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ORIGINAL ARTICLE

Comprehensive transthoracic echocardiographic evaluation of doxorubicin-induced cardiotoxicity: a multimodal imaging approach in an animal model

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Abstract

Aims Anthracycline-induced cardiotoxicity has high incidence rates and causes significant mortality among cancer survivors. Damage to myocardial tissue leads to left ventricular (LV) dilation with systolic dysfunction, typically assessed through echocardiographic measurement of LV ejection fraction (LVEF) and volumes. Early detection is crucial for improving patient outcomes. We aimed to evaluate cardiotoxicity progression and diagnostic performance of different echocardiographic modalities in an animal model.

Methods and results Female Sprague Dawley rats received either intravenous doxorubicin (DOX) injections weekly for 8 weeks (2 mg/kg/week) or saline (control). Transthoracic LV echocardiography was performed before treatment and at 4, 6, and 8 weeks in the treatment course. Two researchers performed and evaluated M-mode, B-mode, and four-dimensional (4D) echocardiography. Bland–Altman plots were created to show the bias and limits of agreement when comparing echocardiographic modalities. Simple linear regression and Pearson correlation were applied to evaluate interobserver variability. Six weeks after the first DOX injection, LVEF, radial LV fractional shortening, LV end-systolic volume, and LV end-diastolic volume were significantly reduced compared with baseline. LV dysfunction and dilation became more pronounced after 8 weeks of DOX treatment. For all parameters, 4D- and M-mode showed the lowest bias and narrowest limits of agreement. The correlation between the researchers' measurements was strong for most parameters.

Conclusion Our rat model of DOX-induced cardiotoxicity demonstrates that volumetric changes are more pronounced. Both 4D- and M-mode imaging techniques proved effective and reliable compared with the standard B-mode approach, with minimal interobserver variability, indicating strong reproducibility.

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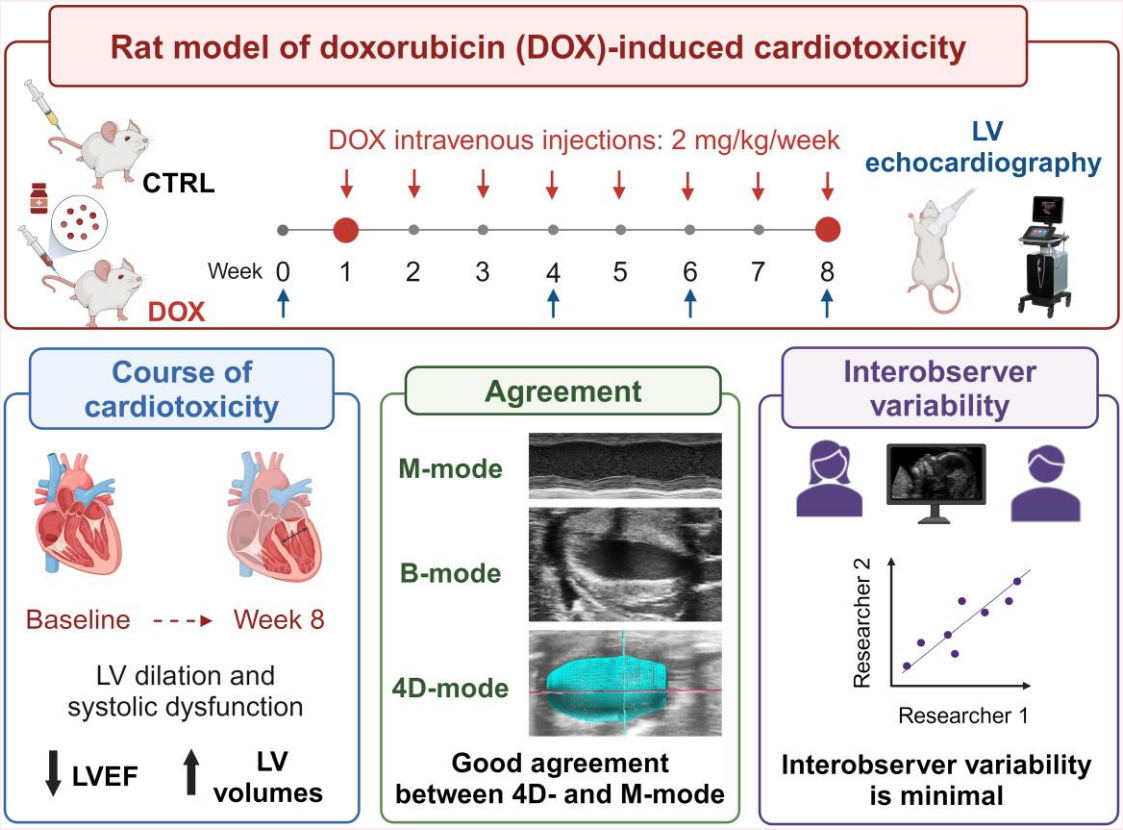
Graphical Abstract



4D, four-dimensional; CTRL, control; DOX, doxorubicin; LV, left ventricle; LVEF, left ventricular ejection fraction. Created with BioRender.com.

Keywords doxorubicin • cardiotoxicity • animal model • echocardiography

Introduction

Anthracyclines like doxorubicin (DOX) rank among the most potent chemotherapeutic agents extensively used to treat various cancers, including breast cancer, lymphomas, sarcomas, and leukaemias. Despite its efficacy, DOX is known for its cardiotoxicity. The pathogenesis of DOX-induced cardiotoxicity involves several mechanisms, with mitochondria identified as the primary subcellular targets.[1] Cardiac damage starts from the first exposure, but the extent is predominantly determined by the cumulative dose of DOX administered.[2] Upon DOX exposure, the heart undergoes structural changes characterized by left ventricular (LV) dilation and a weakened heart muscle with decreased systolic function. This adverse phenotype potentially progresses to heart failure and poses a significant clinical challenge, contributing to high morbidity and mortality among DOX-treated cancer patients and survivors.[3] Given the importance of preserving cardiac function during cancer treatment, various strategies to mitigate anthracycline-induced cardiotoxicity have been explored.[4] However, these efforts have not yet resulted in a universally helpful strategy. The efficacy of cardioprotective strategies decreases with increasing time between damage onset and the initiation of therapy. Early detection of cardiotoxicity in a subclinical stage could prevent cardiomyocyte loss and limit the development of irreversible cardiac injury.[5] Therefore, early detection and accurate monitoring of DOX-induced cardiotoxicity are crucial to improving patient prognosis. Two-dimensional echocardiography (2DE) allows the detection of cardiotoxicity by measuring LV ejection fraction (LVEF). DOX-induced cardiotoxicity has long been defined as a decrease of LVEF by >10% points from baseline to an LVEF < 55%.[6] Although conventional 2DE is the most applied tool, preclinical studies indicate that more innovative imaging methods are needed for cardiac assessment. Three- (3DE) or four-dimensional echocardiography (4DE) is more accurate, demonstrates less variation, and correlates better with magnetic resonance imaging than traditional 2DE.[7] In addition, differences in the accuracy of various echocardiographic modalities (e.g. M-mode and B-mode) and interobserver consistency can lead to misinterpreted data. Preclinical studies show conflicting results regarding which parameters change first after DOX treatment. In addition, there is a lack of studies comparing the effectiveness of different echocardiographic modalities in detecting DOX-induced cardiotoxicity in animal models. Our study aimed to evaluate the onset and progression of cardiac injury and changes in echocardiography parameters over time in a rat model of DOX-induced cardiotoxicity. Moreover, we assessed the diagnostic performance of different echocardiographic modalities and assessed interobserver consistency.

