

Reliability of Echocardiographic Measurements in Infants Exposed to Zika Virus.

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Abstract

Background: Ecocardiography is currently the gold standard imaging method for the diagnosis of most congenital and acquired cardiopathies and for the evaluation of myocardial function and cardiac chamber size and overload. Ecocardiographic measures of left ventricular and right ventricular structure and function are used to define ventricular function and guide medical decisions. The objective of this study is to assess the reproducibility of echocardiographic cardiac chamber measurements in infants in utero exposed to Zika virus.

Methods: Masked cross-sectional diagnostic study with two independent measurements of cardiac chamber dimensions using echocardiography. We studied 50 infants with in utero exposure to Zika virus ranging from 30 to 270 days old of the outpatient clinic of a national reference center for maternal and child health, Rio de Janeiro, Brazil. The analysis included the measurements of the aortic root, left atrium, end diastolic left ventricle, systolic left ventricle, left ventricular ejection fraction, ventricular end diastolic septal thickness, end diastolic thickness of the left ventricular posterior wall and right ventricle. Bland-Altman plots were used to explore differences. Agreement according to intraclass correlation coefficients (ICC) was interpreted as follows: excellent ≥ 0.75 , fair to good $0.4 \leq \text{ICC} < 0.75$, and poor with $\text{ICC} < 0.4$.

Results: All parameters had mean difference of diameters near to zero, excluding left ventricle ejection fraction. Ventricular end-diastolic septal thickness and end-diastolic-thickness of the left ventricular posterior wall showed few unique mean values. Reproducibility was excellent for the aortic root and end diastolic left ventricle ($\text{ICC} = 0.86$ and 0.91 , respectively), fair/good to poor for other parameters and the left ventricle ejection fraction, ventricular end diastolic septal thickness and the end diastolic thickness of the left ventricular posterior wall showed the lowest reproducibility ($\text{ICC} = 0.46, 0.37, 0.53$, respectively).

Conclusions: The minor divergences did not affect the echocardiography results in our sample. However, caution is required to interpret results of measures with low reproducibility.

Background:

Ecocardiography (ECHO) is currently the gold standard imaging method for the diagnosis of most congenital and acquired cardiopathies and for the evaluation of myocardial function and cardiac chamber size and overload. It is a non-invasive and safe test with rapid real-time acquisition of images at low cost and without contraindications. Ecocardiographic measures of left ventricular (LV) and right ventricular (RV) structure and function are used to define ventricular function and guide decisions to the need for indication of inotropic medications, to assess the hemodynamic repercussions of patent ductus arteriosus (PDA) in premature infants and the possible need for pharmacological or surgical closure, to assess the repercussions and indication of closure of pulmonary hyper-flow congenital cardiopathies like ventricular septal defect (VSD) and atrial septal defect (ASD), for pharmacological and surgical treatment of valvular regurgitations like mitral valve insufficiency (MVI) and aortic valve insufficiency (AVI), and to determine the most adequate surgical procedure in cases of complex congenital cardiopathies.[1] Despite

the clinical and research importance placed on pediatric echocardiographic measurements, little has been published about the reliability of these measurements for which multiple raters independently measure the same sample when the true value is not known.[2]

Studies from the 1970s and 1980s provided the normal parameters for cardiac cavitory dimensions until 2000,[3, 4] when the results from a study in 2,036 children were published, redefining the normal ranges for parameters through the production of graphs that used body surface as a criterion and established mean values \pm 2 standard deviations for each measurement.[5]

As with any measurement, echocardiographic quantification is subject to error, so it is important to assess the degree of agreement between two observers. Intraclass correlation coefficient (ICC) estimates the fraction of the total variability of measurements due to inter-individual variations. Variation due to errors should include different components, depending on the study design.[6] The Bland-Altman plot is used to assess agreement between two quantitative measurements. The mean and standard deviation of the measurements are used to calculate the agreement's statistical limits. Bland-Altman is a scatter plot in which the Y axis is the difference between the two paired measurements and the X axis is the mean of these measurements. The diameter of the points is directly proportional to the frequency of inter-examiner agreement.[7, 8]

In previous study, our search group demonstrated cardiac alterations associated with in utero exposure to Zika virus (ZIKV): 10% of patients presented major defects such as ASD, VSD, and PDA.[9]

We study the reliability of cardiac chamber measurements using ECHO in a sample of patients from a cohort of infants *in utero* exposed to ZIKV followed by a Brazilian national reference center to maternal and child health. All the infants were referred for a clinical and echocardiographic evaluation.

