Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.197

RESEARCH ARTICLE

Early Detection of Lapatinib-Induced Cardiotoxicity in Dogs Using Intraventricular Pressure Gradients and Two-Dimensional Speckle-Tracking Echocardiography

Mahmoud A.Y. Helal[1]*†, Ahmed Farag[2]†, Mohamed Elbadawy[3], Tatsuya Usui[4], Kazuaki Sasaki[4], Ryou Tanaka[2]* and Ahmed S. Mandour[5]*†

[1]Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, Qaliobiya 13736, Egypt; [2]Veterinary Teaching Hospital, Tokyo University of Agriculture and Technology, Tokyo, Japan; [3]Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, Qaliobiya 13736, Egypt; [4]Laboratory of Veterinary Pharmacology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan; [5]Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

† These authors contributed equally to this work.

*Corresponding authors: fu0253@go.tuat.ac.jp; dr_mandour@vet.suez.edu.eg; mahmoud.halal@fvtm.bu.edu.eg

ARTICLE HISTORY (25-141)

Received: February 15, 2025
Revised: April 15, 2025
Accepted: April 17, 2025
Published online: April 28, 2025

Key words:
Cardiotoxicity
Diastolic dysfunction
Echocardiography
Lapatinib

ABSTRACT

Lapatinib (tyrosine kinase inhibitor) is commonly used for cancer treatment; it may cause cardiotoxicity through manifesting diastolic dysfunction prior to the onset of systolic impairment. As traditional echocardiographic techniques have the limitations to detect the subclinical cardiotoxicity, hence new interventions are crucial for the timely intervention to treat such toxicities. The current study was planned to investigate the diastolic dysfunction induced by Lapatinib toxicity in cardiac tissues of dogs through non-invasive approaches. For this purpose, two techniques were applied including Intraventricular Pressure Gradients (IVPG) and Two-dimensional Speckle Tracking Echocardiography (2D-STE) on 10 sexually mature female Beagle dogs. Animals were randomly divided into two groups (n=5 each), one group was administered with single toxic dose of Lapatinib (40mg/kg BW) and the other group received a therapeutic dose (30mg/kg BW). IVPG and 2D-STE were applied along with conventional echocardiography before (0-hr), then 4 and 6hrs post-administration of Lapatinib. The results indicated that conventional cardiographs or myocardial strain did not reveal any significant changes in both groups before and after treatments. However, through IVPG, significant reduction in total, basal, mid and mid-to-apical values were observed at post administration time intervals of 4 and 6hrs compared to 0-hr in toxic dose group, revealing early diastolic dysfunction prior to any alteration in strain-based or systolic function. Hence, it was concluded that IVPG can be used for the diagnosis of Lapatinib induced cardiotoxicity prior to any significant change measurable by electrocardiographic techniques in dogs, as well as in other patients treated with tyrosine kinase inhibitors therapy.

To Cite This Article: Helal MAY, Farag A, Elbadawy M, Usui T, Sasaki K, Tanaka R and Mandour AS, 2025. Early Detection of Lapatinib-Induced Cardiotoxicity in Dogs Using Intraventricular Pressure Gradients and Two-Dimensional Speckle-Tracking Echocardiography. Pak Vet J, 45(2): 751-758. http://dx.doi.org/10.29261/pakvetj/2025.197

INTRODUCTION

Cardiotoxicity is a known consequence of exposure to chemotherapeutic agents. Several anticancer drugs have the potential to cause severe cardiotoxicity, which is dependent on the dose and accumulates over time during treatment, posing a serious risk for patients (Lamberti *et al.*, 2014; Chang and Wang, 2018). Patients with cardiotoxicity may show myocardial dysfunctions, including hypertension, heart failure, and structural changes in the heart (Rawat *et al.*, 2021). Therefore, it is essential to find ways and measures to avoid or minimize the cardiovascular side effects associated with cancer treatment while still maintaining its therapeutic benefits for patients (Morelli *et al.*, 2022).

The mechanisms by which anti-cancer drugs cause damage to the heart include the generation of free radicals, leading to cellular damage, and the triggering of immunogenic responses involving antigen-presenting cells within the heart (Kang, 2001). Tyrosine kinase inhibitors

(TKI) have been extensively used for the treatment of different kinds of cancers (Tanaka *et al.*, 2020). Lapatinib is an oral reversible TKI that targets the human epidermal growth factor receptor-2 (HER2) and the epidermal growth factor receptor (EGFR/HER1). The inhibition of HER2 receptors, which are expressed both in cancer cells and cardiac myocytes, leads to oxidative stress, mitochondrial dysfunction, and cell death (Yang *et al.*, 2021). Lapatinib has also been documented to cause adverse cardiac effects which vary from prolongation of QT interval (interval from start of the q wave to the end of the T wave) to reduction of left ventricular ejection fraction, myocardial infarction, acute coronary syndromes, and congestive heart failure (Kerkelä *et al.*, 2006; Fonseca-Alves *et al.*, 2024).

Early identification of cardiotoxicity caused by chemotherapy is still challenging. Diastolic dysfunction is a typical characteristic of many heart diseases and can occur before systolic dysfunction of the left ventricle, leading to heart failure. Early changes in left ventricular diastolic function were found even though the systolic function of the left ventricle was normal during the first month of follow-up after anthracycline (anticancer) therapy (Tassan-Mangina *et al.*, 2006). Traditional approaches are not sufficient, and novel techniques are required to identify diastolic dysfunction at an early stage in individuals who have survived cancer (Shigemitsu *et al.*, 2019).

Recently, there has been great attention in the field of cardiology, both in human and veterinary research, regarding the evaluation of intraventricular pressure differences (IVPDs) and intraventricular pressure gradients (IVPGs) assessed by color M-mode echocardiography (CMME) as non-invasive approaches for the early detection of cardiac dysfunctions, particularly those related to diastolic one (Mandour *et al.*, 2023). This method presents information about velocity along the whole inflow tract from the left atrium (LA) across the mitral valve into the left ventricle (LV) throughout the whole period of diastolic filling rather than a single point, as is the case of conventional pulsed Doppler methods (Matsuura *et al.*, 2022).

Two-dimensional speckle tracking echocardiography (2D-STE) is a sophisticated method that utilizes images of deformation to thoroughly assess myocardial function. Its clinical use is highly promising, as it can provide essential details about regional and global heart muscle function, as well as measure cardiac rotation and synchrony, capabilities not easily achieved with traditional echocardiography (Hamabe *et al.*, 2021). The 2D-STE enables the monitoring of early cardiotoxic effects of chemotherapy through myocardial deformation parameters by measurement of myocardial wall thickness for myocardial strain and strain rate (Calle *et al.*, 2018). An increasing body of literature suggests the utilization of parameters of myocardial deformation to identify early myocardial injury (stage B heart failure) and predict chemotherapy-induced ventricular disorders (Hosono *et al.*, 2024).

