Reference Values and *Z* Scores for Pulsed-Wave Doppler and M-Mode Measurements in Fetal Echocardiography

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Background: Fetal echocardiography is now the standard approach for detailed investigations of fetal cardiac anatomy and function. Available studies proposing reference values for pulsed-wave Doppler (PWD) measurements are often focused on few parameters. Furthermore, the methodology used for validating these proposed reference values is sometimes insufficiently described, and parameters necessary to compute *Z* scores are not always available. Improved definition of reference values with adequate statistical validation is needed for proper interpretation of PWD measurements in a clinical setting. In this study, the authors propose a comprehensive set of reference values and *Z* score equations for fetal PWD and M-mode measurements with thorough assessment of *Z* score quality and validity.

Methods: Women with normal singleton pregnancies between 18 and 39 weeks of gestational age were included. A set of 57 measurements was performed, including PWD, M-mode measurements, and calculation of systolic, diastolic, and global function indices. Several parametric regressions were tested to model each measurement against gestational age. The SD was also modeled to account for heteroscedasticity. *Z* score equations were computed, and the proposed reference values were tested for residual association, residual heteroscedasticity, and departure from the normal distribution.

Results: One hundred four uncomplicated singleton pregnancies with normal fetal hearts were included. Nonlinear relationships with gestational age were found for most measurements. Parametric normalization was successful for most measurements analyzed, and it was possible to compute *Z* score equations with minimal residual association with gestational age, no residual heteroscedasticity, and no significant departure from the normal distribution.

Conclusions: The authors propose a comprehensive set of *Z* score equations for 57 fetal functional measurements, some of which do not have any published reference values. These *Z* score equations will allow echocardiographers to more accurately identify measurements that diverge from normal and thus detect earlier potential alterations in fetal heart function. (J Am Soc Echocardiogr 2016;■:■-■.)

Keywords: Fetal echocardiography, *Z* score, Reference values, Fetal heart function

Fetal echocardiography is indicated in a variety of obstetric circumstances, including family history of congenital heart disease, pregestational diabetes, fetal arrhythmias, and chromosomal anomalies.[1,2] In addition to delineating the cardiac anatomy, it allows a thorough assessment of the physiology and function of the fetal heart. As the fetus matures, blood velocities and related echocardiographic measurements change. Several authors have published reference values for various functional measurements in the fetus.[3-23] However, the available studies proposing reference values often focused on few functional parameters. Furthermore, the methodology used for the validation of those values is sometimes insufficiently described.

In this study, we aimed to determine comprehensive reference values and parametric *Z* score equations for several functional fetal heart measurements between 18 and 39 weeks of gestation. To do this, we used a standardized normalization protocol with thorough analysis of the normalized measurements for residual biases and adequacy *Z* score distribution.[24,25]

METHODS

Population

This was a cross-sectional study targeting pregnant women attending the fetal echocardiography clinic of the Sainte-Justine

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Abbreviations
GA = Gestational age
IVC = Inferior vena cava
LV = Left ventricular
PWD = Pulsed-wave Doppler

Mother and Child University Hospital Center (Montreal, Qc, Canada). Referral indications were mostly for family history of congenital heart disease, suspicion of abnormal cardiac anatomy during obstetric ultrasound screening, or suspicion of fetal arrhythmia. Between 2002 and 2008, women with singleton pregnancies from 18 to 39 weeks of gestation that met the following criteria were eligible: (1) normal fetal heart anatomy and function as determined by the attending fetal cardiologist, (2) well-positioned fetus with adequate acoustic windows, and (3) fetus in presumed sinus rhythm without documented tachyarrhythmia. Exclusion criteria were (1) chromosomal abnormalities (documented or suspected), (2) intrauterine growth restriction or macrosomia, (3) pregestational maternal diabetes or gestational diabetes with insulin treatment, (4) pregestational hypertension and pre-eclampsia, (5) oligohydramnios or polyhydramnios, (6) any extracardiac fetal anomalies, and (7) any other maternal or fetal condition that can affect hemodynamic status. Women were initially screened by the sonographer, and if they met the inclusion criteria, all images were stored for further analysis (see the following discussion). Participants' medical charts were also reviewed after delivery to double-check for inclusion and exclusion criteria. Gestational age (GA) was estimated using first-trimester ultrasound or, if no ultrasound study was available, last menstrual period. Clinical data were collected and managed using REDCap electronic data capture tools hosted at Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke.[26] This study was approved by the local institutional ethics board.

