistical program (G*Power test 3.1) with the estimated standard deviation of the percentage of the LA fractional shortening (LA-FS) from a previous study [6], an expected 80% power, and a at 0.05, which was enough to find a minimum difference of LA-FS between two groups.

Information on all cats, including breed, sex, age, body weight, body condition score, and clinical findings was recorded. All cats were subject to a complete physical examination, systolic blood pressure measurement and blood collection for complete blood count, blood chemistry and total T4 measurements. Cardiac examination including echocardiography, electrocardiography and thoracic radiography was performed in all cats. No cats had received medications before being enrolled in the study.

**Inclusion and exclusion criteria**

As inclusion criteria, the control group consisted of healthy cats that had left ventricular wall thickness of < 6 mm and left atrial diameter <16 mm assessed by echocardiography and had no other systemic illness. Cats with left ventricular wall thickness of ≥ 6 mm in at least one region during end diastole [21] were recruited into the HCM group.

For exclusion criteria, cats affected with other cardiomyopathies including restrictive cardiomyopathy, dilated cardiomyopathy or unclassified cardiomyopathy were excluded. Restrictive cardiomyopathy was identified by left atrial or biatrial enlargement, normal left ventricular wall thickness, normal or decreased systolic function, and restrictive ventricular filling pattern with pulsed wave Doppler echocardiography [22]. Dilated cardiomyopathy was characterized by cardiac chamber dilatation (left ventricular end diastolic dimension > 17 mm) with wall thinning and systolic dysfunction (left ventricular fractional shortening less than 35 % and left ventricular end systolic dimension > 12 mm ) [23-25]. Cats that could not be categorized into the above-mentioned forms of cardiomyopathy were classified as unclassified cardiomyopathy. Cats with renal disease (creatinine >2.0 mg/dL), systemic hypertension (systolic blood pressure >160 mmHg), hyperthyroidism (serum total T4 concentration >4 µg/dl) [21] and any systemic diseases were excluded from the study.
Conventional echocardiography

Two-dimensional and M-mode echocardiography were performed by an investigator (SS). An ultrasound machine (Eko7, Samsung Medison, Seoul, South Korea) with a 4-12 MHz phased array transducer was used. Two-dimensional and M-mode echocardiography was performed on the right parasternal long-axis four-chamber view, to measure the chamber size and wall thickness. The M-mode cursor placed perpendicular to the interventricular septum and left ventricular wall below the tips of the mitral valves at the largest ventricular chamber size. Left ventricular internal dimension at end-diastole and end-systole, interventricular septum thickness at end-diastole and end-systole, left ventricular posterior wall thickness at end-diastole and end-systole were recorded. The ratio of LA to aorta dimension was measured during first diastolic frame of aortic valve closure from a right parasternal short axis view [3].

Pulsed-wave Doppler and tissue Doppler imaging were used for assessing left ventricular diastolic function. Transmitral flow velocities were measured from the left apical four-chamber view. The gate was placed at the tips of the mitral valve leaflets when they were wide open [26]. Peak velocity of early diastolic transmitral flow, peak velocity of late transmitral flow and the ratio of peak velocity of early diastolic to late diastolic transmitral flow was recorded. Isovolumic (or isovolumetric) relaxation time was measured from the left apical five-chamber view by placing the gate in the left ventricular outflow tract near the anterior mitral valve leaflet to reveal both aortic ejection flow and left ventricular inflow [27, 28]. Pulmonary vein flow velocities were measured in the right parasternal short-axis view [29]. Peak velocity of systolic and diastolic pulmonary vein flow, and flow reversal at atrial contraction were recorded. The ratio of peak velocity of systolic to diastolic pulmonary vein flow were calculated. The myocardial motion along the longitudinal axis of the heart was investigated by placing the gate on the subendocardial portions of the lateral corner of the mitral annulus [30]. Peak velocity of early and late diastolic mitral annular motion and the ratio of peak velocity of early to late diastolic mitral annular motion as determined by pulsed-wave Doppler echocardiography were recorded. The ratio of peak velocity of early diastolic transmitral flow to peak velocity of early diastolic mitral annular motion were calculated [31].
The LA diameter (LAD) was measured on the right parasternal long-axis four-chamber view parallel with the mitral annulus [32]. The maximal and minimal LAD (LADmax and LADmin) were measured. The LADmax was measured at end-systole (one frame before opening of mitral valve), and the LADmin was measured at peak atrial contraction (one frame before closure of mitral valve) [10]. Changes of the LAD were expressed as the percentage of fractional shortening of the left atrium (LA-FS) by the formula (LADmax-LADmin/LADmax) x100. The left apical four-chamber view was used to measure the maximal LA volume during atrial end-diastole and minimal LA volume during atrial end-systole. The percentage of LA ejection fraction (LA-EF) was calculated by an ultrasound machine automated software [3]. The LA area change (FAC) was measured by tracing the LA endocardial border during LA end diastolic and systolic phases on left apical four-chamber view. Left atrial maximal area (LAAmax) and minimal area (LAAmin) were measured in cm². Then, FAC was then calculated with the formula FAC = [(LAAmax – LAAmin)/LAAmax] x 100 [5, 7]. The measurements of LA-EF and FAC were performed on the same cardiac cycle as 2D-STE.

