Comparison of 2D versus M-mode echocardiography for assessing fetal myocardial wall thickness

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ABSTRACT

Objective: M-mode and 2D have been proposed for evaluating fetal myocardial thickness. However, studies comparing the performance of both modalities are lacking. We aimed to compare 2D versus M-mode reproducibility for assessing myocardial wall thicknesses.

Methods: A prospective study including 45 healthy fetuses from low-risk pregnancies evaluated between 18 and 41 weeks of gestation. Left and right ventricular free-wall and septal myocardial thicknesses were measured at end-diastole (ED) and end-systole (ES) in transverse 4-chamber view using 2D and M-mode. Intra- and interobserver reproducibility was evaluated by the concordance correlation coefficient (CCC). Both techniques were compared by \textit{t}-test of the CCC.

Results: 2D and M-mode demonstrated excellent and similar intraobserver repeatability, with the best concordance in ES septal thickness (M-mode CCC 0.956 versus 2D-mode CCC 0.914). Interobserver reproducibility demonstrated also a high concordance, optimal in ES left ventricular free wall (M-mode 0.925 versus 2 D 0.855). Comparison of both techniques demonstrated a high concordance in all measurements, except for ED septal thickness with better reproducibility using M-mode (CCC 0.954 versus 0.847, \(p = .017\)).

Conclusions: 2D and M-mode can be used in a reproducible manner for measuring fetal myocardial thickness, with a slightly better performance of M-mode for assessing ED septal wall thickness.

Introduction

Fetal echocardiography has evolved remarkably during last decades. Initially, its use was limited to assess cardiac structural integrity [1–3]. However, advances in ultrasound and incorporation of new myocardial imaging techniques permitted a more accurate evaluation of fetal cardiac structure and function. Recently, different patterns of cardiac dysfunction and remodeling have been described in fetal diseases such as twin-twin transfusion syndrome, [4,5] intrauterine growth restriction, [6–10] congenital heart disease, [11,12] maternal diabetes, [13–16] the use of antiretroviral drugs [17], or assisted reproductive technologies [18,19]. An accurate and reproducible measurement of atrial and ventricular structures is essential for an adequate evaluation of adaptive changes in fetal cardiac shape and structure, also known as fetal cardiac remodeling.

Initial studies standardizing fetal cardiac morphometry were based in M-mode [1,20] and allowed the description of ventricular hypertrophy (i.e. septal hypertrophy in fetuses of diabetic mothers [13–16]) or dilatation (i.e. antenatal dilated cardiomyopathy [21,22]). Later, improvement of ultrasound resolution and its widespread use in obstetrics have led to the incorporation of 2 dimensional (2D) mode as a fundamental part of fetal echocardiography including its use for cardiac morphometry [23,24]. However, the technique proposed for the measurement of myocardial thickness differs across and within studies, [23–25] with no international consensus so far. Additionally, no previous studies have directly compared 2D and M-mode for measuring of fetal ventricular wall thickness.

The objective of the present study was to evaluate and compare the reproducibility of 2D versus M-mode.
for the measurement of fetal myocardial thicknesses. For this, a reproducibility study was conducted in a low-risk population of fetuses at 18–41 weeks of pregnancy.

Materials and methods

Study population

A prospective study including uncomplicated singleton pregnancies between 18+0 and 41+0 weeks recruited from low-risk pregnancies attended at BCNatal in Barcelona between October 2015 and October 2016. A random selection of fetal echocardiographies from a larger cohort was obtained for this study, and only pregnancies with good quality images were used for final analysis. Exclusion criteria included cases with pregestational diabetes mellitus, maternal hypertrophic or dilated cardiomyopathy, preeclampsia, maternal HIV, stillbirth, intrauterine growth restriction, preterm delivery, major congenital birth defect, aneuploidies, or genetic syndromes. Gestational age was calculated according to first trimester’s crown-rump length. Birthweight centiles were calculated according to local standards [26]. The study protocol was approved by hospital’s Ethical Committee and written consent form was obtained from all participants.