During passive filling of heart, the atrioventricular pressure gradient is created by unwinding of the ventricle and elastic recoil, which draws the blood from the atrium into the ventricle. Imatinib belongs to TKI drugs and has been found effective in reducing the isovolumetric relaxation, which is evident through a decrease in the –dP/dt peak amplitude. The term "-dP/dt peak amplitude" refers to the maximal rate of decrease in pressure (dP) over time (dt) during the early phase of ventricular diastole (relaxation), providing information about ability of the heart to relax and fill with blood. This is a diastolic parameter determined through catheterization (Chiba *et al.*, 2022) In contrast, IVPGs are non-invasive tools, having a potential to detect the diastolic changes. We hypothesize that Lapatinib induces early and subclinical cardiotoxicity in dogs, primarily affecting diastolic function before the onset of overt systolic dysfunction. This cardiotoxicity can be detected using advanced echocardiographic techniques, such as IVPG and 2D-STE, which are more sensitive than conventional methods in identifying early diastolic impairment.

The aim of this study was to evaluate the early cardiotoxic effects of Lapatinib on cardiac function in dogs using non-invasive echocardiographic techniques. Specifically, the sensitivity of conventional echocardiography was compared with the advanced echocardiographic techniques, in detecting early diastolic dysfunction induced by Lapatinib. By identifying these early changes, we thought to establish IVPG and 2D-STE as potential diagnostic tools for the timely detection of cardiotoxicity in patients undergoing TKI therapy, thereby enabling early intervention to prevent long-term cardiovascular complications in these individuals.

MATERIALS AND METHODS

Ethical approval: All experimental procedures and animal care followed the guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Tokyo University of Agriculture and Technology, Japan for using animals in experimental studies (Ethical approval number: R04-218).

Animals and study protocol: A total of 10 adult female, clinically healthy Beagle dogs, aged 2-3 years and weighing 8-10kg, were selected for this study. To ensure their healthy status, complete clinical and laboratory examination of dogs was carried out prior to experimental trial. Based on clinical examination, electrocardiography and echocardiography, all the animals were declared free from any kind of cardiac disorder. Animals were randomly divided into two groups (n=5 each), and were orally administered Lapatinib (Tykerb® containing 250mg Lapatinib ditosylate, Novartis). The first group received a single toxic dose of Lapatinib (40mg/kg BW), while the other group received a single therapeutic dose (30mg/kg BW). The Lapatinib dosages were selected on the basis of research findings regarding its dosing, pharmacokinetics and safety aspects in dogs (Maeda *et al.*, 2022; Yu *et al.*, 2024). The echocardiographic evaluations were carried out following the method described by Bence *et al.* (2005) and endorsed by Abdelgalil and Alkahtani (2023) at three distinct intervals; Pre (before Lapatinib administration), Post-1 (4hrs post Lapatinib administration) and Post-2 (6hrs post Lapatinib administration). After the completion of trial, the animals were kept at the Laboratory of Veterinary Surgery under standard housing conditions for the recommended 7 days withdrawal period (Lambertini *et al.*, 2019) and then used for another experiment. No animal was euthanized for the current experiment.

Conventional echocardiography: For echocardiography, ProSound alpha 10 ultrasonography system with a sector probe of 5MHz (Hitachi Aloka Medical, Tokyo, Japan), and having an attached lead II electrocardiogram for measuring the duration of variables for electrocardiography, was used. All the animals were evaluated thoroughly through different electrocardiographic modes (two dimensional, spectral Doppler, M-Mode, and Tissue Doppler Imaging-TDI) to record standard parasternal (left and right) views. Following measurements were recorded: 1) LV end-diastolic (LVIDd) and end-systolic (LVIDs) diameters; 2) diastolic and systolic interventricular septal thickness (IVSd, IVSs); 3) diastolic and systolic LV free wall (LVPWd, LVPWs) thickness; 4) ejection fraction (EF); 5) fractional shortening (FS); 6) aortic root (Ao) diameter; and 7) left atrial (LA) dimensions. Pulsed-wave Doppler echocardiography was used to evaluate the right ventricular outflow tract (RVOT) through pulmonary artery. The aortic blood flow evaluation was done from left apical five-chamber view through the left transthoracic echocardiography, which was started from the parasternal apical four-chamber view. Diastolic indices were assessed using the Doppler function of mitral inflow and TDI including early (E) and late (A) mitral inflow velocities, E/A ratio and declaration time. Following readings were recorded: 1) Early and late diastolic myocardial velocities (Em & Am), using pulsed TDI at both lateral and septal annuli; 2) Ratios of early mitral inflow with early tissue velocity (E/Em), and early tissue velocity with annular tissue velocities (Em/Am).

Assessment of IVPG by color M-mode echocardiography: The intraventricular pressure gradients (IVPG) assessment was done following the method described by Kobayashi *et al.* (2017). Briefly, IVPG was calculated through the left parasternal longitudinal apical four chamber view by using the color M-mode echocardiography (CMME) image. For the precision, the CMME sweep speed was set at 50-100mm/s, consistent with standard settings for high-temporal-resolution M-mode imaging in cardiovascular studies. This range ensured optimal visualization of diastolic flow propagation while minimizing motion artifacts. Later, MATLAB (The MathWorks, Natick, MA, USA) software was used to assess IVPG and calculated by using the IVPD divided by the LV length. The LV was divided into three segments including basal, middle and apical regions. The mid-apical IPVG was measured using 2/3rd segment of LV length on apex side, validated by Yotti *et al.* (2005), through a comparison of IVPD derived from CMME with the temporal IVPD (obtained through catheterization). This IVPG analysis was carried out at the end of the expiratory phase for three consecutive heartbeats and mean values were used for statistical analysis.

The IVPG workflow has been illustrated in Fig. 1 as follows: The left parasternal apical four-chamber echocardiographic view is shown in Fig. 1A. Raw color M-mode echocardiography (CMME) images of mitral inflow, with the color scale representing blood flow velocity, are shown in Fig. 1B. Fig. 1C shows the MATLAB-processed IVPG curves for basal, mid and apical segments; total and mid-to-apical IVPG were derived from these curves mathematically. Fig. 1D shows the temporal distribution of IVPG (mmHg/mm) during diastole, highlighting systolic and early diastolic phases.