Two-dimensional speckle tracking echocardiography (2D-STE)

Two-dimensional speckle tracking echocardiography of the left apical four-chamber view was used to analyze the longitudinal deformation of the left atrium. Two-dimensional echocardiographic images were recorded at 100 frames/s for consecutive three cardiac cycles and three sceneries and stored these in the Digital Imaging and Communications in Medicine format. Offline analysis was performed in images with good quality from each cat. The investigator was blinded to the group where the cats were allocated. The LA wall, including the interatrial septum, the lateral wall and the atrial roof were tracked along during end-diastole. After automatic tracking, manual editing was performed to correct software errors in the region of interest. The ultrasound machine computer software (Strain 2.0 with Bull’s Eye) calculated the LA strain. The mean values of the measurements from three consecutive cardiac cycles were used in all analyses. Six speckle segments were analyzed in each cat. The strain of each segment (as percentages) was plotted on the y-axis versus time (in seconds) on the x-axis over an entire cardiac cycle (Fig. 1). The different colored graphs represent strains from different segments. The white dotted line is the average strain. The peak atrial longitudinal strain (PALS) was
manually measured from the peak strain of the average strain value at the end of reservoir phase [7].

**Measurement variability**

The data from six randomly selected cats in the control group were used for calculating the variability of PALS. For the intra-observer variability, the measurement data from the same operator repeated on two different days (seven days apart) were used. Measurements were performed in the same cardiac cycle from the same cine loop. The inter-observer variability was calculated from measurements of two operators with different levels of experience in echocardiography. The variability was quantified as the coefficient of variation (CV) by the formula, \( \%CV = \frac{\text{standard deviation}}{\text{mean}} \times 100 \). The degree of repeatability was determined as follows: CV <5%, very low variability; 5-15%, low variability; 16-25% moderate variability; or >25% high variability [33].

**Statistical analysis**

Statistical analyses were performed using a commercially available software (SPSS version 22, Inc, Chicago, IL, USA). Descriptive statistics were used to describe the characteristics of the cats including sex, breed, age, body weight, systolic blood pressure and heart rate. The normality of data was assessed with the Shapiro-Wilks normality test. Comparisons between the two groups (the control and HCM cats) were performed by using the independent student t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. The categorical data were compared by using Fisher’s exact test. Comparisons of 3 subgroups, including control and HCM cats with LAD <16 mm, and the HCM cats with LAD >16 mm, were performed by using the one-way Analysis of Variance. The multiple comparisons were performed by the Bonferroni method. An Analysis of Covariance model was used to test the fixed effects of sex, breed, and age, as covariates on conventional and 2D-STE-derived echocardiographic variables. The correlations between PALS and LA-FS, LA-EF, and FAC, assessed by conventional echocardiography, were tested by the Pearson’s correlation coefficient. A value of \( P <0.05 \) was considered significant.

**Results**

A total of 37 cats were included in the study, including 20 control cats and 17 HCM cats. The general
characteristics of the control and HCM groups are summarized in Table 1. Age, body weight, heart rate and systolic blood pressure did not differ significantly between the control and HCM groups. Male and domestic shorthair cats were over-represented, but the number of cats in each sex and breed did not differ significantly between the control and HCM groups. Five of 17 cats in the HCM group had systolic anterior motion assessed by echocardiography. None of cats recruited to the study had mitral valve dysplasia.