Methods

Animal model and study design

All animal experiments follow the EU Directive 2010/63/EU for animal testing and are approved by the local ethical committee (Ethical Commission

for Animal Experimentation, UHasselt, Diepenbeek, Belgium, ID 201942 and ID 202154). Animals were group-housed in a standard cage with cage enrichment at the conventional animal facility of UHasselt and fed a standard pellet diet with water available *ad libitum*. The environmental conditions were rigorously controlled (i.e. 22°C temperature and 22–24% humidity). Six-week-old female Sprague Dawley rats (Janvier Labs, Le Genest-Saint-Isle, France) were randomly allocated into two groups. The first group received DOX (2 mg/kg, $n = 14$, Accord Healthcare B.V., Utrecht, The Netherlands), intravenously injected weekly for 8 weeks (16 mg/kg cumulative dose). The model adheres to the current guidelines.[8] The second group received an equal volume of 0.9% saline as a control group (CTRL, $n = 14$). Echocardiography was performed at baseline and 4, 6, and 8 weeks after the first injection. The study was conducted in two cohorts, with echocardiography performed in all animals at baseline and Week 8. Echocardiography at Week 4 and Week 6 was conducted in part of the animals ($n = 7$ at both time points). Rats were euthanized with an overdose of sodium pentobarbital (150 mg/kg intraperitoneal, Dolethal, Vetoquinol, Aartselaar, Belgium) in Week 9 of the study.

Echocardiography

LV transthoracic echocardiography was performed using a Vevo® 3100 high-resolution imaging system with a 21 MHz MX250 transducer (FUJIFILM VisualSonics, Inc., Amsterdam, The Netherlands) as described before.[9] Parasternal long-axis images were acquired in single-plane B-mode with ECG (electrocardiogram)-gated kilohertz visualization to measure longitudinal LV fractional shortening (LVFS), LV end-systolic volume (LVESV), and LV end-diastolic volume (LVEDV). Pulsed-wave (PW) Doppler and tissue Doppler modes were used to measure the ratio of peak flow velocity in early vs. late filling (E/A) and of peak mitral flow vs. annular velocity (E/E′). Parasternal short-axis images were acquired in M-mode to measure radial LVFS. 4DE was performed to measure LVESV, LVEDV, LVEF, and LV cardiac output (LVCO). All analyses were performed using Vevo® LAB (Vevo® LAB software, version 5.6.1, FUJIFILM VisualSonics, Inc.). Two independent and blinded researchers analysed the echocardiographic images.

Strain measurement

To perform strain analysis of the LV, mid-ventricular parasternal short- and long-axis B-mode cine loops were imported into the Vevo® Strain software (version 5.6.1, FUJIFILM VisualSonics, Inc.). The most optimal cardiac cycles were selected to assess LV global circumferential strain (LVGCS, short-axis), LV peak radial strain (long-axis), and LV global longitudinal strain (LVGLS, long-axis). The endocardial border was manually traced, and simultaneous epicardial tracing was performed automatically to obtain 48 sampling points, dividing the LV into six myocardial segments. In each segment, peak strain was analysed.

Random missing values of echocardiographic parameters are due to poor image quality or deceased animals.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad software, version 10.2.1). The normal data distribution was tested with the D'Agostino and Pearson normality test. Repeated measures mixed-effects analysis was performed for parameters assessed at multiple time points. When data were not normally distributed, a Mann–Whitney test was used to compare values between two time points. The parametric one-way ANOVA test was performed to compare parameters measured with different echocardiographic modalities at Week 8. Bonferroni *post hoc* tests were performed for multiple comparisons. The Bland–Altman comparison method evaluated the agreement between the echocardiographic modalities by plotting the difference in the measurements of the two modalities against their average. The mean of the differences (bias) and 95% limits of agreement were calculated. Measurements of two researchers were compared with parametric unpaired *t*-tests to evaluate interobserver variability. Simple linear regression models were applied to correlate the measurements. Pearson correlation coefficients (r) were determined. Outliers were identified using the ROUT method with a maximum desired false discovery rate of 1%, with this criterion established *a priori*. Data are expressed as the mean ± SEM. $P < 0.05$ was considered statistically significant.

Results

After 6 weeks, DOX affects LV systolic function, strain, and volumes

Echocardiography of the LV was performed at baseline and after 4, 6, and 8 weeks of DOX treatment to measure parameters of LV systolic function, diastolic function, strain, and volumes. 4D-, B-, and M-modes were used to measure LVEF and LV cardiac index, longitudinal LVFS, and radial LVFS, respectively (Supplementary data online, *Tables S1* and *S2*). Four weeks of DOX treatment did not affect systolic function compared with baseline and CTRL (*Figure 1*). At Week 6, DOX-treated rats showed significantly reduced LVEF and radial LVFS compared with baseline (LVEF: −7% and radial LVFS: −14%; both $P < 0.05$) (*Figure 1A* and *C*). Longitudinal LVFS and LV cardiac index were not significantly affected at this time point (*Figure 1B* and *D*). From Week 6 onward and after 8 weeks of DOX treatment, all parameters of systolic function were significantly reduced compared with baseline (LVEF: −30%; $P < 0.0001$, longitudinal LVFS: −43%; $P < 0.0001$, radial LVFS: −39%; $P < 0.0001$, LV cardiac index: −25%; $P < 0.001$). LV cardiac index significantly differed for CTRL rats between 6 and 8 weeks ($P < 0.05$). Significant differences between the CTRL and DOX groups were found only for Week 8, with LVEF, longitudinal LVFS, and radial LVFS significantly decreased in DOX rats (all $P < 0.0001$). Representative echocardiographic images obtained at Week 8 can be found in supplement (Supplementary data online, *Figure S1*).

Volumetric measurements were performed with 4DE and are shown in *Figure 2*. Compared with baseline, DOX-treated rats showed no change in LV volumes at Week 4, while a significant increase in LVESV (+87%; $P < 0.01$) and LVEDV (+30%; $P < 0.0001$) was observed at Week 6, which was further increased at Week 8 for LVESV (+296%; $P < 0.0001$ vs. baseline) (*Figure 2A* and *B*). Also, in CTRL rats, LVEDV significantly differed between time points. LVESV and LVEDV were significantly different between CTRL and DOX groups at Week 6 ($P < 0.05$ and $P < 0.01$, respectively) and were more pronounced at Week 8 ($P < 0.0001$).