The IVPG was segmented into basal, mid, and apical regions (Fig. 1C). Total IVPG represented the cumulative gradient across all segments, while mid-to-apical IVPG was calculated as the mean of mid and apical values. These derived metrics were used for statistical comparisons.

Two-dimensional Speckle tracking echocardiography: The 2D-STE was used to obtain motion images from the left parasternal long-axis view. The frame rates used were between 70 and 110 frames per second. Recorded cine loops were saved for further offline analysis using DAS-RS1 software 1.1v by Hitachi Aloka Medical in Tokyo, Japan (Hamabe *et al.*, 2021). Circumferential and radial strains and strain rates were acquired from the left parasternal short axis view at the papillary muscles level, while the longitudinal strain was obtained from the left apical four-chamber view. Following the acquisition, offline analysis of different types of software was used to measure the global longitudinal strain (GLS), circumferential strain and strain rates, and radial strain and strain rates.

At least three videotapes of optimal imaging quality containing three consecutive cardiac cycles were selected from each dog for 2D-STE analysis. The average frame rate for analyzed images was 79.8±10.5 per second. The left ventricular wall from the short axis and long axis was automatically divided into six segments. By the short axis method (Hamabe *et al.*, 2013), radial and circumferential segmentation with an angular interval of 60° divided the LV into 6 segments: anterior, anterior septal, anterior lateral, inferior, inferior septal, and inferior lateral. From the long axis, the left ventricle was divided into basal anteriolateral, mid-anteriolateral, apical lateral, apical septal, mid inferioseptal, and basal inferioseptal segments. Segmental peak longitudinal, circumferential, and radial strains and strain rates and the global peak systolic strains, which were the average of the 6 segments obtained from the same frame, were calculated. Three different ways that were used to measure the heart muscle movement and changes in shape during each heartbeat are shown in three parts of Fig. 2. Fig. 2A (Radial Strain) shows how much the heart wall thickens inward during contraction. Fig. 2B (Circumferential Strain) demonstrates how the heart muscle squeezes together along its circular diameter. Fig. 1C (Longitudinal Strain) displays how the heart shortens from top (base) to bottom (apex) during pumping.

Statistical analysis: Data thus collected were subjected to statistical analysis through GraphPad (version 8.0, GraphPad Software Inc., San Diego, California). A two-way ANOVA was used to compare the functional parameters between the therapeutic and toxic dose groups at two intervals (post treatment 4 and 6hrs). Tukey's post-hoc test was applied to compare the mean±SD values and $P<0.05$ was considered as significant. Shapiro-Wilk test was applied for data normality assessment.

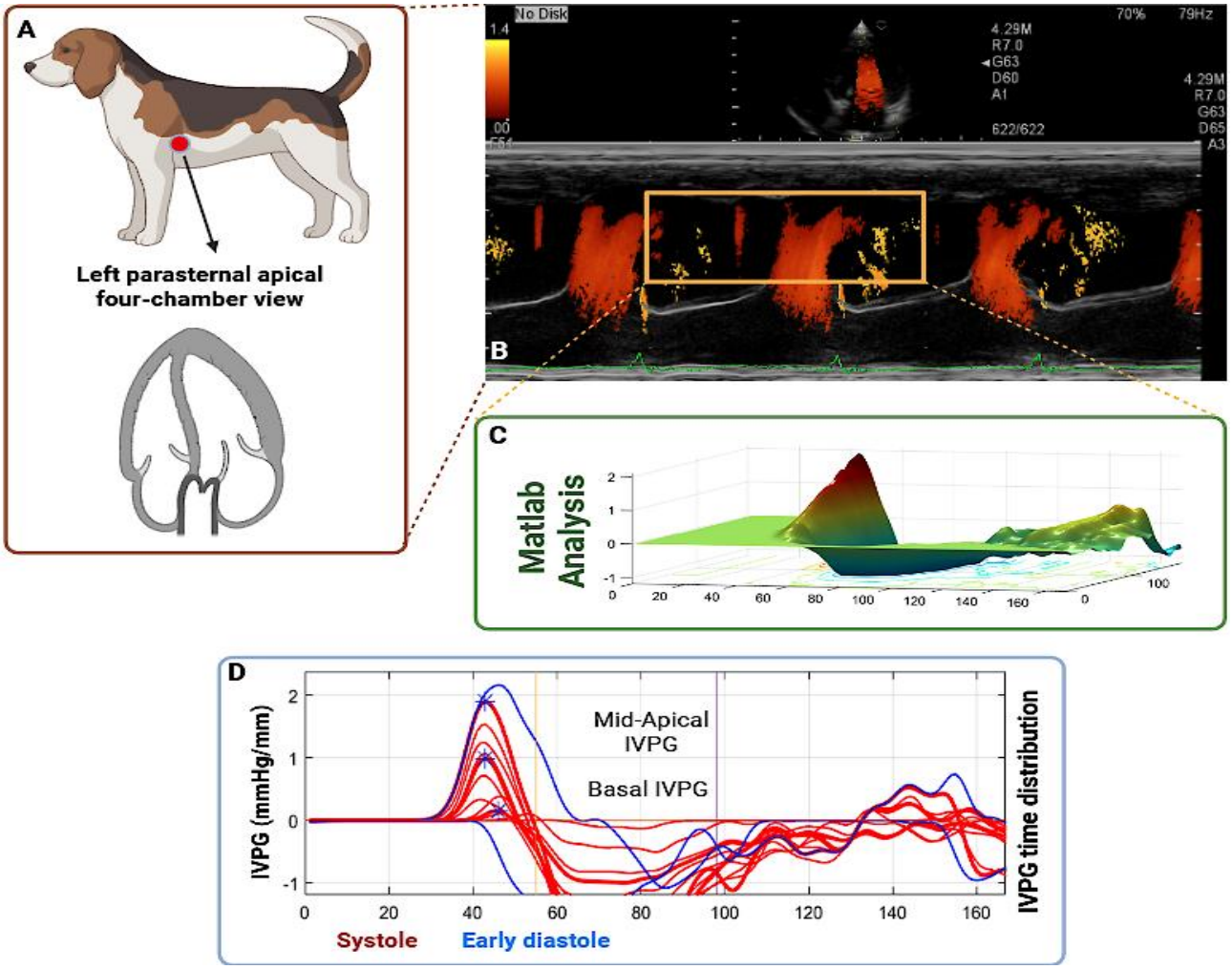

Fig. 1: Intraventricular pressure gradient (IVPG) analysis workflow. A): Left parasternal apical four-chamber echocardiographic view in a Beagle dog. The red dashed line indicates the scan plane for IVPG measurement. B): Raw color M-mode echocardiogram (CMME) of mitral inflow, with color scale representing blood flow velocity (red: diastolic inflow toward the LV). The vertical axis shows LV depth (mm), and the horizontal axis displays time (ms). C): MATLAB-processed IVPG curves, IVPG curves showing pressure gradients (Y-axis: mmHg/mm) across LV segments (basal, mid, apical) during the cardiac cycle (X-axis: time in milliseconds, aligned with ECG R-wave). D): Time-distributed IVPG (Y-axis: mmHg/mm) throughout the cardiac cycle (X-axis: time in ms). The blue asterisk refers to basal, mid and apical IVPG while total IVPG and mid-to-apical IVPG were calculated mathematically.