Table 1
The general characteristics of cats in the control and hypertrophic cardiomyopathy groups

| Variable                  | Control (N = 20) | HCM (N = 17) | p-value |
|---------------------------|------------------|--------------|---------|
| Age (year)                | 5.05 ± 3.03      | 5 ± 3.43     | 0.963   |
| Body weight (Kg)          | 4.47 ± 0.92      | 4.25 ± 1.04  | 0.497   |
| Sex                       |                  |              |         |
| Male                      | 14               | 10           | 0.512   |
| Female                    | 6                | 7            |         |
| Heart rate (bpm)          | 206 ± 20         | 209 ± 31     | 0.798   |
| Systolic blood pressure (mmHg) | 128 ± 20 | 116 ± 20   | 0.073   |
| Breed                     |                  |              |         |
| Domestic Shorthair        | 10               | 10           | 0.843   |
| American shorthair        | 5                | -            |         |
| Sphinx                    | 2                | -            |         |
| Scottish fold             | 1                | -            |         |
| Siamese                   | 1                | 1            |         |
| Exotic shorthair          | 1                | 1            |         |
| Persian                   | -                | 1            |         |
| Khao Manee                | -                | 1            |         |

Age, body weight, heart rate and systolic blood pressure are expressed as mean ± standard deviation. Sex and breeds are expressed as the number of cats. Numerical data were compared by using the independent student t-test. Categorical data were compared by using Fisher’s Exact test.

The result of the conventional echocardiography showed an increase of interventricular septum thickness at end-diastole (P < 0.001), left ventricular posterior wall thickness at end-diastole (P < 0.001), interventricular septum thickness at end-systole (P = 0.01), left ventricular posterior wall thickness at end-systole (P = 0.026), LAD and the ratio of LA to aorta dimension in the HCM group (P < 0.001) compared to the control group, while left ventricular internal dimension at end-diastole were significantly lower in the HCM group than in the control group (P = 0.027). The pulsed-wave Doppler echocardiography demonstrated that peak velocity of early diastolic transmitral flow (P = 0.002), peak velocity of systolic pulmonary vein flow (P = 0.001), and peak velocity of diastolic pulmonary vein flow (P = 0.006) were significantly lower in the HCM group than the control group. The pulsed-wave echocardiography and tissue Doppler imaging were not significantly different between the two
groups. The LA-FS (P < 0.001), LA-EF (P = 0.001) and FAC (P < 0.001) were significantly lower in the HCM group than in the control group (Table 2).

Table 2
Comparison of conventional echocardiographic values in the control and hypertrophic cardiomyopathy groups

| Variable      | Control (N = 20) | HCM (N = 17) | p-value     |
|---------------|-----------------|--------------|-------------|
| Size and structure |              |              |             |
| IVSd (mm)     | 4.24 ± 0.89     | 7.12 ± 1.14  | < 0.001*    |
| LVId (mm)     | 14.31 ± 1.65    | 12.38 ± 3.03 | 0.027*      |
| LVPWd (mm)    | 3.5 ± 0.59      | 5.56 ± 1.64  | < 0.001*    |
| IVSs (mm)     | 7.18 ± 1.40     | 8.63 ± 1.86  | 0.01*       |
| LVIDs (mm)    | 6.35 ± 1.63     | 6.06 ± 2.38  | 0.669       |
| LVPWs (mm)    | 7.01 ± 0.92     | 8.24 ± 1.94  | 0.026*      |
| LA (mm)       | 12.22 ± 1.42    | 15.55 ± 2.81 | < 0.001*    |
| Ao (mm)       | 8.66 ± 1.3      | 8.3 ± 1.73   | 0.476       |
| LA:Ao         | 1.44 ± 0.28     | 1.92 ± 0.44  | < 0.001*    |
| LV function   |                |              |             |
| FS%           | 55.58 ± 10.22   | 51.38 ± 10.79| 0.233       |
| E (m/s)       | 0.83 ± 0.19     | 0.62 ± 0.18  | 0.002*      |
| A (m/s)       | 0.61 ± 0.12     | 0.53 ± 0.29  | 0.346       |
| E:A           | 1.34 ± 0.31     | 1.46 ± 0.74  | 0.09        |
| IVRT (m/s)    | 45.7 ± 6.64     | 47.4 ± 9.12  | 0.504       |
| S (cm/s)      | 0.46 ± 0.09     | 0.31 ± 0.13  | 0.001*      |
| D (cm/s)      | 0.35 ± 0.06     | 0.26 ± 0.12  | 0.006*      |
| AR (cm/s)     | 0.14 ± 0.03     | 0.16 ± 0.05  | 0.367       |
| S:D ratio     | 1.25 ± 0.2      | 1.2 ± 0.22   | 0.464       |
| E (m/s)       | 0.11 ± 0.03     | 0.08 ± 0.04  | 0.056       |
| A (m/s)       | 0.08 ± 0.03     | 0.07 ± 0.04  | 0.54        |
| S (m/s)       | 0.07 ± 0.02     | 0.07 ± 0.03  | 0.625       |
| E:A ratio     | 1.33 ± 0.31     | 1.15 ± 0.39  | 0.16        |
| E:E′ ratio    | 7.8 ± 2.92      | 9.7 ± 6.8    | 0.285       |
| LA function   |                |              |             |
| LA-FS (%)     | 27.27 ± 6.94    | 14.95 ± 8.23 | < 0.001*    |
| LA-EF (%)     | 69.10 ± 13.64   | 46.09 ± 21.48| 0.001*      |
| FAC (%)       | 63.73 ± 11.30   | 35.54 ± 18.07| < 0.001*    |