Methods:

We performed a blinded cross-sectional diagnostic study with two independent measurements of cardiac dimensions using ECHO of infants born between November 2015 and January 2017 who were followed at our outpatient Pediatric Infectious Disease Clinic at the Fernandes Figueira Institute (IFF-FIOCRUZ). The infants who received echocardiograms were born either at IFF or other institutions with positive ZIKV maternal polymerase chain reaction (PCR) results during pregnancy or positive PCR results at birth. Our institution is a national research referral center for high risk pregnancies and high risk infants. For this reason, during the Zika epidemic we were the major referral center in Rio de Janeiro for cases of suspected maternal or infant ZIKV infection.

The study was approved by the Institutional Review Board of the Brazilian National Institute of Infectious Diseases (INI-FIOCRUZ – CAAE 62728516.2.0000.5262). All parents or guardians provided written informed consent. All data analyzed were anonymized. The tests were performed by two of the three pediatric cardiologists at the Department of Pediatric Cardiology of IFF, using an Acuson X300 echocardiography system, blinded in relation to the previous tests. The doubles were chosen at random.

All pediatric cardiologists are certified specialists in Pediatric Cardiology by the Brazilian Society of Cardiology and Brazilian Society of Pediatrics and have at least 15 years of practice in the specialty. No patient required sedation to undergo the tests, which were repeated by the respective examiners with a maximum interval of 15 days. The tests were performed during the normal patient flow in the Pediatric Cardiology Outpatient Department of IFF, and the cavitory measurements were taken. The cardiological evaluation took place between 30 and 270 days of life and included measurements of aortic root, left atrium, end-diastolic left ventricle, systolic left ventricle, right ventricle, left ventricular ejection fraction, ventricular end-diastolic septum thickness, and end diastolic thickness of the left ventricular posterior wall. Measurements were taken at the cross-sections defined in the echocardiography guidelines, namely the unidimensional mode of the parasternal longitudinal section of the long axis of the left ventricle at the level of the aortic valve leaflets to measure the aorta and left atrium, and for the others, the parasternal section of the short axis of the left ventricle at the level of the mitral valve leaflets.[1]

The analysis was performed in Med Calc 17.9 and the free software R 3.5.1 [10] in the BlandAltmanLeh and nopaco packages. The median values expressed in centimeters with the respective interquartile ranges (IQR) were summarized in tables and compared with the normal values according to body surface which does not change in 15 days.[5] Due to rejection of the normality of measurements according to the boxplot and Shapiro-Wilk test, the measurements were also compared between the two observers using the Wilcoxon non-parametric test. Intraclass correlation coefficients (ICC) were calculated (measures of unique consistency and means), with the respective 95% confidence intervals (95% CI). Interpretation of ICC values was as follows: excellent reliability when ≥ 0.75 , fair to good with $0.4 \leq \text{ICC} < 0.75$, and poor when $\text{ICC} < 0.4$. [6] Bland-Altman plots were produced to explore the distribution of each ECHO measurement considering the size of the differences between the two independent evaluations, using as the reference the mean of the differences and limits determined by two standard deviations below and above the mean. Each point's size is determined by the value's frequency. Few unique observations and concentration of points in certain mean values can lead to bad overall agreement parameters, as for example ICC.

Results:

We performed two echocardiograms with maximum interval of 15 days in 50 infants of the 120 patients who were brought by their parents or guardians to the pediatric clinic for a cardiac consult and performance of an echocardiogram because of the *in utero* exposure to ZIKV. No infant had a diagnosis of congenital cardiopathy with hemodynamic repercussions. The mean age of the 50 infants was 111 days (95% CI = 94.7-127.3), with body surface varying from 0.2 to 0.3 m²; 60% were girls, and 86% were term infants.