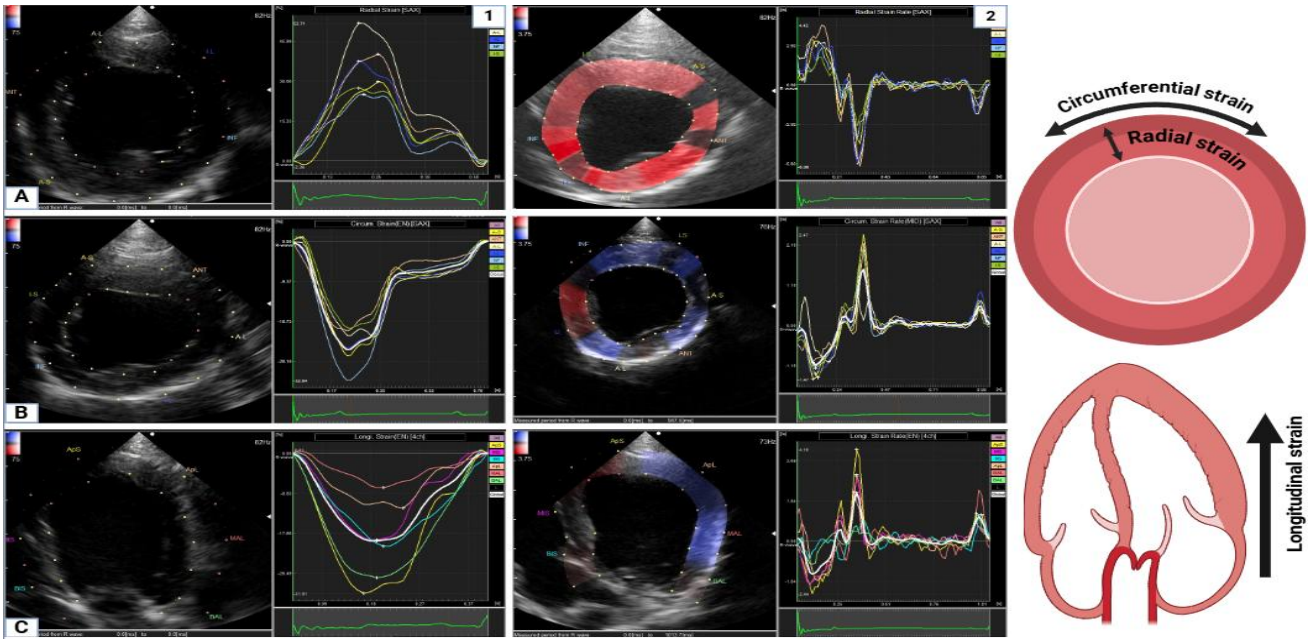

Fig. 2: Myocardial strain analysis across three key axes. A (Radial): Measures thickening/thinning of the LV wall (strain, %) and its rate (strain rate, s^{-1}). B (Circumferential): Assesses shortening/lengthening along the circular LV short-axis (strain, %) and its rate. C (Longitudinal): Evaluates base-to-apex shortening (strain, %) and its rate in the long-axis view. Column 1: Strain magnitude (%) per segment. Column 2: Strain rate (speed of deformation, s^{-1}).

RESULTS

Conventional echocardiography: Non-significant differences were observed in both the toxic and therapeutic groups in all the heart function parameters recorded through conventional echocardiography. The detailed data is presented in Table 1.

Color M-mode echocardiography: The CMME was conducted effectively in all dogs. Standard spectral mitral inflow waves were observed in all cases, depicting the mitral valve opening and closure. The analysis of CMME data included the computation of five distinct parameters: total, basal, mid-to-apical, mid, and apical, which were obtained using MATLAB.

Descriptive statistics for CMME variables in both groups are shown in Table 2. Within the same group, specifically in the toxic group, significant changes were observed over time. Total IVPD and basal IVPD, total IVPG and basal IVPG, Mid to apical IVPG and Mid IVPG exhibited significant ($P<0.05$) reductions at 4 and 6hrs compared to baseline values (0-hr). However, the differences in mid to apical IVPD, mid IVPD, apical IVPD and apical IVPG among three time points in toxic group were statistically non-significant. Similarly, non-significant differences were observed at both time intervals post administration compared to baseline values for all parameters in the therapeutic dosage group of dogs (Table 2).

Two-Dimensional speckle tracking echocardiographic measurements: In our assessment of heart function through 2D-STE, it was found that there were non-significant changes in all parameters among three time points in the toxic, as well as therapeutic group, as has been illustrated in Table 3.

DISCUSSION

In the current study, advanced echocardiographic techniques, specifically IVPG and 2D-STE were used to investigate the early hemodynamic effects of Lapatinib, a tyrosine kinase inhibitor (TKI). Our findings showed significant reductions in IVPG parameters at 4 and 6 hrs compared to 0-hr in the toxic group, indicating that Lapatinib causes early diastolic dysfunction. However, conventional echocardiography and speckle-tracking-derived myocardial deformation indices did not show any significant differences between the toxic and therapeutic groups.