A peak velocity of early diastolic transmitral flow, A peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler, Ao Aorta, AR peak velocity of pulmonary vein flow reversal at atrial contraction, D peak velocity of diastolic pulmonary vein flow, E peak velocity of early diastolic transmitral flow, E′ peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler, E:A ratio of E to A, E:A′ ratio of E to A, E:E′ ratio of E to E′, FAC left atrial fractional area change, FS left ventricular fractional shortening, HCM hypertrophic cardiomyopathy, IVRT isovolumic (or isovolumetric) relaxation time, IVSd interventricular septum thickness at end-diastole, IVSs interventricular septum thickness at end-systole, LA left atrium, LA:Ao left atrial and aorta ratio, LA-FS left atrial fractional area change, LA-EF left atrial fractional shortening, LVId left ventricular internal dimension at end-diastole, LVId left ventricular internal dimension at end-systole, LVPWd left ventricular posterior wall thickness at end-diastole, LVPWs left ventricular posterior wall thickness at end-systole, S peak velocity of systolic pulmonary vein, S′ peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler, S:D ratio of S to D

Data are expressed as mean ± standard deviation.

* indicate statistical significance at p < 0.05 assessed by the independent student t-test.

The PALS was significantly lower in the HCM group than the control group (P < 0.001). The longitudinal strain of all six LA regions was significantly reduced in the HCM group compared to the normal group, except the septal-roof and the lateral-roof of the LA (Table 3) (Fig. 2).
Table 3

Two-dimensional echocardiographic data of cats in the control and hypertrophic cardiomyopathy groups.

| Variable | Control (N = 20) | HCM (N = 17) | p-value |
|----------|------------------|--------------|---------|
| PALS     | 27.25 [21.18,32.97] | 13.33 [5.53,17.32] | < 0.001* |
| Longitudinal strain of each left atrial region | | | |
| Septal - base | 24.01 [15.84,40.74] | 12.30 [5.22,19.01] | 0.006* |
| Septal - mid | 32.12 [25.67,54.54] | 17.07 [6.48,24.82] | < 0.001* |
| Septal - roof | 17.18 [6.22,29.59] | 7.57 [3.23,13.13] | 0.063 |
| Lateral - roof | 18.43 [8.66,32.19] | 4.00 [-0.22,28.47] | 0.067 |
| Lateral - mid | 23.30 [18.17,35.77] | 15.83 [9.72,25.42] | 0.038* |
| Lateral - base | 37.42 [13.60,46.80] | 10.87 [7.22,17.23] | 0.002* |

PALS peak atrial longitudinal strain, HCM hypertrophic cardiomyopathy
Data are expressed as median and 25th, 75th percentiles.
* indicate statistical significance at p < 0.05 by Mann-Whitney U Test.

An assessment of the LA function of 3 subgroups [control group (n = 20), HCM cats with a LA diameter < 16 mm (n = 9), and HCM cats with a LA diameter ≥ 16 mm (n = 8)] was compared (Table 4). The results showed that LA-FS (P < 0.001), LA-EF (P = 0.001), FAC (P < 0.001) and PALS (P < 0.001) in both HCM cat subgroups were significantly lower than in the control group. However, the values of these variables were not significantly different between HCM cat subgroups.

Table 4

Assessment of left atrial function of 3 subgroups of cats.

| Variables | Control (N = 20) | HCM with LAD < 16 mm (N = 9) | HCM with LAD ≥ 16 mm (N = 8) | p-value |
|-----------|------------------|-----------------------------|-----------------------------|---------|
| LA-FS (%) | 25.27 [23.30,31.67] | 17.48 [8.68, 19.67]* | 14.26 [6.38, 20.00]* | < 0.001* |
| LA-EF (%) | 64.97 [59.75,83.05] | 36.53 [28.07, 75.9]* | 39.87 [25.45, 60.42]* | < 0.001* |
| FAC (%)  | 63.28 [57.46, 72.30] | 29.57 [14.66, 29.57]* | 32.09 [24.07, 52.39]* | < 0.001* |
| PALS (%) | 27.25 [21.18, 32.97] | 8.45 [5.93, 21.57]* | 14.50 [5.93, 18.34]* | < 0.001* |