Diastolic function parameters remained unchanged in the DOX group at all time points and were similar to the CTRL group (see Supplementary data online, *Figure S2A* and *B*). CTRL rats showed a significant −E/E′ reduction at Week 4 and Week 8 compared with baseline ($P < 0.05$ and $P < 0.01$; Supplementary data online, *Figure S2B*). Furthermore, survival for CTRL and DOX groups represented as a Kaplan-Meier plot in supplement (Supplementary data online, *Figure S3*).

Strain measurements were performed with B-mode and are shown in Supplementary data online, *Figure S4*. GCS shows a significant decrease in DOX animals between baseline and Weeks 4 or 6 ($P < 0.001$ and $P < 0.0001$) (see Supplementary data online, *Figure S4B*). Four and 6 weeks of DOX treatment did not affect GLS and LV peak radial strain compared with baseline and CTRL (see Supplementary data online, *Figure S4A* and *C*). At Week 8, DOX-treated rats showed significantly reduced GLS, GCS, and LV peak radial strain compared with baseline (GLS: −36%; $P < 0.0001$, GCS: −36%; $P < 0.0001$, peak radial strain: −48%; $P < 0.0001$) (see Supplementary data online, *Figure S4*). GLS and GCS significantly differed for DOX-treated rats between 4 and 8 weeks (both $P < 0.05$), and GLS was more pronounced between 6 and 8 weeks ($P < 0.0001$) (see Supplementary data online, *Figure S4A* and *B*). DOX-treated rats significantly differed for GCS and LV peak radial strain between Weeks 6 and 8 (GCS $P < 0.05$ and LV peak radial strain $P < 0.01$) (see Supplementary data online, *Figure S4B* and *C*). Significant differences between the CTRL and DOX groups were found for Weeks 6 and 8 with GCS ($P < 0.01$ and

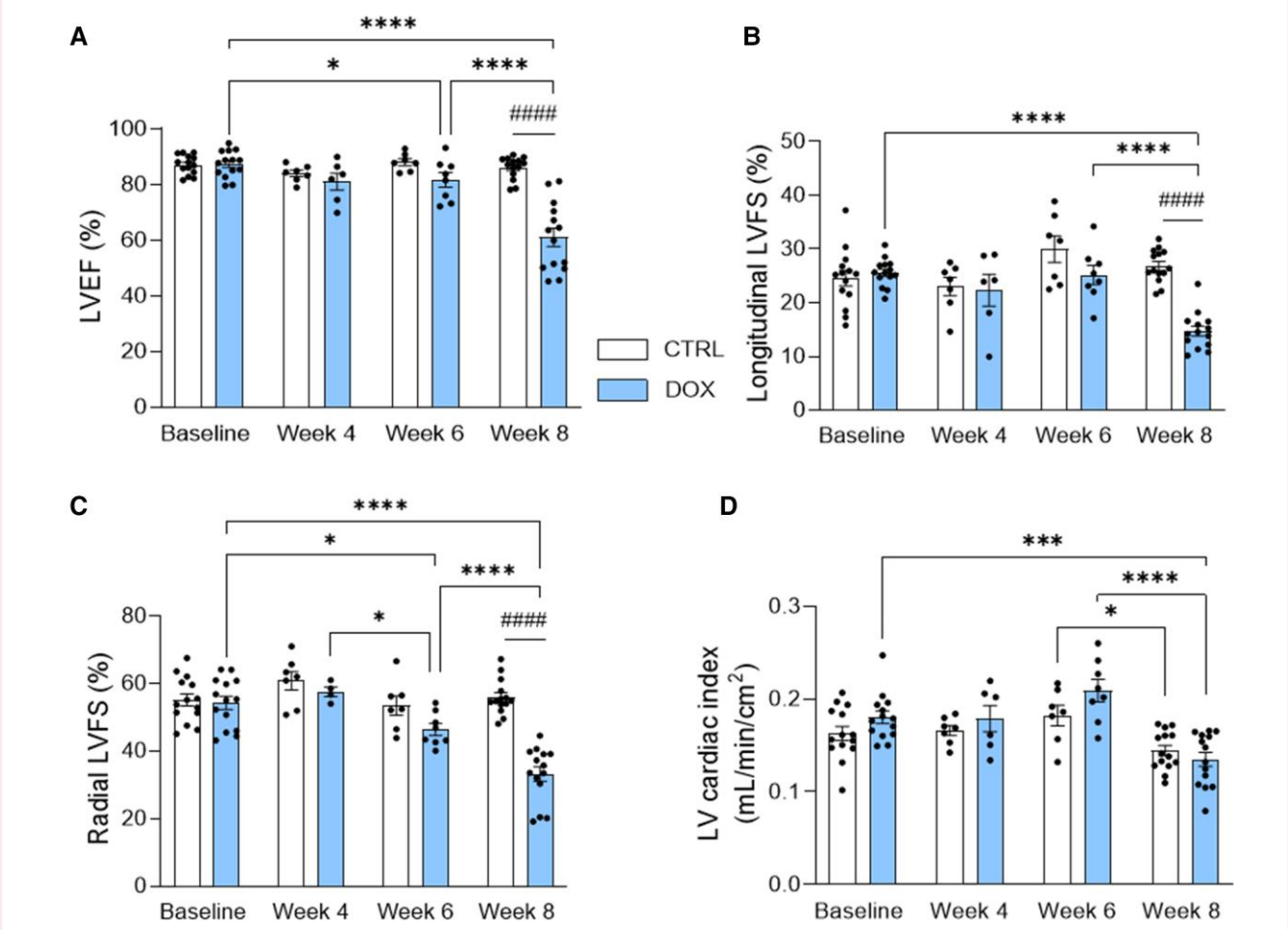


Figure 1 LV systolic function over time. LVEF (*A*), longitudinal LVFS (*B*), radial LVFS (*C*), and LV cardiac index (*D*) measured in CTRL and DOX animals (both $n = 14$). The LV cardiac index is calculated by normalizing LVCO to body surface area. Data are shown as mean ± SEM. For CTRL: $n = 14$ (baseline, Week 8) and $n = 7$ (Week 4, Week 6). For DOX: $n = 14$ (baseline, Week 8), $n = 4/6$ (Week 4), and $n = 8$ (Week 6). $^{*}P < 0.05$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. $^{\#\#\#\#}P < 0.0001$. CTRL, control; DOX, doxorubicin; LV, left ventricular; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

$P < 0.0001$) and for Week 8 with GLS and LV peak radial strain decreased in DOX rats (both $P < 0.0001$) (see Supplementary data online, *Figure S4*).