Table 1 showed the medians calculated for the measurements performed by observers 1 and 2.

The medians for the measurements were mostly identical. All the median measurements were within normal ranges.

Table 1

Dimensions (in centimeters) of two independent echocardiographic measurements in 50 infants (medians and interquartile ranges) with *in utero* exposure to ZIKV, and respective normal values.

| Parameter (Diameter) | Appraiser 1 | | Appraiser 2 | | p value † | Normal values | |
|---|---------------------|---------------------|---------------------|---------------------|-----------|---------------------|---------------------|
| | Median (IQ*) | Mean (95%CI) | Median (IQ*) | Mean (95%CI) | | Mean (95%CI ‡) | Mean (95%CI ‡) |
| | | | | | | Body surface | |
| | | | | | | 0.25 m ² | 0.30 m ² |
| Aortic root (cm) | 1.10 (1.00-1.20) | 1.09 (1.04-1.14) | 1.10 (1.00-1.20) | 1.11 (1.06-1.16) | 0.401 | 1.04 (0.80-1.28) | 1.13 (0.9-1.36) |
| Left atrium(cm) | 1.40 (1.20-1.60) | 1.40 (1.32-1.47) | 1.40 (1.30-1.60) | 1.42 (1.35-1.49) | 0.688 | 1.40 (1.05-1.75) | 1.53 (1.15-1.91) |
| End-diastolic left ventricle (cm) | 2.10 (1.90-2.40) | 2.10 (1.99-2.21) | 2.10 (1.90-2.40) | 2.10 (2.00-2.20) | 0.694 | 2.0 (1.64-2.36) | 2.29 (1.8-2.58) |
| Systolic left ventricle (cm) | 1.20 (1.00-1.30) | 1.18 (1.11-1.25) | 1.30 (1.10-1.40) | 1.25 (1.19-1.32) | 0.052 | 1.32 (1.02-1.62) | 1.48 (1.08-1.88) |
| Ventricular end-diastolic septal thickness (cm) | 0.40 (0.30-0.40) | 0.38 (0.36-0.41) | 0.40 (0.30-0.40) | 0.38 (0.36-0.41) | 0.596 | 0.38 (0.24-0.52) | 0.39 (0.25-0.53) |
| End-diastolic thickness of the left ventricular posterior wall (cm) | 0.40 (0.30-0.40) | 0.37 (0.34-0.39) | 0.40 (0.30-0.40) | 0.35 (0.33-0.38) | 0.260 | 0.36 (0.26-0.46) | 0.41 (0.28-0.54) |
| Right ventricle (cm) | 0.90 (0.73-1.00) | 0.89 (0.84-0.95) | 0.90 (0.80-0.90) | 0.87 (0.83-0.91) | 0.381 | 0.87 (0.42-1.32) | 0.87 (0.42-1.32) |
| Left ventricle ejection fraction (%) | 76.5 (69.0-82.8) | 75.7 (73.2-78.2) | 69.5 (66.0-74.8) | 70.4 (68.3-72.5) | 0.003 | > 55 | > 55 |

*Interquartile ranges; † Wilcoxon test (p value < 0,05); ‡ Confidence interval.

Table 2 showed ICC for the measurements performed by observers 1 and 2.

Table 2

Reliability of target parameters in two independent echocardiographic measurements, expressed as intraclass correlation coefficients (ICC) and respective 95% confidence intervals in 50 infants with *in utero* exposure to ZIKV.

| Parameter (diameter in cm) | Single | | Interpretation |
|--|--------|----------------|----------------|
| | ICC | 95% CI | |
| Aortic root | 0.75 | 0.60 0.85 | Excellent |
| Left atrium | 0.43 | 0.17 0.63 | Fair / Good |
| End-diastolic left ventricle | 0.83 | 0.72 0.90 | Excellent |
| Systolic left ventricle | 0.44 | 0.19 0.64 | Fair / Good |
| Ventricular end-diastolic septal thickness | 0.23 | -0.049 0.48 | Poor |
| End-diastolic-thickness of the left ventricular posterior wall | 0.36 | 0.09 0.58 | Poor |
| Right ventricle | 0.40 | 0.14 0.61 | Fair / Good |
| Left ventricle ejection fraction | 0.19 | -0.04 0.09 | Poor |