This study included only uncomplicated pregnancies, and as such, most of the deliveries occurred out of our tertiary center. Newborns delivered at other institutions who were not referred back to us for clinical suspicion of heart disease were considered normal and were not brought back for postnatal echocardiography.

Echocardiographic Measurements

Two experienced fetal sonographers acquired all anatomic and functional measurements under the supervision of two pediatric cardiologists (J.-L.B. and J.-C.F.). Acuson Sequoia echocardiographic machines equipped with appropriate transducers were used (Siemens Medical Solutions USA, Malvern, PA). Measurements were made off-line on the source images directly on the echocardiographic machine by only one experienced sonographer under the supervision of a pediatric cardiologist (J.-L.B.). Each parameter was measured and averaged over three consecutive cardiac cycles. Thirty-nine pulsed-wave Doppler (PWD) parameters (29 velocities and 10 calculated measurements) and 18 M-mode parameters (14 measurements and four calculated values) were acquired.

A complete list of measurements and calculation for PWD-derived parameters is presented in Table 1. Figures 1 and 2 show diagrams of image planes and cursor placement. Images were adjusted to have the measured structure as perpendicular to the ultrasound beams as possible. All PWD flow recordings were obtained with an insonation angle of $<30^\circ$ between the cursor and the direction of blood flow. Angle correction was not used. PWD interrogations of the tricuspid, mitral, pulmonary, and aortic valves were obtained. We also obtained Doppler flow measurements from the ductus arteriosus, aortic isthmus, ductus venosus, and inferior vena cava (IVC). From these Doppler flow interrogations, we measured peak velocities, velocity-time integral, pulsatility indices, and myocardial performance index. We also calculated aortic, main pulmonary artery, and ductal flow using the velocity-time integral and the diameter of the corresponding structure. Whenever possible, we avoided sampling Doppler velocities during periods of fetal breathing. Fused inflow E and A waves were excluded from the analysis.

M-mode measurements included the right ventricular anterior wall, left ventricular (LV) posterior walls, ventricular chambers, and interventricular septum in diastole and in systole. These measurements were obtained from two different views: short-axis as well as four chamber views. Right ventricular and LV fractional shortening were calculated in both short-axis and four-chamber views [(difference of end-diastolic and end-systolic dimension) divided by end-diastolic dimension, expressed in percentage]. Two-dimensional measurements of the pulmonary valve and the aortic valve were also acquired to compute cardiac outputs.

Parametric Normalization and *Z* Scores

Z scores were computed using a previously described standardized approach.[24,25] Each echocardiographic measurement was normalized for GA. Four regression models were empirically tested: linear ($y = ax + b$), allometric ($y = ax^b$), second-order polynomial ($y = ax^2 + bx + c$), and third-order polynomial ($y = ax^3 + bx^2 + cx + d$). Polynomial models were considered only when more simple models did not achieve adequate fit. Selection of the final model was based on visual inspection of the normalized residual values and review of fit diagnostic aids (plots of residuals over predicted values, plots of residuals over quantiles, and "proportion-less" plots). Fit plots of the normalized residual values over GA by linear and polynomial regression were reviewed to detect potential residual association. Mathematical transformation of the echocardiographic parameters was considered only if the distribution of the normalized residual values suggested significant departure from the normal distribution. The model retained for each echocardiographic measurement was used to calculate the measurement's predicted mean.

Preliminary analysis showed that heteroscedasticity was present (the SD of the normalized residual was not constant across the range of GA). Modelization of the SD against GA was performed as previously described.[24,27] Briefly, we estimated the rate of increasing SD using linear regression of the absolute value of the normalized residual values. Predicted SD was calculated by multiplying the predicted absolute values of the SD mean by $\sqrt{(2/\pi)}$ (the mean of a half standard normal distribution). *Z* scores were then calculated as follows:

$$Z\ \text{score} = \frac{\text{Observed value} - \text{Predicted mean}}{\text{Predicted SD}}$$

Standard equations for predicted means and *Z* scores according to the regression models are listed in Table 2. The validity of all *Z* scores was thoroughly assessed, and a detailed description of *Z* score assessments is provided in the Appendix.

Statistical Analysis

We used SAS for Windows version 9.3 for all analyses (SAS Institute, Cary, NC). We used PROC NLIN for allometric models and PROC GENMOD for all other models. Mean *Z* scores were compared using Student's *t* test. The *t* statistic was used to estimate *P* values for residual association. Departure from a normal distribution