Study protocol and echocardiography

Demographic and perinatal data were obtained by parental questionnaire and review of hospital records. All participants underwent a single fetal cardiac ultrasound evaluation using a Siemens® Sonoline Antares machine (Siemens Medical Systems, Malvern, PA), with a 2–6 MHz linear curved-array probe and a 2–10 MHz phased-array cardiology probe. A standard fetal echocardiography was first conducted to rule out the presence of any cardiac structural anomaly. Then, M-mode and 2D were applied for measuring myocardial wall thickness at septal and free walls of left and right ventricles.

For 2D evaluation, a movie clip of a lateral four-chamber view, including at least three complete heart cycles without fetal movements, was stored for off-line analysis. Myocardial wall thickness was measured at the level of the atrioventricular valves excursion (Figure 1(a)). For the left and right ventricular walls, caution was taken not to include the chordae tendineae to avoid a false thicker wall. Heart cycle periods were identified with the use of cine-loop. End-diastole was considered at the largest ventricular distention just after the atrioventricular valves closure and end-systole period at the smallest ventricular cavity just before the atrioventricular valves open.

For M-mode measurements, a four-chamber lateral view was obtained, and M-mode was applied and adjusted to include only the fetal heart. The position of the M-mode line was located perpendicular to the septal wall at the level of the atrioventricular valves excursion (Figure 1(b)). The M-mode image obtained was considered adequate for analysis if at least three consecutive heart cycles without fetal movements were present. End-diastole was considered at the largest ventricular cavity diameter just after the closure of the AV valves and end-systole at the narrowest ventricular cavity just before the opening of the AV valves. Myocardial thickness was measured positioning the calipers following the edges of endo and epicardium.

All measurements were performed off-line by two observers (L.G-O. and A.S-M.) for the interobserver reproducibility. For the intraobserver repeatability, a second analysis was performed by a single observer, at least 1 week after the first measurement.

Sample size estimation

To determine the adequate sample size for repeatability and reproducibility with continuous variables, the formula proposed by Bonnet et al. was used [27]. For an estimated Concordance Correlation Coefficient (CCC) between 0.8 and 0.85, a sample size of 42 pairs were needed.

Statistical analysis

Stata® 14.2 (Stata Corp, College Station, TX) was used for the statistical analysis. p values less than .05 were considered as significant. For continuous variables, a Shapiro–Wilk test was used for determination of the distribution. Data were expressed as mean (standard deviation) for normal distributions or median (inter-quartile range) for non-normal distributions. Categorical variables were expressed as absolute numbers and percentages.

Inter and intraobserver reproducibility was assessed using the Concordance Correlation Coefficient (CCC) [28]. Limits of agreement and standard error were calculated for all comparisons and Bland–Altman plots were obtained [29]. For 2D versus M-mode comparison, CCC coefficients were compared with a t-test analysis. To evaluate the impact of gestational age on intra and interobserver analyses, the difference between pairs/mean between pairs ratio was plotted and assessed with a linear regression analysis.
Figure 1. Description of landmarks for ventricular walls assessment. (a) Ventricular walls measurement by 2 dimensional (2D)-mode in four-chamber lateral view at end-diastole. Left image: 2D-ultrasound; right image: descriptive scheme of 2D measurement. (b) Ventricular walls measurement by M-mode in four-chamber lateral view at end-diastole. Upper-left image: 2D-ultrasound. White thick line corresponds to reference M-line. Lower-left image: M-mode representation of the reference M-line. Lower-right image: descriptive scheme of M-mode measurement. RV: right ventricle; S: septal wall; LV: left ventricle; CT: chordae tendinae; 1: left ventricular wall thickness at end-systole; 2: septal wall thickness at end-systole; 3: right ventricular wall thickness at end-systole; 4: left ventricular wall thickness at end-diastole; 5: septal wall thickness at end-diastole; 6: right ventricular wall thickness at end-diastole.
Table 1. Maternal and perinatal characteristics of the study population.

| Variable                      | Value         |
|-------------------------------|---------------|
| Maternal characteristics      |               |
| Age, years                    | 32.5 ± 4.63   |
| Body mass index, kg/m²        | 22.7 ± 2.86   |
| Chronic diseases              | 0             |
| Smoking habit                 | 4 (8.9)       |
| Nulliparous                   | 24 (53.3)     |
| Cesarean section              | 1 (2.2)       |
| Gestational age at birth, weeks | 40.3 (39.5–41.0) |
| Birthweight, grams            | 3485 (3320–3841) |
| Birthweight percentile        | 70.5 (22.5–86.0) |
| Five-minutes APGAR <7         | 0             |

Data expressed as mean ± standard deviation, median (interquartile range) or n (%).