Table 1: Assessment of cardiac structure and function using conventional echocardiography at different times in toxic and therapeutic groups (mean±SD)

Group		Toxic			Therapeutic		
Time (hrs)		zero	4	6	zero	4	6
	Parameters						
	HR (beats/min	118.8±24.7	116.8±18.4	115.0±9.38	105.5±26.3	103.3±11.23	120.3±25.2
	EDV(ml)	29.0±5.43	33.0±8.46	32.4±7.83	34.0±11.5	24.8±6.94	24.3±8.26
	ESV(ml)	9.62±2.7	10.5±4.11	11.1±3.5	10.4±6.42	6.23±2.46	6.55±5.19
	SV(ml)	19.6±3.36	22.2±7.29	20.8±4.49	23.8±5.63	18.5±4.39	17.8±3.56
	FS (%)	31.3±4.7	32.1±6.92	29.9±2.51	36.1±8.80	37.95±5.8	39.7±10.7
	LA/Ao ratio	1.35±0.19	1. 47±0.21	1.37±0.18	1.55±0.26	1.43±0.17	1.54±0.1
Pulmonary Artery	Pul PV (cm/s)	66.9±17.4	65.9±10.5	64.4±19.5	85.6±11.58	84.18±8.18	85.8±7.07
Aortic flow	PV (cm/s)	73.0±27.2	76.6±5.72	88.5±15.6	73.3±13.0	86.13± 4.44	98.4±15.5
	E/A ratio	1.47±0.24	1.80±0.61	1.62±0.43	1.30±0.050	1.47± 0.17	1.17±0.28
	Dect (ms)	126±43.1	114.8±18.2	110±21.2	126.5±45.7	106.5± 17.5	106±18.97
	Sm (cm/s)	7.62±1.38	6.38±1.91	7.42±2.33	8.00±1.74	8.17± 0.59	8.5±0.95
TDI septal	Em (cm/s)	8.22±1.13	8.06±1.63	8.18±0.81	7.70±0.99	7.23± 0.45	7.05±0.57
	Am (cm/s)	8.06±2.28	7.66±1.76	6.5±1.27	9.60±2.06	7.37± 1.28	7.85±1.97
	Em/Am ratio	1.1±0.39	1.09±0.28	1.29±0.26	0.84±0.20	1.03± 0.23	0.94±0.17
	E/Em ratio	8.24±0.89	8.20±1.66	8.12±2.86	7.93±1.45	7.86± 0.51	8.0±0.48
TDI lateral	Em(cm/s)	8.8±1.25	8.82±1.92	9.74±0.84	8.00±0.59	8.55±0.56	7.63±1.31
	Am(cm/s)	8.89±1.01	10.4±2.45	10.0±2.2	7.65±1.58	10.1± 3.1	9.85±3.03
	Em/Am ratio	0.99±0.18	0.87±0.23	1.0±0.18	1.11±0.29	0.94±0.35	0.82±0.16
	E/Em ratio	7.26±1.39	7.35±1.58	5.08±2.23	7.73±0.93	6.79±0.55	8.04±1.69

TDI, tissue Doppler imaging; HR, heart rate; EDV, left ventricle end-diastolic volume; ESV, left ventricle end-systolic volume; SV, stroke volume; FS, fractional shortening; LA/Ao, the left atrium/ aortic diameter ratio; Pul PV, pulmonary artery peak velocity; PV, peak velocity; E/A early to late diastolic velocity metralis ratio, Dect deceleration time, Sm systolic mitral annular velocity, Em, early diastolic mitral annular velocity; Am, late diastolic mitral annular velocity; Em/Am, Early to late diastolic velocity of the LV wall; E/Em, early diastolic velocity mitralis to early diastolic velocity of the LV wall ratio.

Table 2: Assessment of Intraventricular pressure difference (IVPD, mmHg) and gradients (IVPG, mmHg/mm) indices at different times in toxic and therapeutic groups (mean±SD)

Group	Toxic			Therapeutic		
Time (hrs)	zero	4	6	zero	4	6
Total IVPD	1.40±0.40	0.60±0.17*	0.57±0.14*	1.50±0.24	1.30±1.80	1.40±0.22
Basal IVPD	0.87±0.26	0.40±0.23*	0.29±0.2*	0.70±0.14	0.63±0.22	0.71±0.24
Mid to apical IVPD	0.57±0.20	0.20±0.16	0.29±0.13	0.49±0.27	0.47±0.047	0.47±0.047
Mid IVPD	0.54±0.18	0.20±.016	0.27±0.11	0.52±0.27	0.40±0.068	0.56±0.39
Apical IVPD	0.032±0.042	0.00±0.012	0.02±0.19	0.018±0.018	0.01±0.38	0.012±0.022
Total IVPG	1.1±0.33	0.52±0.16*	0.50±0.075*	1.10±0.11	0.82±0.19	1.00±0.11
Basal IVPG	0.67±0.18	0.25±0.16*	0.24±0.15*	0.73±0.072	0.70±0.027	0.71±0.23
Mid to apical IVPG	0.45±0.19	0.15±0.18*	0.26±0.13*	0.34±0.17	0.35±0.044	0.35±0.044
Mid IVPG	0.43±0.18	0.20±0.17*	0.24±0.11*	0.36±0.17	0.3±0.06	0.41±0.28
Apical IVPG	0.046±0.032	0.031±0.012	0.018±0.015	0.049±0.032	0.048±0.029	0.028±0.019

*Significant difference from respective values at zero hour in toxic group ($P<0.05$); IVPD: mmHg (pressure difference); IVPG: mmHg/mm (pressure gradient per unit length).

Table 3: Two-dimensional speckle tracking echocardiographic measurements at different times in toxic and therapeutic groups (mean±SD)

Group	Toxic			Therapeutic		
Time (hrs)	zero	4	6	zero	4	6
Radial strain [%]	35.60 ±2.71	31.50±2.46	31.90±6.75	24.60±2.34	24.70±5.02	26.00±5.26
Radial strain rate [/s]	2.93 ±20.50	2.85±0.24	2.76±0.61	2.42±0.16	2.36±0.44	2.72±0.52
Circum. strain (EN) [%]	-24.30±3.15	-19.30±1.31	-20.6±2.15	-20.0±1.28	-19.4±4.06	-20.8±1.46
Circum. strain (ep) [%]	-4.78±0.9	-3.94±0.64	-4.98±1.63	-6.78±0.69	-6.1±0.76	-6.3±0.48
Circum. strain (MID) [%]	-12.8±1.69	-10.3±0.59	-11.6±1.75	-12.4±0.93	-11.8±1.98	-12.6±0.36
Circum. strain sate (EN) [/s]	-2.08 ±0.14	-1.71±0.21	-1.8±0.23	-1.8±1.17	-1.78±0.24	-2.01±0.12
Circum. strain rate (ep) [/s]	-0.66±0.12	-0.53±0.11	-0.59±0.15	-0.76±0.07	-0.67±0.05	-0.73±0.16
Circum. strain rate (MID) [/s]	-1.25±0.11	-0.96±0.19	-1.09±0.13	-1.18±0.11	-1.12±0.1	-1.27±0.12
Longi. strain (EN) [%]	-14.8±2.96	-16.8±3.46	-15.6±2.85	-16.5±2.31	-16.8±2.63	-16.7±3.84
Longi. strain rate (EN) [/s]	-1.36±0.33	-1.61±0.36	-1.45±0.33	-1.68±0.24	-1.69±0.3	-1.51±0.23

Strain was measured across three myocardial layers (endocardial [EN], mid-wall [MID], epicardial [ep]) to assess regional deformation; EN: Endocardial layer (inner heart wall); ep: Epicardial layer (outer heart wall); MID: Mid-myocardial layer (middle heart wall).