Diameters of aortic root and end-diastolic left ventricle had low differences between two observers, with mean difference near to zero and mean values varied up to 0.5 cm (Fig. 1). Medians of aortic root and end-diastolic left ventricle (1.1 cm and 2.1 cm) did not show differences between two evaluations ($p > 0.05$, Table 1). Also, it was observed excellent agreement for these two measurements (Table 2).

The Bland Altman plot of diameter of systolic left ventricle showed low differences between two observers, with mean difference near to zero and mean values varied up to 0.6 cm (Fig. 1). Medians of systolic left ventricle (1.2 and 1.1 cm) did not show differences between two measurements ($p > 0.05$, Table 1). The agreement was considered good (Table 2).

Although the mean difference in diameter of ventricular end-diastolic septal thickness and end-diastolic-thickness of the left ventricular posterior wall are near to zero, few unique mean values were observed (Fig. 1). Medians of ventricular end-diastolic septal thickness and end-diastolic-thickness of the left ventricular posterior wall (0.4 and 0.4 cm) did not show differences between two observers ($p > 0.05$, Table 1). These measurements were the lowest structures when compared with others (Table 1). The agreement was considered poor (Table 2). Other measurement with low agreement between observers

was left ventricle ejection fraction (Table 2). The medians were higher 7 percentage points when compared by the two observers ($p < 0.05$, Table 1).

Discussion:

This is one of the first studies on the reliability of echocardiographic parameters in infants. The most reliable parameters were the diameters of aortic root and end-diastolic left ventricle, while the other parameters varied from good/fair to bad, especially those with smaller dimensions, namely ventricular end-diastolic septal thickness and end-diastolic thickness of the left ventricular posterior wall. However, the disagreements occurred in approximately 0.5% of patients.

The small divergences did not affect the echocardiographic test reports, which could be explained by the fact that differences of 1 mm altered the reliability of two measures but did not exceed the normal range for the cavitory measurements assessed in this study.

Since the system calculates left ventricle ejection fraction as the ratio between end-diastolic left ventricle and systolic left ventricle, it was mainly affected by the discrepancies in the measurements of the systolic left ventricle.

Medical consensuses and guidelines use clinical and complementary test criteria to orient diagnosis and treatment decisions. This involves assessing the parameters' diagnostic accuracy by measuring the sensitivity and specificity, with reliability as a prerequisite for accuracy.[10]

Echocardiography is an easy test to perform, and its use as a parameter to guide clinical or surgical approaches requires good reliability of measurements obtained by different observers. However, there is little information in the literature on the reliability of the parameters obtained with echocardiography and that are used in cardiology guidelines to indicate closure of the ductus arteriosus, VSD, and ASD or valvuloplasty in cases of valvular insufficiencies such as rheumatic mitral and aortic valve insufficiencies.

Geelhoed et al found good reliability for echocardiographic measurements of left heart structures in 28 healthy children with median age of 7.5 years.[12] Another study assessing reliability of left ventricular systolic function of 59 potential pediatric heart donors with less than 18 years reported that ICC between measurements done by local hospitals and by trained pediatric cardiologist at a pediatric heart center was 0,59 (fair to good) and in this study, 20% of echocardiograms with LV dysfunction by local measurements turned out to have normal function when measured in the central laboratory.[13] Lipshultz et al compared measurements of LV dimension and wall thickness obtained on 735 children of HIV-mothers at 10 clinical sites and concluded that they differed so much that a central echocardiographic facility is needed to provide consistent and reliable data for research studies and clinically meaningful results.[14]None of these studies evaluated infants.