Table 2. Mean values for fetal myocardial wall thicknesses using 2D and M-mode.

| Variable                      | M-MODE        | 2D            | p value |
|-------------------------------|---------------|---------------|---------|
| Septum (d)                    | 3.08 ± 0.96   | 2.87 ± 0.87   | .27     |
| Septum (s)                    | 4.06 ± 1.23   | 3.63 ± 1.03   | .07     |
| LV free wall (d)              | 2.77 ± 0.84   | 2.55 ± 0.80   | .21     |
| LV free wall (s)              | 3.38 ± 0.94   | 3.08 ± 0.91   | .13     |
| RV free wall (d)              | 2.61 ± 0.79   | 2.66 ± 0.85   | .79     |
| RV free wall (s)              | 3.21 ± 0.98   | 3.13 ± 0.90   | .70     |

All the measurements in mm and expressed as mean ± SD. p value calculated by t-test comparison of mean values by 2D versus M-mode. LV: left ventricular; RV: right ventricular; (d): end-diastolic; (s): end-systolic; s: septum.

Results

Study population

Image dataset of 45 patients were used. Maternal and perinatal characteristics of patients are described in Table 1. Mean maternal age and birthweight were 32.5 years and 3485 g, respectively. Median gestational age at ultrasound was 29 weeks with the following patient’s distribution: 18–25 weeks (n = 15), 26–33 weeks (n = 15) and 34–41 weeks (n = 15).

M-mode and 2D reproducibility

Mean values of the fetal ventricular walls were similar between M-mode and 2D-mode (Table 2). Intraobserver analysis demonstrated good repeatability of both septal and left ventricular walls, irrespective of the technique used, with the best concordance in end-systolic septal thickness (M-mode CCC 0.956 and 2D CCC 0.914) and worse performance at the end-diastolic right free ventricular wall (M-mode CCC 0.691 and 2D CCC 0.783) (Table 3). Both techniques showed a similar interobserver reproducibility (Table 4, Figure 2). Comparison of M-mode versus 2D reproducibility showed a similar performance of both techniques, except for a significantly better intraobserver reproducibility in end-diastolic septal wall thickness by M-mode as compared to 2D-mode (Table 5, Figure 2). As presented in Figure 51, gestational age had no impact on M-mode and 2D-mode in both intraobserver and interobserver analysis (β coefficient ≈0; p > .1 for all).

Discussion

This study shows that M-mode and 2D are both highly reproducible methods for assessing fetal myocardial wall thicknesses, with slightly better performance of M-mode for end-diastolic septal wall thickness.

Regarding M-mode, our data is in agreement with previous studies reporting a good reproducibility for measuring ventricular wall thickness at end-diastole [20,30–32]. For instance, Veille et al. described a low intra and interobserver variability of left and right free-walls with M-mode, with values of ±5.1% and ±4.5%,
respectively [20]. In addition, St. John Sutton et al. reported a high interobserver correlation of 0.94 for left and right ventricular walls [30]. Regarding septal wall, Macklon et al. performed an intraclass correlation analysis in gestational diabetes, with values of 0.71 for both intra and interobserver reproducibility in fetal septal wall thickness [31]. The results from the present study also demonstrate a very good reproducibility by M-mode for both free and septal myocardial walls at end-diastole. We further demonstrate that intra and