4D- and M-modes show the best agreement to evaluate LV systolic function and volumes

Multimodal echocardiography assessed LV systolic function and volumes in DOX-treated rats at Week 8. LVEF and LVCO were not significantly different between modalities (*Figure 3A and B*). The variance of LVEF was similar with each modality, while for LVCO, it was markedly greater with B-mode compared with other modalities. For LVEF, the bias was low and the limits of agreement were narrow for all comparisons, with 4D- and M-modes showing the best agreement [bias 1.69 (−12.81; 16.20)] (*Figure 3C*). Furthermore, the agreement for LVCO was best between 4D- and M-modes [bias 6.90 (−12.28; 66.07)], with a notable difference from the other comparisons (*Figure 3D*). When including B-mode in the comparison, the bias increased and the agreement limits broadened.

Regarding volumes, LVEDV was significantly higher when measured with B-mode compared with M-mode ($P < 0.01$), and a similar trend was observed for LVESV ($P = 0.07$) (*Figure 4A and B*). Again, Bland–Altman plots indicated the lowest bias and narrowest limits of agreement for comparing 4D- and M-mode (*Figure 4C and D*).

The interobserver variability of echocardiographic measurements is minimal

As shown in *Figure 5*, both researchers showed a strong correlation for LVEF (Pearson $r = 0.93$; $P < 0.0001$) and LVCO (Pearson $r = 0.79$; $P = 0.0009$) (*Figure 5A and B*). In contrast, longitudinal LVFS was weakly correlated between both researchers (Pearson $r = 0.17$), suggesting substantial interobserver variability (*Figure 5C*). For radial LVFS, a strong correlation was observed (Pearson $r = 0.81$; $P = 0.0004$) (*Figure 5D*).

Regarding volumes measured with 4DE, both researchers showed a strong correlation for LVESV (Pearson $r = 0.97$; $P < 0.0001$) (*Figure 6A*) and LVEDV (Pearson $r = 0.86$; $P < 0.0001$) (*Figure 6B*). In contrast, the correlation was weak for LVESV measured with B-mode (Pearson $r = 0.43$) (*Figure 6C*). For LVEDV, the correlation remains consistent with that observed for 4DE (*Figure 6D*).

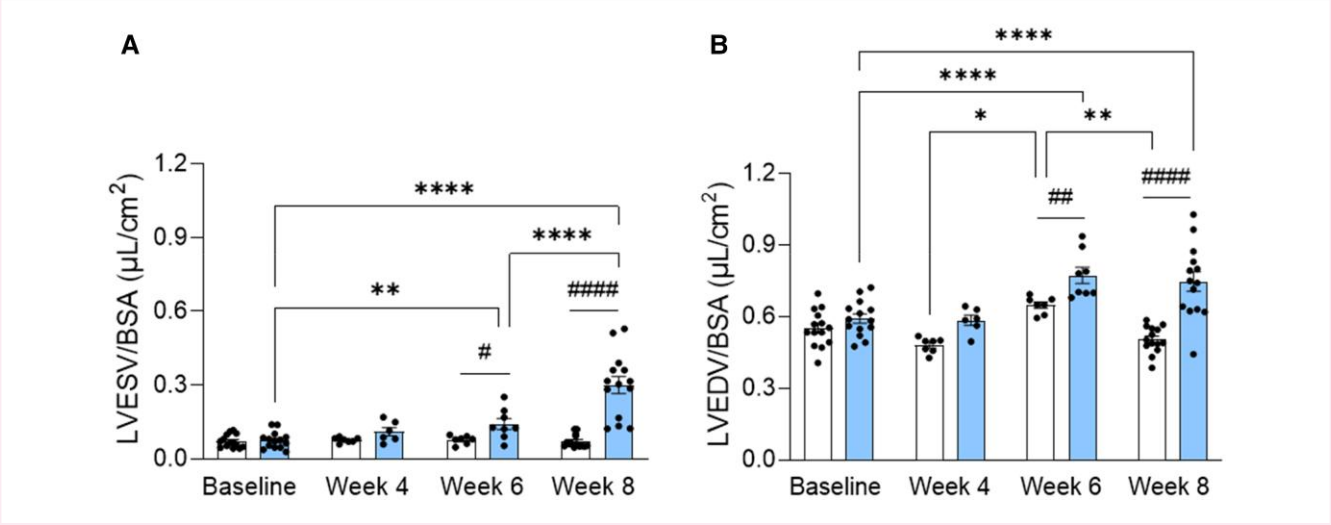


Figure 2 LV volumes over time. LVESV (*A*) and LVEDV (*B*) measured with 4D-mode in CTRL and DOX animals (both $n = 14$). Data are shown as mean ± SEM. For CTRL: $n = 14$ (baseline, Week 8) and $n = 7$ (Week 4, Week 6). For DOX: $n = 14$ (baseline, Week 8), $n = 6$ (Week 4), and $n = 8$ (Week 6). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.0001$. $^{\#\#}P < 0.01$ and $^{\#\#\#\#}P < 0.0001$. BSA, body surface area; CTRL, control; DOX, doxorubicin; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume.

Discussion

In this study, we conducted a comprehensive echocardiographic evaluation of a rat model of DOX-induced cardiotoxicity, demonstrating the superior reproducibility of 4D- and M-mode over B-mode for assessing disease progression.