The low reliability of a test limits the generalization of approaches and recommendations in guidelines and consensus.[15, 16] As an example, the score proposed by McNamara and Sehgal for closure of the PDA in premature infants included clinical criteria and echocardiographic measurements. Among the measurements tested, those with the highest predictive value for indication of closure of a hemodynamically significant PDA were the size of aortic root and left atrium, but they were also the parameters with the lowest inter-examiner reliability.[15] In our study, the size of aortic root showed excellent reliability, while reliability of left atrium was fair. This suggests that the use of this score or any other to indicate closure of PDA may be compromised by the difficulty in reproducing these measurements by different observers and in distinct settings. Schwarz et al. (2016) studied the reliability of echocardiographic parameters for the diagnosis of PDA in low birth weight premature infants and found bad reliability for the size of aortic root and left atrium, while the reliability was somewhat better for other parameters (short axis of the ductus arteriosus, resistance index of the celiac artery and anterior cerebral artery, among others)but some of these parameters were not assessed in our study.[17]

Alterations in the Bland-Altman plot may occur due to the limited number of unique observations and the concentration of points at given mean values, which can lead to low overall agreement indices (e.g., ICC). ICC also considers normal data and is affected by the data's deviation from normality and variability, as is the Bland-Altman plot. Due to the non-normality of four of the parameters studied in the current sample (systolic left ventricle, ventricular end-diastolic septal thickness and end-diastolic-thickness of the left ventricular posterior wall and right ventricle), caution is recommended when interpreting the plots, as well as the reliability results (ICC).

This study has limitations. The sample size is small but was similar to or larger than other studies on this topic in the literature and the observers have high and homogeneous level of training.

Conclusions:

Although we found variable reliability for all the cardiac measurements, the minor divergences did not affect the echocardiography results in our sample. However, caution is required to interpret results of measures with low reliability (ventricular end-diastolic septal thickness, end-diastolic-thickness of the left ventricular posterior wall and left ventricle ejection fraction).

List Of Abbreviations:

ECHO: Echocardiography

LV: Left ventricle

RV: Right ventricle

PDA: Patent ductus arteriosus

VSD: Ventricular septal defect

ASD: Atrial septal defect

MVI: Mitral valve insufficiency

AVI: Aortic valve insufficiency

IQR: Interquartile ranges

ICC: Intraclass correlation coefficient

PCR: Polymerase chain reaction

Declarations

Ethics approval and consent to participate: The study was approved by the Institutional Review Board of the Brazilian National Institute of Infectious Diseases (INI-FIOCRUZ – CAAE 62728516.2.0000.5262). All parents or guardians of minors included in this study provided written informed consent.

Consent for publication: Not applicable.

Data availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests" in this section.

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Authors' contributions: All authors contribute to the conception of the study.DHGO,SRLP,RVCO,TMR,LMLS and RO conducted the analysis and wrote the first draft. DHGO, CVBF and MFMP performed the exams and provided critical editorial input. All authors read and approved the final manuscript.