![Figure 2. Bland–Altman plots for intra and interobserver reproducibility of left, right, and septal walls thickness with M-mode versus 2D-mode. Upper figures: M-mode versus 2D-mode for septal thickness at end-diastole with intra (left) and interobserver (right) analysis; Middle figures: M-mode versus 2D-mode for left ventricular thickness at end-diastole with intra (left) and interobserver (right) analysis; bottom figures: M-mode versus 2D-mode for right ventricular thickness at end-diastole with intra (left) and interobserver (right) analysis.](image-url)
interobserver reproducibility is also satisfactory when assessed at end-systole, which might be potentially useful for assessing myocardial thickening. In addition, both 2D and M-mode showed a better repeatability at septal and left ventricular free walls as compared to right free wall – potentially more challenging to assess due to its trabeculation or complex shape. Interestingly, a recent study of Tedesco et al. assessed the inter and intraobserver reproducibility of ventricular walls thickness based on STIC-M mode analysis. This study reported CCC for septal thickness of 0.85 (95% LoA – 0.056 to 0.044) and 0.86 (95% LoA – 0.02 to 0.03) for intra and interobserver reproducibility, similar to the performance achieved by conventional M-mode [32].

Although most studies report good reproducibility, the methodology reported to measure myocardial thickness with M-mode is variable. While many studies used only a lateral four-chamber view approach, [33,34] others also use short axis view, in cases of sub-optimal visualization of fetal heart [1]. Even more, despite that M-line is commonly placed using the open M-mode [32], some groups have pitted that M-line is commonly placed using the open M-mode [32].

Regarding 2D, we also confirm previous data reporting good inter and intraobserver reproducibility for ventricular walls with intra and interobserver variabilities of <<5–7% for both ventricles [25]. Again, the methodology used for measuring fetal myocardial walls thickness –mainly in septum – by 2D varies among studies [15,23,25,36]. While, some studies reported measurements in an apical four-chamber view at end-diastole below coapted of atrioventricular leaflets, [23] others used a lateral four chamber view [15]. In addition, the proposed place to measure the septum also varies from using atrioventricular leaflets as a reference landmark [25] to a midseptum approach [36]. In the present study, we propose a lateral four-chamber view to achieve a better identification of the chordae tendinae. Furthermore, all measurements were performed at the level of the atrioventricular valves excursion to avoid the inclusion of the valve in the wall thickness. The use of the atrioventricular valves excursion should allow us a better inter and intraobserver reproducibility, because of a well-defined reference landmark. Furthermore, based on our experience, we consider that the exclusion of chordae tendinae is very important to avoid a false overestimation of lateral walls thickness, which are difficult to identify during end-diastole, and commonly measured as part of the lateral wall. To avoid this, the use of cine-loop is useful to track the wall displacement. Another important aspect to consider is that ultrasound is characterized by a better axial than lateral resolution, which allows a good definition of close structures [24].

Despite many studies having previously evaluated the performance of M-mode and 2D, to our

| Variable                  | CCC   | Standard error | 95% Confidence interval | 95% limit of agreement | p value* |
|---------------------------|-------|----------------|-------------------------|------------------------|----------|
| Interobserver             |       |                |                         |                        |          |
| Septum (d)                | 0.809 | 0.036          | 0.738 to 0.879          | –0.986 to 1.235        | .70      |
| Septum (s)                | 0.763 | 0.041          | 0.682 to 0.845          | –1.131 to 1.758        | .56      |
| LV free wall (d)          | 0.780 | 0.040          | 0.702 to 0.857          | –0.763 to 1.261        | .25      |
| LV free wall (s)          | 0.793 | 0.037          | 0.721 to 0.855          | –0.666 to 1.377        | .13      |
| RV free wall (d)          | 0.757 | 0.045          | 0.668 to 0.846          | –1.109 to 1.138        | .23      |
| RV free wall (s)          | 0.804 | 0.037          | 0.732 to 0.877          | –0.949 to 1.286        | .30      |
| Intraobserver             |       |                |                         |                        |          |
| Septum (d)                | 0.842 | 0.043          | 0.758 to 0.925          | –0.720 to 1.146        | .017     |
| Septum (s)                | 0.810 | 0.052          | 0.708 to 0.912          | –0.991 to 1.284        | .14      |
| LV free wall (d)          | 0.784 | 0.057          | 0.673 to 0.895          | –0.773 to 1.209        | .7       |
| LV free wall (s)          | 0.839 | 0.042          | 0.756 to 0.922          | –0.579 to 1.175        | .37      |
| RV free wall (d)          | 0.769 | 0.062          | 0.648 to 0.891          | –1.134 to 1.040        | .3       |
| RV free wall (s)          | 0.821 | 0.049          | 0.725 to 0.917          | –1.017 to 1.173        | .55      |

CCC: concordance correlation coefficient between M-mode and 2D; (d): end-diastole; (s): end-systole; LV: left ventricular; RV: right ventricular.