Volume changes are more pronounced following DOX treatment

The prognosis of a cancer patient suffering from or at high risk of developing anthracycline-induced cardiac injury is highly dependent on early discovery.[10] While clinical symptoms may not appear until cardiac reserves are depleted, mild dysfunction may exist. Transthoracic echocardiography is a non-invasive, safe, and reliable tool for monitoring cardiac function in rodent cardiotoxicity models.[11] In our study, we confirmed the onset of cardiotoxicity starting at 6 weeks post-injection (i.e. cumulative dose of 12 mg/kg), characterized by elevated LV volumes, impaired LV systolic function parameters, and strain parameters, resembling the clinical phenotype seen in DOX-treated patients. While volumetric measurements uniformly changed at Week 6, not all systolic function parameters showed early alterations as LVEF often fails to demonstrate early myocardial damage.[7] Growing evidence suggests that strain may be more sensitive to detect early myocardial changes.[12] Systematic reviews indicate that a decline in GLS precedes LVEF decline, is associated with higher cardiotoxicity risk, and is more accurate in detecting early LV dysfunction.[13,14] In contrast, discrepancies exist in preclinical rodent studies due to variations in measurement techniques and animal models.[15,16] Notably, evidence suggests that GCS may precede GLS in detecting early chemotherapy-induced cardiotoxicity, particularly under conditions of spherical remodelling.[17] In our study, GCS demonstrated superior sensitivity, detecting cardiotoxicity after 4 weeks of the first DOX injection compared with 8 weeks for GLS. This highlights the complementary role of GCS, particularly in capturing circumferential shortening and providing a more sensitive and comprehensive assessment of subclinical cardiac dysfunction. In addition, given the increased severity of systolic dysfunction and LV dilation at Week 8 in this study, follow-up studies are warranted to assess further progression of cardiotoxicity in our model. Multiple studies have reported similar systolic and volumetric impairment in rodents treated with DOX. While Chan *et al.*[18] showed a significant decrease in LVFS and LVEF and an increase in LVESV in mice treated with a cumulative dose of 24 mg/kg DOX relative to CTRL, others reported similar decreases in systolic function parameters at lower cumulative DOX doses.[19–21] However, in some studies, systolic function was unaltered at these doses. While Ohlig *et al.*[22] showed no significant changes in LVEF and LVFS after 18 mg/kg DOX, Chakouri *et al.*[23] only showed a decrease in LVEF and LVFS 1 month after the last injection in rats treated with a cumulative dose of 12.5 mg/kg DOX. Although DOX-induced cardiotoxicity is primarily associated with the deterioration of systolic function, it can also affect diastolic function,[24] which was not observed in our study. Variations in cardiac load, cardiac rhythm, age, and differences in measurement techniques may account for the conflicting results in studies.[6] Discrepancies across studies may also be explained by variations in the treatment regimen (i.e. cumulative dose and duration). Noteworthy, we performed 4DE to evaluate the progressive deterioration of cardiac function over time whereas other studies mainly relied on M- or B-mode echocardiography. Stegmann *et al.*[25] demonstrated that 4DE is as precise as 4D CMR and highly reproducible, emphasizing its status as the preferred method for cardiac assessment. Finally, radial LVFS was measured using M-mode imaging, while longitudinal LVFS was assessed with B-mode imaging. In our model, B-mode has proven to be the least ideal imaging modality, while M-mode would be a better choice for our model (and 4D imaging being the optimal choice). Therefore, it is likely that the earlier detection of changes in radial LVFS may result from the higher sensitivity of M-mode imaging compared with B-mode, rather than a true mechanistic difference.

The reproducibility of echocardiography: B-mode echocardiography is not as suitable to evaluate DOX-induced cardiotoxicity in a rat model

Echocardiography offers a reliable, cost-effective, and widely accessible technique for evaluating cardiac function in humans and small animals.

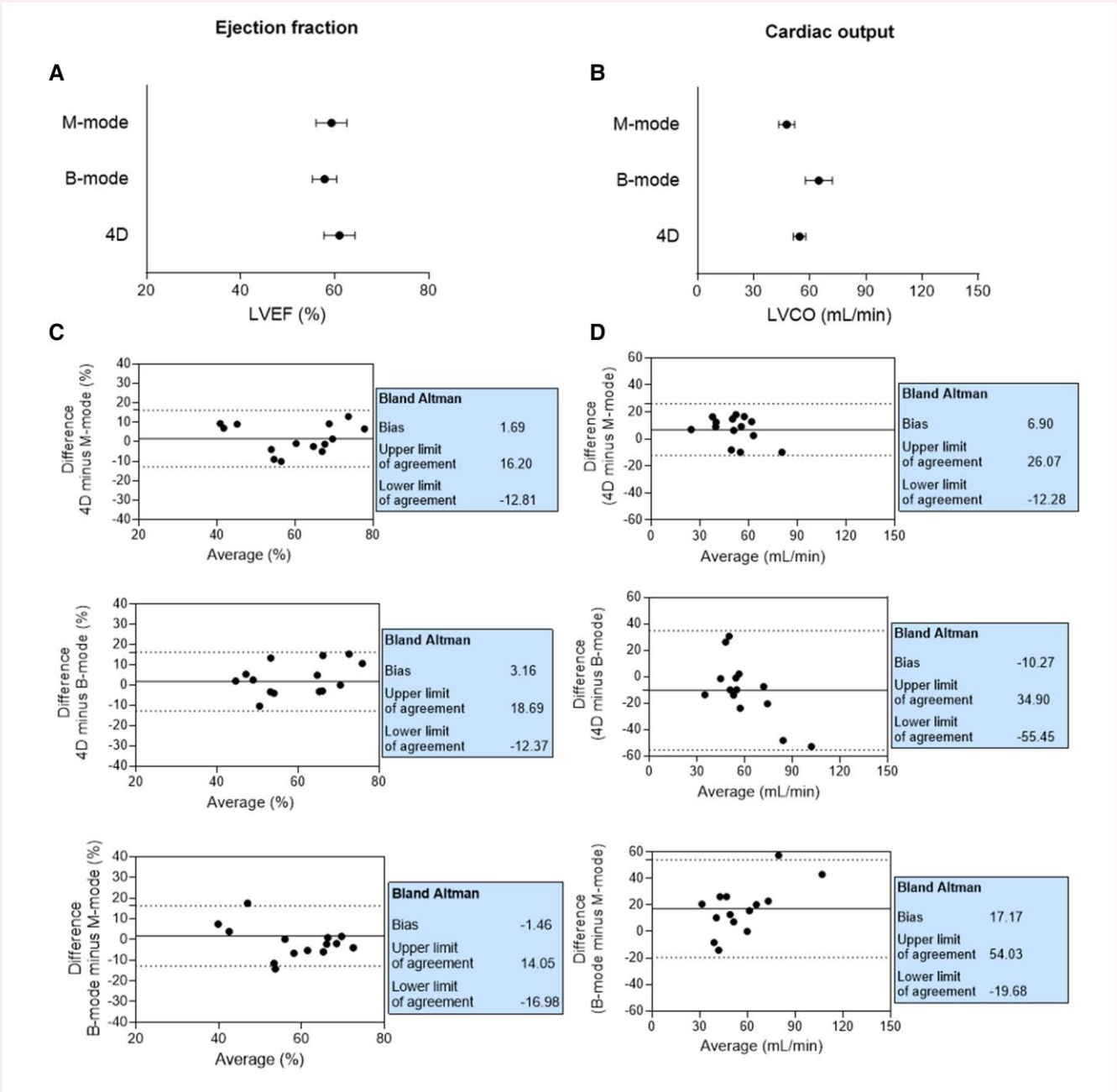


Figure 3 Agreement between echocardiographic modalities for assessing LV systolic function. Comparison of LVEF (*A*) and LVCO (*B*) measured after 8 weeks of treatment with different echocardiographic modalities in DOX animals ($n = 14$). Bland–Altman comparisons of different modes for LVEF (*C*) and LVCO (*D*). For (*A*) and (*B*), data are shown as mean ± SEM. LVEF, left ventricular ejection fraction; 4D, four-dimensional; LVCO, left ventricular cardiac output.