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Figures

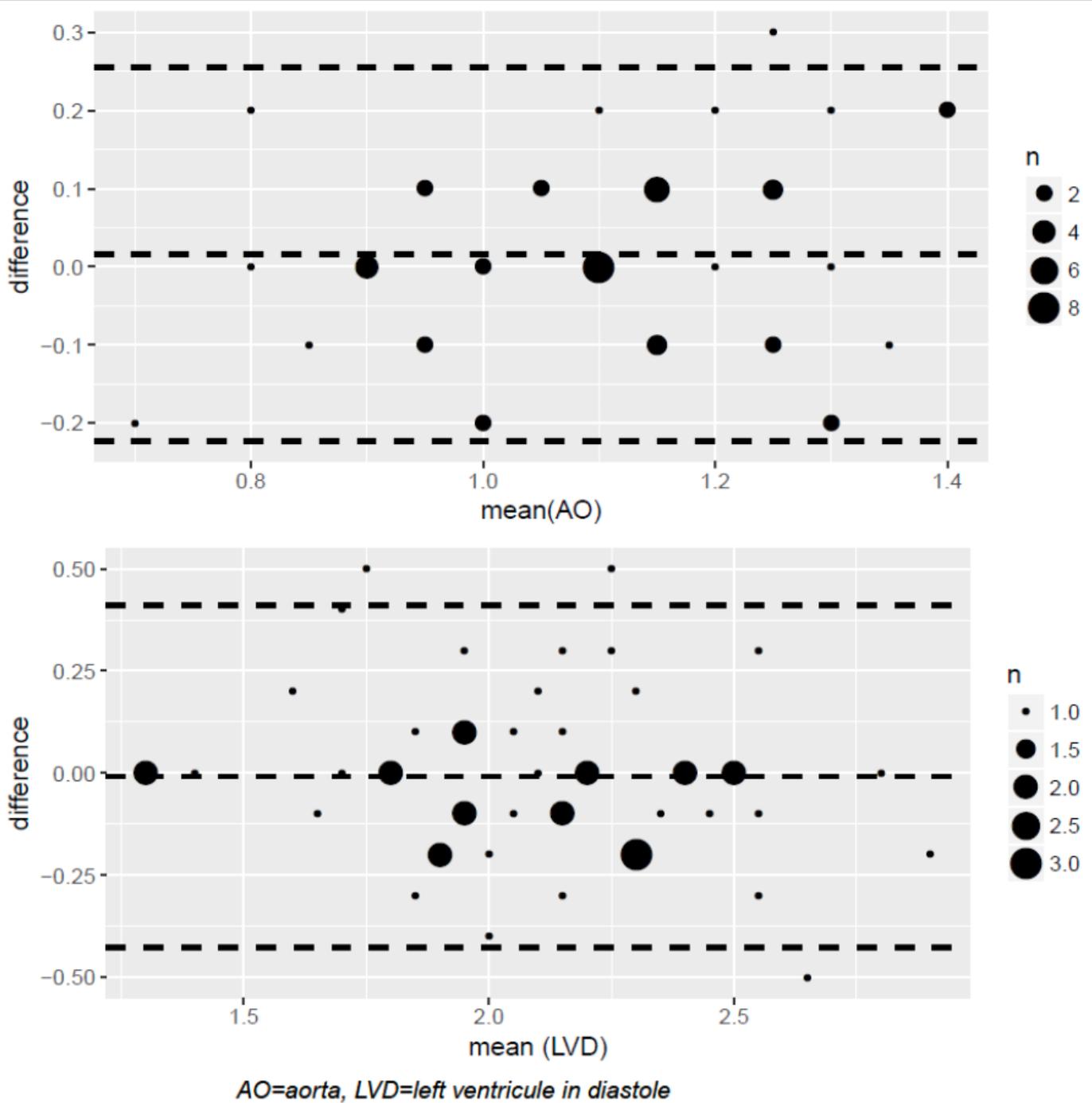


Figure 1

Bland-Altman plots of measurements of aorta and left ventricle in diastole diameter in 50 infants with in utero exposure to ZIKV. Rio de Janeiro, Brazil, 2018. Bland-Altman plot of ECHO measurements with excellent agreement by two pediatric cardiologists. Brazil, 2018. Note:AO=aortic root, EDLV=end-diastolic left ventricle, SD=standard Deviation, CE1=cardiological evaluation 1 and CE2=cardiological evaluation 2. Excellent agreement (Mean difference between CEs near to zero and low differences).