*p value calculated by t-test comparing CCC coefficients of M-mode and 2D.
knowledge, this is the first study focused in a direct comparison of both techniques. Our study demonstrates a similar reproducibility for M-mode and 2D when assessing ventricular wall thicknesses. Interestingly, M-mode appears to have a slightly better repeatability for septal wall thickness at end-diastole. This finding could be related to a better identification of the limits of ventricular structures across the cardiac cycle when M-mode is applied.

From a clinical perspective, the assessment of ventricular walls thickness in fetal life has gained relevance since the description of antenatal cardiac remodeling as an adaptive mechanism to compensate a noxa [37]. Several antenatal insults, such as hypoxia, lack of nutrients, infection or inflammation could affect the developing heart that adapts by changing its shape and function [17,38]. For example, maternal diabetes mellitus has been widely described to associate fetal myocardial hypertrophy (particularly thickening of the basal septal wall) [31]. More recently, fetal cardiac hypertrophy has also been related to the use of the maternal antiretroviral treatment with zidovudine in HIV infected pregnancies [17]. Intrauterine growth restriction has also been associated with changes in fetal cardiac shape – more spherical ventricles – together with myocardial hypertrophy in the more severe cases [6,10].

Current guidelines for echocardiography in adult’s population proposed the use of septal end-diastolic thickness and the estimated ventricular mass (either with M-mode or 2D-mode) for assessing and quantifying the presence of cardiac hypertrophy [35]. ISUOG Guidelines for fetal heart assessment mention the use of M-mode as a good method for ventricular walls thickness, but the optimal methodology is not detailed [39]. Based on our results, we propose a well-defined technique for measuring fetal myocardial wall thickness in order to achieve an optimal performance for assessing fetal myocardial thickness. In summary, a lateral four-chamber view should be obtained for both 2D and M mode, with clear visualization of the excursion of both AV valves. For 2D approach, walls thickness should be measured at the level of the atrioventricular valves excursion, with the largest ventricular distension for end-diastole just after the atrioventricular valves closure, and the smaller volume for end-systole, just before the atrioventricular valves opening (Figure 1). For M-mode, the reference M-line should be applied perpendicular to the septum, also at the level of the atrioventricular valves excursion, and measurements considering the widest and narrowest ventricular diameter (Figure 2). In addition, we believe that both, 2D or M-mode could be used depending of the operator’s experience or preferences, although a slightly better performance could be achieved by M-mode.

The present study has several strengths and limitations that merit comment. Firstly, we used a rigorous and well-defined technique for both M and 2D mode measurements. The definition of a well-defined method, using clear anatomic references in both M-mode and 2D-mode might be optimal for developing reference ranges, and for clinical application in conditions leading to fetal cardiac remodeling. Secondly, to our knowledge, this is the first study focused in comparing the use of 2D and M-mode in the same population, both at end-diastole and end-systole, and demonstrating for the first time a slightly better performance of M-mode as compared to 2D for septal wall thickness. However, this study has limitations. We acknowledge that the use of normal healthy pregnancies could affect the external validation in fetal conditions related to cardiac affection. Future studies are warranted to confirm and validate our results in fetal or maternal pathologies.

In conclusion, our study demonstrated that both M-mode and 2D-mode are good and reproducible techniques for the assessment of fetal ventricular walls thickness, with a slightly better performance of M-mode for septal wall thickness. The choice could be based on the operator’s experience and the fetal or maternal condition.

Disclosure statement

The authors report no declarations of interest.

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