Multiple echocardiographic modalities are available, including M-mode, B-mode, and 3D/4D-modes.[26] However, a major limitation of M- and B-mode is that they depend on geometric assumptions to reconstruct the physiologically irregular shape of the heart.[27] Moreover, small errors during the analyses have major implications for the accuracy of volumetric calculations. Therefore, 3D/4D-mode echocardiography should be the most reliable method and the prevailing choice for functional and volumetric measurements of the LV as it does not rely on geometrical assumptions. 4DE also strongly agrees with CMR, confirmed in preclinical studies with rodents[25,28] and humans.[29,30] However, a survey from the European Association of Cardiovascular Imaging in 2020 revealed that using two-dimensional (2D) rather than 3D/4D echocardiography remains frequently used in the clinic.[31] Moreover, echocardiography can show variable reproducibility due to different echocardiographic modalities or operators.[32] In addition, direct comparisons of 4D-, B-, and M-mode for systolic, diastolic, and volumetric parameters, particularly in preclinical models of DOX-induced cardiotoxicity, are limited. Our study is the first to present a head-to-head comparison of these modalities. We used the Bland–Altman comparison method to evaluate agreement and assessed interobserver variability by correlating the

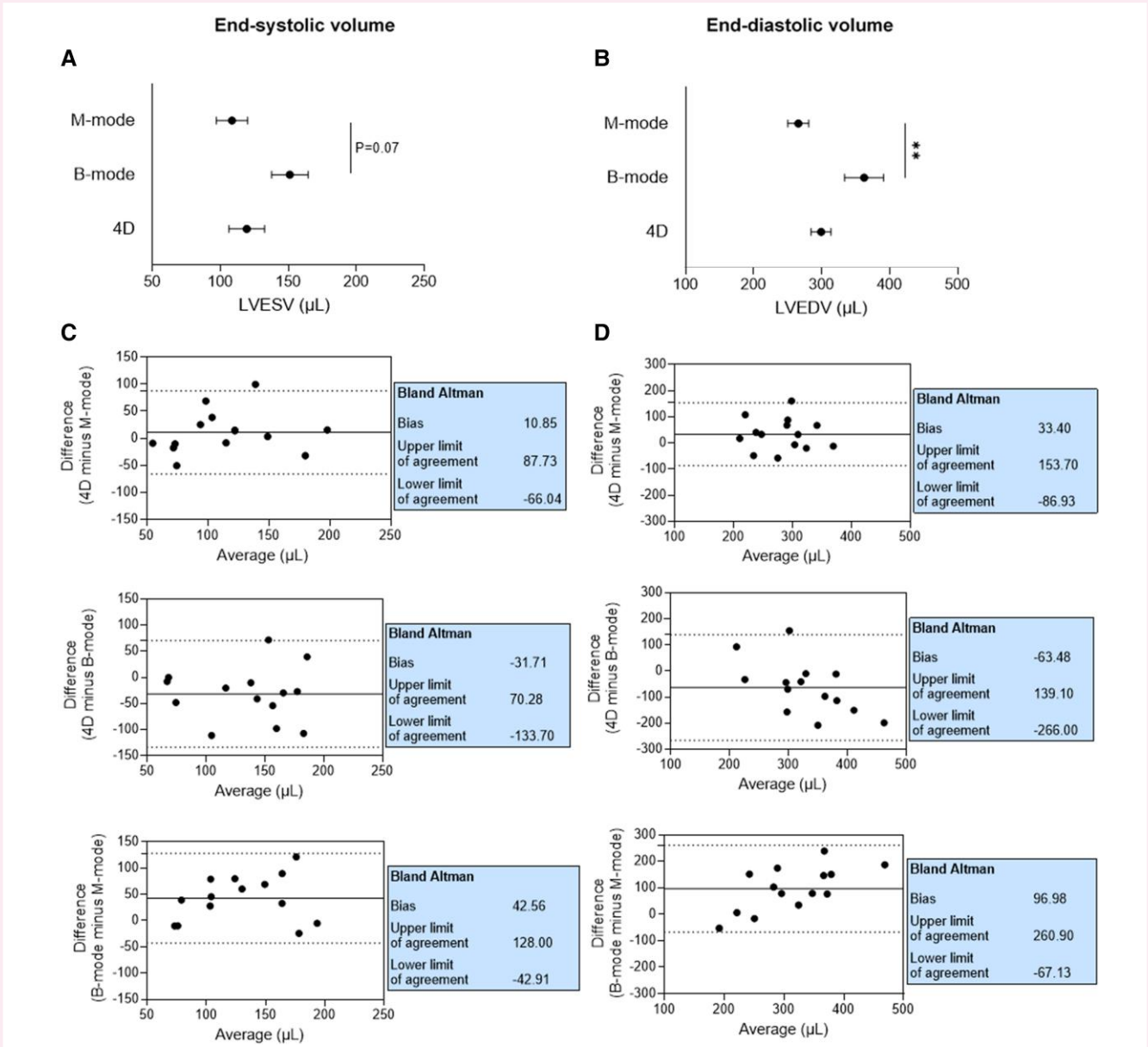


Figure 4 Agreement between echocardiographic modalities for assessing LV systolic volumes. Comparison of LVESV (*A*) and LVEDV (*B*) measured after 8 weeks of treatment with different echocardiographic modalities in DOX animals ($n = 14$). Bland–Altman comparisons of different modes for LVEF (*C*) and LVCO (*D*). For (*A*) and (*B*), data are shown as mean ± SEM. $^{**}P < 0.01$. 4D, four-dimensional; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume.

measurements by two researchers as recommended by Bunting *et al.*[32] Our study shows that all echocardiographic modalities provide comparable results when assessing LV systolic function parameters, with Bland–Altman plots demonstrating low bias and narrow limits of agreement. However, B- and M-mode showed low agreement for volumetric measurements, and using B-mode generally worsened agreements. Indeed, 4D- and M-mode echocardiography showed the best agreement for all parameters of LV systolic function and volumes, with the lowest bias and narrowest limits of agreement. These findings indicate that B-mode data are less reliable for monitoring DOX-induced cardiotoxicity than 4D- and M-mode data. Previous studies have demonstrated that 4DE significantly enhances the accuracy and reproducibility of LV function quantification compared with B-mode echocardiography,[25,33] as it minimizes motion artefacts through ECG and respiratory gating. Although guidelines indicate that B-mode is preferable over M-mode echocardiography,[15] our results demonstrate less variability and a higher reproducibility for M-mode than B-mode. This could be explained by the fact that no modified Simpson's rule was performed. We acquired single-plane B-mode images from a parasternal long-axis view. However, Zacchigna *et al.*[15] also proposed that the modified Simpson's method allows measurements of LV volumes with higher accuracy. This method combines B-mode images from a parasternal long-axis view with images from three short axes (i.e. base, mid, and apex) to assemble a 3D reconstruction of the LV. It is more accurate in