FAC left atrial fractional area change, HCM hypertrophic cardiomyopathy, LAD left atrial diameter, LA-EF the percentage of left atrial ejection fraction, LA-FS the percentage of left atrial fractional shortening, PALS peak atrial longitudinal strain
Data are expressed as median [25th percentile and 75th percentile]
* indicate statistical significance at p < 0.05 of 3 subgroups.
The significance difference was assessed by the Kruskal-Wallis test.
* indicate significant difference compared with the control group

The correlations between the peak atrial longitudinal strain and echocardiographic values assessed by conventional echocardiography in entire population showed weak positive correlations between PALS and left ventricular internal dimension at end-diastole (r = 0.362, P = 0.028), peak velocity of early diastolic transmitral flow (r = 0.41, P = 0.012), peak velocity of systolic pulmonary vein flow (r = 0.41, P = 0.013), peak velocity of diastolic pulmonary vein flow (r = 0.341, P = 0.042), and peak velocity of early diastolic mitral annular motion as determined by pulsed-wave Doppler echocardiography (r =
Weak negative correlations between PALS and left ventricular posterior wall thickness at end-systole \( (r=-0.379, P=0.021) \) and interventricular septum thickness at end-systole \( (r=-0.393, P=0.016) \). Moderate negative correlations between PALS and interventricular septum thickness at end-diastole \( (r=-0.563, P<0.001) \) and left ventricular posterior wall thickness at end-diastole \( (r=-0.516, P=0.001) \) were observed. There was no significant correlation between PALS and LAD (Table 5). There was also no correlation between PALS and heart rate \( (r=-0.042, P=0.803) \). 

The correlation analysis of data of all cats demonstrated a highly positive correlation between PALS and LA-EF \( (r=0.797, P<0.001) \) as well as PALS and FAC \( (r=0.746, P<0.001) \). The peak atrial longitudinal strain moderately correlated with LA-FS \( (0.538, P=0.001) \) (Fig. 3).
LA-EF. However, FAC was affected by breeds (domestic shorthair or pure breeds) (P = 0.04). The intra-observer and inter-observer measurements of PALS variability were 4.17% and 14%, respectively.

Discussion
We undertook this study to assess LA function of control cats and cats affected with HCM, using 2D-STE. The first major finding of this study is that 2D-STE can detect changes in LA function. The second was that the change in LA function can be detected in cats with HCM, despite the LA size is not yet being enlarged. Lastly, 2D-STE values correlate well with values of LA function variables, including LA-FS, LA-EF and FAC assessed, by conventional echocardiography.

Two-dimensional speckle tracking echocardiography is an echocardiographic technique that can be used to assess LA longitudinal strain in dogs [5, 6, 9] and humans [7, 8, 15, 34]. Few studies using 2D-STE to assess left ventricular function in cats with HCM have been published [16–20]. This technique has been used to evaluate left ventricular myocardial function with adequate repeatability [16–18]. To our knowledge, no study focusing on assessment of LA function in feline HCM using 2D-STE has been reported. The present study demonstrated that 2D-STE may be used to evaluate changes in LA function in HCM cats with high repeatability and reproducibility. Intra- and inter-observer variability of PALS were clinically acceptable (CV < 15%), and similar results have been reported in humans [15] and dogs [5, 9].

The peak atrial longitudinal strain, assessed by 2D-STE, provides information on the longitudinal deformation of the left atrium during the reservoir phase [7, 8]. The result of this study showed that PALS was lower in the HCM group than the control group. In addition, LA-FS, LA-EF and FAC, conventional echocardiographic parameters for assessing LA contraction, were found to be lower in the HCM group than the control group. Based on these findings, it is likely that HCM cats have not only left ventricular diastolic dysfunction but also impaired LA function. This result is in agreement with a previous study reporting a reduction of LA function in cats affected with HCM [10]. The present study showed that the LA roof had the lowest longitudinal strain. The LA roof is closed to the mediastinum, which may limit the movement of this region [14]. The longitudinal strains of all LA
regions were decreased in the HCM group, suggesting that global LA reservoir functional changes in cats affected with HCM. In this study, only one peak of atrial longitudinal strain was found in cats which is different from that found in humans and dogs [5, 7]. This finding may occur secondary to rapid heart rate in cats.