A major finding that indicates Lapatinib-induced early diastolic dysfunction in the toxic group is the significant decrease in the total, basal, mid-to-apical and mid portions of IVPG. For assessing diastolic function, IVPG has been validated as a sensitive non-invasive technique (Elhaieg *et al.*, 2023; Farag *et al.*, 2023; Mandour *et al.*, 2023). It evaluates the effectiveness of diastolic relaxation by measuring the pressure differences between various left ventricular areas during diastole. In the current study, both total and basal IVPG values were significantly ($P<0.05$) decreased at 4 and 6hr post Lapatinib administration in dogs, however these findings were quite similar to those already reported in the literature, which indicate that diastolic function can be potential indicator for cardiotoxicity induced by chemotherapeutic agents (Tassan-Mangina *et al.*, 2006). Previously, such results have been reported to anthracyclines (chemotherapeutic agent) post exposure and a significant reduction in IVPG value associated with diastolic function has been documented in cancer patients (Shigemitsu *et al.*, 2019; Matsuura *et al.*, 2021; Hosono *et al.*, 2024). This decrease in IVPG can be an indication of increased left ventricular stiffness or impaired active relaxation, both factors are important for the onset of HFpEF (heart failure with preserved ejection fraction). Another important finding reported by Chiba *et al.* (2022) cannot be ignored, where authors have reported impaired relaxation of the LV, as demonstrated by decreased dp/dt and observed through catheterization following imatinib administration, which is another tyrosine kinase inhibitor with similar properties and clinical uses as those of Lapatinib.

In the current study, in toxic group both total and mid-to-apical IVPG values were significantly reduced ($P<0.05$) post Lapatinib administration, which is indicative of early diastolic dysfunction. This decrease can be attributed to the mechanism that basal IVPG is more dependent on left atrial pressure, whereas mid-to-apical IVPG is mainly influenced by LV active suction (Ohara *et al.*, 2012; Iwano *et al.*, 2015). Active relaxation of the heart and elastic recoil are critical for its proper filling during early diastole. The elastic recoil can be defined as the energy stored during systole and then released to produce the suction force which draws blood from left atrium to LV. This energy is directly linked to LV deformation because the strain assessment in both directions (longitudinal and circumferential) correlates with the mid-to-apical IVPG (Ohara *et al.*, 2012; Shigemitsu *et al.*, 2019). The cardiotoxicity induced by Lapatinib may interfere both with elastic recoil and active relaxation that leads to significant reduction in IVPG parameters in the toxic group, which is evident from this study.

In cardiotoxicity, basal IVPG is strongly associated with LA pressure than LV elastic recoil (Matsuura *et al.*, 2021). However, in the current study, it was interesting to note that the toxic group had shown decrease in basal IVPG. This decrease is supported by the non-significant changes in mitral E/A ratio due to the reason that early diastolic dysfunction was induced by decreased elastic recoil at the base level rather than higher LA pressure (Nagueh, 2020). This reduction in elastic recoil can be attributed to the lower capacity of LV to relax effectively. However, conventional echocardiographic parameters are unable to detect such impairments at this early stage (Nguyen *et al.*, 2023). In toxic group, a significant decrease in IVPG segment has been attributed to the early impact of Lapatinib administration on LV diastolic functions (Guerra and Leite-Moreira, 2018). The elastic recoil and diastolic suction can be reduced due to the diastolic mechanics which are influenced by the Lapatinib toxic effects on heart prior to the onset of systolic dysfunction (Matsuura *et al.*, 2021). Such findings suggest that the IVPG has a potential to be used as a diagnostic tool to identify such cardiac impairments and timely interventions to stop any consequences leading to heart failure.

It is worth mentioning that IVPG has shown more significant changes during assessments than IVPD. To exclude the influence of heart size, IVPG can be computed mathematically through dividing IVPD by LV length, which make IVPG more reliable and accurate tool to determine the cardiotoxicity. However, it is believed that it should be related to suppression of the HER2/neu receptor that is deemed necessary for the healthy heart functioning (Chien, 2006; Choi and Chang, 2017). HER2 receptors are expressed both in cancer cells and cardiac myocytes and lead to survival pathways through the PI3k/Akt signaling cascade. In the current study, it has been observed that Lapatinib therapy may cause the cardiac damage through blocking the expression of HER2 receptors, which results in early diastolic dysfunction along with oxidative stress, mitochondrial dysfunction and cell death (Yang *et al.*, 2021). Similar results have been reported in literature following the HER2-targetting treatments (trastzumb and Imatinib), demonstrating the cardiotoxic potential of such chemotherapeutic agents (Klement *et al.*, 2012; Chiba *et al.*, 2022; Wu *et al.*, 2022; Xie *et al.*, 2024).

The administration of a therapeutic dose of Lapatinib in the current experiment did not affect either the novel or standard echocardiographic results due to the absence of

clinically significant adverse cardiac effects under Lapatinib therapeutic dose, which coincides with the results of Dogan *et al.* (2012). Despite the significant decrease in IVPG, our investigation did not find any significant differences among three time points in the toxic and therapeutic groups in conventional echocardiographic parameters, such as chamber dimensions, fractional shortening (FS), and left ventricular ejection fraction (EF). These findings support the hypothesis that at the early stage of cardiotoxicity, systolic function, as measured by these conventional measures, remains unchanged (Tassan-Mangina *et al.*, 2006; Matsuura *et al.*, 2021). Traditional echocardiographic techniques may not be sensitive enough to detect early subclinical cardiotoxicity because diastolic dysfunction, as shown by the IVPG data, often develops before obvious systolic and diastolic impairment. As a result, the negative effects of the medication may potentially be more noticeable by IVPG with repeated dosage.