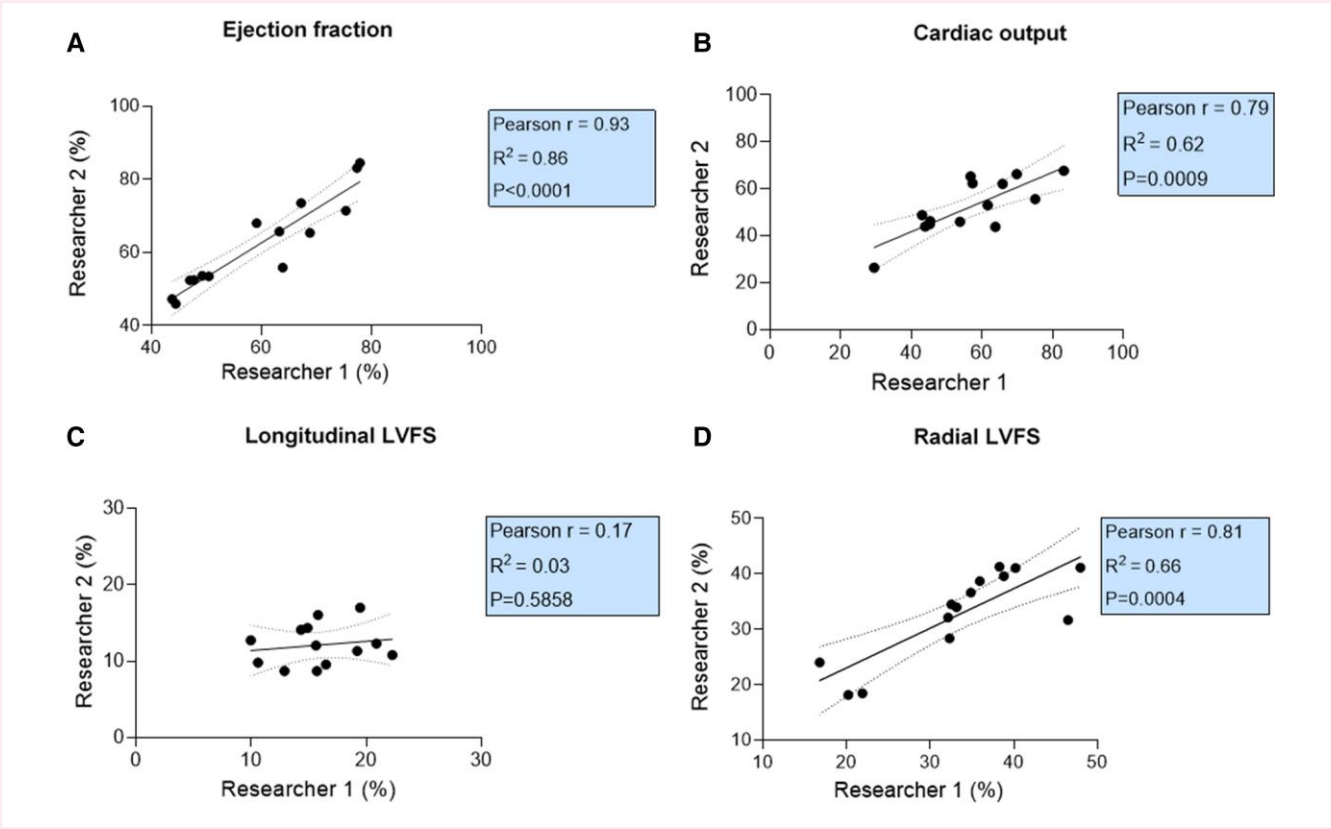


Figure 5 Interobserver variability for LV systolic function. Correlation between the measurements of both researchers for LVEF (*A*), LVCO (*B*), longitudinal LVFS (*C*), and radial LVFS (*D*) in DOX animals after 8 weeks of treatment ($n = 14$). LVEF, left ventricular ejection fraction; LVCO, left ventricular cardiac output; LVFS, left ventricular fractional shortening.

quantifying volumes in pathological conditions. Therefore, future analyses should incorporate this method for improved accuracy.

The researchers' measurements are consistent in our study, supporting the reliability of the findings

Next to the agreement between echocardiographic techniques, the variability between the investigators who examined the parameters is crucial when assessing echocardiography's reproducibility in an animal model. For most systolic function and volumetric parameters, the correlation between the researchers' observations in our study was very strong, indicating minimal interobserver variability, which was not the case using B-mode. These findings further confirm that B-mode echocardiography, which relies on geometrical assumptions, is less appropriate for evaluating DOX-induced cardiotoxicity. The study of Stegmann *et al.*[25] showed an excellent agreement for interobserver measurements with 4DE, confirming our results, and showed a higher interobserver reproducibility with 4DE. In addition, our findings are consistent with a study investigating the reproducibility of echocardiographic techniques for assessing cardiac function in breast cancer patients undergoing chemotherapy. Indeed, Thavendiranathan *et al.*[34] compared 2D Simpson's method with multidimensional echocardiography (3DE) and tested the interobserver variability in assessing LVEF and volumes. In line with our results, multidimensional echocardiography showed significantly lower variability in time than the other methods. Moreover, the interobserver variability was the lowest for multidimensional echocardiography, indicating that 3DE/4DE is the most reproducible technique for LV systolic function and volume measurements. Finally, in patients with cardiovascular diseases, multidimensional echocardiography showed good interobserver variability in assessing LV function and dimensions.[35] Therefore, 3DE/4DE of LVEF can reduce interobserver variability in patients undergoing cancer therapy and rodent models of cardiotoxicity.[36] These findings underscore the superiority of 4D imaging as the optimal approach for obtaining reliable results in preclinical DOX-induced cardiotoxicity studies, even when different researchers perform the scans.

Clinical relevance of the study

The functional and morphological changes following anthracycline chemotherapy resemble that of the LV dilated cardiomyopathy, characterized by dilation and a progressive decline of systolic dysfunction.[37] This phenotype, together with an increased interstitial fibrosis, was also shown in our animal model.[9]

Regarding our animal model, the rats received repeated doses of DOX, the most commonly used anthracycline drug known for its association with cardiotoxicity, for multiple weeks to gradually develop myocardial changes over time and mimic the chronic nature of cardiotoxicity. In addition, the intravenous injection route contributes to our animal model's translatability as it mirrors clinical protocols and results in pharmacokinetics like in patients.[38] In contrast, most preclinical studies include a rodent model in which DOX is administered via peritoneal