Interestingly, the present study showed that changes in PALS, LA-FS, LA-EF and FAC were found in HCM cats with both normal and enlarged LA size. This finding suggests that the poor performance of the LA function in HCM cats is not dependent on the LA size. Moreover, the change in LA function may occur before the LA structural change.

The peak atrial longitudinal strain assessed by 2D-STE correlated with LA function parameters assessed by conventional echocardiography. This result suggests that PALS offers an additional method for evaluating LA function. A previous study suggested that 2D-STE is a less load-dependent and higher repeatability method than conventional echocardiography [7]. Based on ANCOVA of data from this study, PALS was not affected by age, breeds or sex. However, breeds affected FAC, when assessed by conventional echocardiography. These findings suggest that PALS may be a more suitable technique for evaluating LA function in cats than parameters assessed by conventional echocardiography.

Interestingly, PALS also correlated with left ventricular wall thickness and chamber size and diastolic function parameters assessed by conventional echocardiography and tissue Doppler imaging. This finding suggests the relationship between left ventricular structural and functional changes and LA reservoir function. The PALS and peak velocity of early diastolic transmitral flow were decreased in the HCM group. A decrease in peak velocity of early diastolic transmitral flow is associated with an increase in left ventricular filling pressure. These findings suggest that a decrease in LA reservoir phase function may be a consequence of an increase in left ventricular filling [38].

The present study has some limitations that should be considered. First, the number of cats used in this study was relatively small. Studies with a larger number of cats should be performed before adapting 2D-STE for use in clinical routine. Second, 2D-STE software was created for analysis of left ventricular function in humans; therefore, it may have some limitations for LA function assessment in
cats. Third, during assessment, it was challenging to trace the thin feline LA wall and therefore also some undesired tracking of extracardiac structure (like mediastinum) could have occurred; however, increasing the size by increasing the dept and gain of images may help to visualize the LA wall.

Fourth, myocardial wall dropout in the interatrial septum and pulmonary vein inlets may affect the tracking procedure. Tracing the LA cavity before the atrial contraction may help to visualize these areas better [14]. Lastly, 2D-STE requires high-quality images that are difficult to obtain in cats with a very fast heart rate. The 2D-STE software used in the present study does not allow analysis in subjects with heart rate more than 240 beats/minute. Therefore, cats had to be handled in low-stress conditions to help maintain a low heart rate. The use of ultrasound machine system with higher frame rate may solve this problem [35]. The correlation between PALS and heart rate was not found in the present study suggesting that more concerns have to be taken for effects of heart rate on image and tracking quality.

Conclusions
Assessment of the PALS of LA assess by 2D-STE is feasible to evaluate changes in LA function of HCM cats. This method has accurate repeatability and reproducibility and may be useful for the diagnosis and management of cats affected with HCM. The method may be improved further by the development of specialized software for veterinary use. Studies with a larger number of HCM cats should be performed to confirm the advantage of 2D-STE in assessment LA function before the technique can be applied into routine clinical use.

Abbreviations
2D-STE
Two-dimensional speckle tracking echocardiography; CV:Coefficient of variation; FAC:Fractional area change; HCM:Hypertrophic cardiomyopathy; LA:Left atrium (atrial); LA-EF:The percentage of the LA ejection fraction; LA-FS:The percentage of the LA fractional shortening; LAA:Left atrial area; LAD:Left atrial diameter; PALS:Peak atrial longitudinal strain.

Declarations
Acknowledgements:
The authors would like to thank the Small Animal Hospital, Faculty of Veterinary Science,
Chulalongkorn University for supporting data and facilities and Dr. Vachira Hunprasit for the statistical assistance.

**Funding**

This study was supported by the 90th Anniversary of Chulalongkorn University, Rachadapisek Sompote Fund and the 72nd anniversary of his Majesty King Bhumibala Aduladeja Fund, Graduate School, Chulalongkorn University, Thailand.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Author’s contributions**

Study conception and design: SDS; Acquisition of data: AK and SDS; Analysis of and interpretation of data: AK and SDS; Drafting of manuscript: AK and SDS; Critical revision: SDS; All authors read and approved the final manuscript.

**Ethics approval**

The study protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University, Thailand (Animal Use Protocol No.1831073). The owner gave consent for their pets to be part of this study.

**Consent for publication**

Written informed consent was obtained from the cats’ owners for publication of this manuscript.

**Competing interests**

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

**References**

1. Paige CF, Abbott JA, Elvinger F, Pyle RL. Prevalence of cardiomyopathy in apparently healthy cats. J Am Vet Med Assoc. 2009;234(11):1398-403.