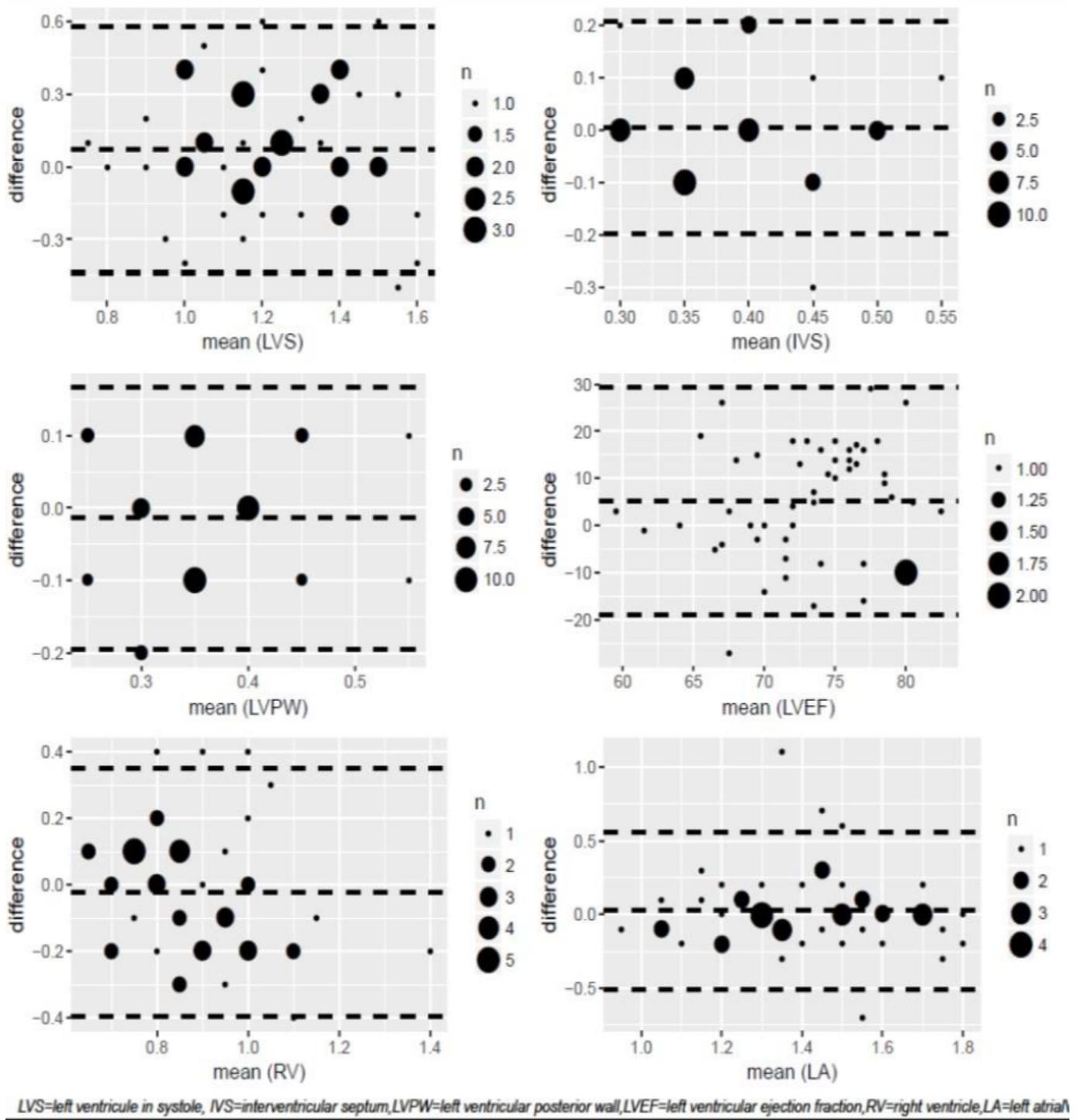


Figure 2

Bland-Altman plots of measurements of LVS, IVS, LVPW, LVEJ, RV and LA in 50 infants with in utero exposure to ZIKV. Rio de Janeiro, Brazil, 2018. Bland-Altman plot of ECHO measurements with good to poor agreement by two pediatric cardiologists. Brazil, 2018. Note: SLV= systolic left ventricle, EDVS= end diastolic ventricular septum, EDPW= end-diastolic-thickness of the left ventricular posterior wall, LVEF=left ventricular ejection fraction, RV=right ventricle, LA= left atrium, CE1= cardiological evaluation

1, CE2= cardiological evaluation 2, SD=standard deviation. LA: Good agreement (Mean difference between CEs near to zero). EDPW and EDVS: Poor agreement (Mean difference between CEs near to zero with few unique values). SLV and RV: Fair to good agreement (Mean difference between CEs near to zero but with some differences higher than 0.4 mm) and LVEF: Poor agreement (High variability of differences between the two CEs).