Results of the present study also revealed that global longitudinal strain, circumferential strain, and radial strain did not differ between the toxic and therapeutic doses of Lapatinib, based on 2D-STE, which measures myocardial deformation using strain and strain rate. In patients receiving chemotherapy, the global longitudinal strain (GLS) has been recognized as a sensitive indicator of early cardiac impairment (McGregor *et al.*, 2021). However, the acute consequence of Lapatinib exposure may be the reason for the lack of meaningful results in 2D-STE in the present study, indicating that myocardial deformation may take longer to appear in conditions involving short-term toxicity. The absence of significant changes in systolic function or myocardial strain further underscores the utility of IVPG as a more sensitive indicator of early cardiotoxic effects. Therefore, incorporating IVPG into routine surveillance protocols could enhance the early detection of cardiotoxicity in patients receiving Lapatinib or other similar anticancer drugs.

In the present study, the techniques like intraventricular pressure gradients (IVPG) and two-dimensional speckle-tracking echocardiography (2D-STE) were chosen because they offer superior sensitivity for detecting early and subclinical cardiotoxicity compared to conventional cardiac markers. Traditional markers, such as ejection fraction or biomarkers like troponin, often identify cardiac dysfunction only after a remarkable damage has occurred. In contrast, IVPG and 2D-STE provide detailed insights into diastolic function and myocardial deformation, enabling the detection of subtle changes in cardiac mechanics before overt systolic dysfunction or structural damage becomes apparent. These advanced techniques are particularly valuable for early intervention, which is critical in minimizing long-term cardiovascular risks associated with Lapatinib and other chemotherapeutic agents. Thus, their use aligns with our goal of identifying cardiotoxicity at the earliest possible stage.

Despite some valuable results, there were certain key limitations in the current study. Firstly, the impact of long-term administration of Lapatinib on cardiac function to investigate the chronic nature of Lapatinib treatment in clinical practice could not be investigated. Administration of a single dose of Lapatinib impaired the left ventricle diastolic function and increased the period of ventricular repolarization within 30 min after administration; however, the time course of cardiotoxic effects of multiple-dose administration of Lapatinib could not be monitored. Secondly, continuous monitoring of plasma concentrations of cardiac biomarkers such as cardiac troponin I (cTnI), N-terminal pro B-type natriuretic peptide (NT-proBNP), Creatine Kinase (CK), AST, or LDH could not be conducted. These cardiac biomarkers are known to serve as important indicators of myocardial injury and stress.

While echocardiography can have limitations related to dog size and positioning, these challenges were mitigated in this study through careful standardization and the use of advanced techniques. Beagles, the breed used in this study, are well-suited for echocardiography due to their consistent size and anatomy, which facilitate reliable imaging. Additionally, all echocardiographic measurements were performed by experienced operators using high-resolution equipment, ensuring optimal image quality and repeatability. To further enhance reliability, multiple cardiac cycles were analyzed, and mean values were used for statistical analysis. While limitations exist, the combination of standardized protocols, experienced personnel, and advanced imaging techniques (IVPG and 2D-STE) ensured that the results were both reliable and reproducible, supporting the validity of our findings.

Conclusions: In conclusion, the present study demonstrates that while conventional echocardiographic and speckle-tracking parameters remain unchanged, IVPG is a sensitive marker of early diastolic dysfunction following Lapatinib administration. The incorporation of IVPG into routine cardiotoxicity monitoring may improve early detection and allow for timely therapeutic interventions, ultimately reducing the cardiovascular risk associated with Lapatinib therapy.

Competing interests: The authors declare no competing interest.

Authors contribution: MAYH, AF, ASM, ME, TU, KS and RT designed the study. MAYH, AF and ASM carried out investigations under the supervision of RT. MAYH, AF and ASM collected data. MAYH, AF, ASM, ME, TU and KS prepared the manuscript. All authors critically reviewed the article and approved the submitted version.

REFERENCES

Abdelgalil AA and Alkahtani HM, 2023. Lapatinib: A comprehensive profile. In: "Brittain HG (ed), Profiles of Drug Substances, Excipients and Related Methodology". Elsevier pp:135–166.

Bence AK, Anderson EB, Halepota MA, *et al.*, 2005. Phase I pharmacokinetic studies evaluating single and multiple doses of oral GW572016, a dual EGFR-ErbB2 inhibitor, in healthy subjects. Invest New Drugs 23:39–49.

Calle MCA, Sandhu NP, Xia H, *et al.*, 2018. Two-dimensional speckle tracking echocardiography predicts early subclinical cardiotoxicity associated with anthracycline-trastuzumab chemotherapy in patients with breast cancer. BMC Cancer 18(1):1037. https://doi.org/10.1186/s12885-018-4935-z.

Chang VY and Wang JJ, 2018. Pharmacogenetics of chemotherapy-induced cardiotoxicity. Curr Oncol Rep 20:52.

Chiba K, Kambayashi R, Onozato M, *et al.*, 2022. Imatinib induces diastolic dysfunction and ventricular early-repolarization delay in the halothane-anesthetized dogs: Class effects of tyrosine kinase inhibitors. J Pharmacol Sci 150:154–62.

Chien KR, 2006. Herceptin and the heart—a molecular modifier of cardiac failure. N Engl J Med 354:789–90.

Choi HD and Chang MJ, 2017. Cardiac toxicities of Lapatinib in patients with breast cancer and other HER2-positive cancers: a meta-analysis. Breast Cancer Res Treat 166:927–936.

Dogan E, Yorgun H, Petekkaya I, *et al.*, 2012. Evaluation of cardiac safety of Lapatinib therapy for ErbB2-positive metastatic breast cancer: A single center experience. Med Oncol 29:3232–3239.

Elhaieg A, Farag A, Elfadadny A, *et al.*, 2023. Effect of experimental periodontitis on cardiac functions: a comprehensive study using echocardiography, hemodynamic analysis, and histopathological evaluation in a rat model. Front Vet Sci 10: 1327484. https://doi.org/10.3389/fvets.2023.1327484.

Farag A, Mandour AS, Kaneda M, *et al.*, 2023. Effect of trehalose on heart functions in rats model after myocardial infarction: assessment of novel intraventricular pressure and heart rate variability. Front Cardiovasc Med 10:1182628. https://doi.org/10.3389/fcvm.2023.1182628.

Fonseca-Alves CE, Leis-Filho AF, Lacerda ZA, *et al.*, 2024. Lapatinib antitumor effect is associated with PI3K and MAPK pathway: An analysis in human and canine prostate cancer cells. PLoS One 19:e0297043.