2. Payne J, Borgeat K, Connolly D, Boswood A, Dennis S, Wagner T, et al. Prognostic
indicators in cats with hypertrophic cardiomyopathy. J Vet Intern Med. 2013;27(6):1427-36.

3. Abbott JA, MacLean HN. Two-dimensional echocardiographic assessment of the feline left atrium. J Vet Intern Med. 2006;20(1):111-9.

4. Silva A, Muzzi R, Oberlender G, Nogueira R, Muzzi L. Feline hypertrophic cardiomyopathy: an echocardiographic approach. Arch Med Vet. 2013;45(1).

5. Baron Toaldo M, Romito G, Guglielmini C, Diana A, Pelle N, Contiero B, et al. Assessment of Left Atrial Deformation and Function by 2-Dimensional Speckle Tracking Echocardiography in Healthy Dogs and Dogs With Myxomatous Mitral Valve Disease. J Vet Intern Med. 2017;31(3):641-9.

6. Caivano D, Rishniw M, Birettoni F, Patata V, Giorgi M, Porciello F. Left atrial deformation and phasic function determined by two-dimensional speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Cardiol. 2018;20(2):102-14.

7. Cameli M, Lisi M, Righini FM, Mondillo S. Novel echocardiographic techniques to assess left atrial size, anatomy and function. J Cardiovasc Ultrasound 2012;10(1):4.

8. Cianciulli TF, Saccheri MC, Lax JA, Bermann AM, Ferreiro DE. Two-dimensional speckle tracking echocardiography for the assessment of atrial function. World J Cardiol. 2010;2(7):163.

9. Dermlim A, Nakamura K, Morita T, Osuga T, Nisa K, Sasaoka K, et al. The Repeatability and Left Atrial Strain Analysis Obtained via Speckle Tracking Echocardiography in healthy Dogs. J Vet Cardiol. 2019;23:69-80.

10. Linney C, Dukes-McEwan J, Stephenson H, López-Alvarez J, Fonfara S. Left atrial size, atrial function and left ventricular diastolic function in cats with hypertrophic cardiomyopathy. J Small Anim Pract. 2014;55(4):198-206.
11. Vizzardi E, D'aloia A, Rocco E, Lupi L, Rovetta R, Quinzani F, et al. How should we measure left atrium size and function? J Clin Ultrasound. 2012;40(3):155-66.

12. Coelho M, Muzzi R, Abreu C, Schulien T, Muzzi L, Oliveira L, et al. Assessment of left atrial function in dogs with myxomatous mitral valve disease by biplane simpson’s method. Arq Bras Med Vet Zoo. 2018;70(5):1349-54.

13. Todaro M, Choudhuri I, Belohlavek M, Jahangir A, Carerj S, Oreto L, et al. New echocardiographic techniques for evaluation of left atrial mechanics. Eur Heart J Cardiovasc Imaging. 2012;13(12):973-84.

14. Rimbaş RC, Dulgheru RE, Vinereanu D. Methodological gaps in left atrial function assessment by 2D speckle tracking echocardiography. Arq Bras Cardiol. 2015;105(6):625-36.

15. Ahmed MK, Soliman MA, Reda AA, El-Ghani RSA. Assessment of left atrial deformation properties by speckle tracking in patients with systolic heart failure. Egypt Heart J. 2015;67(3):199-208.

16. Suzuki R, Mochizuki Y, Yoshimatsu H, Teshima T, Matsumoto H, Koyama H. Determination of multidirectional myocardial deformations in cats with hypertrophic cardiomyopathy by using two-dimensional speckle-tracking echocardiography. J Feline Med Surg. 2017;19(12):1283-9.

17. Takano H, Isogai T, Aoki T, Wakao Y, Fujii Y. Feasibility of radial and circumferential strain analysis using 2D speckle tracking echocardiography in cats. J Vet Med Sci. 2015;77(2):193-201.

18. Sugimoto K, Fujii Y, Sunahara H, Aoki T. Assessment of left ventricular longitudinal function in cats with subclinical hypertrophic cardiomyopathy using tissue Doppler imaging and speckle tracking echocardiography. J Vet Med Sci. 2015;77(9):1101-8.

19. Visser LC, Sloan C, Stern JA. Echocardiographic assessment of right ventricular size
and function in cats with hypertrophic cardiomyopathy. J Vet Intern Med. 2017;31(3):668-77.

20. Spalla I, Boswood A, Connolly DJ, Luis Fuentes V. Speckle tracking echocardiography in cats with preclinical hypertrophic cardiomyopathy. Journal of Veterinary Internal Medicine. 2019;33(3):1232-41.