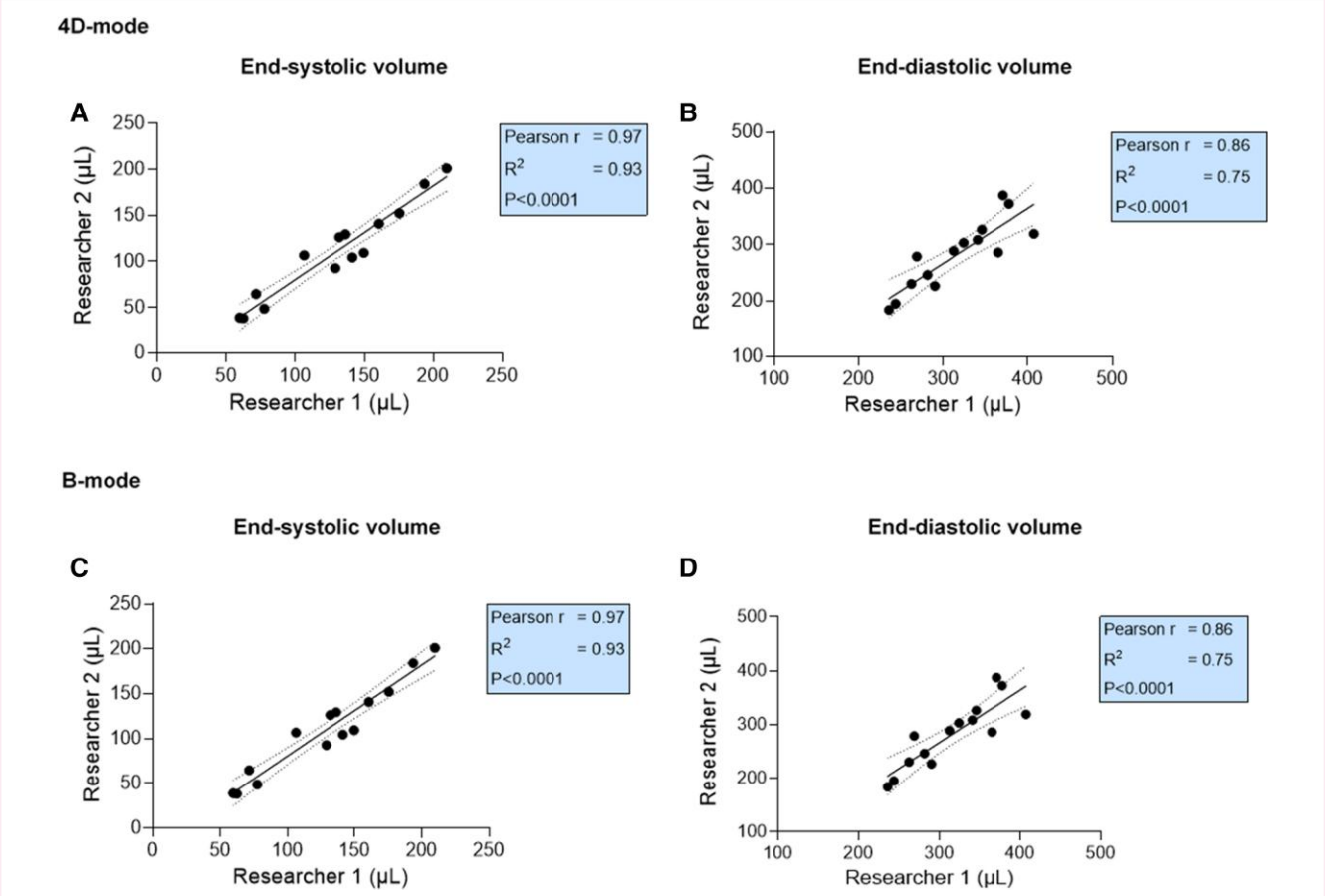


Figure 6 Interobserver variability for LV systolic volumes. Correlation between both researchers for LVESV and LVEDV measured in 4D-mode (*A* and *B*) and B-mode (*C* and *D*) in DOX animals after 8 weeks of treatment ($n = 14$). 4D, four-dimensional; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume.

injections as did by O'Connell *et al.* and Hayward *et al.*, which is inconsistent with the treatment in cancer patients. This results in a lack of reproducibility and substantial variation among different studies.[39] Finally, the cumulative DOX dose of 16 mg/kg in our study equals the clinical dose of 592 mg/m^2, which is administered to patients with advanced cancer and is in line with the doses used in other preclinical studies.[40]

Limitations of the study

This study includes some limitations. First, the healthy rats used do not fully replicate the complex pathophysiological conditions seen in cancer patients with DOX-induced cardiotoxicity and multiple comorbidities. Future research should involve a tumour-induced rat model to validate our findings. Second, echocardiographic measurements were performed 8 weeks after the initial DOX injection. Long-term studies should tightly monitor changes in LV function and volumes as cardiotoxicity may still worsen even after treatment cessation. Moreover, the reproducibility of the different echocardiographic modes is only measured immediately after the last DOX injection. A temporal variability test would better capture the accuracy over time.[34]

Conclusion

We conclude that LV volumetric changes were more pronounced than systolic dysfunction in our rat model of DOX-induced cardiotoxicity. In addition, 4DE has shown to be an effective approach, while B-mode appears less suitable. These findings underscore the importance of applying appropriate methods for detecting DOX-induced cardiotoxicity in rodent models which is crucial for testing potential cardioprotective agents before translation to larger models or human studies.

Supplementary data

Supplementary data are available at *European Heart Journal – Imaging Methods and Practice* online.

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Conflict of interest: None declared.

Data availability

No new data were generated or analysed in support of this research.

Lead author biography

Prof. Virginie Bito obtained her PhD in Biomedical Sciences from KU Leuven (Belgium) in 2004. Her doctoral research, titled "Mechanisms of excitation-contraction coupling in myocytes from normal and chronically ischemic pig myocardium", explored the fundamental mechanisms underlying cardiac remodelling in chronic ischaemia. Following 8 years of post-doctoral research, she was appointed associate professor at Hasselt University in 2012. With no pre-existing infrastructure or expertise in fundamental cardiology at the institute, she established her own research group from the ground up, pioneering this new domain within the university. Initially embedded within the *Physiology* research group, she is now a core member of the *Cardiac and Organ Systems* research group at BIOMED. Prof. Bito's research focuses on developing and characterizing novel therapeutic strategies to enhance cardiac function in pathological conditions such as myocardial infarction, diabetes, and anthracycline-induced cardiotoxicity. Her work includes interventions such as exercise therapy, anti-AGE therapies, and stem cell-based approaches. With a strong emphasis on fundamental research, she investigates molecular and cellular mechanisms of heart failure using preclinical animal models. She currently leads a research team of five members, supervising four PhD students-two in Belgium and two in the Democratic Republic of Congo (DRC)-alongside one post-doctoral researcher. In addition to her scientific research, she is actively involved in teaching at Hasselt University, contributing to courses for medical, biomedical, and biology students at both bachelor's and master's levels. Prof. Bito plays an important role in international academic collaboration, particularly through VLIR-UOS projects. She leads a VLIR-IUC sub-project with the Université Moulay Ismail (Morocco), serves as the promoter of a VLIR-TEAM project with the Université de Kindu (DRC), and coordinates a large VLIR-IUC project with the University of Lubumbashi (DRC). Beyond her research and academic leadership, Prof. Bito has held key administrative roles at UHasselt. Since 2013, she has chaired the Ethical Committee for Animal Experimentation, ensuring responsible and ethical research practices. In 2020, she was appointed Vice-Director of BIOMED, further contributing to the strategic development of the institute. Through her leadership in research, education, and international collaboration, Prof. Bito continues to make significant contributions to the fields of cardiovascular science and global health partnerships.

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