Guerra M and Leite-Moreira AF, 2018. Relevance of intraventricular pressure gradients in left ventricular diastolic and systolic function: clinical implications. Rev Port Cir Cardiotorac Vasc 25(1-2):19–26.

Hamabe L, Fukushima R, Kawamura K, *et al.*, 2013. Evaluation of changes in left ventricular myocardial function observed in canine myocardial dysfunction model using a two-dimensional tissue tracking technique. J Vet Sci 14:355–62.

Hamabe L, Mandour AS, Shimada K, *et al.*, 2021. Role of two-dimensional speckle-tracking echocardiography in early detection of left ventricular dysfunction in dogs. Animals 11:2361.

Hosono Y, Takahashi K, Shigemitsu S, *et al.*, 2024. Assessment of anthracycline-induced cardiotoxicity in childhood cancer survivors during long-term follow-up using strain analysis and intraventricular pressure gradient measurements. Heart Vessels 39:105–116.

Iwano H, Kamimura D, Fox E, *et al.*, 2015. Altered spatial distribution of the diastolic left ventricular pressure difference in heart failure. J Am Soc Echocardiogr 28:597–605.

Kang YJ, 2001. Molecular and cellular mechanisms of cardiotoxicity. Environ Health Perspect 109:27–34.

Kerkelä R, Grazette L, Yacobi R, *et al.*, 2006. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 12(8):908–16. doi: 10.1038/nm1446.

Klement GL, Goukassian D, Hlatky L, *et al.*, 2012. Cancer therapy targeting the HER2-PI3K pathway: potential impact on the heart. Front Pharmacol 3:113.

Kobayashi M, Takahashi K, Yamada M, *et al.*, 2017. Assessment of early diastolic intraventricular pressure gradient in the left ventricle among patients with repaired tetralogy of Fallot. Heart Vessels 32:1364–74.

Lamberti M, Giovane G, Garzillo EM, *et al.*, 2014. Animal models in studies of cardiotoxicity side effects from antiblastic drugs in patients and occupational exposed workers. Biomed Res Int 2014:240642.

Lambertini M, Martel S, Campbell C, *et al.*, 2019. Pregnancies during and after trastuzumab and/or lapatinib in patients with human epidermal growth factor receptor 2–positive early breast cancer: Analysis from the NeoALTTO (BIG 1-06) and ALTTO (BIG 2-06) trials. Cancer 125(2):307–16.

Maeda S, Sakai K, Kaji K, *et al.*, 2022. Lapatinib as first-line treatment for muscle-invasive urothelial carcinoma in dogs. Sci Rep 12:4(2022). https://doi.org/10.1038/s41598-021-04229-0.

Mandour AS, Farag A, Helal MAY, *et al.*, 2023. Non-invasive assessment of the intraventricular pressure using novel color M-mode echocardiography in animal studies: Current status and future perspectives in veterinary medicine. Animals (Basel) 13(15):2452. https://doi.org/10.3390/ani13152452.

Matsuura K, Shiraishi K, Mandour AS, *et al.*, 2021. The utility of intraventricular pressure gradient for early detection of chemotherapy-induced subclinical cardiac dysfunction in dogs. Animals (Basel) 11:1122.

Matsuura K, Bach MBT, Takahashi K, *et al.*, 2022. Non-invasive assessment of left ventricular relaxation property using color M-mode-derived intraventricular pressure gradients in cats. J Vet Cardiol 41:236–48.

McGregor P, Moura FA, Banchs J, *et al.*, 2021. Role of myocardial strain imaging in surveillance and management of cancer therapeutics-related cardiac dysfunction: a systematic review. Echocardiography 38:314–28.

Morelli MB, Bongiovanni C, Da Pra S, *et al.*, 2022. Cardiotoxicity of anticancer drugs: Molecular mechanisms and strategies for cardioprotection. Front Cardiovasc Med 9:847012.

Nagueh SF, 2020. Left ventricular diastolic function: Understanding pathophysiology, diagnosis, and prognosis with echocardiography. JACC Cardiovasc Imaging 13:228–44.

Nguyen VA, Nguyen DT, Ong LTT, *et al.*, 2023. Early detection of reduced left ventricular systolic function by 2D speckle tracking echocardiography in patients with primary mitral regurgitation in a Vietnamese cohort. Glob Cardiol Sci Pract 2023:e202326.

Ohara T, Niebel CL, Stewart KC, *et al.*, 2012. Loss of adrenergic augmentation of diastolic intra-LV pressure difference in patients with diastolic dysfunction: Evaluation by color M-mode echocardiography. JACC Cardiovasc Imaging 5:861–70.

Rawat PS, Jaiswal A, Khurana A, *et al.*, 2021. Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. Biomed Pharmacother 139:111708.

Shigemitsu S, Takahashi K, Yazaki K, *et al.*, 2019. New insight into the intraventricular pressure gradient as a sensitive indicator of diastolic cardiac dysfunction in patients with childhood cancer after anthracycline therapy. Heart Vessels 34:992–1001.

Tanaka Y, Watanabe M, Saeki K, *et al.*, 2020. Evaluation of the proper dosage of lapatinib and its safety in dogs. Transl Regul Sci 2:68–71.

Tassan-Mangina S, Codorean D, Metivier M, *et al.*, 2006. Tissue Doppler imaging and conventional echocardiography after anthracycline treatment in adults: Early and late alterations of left ventricular function during a prospective study. Eur J Echocardiogr 7:141–46.

Wu Q, Bai B, Tian C, *et al.*, 2022. The molecular mechanisms of cardiotoxicity induced by HER2, VEGF, and tyrosine kinase inhibitors: An updated review. Cardiovasc Drugs Ther 36(3):511-24. https://doi.org/10.1007/s10557-021-07181-3.

Xie S, Sun Y, Zhao X, *et al.*, 2024. An update of the molecular mechanisms underlying anthracycline induced cardiotoxicity. Front Pharmacol 15:1406247.

Yang Z, Wang W, Wang X, *et al.*, 2021. Cardiotoxicity of epidermal growth factor receptor 2-targeted drugs for breast cancer. Front Pharmacol 12:741451.

Yotti R, Bermejo J, Antoranz JC, *et al.*, 2005. A noninvasive method for assessing impaired diastolic suction in patients with dilated cardiomyopathy. Circulation 112:2921–29.

Yu TW, Yamamoto H, Morita S, *et al.*, 2024. Comparative pharmacokinetics of tyrosine kinase inhibitor, lapatinib, in dogs and cats following single oral administration. J Vet Med Sci 86:317–21.