21. Nelson RW, Couto CG. Small Animal Internal Medicine. Fifth ed. St.Louis , Missouri: Elsevier - Health Sciences Division; 2009. 145-58,764 p.

22. Fox PR, Basso C, Thiene G, Maron BJ. Spontaneously occurring restrictive nonhypertrophied cardiomyopathy in domestic cats: a new animal model of human disease. Cardiovascular Pathology. 2014;23(1):28-34.

23. MacDonald K, Ettinger SJ, Feldman EC. Myocardial disease: feline. Textbook of veterinary internal medicine : Diseases of the Dog and the Cat. 2. 7 ed. St. Louis: Saunders Elsevier; 2010. p. 1328-41.

24. Tilley LP, SMITH F, Oyama M, Sleeper M. CHAPTER 8 Feline cardiomyopathy. Manual of canine and feline cardiology Missouri: Saunders Elsevier. 4th ed. Missouri: Saunders Elsevier; 2008. p. 168.

25. Pion PD, Kittleson MD, Rogers QR, Morris J. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science. 1987;237(4816):764-8.

26. Disatian S, Bright J, Boon J. Association of age and heart rate with pulsed-wave Doppler measurements in healthy, nonsedated cats. J Vet Intern Med. 2008;22(2):351-6.

27. Boon JA. CHAPTER FOUR Evaluation of Size, Function, and Hemodynamics. Veterinary Echocardiography. second ed: John Wiley & Sons; 2011. p. 234-46,569-80.

28. Schober KE, Maerz I. Assessment of left atrial appendage flow velocity and its relation to spontaneous echocardiographic contrast in 89 cats with myocardial
disease. J Vet Intern Med. 2006;20(1):120-30.

29. Santilli RA, Bussadori C. Doppler echocardiographic study of left ventricular diastole in non-anaesthetized healthy cats. Vet J. 1998;156(3):203-15.

30. Koffas H, Dukes-McEwan J, Corcoran B, Moran C, French A, Sboros V, et al. Colour M-mode tissue Doppler imaging in healthy cats and cats with hypertrophic cardiomyopathy. J Small Anim Pract. 2008;49(7):330-8.

31. Koffas H, Dukes-McEwan J, Corcoran B, Moran C, French A, Sboros V, et al. Pulsed tissue Doppler imaging in normal cats and cats with hypertrophic cardiomyopathy. J Vet Intern Med. 2006;20(1):65-77.

32. Schober KE, Maerz I, Ludewig E, Stern JA. Diagnostic accuracy of electrocardiography and thoracic radiography in the assessment of left atrial size in cats: Comparison with transthoracic 2-dimensional echocardiography. J Vet Intern Med. 2007;21(4):709-18.

33. Bland M. Clinical measurement. An introduction to medical statistics. 3. 3rd ed. Oxford, United Kingdom 2000. p. 269-94.

34. Roșca M, Lancellotti P, Popescu BA, Piérard LA. Left atrial function: pathophysiology, echocardiographic assessment, and clinical applications. Heart. 2011;97(23):1982-9.

35. Joos P, Porée J, Liebgott H, Vray D, Baudet M, Faurie J, et al. High-frame-rate speckle-tracking echocardiography. IEEE transactions on ultrasonics, ferroelectrics, and frequency control. 2018;65(5):720-8.

Figures
Figure 1

The image of the left apical four-chamber view of the left atrial strain profile of a hypertrophic cardiomyopathy cat. A region of interest is manually drawn to include the left atrial wall. The automatic software system divided the left atrial wall into 6 different segments with different colors. A white dotted line is presented as the mean of strain value of the left atrium. LA= left atrium; LV= left ventricle; PALS= peak atrial longitudinal strain (white arrow); RA= right atrium; RV= right ventricle.
Figure 2

The Box and Whisker plot shows median and 25 to 75 percentile range of the atrial longitudinal strain in each region between the normal (blue) and hypertrophic cardiomyopathy (HCM) groups (orange).
Correlations between PALS and other LA function parameters and LA diameter using Pearson’s correlation coefficient; a. PALS and LA-FS ($r=0.538$, $P=0.001$) b. PALS and LA-EF ($r=0.797$, $P<0.001$) c. PALS and FAC ($r=0.746$, $P<0.001$) d. PALS and LAD ($r=-0.248$, $P=0.139$) in the control (white) and hypertrophic cardiomyopathy groups (blue). PALS = peak atrial longitudinal strain; LA-FS = left atrial fractional shortening LA-EF = left atrial ejection fraction; FAC = left atrial fractional area change; LAD = left atrial diameter.