Defining the relationship between fetal Doppler indices, abdominal circumference and growth rate in severe fetal growth restriction using functional linear discriminant analysis

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The relationship between Doppler measurements, size and growth rate in fetal growth restriction has not been defined. We used functional linear discriminant analysis (FLDA) to investigate these parameters taking account of the difficulties inherent in exploring relationships between repeated observations from a small number of cases. In 40 fetuses with severe growth restriction, serial abdominal circumference (AC), umbilical, middle cerebral artery (MCA) and ductus venosus Doppler pulsatility index measurements were recorded. In 11 singleton fetuses with normal growth, umbilical artery pulsatility index only was measured. Data were expressed as $z$-scores in relation to gestation and analysed longitudinally using FLDA. In severe growth restriction, the Spearman correlation coefficients between umbilical artery pulsatility index and AC $z$-score, MCA pulsatility index and AC $z$-score and ductus venosus pulsatility index $z$-score and AC $z$-score were, respectively: $0.36, p = 4.4 \times 10^{-2}$; $0.70, p = 1.1 \times 10^{-17}$ and $-0.50, p = 8.1 \times 10^{-4}$. No relationship was seen between Doppler parameters and growth rate. There was no relationship between umbilical artery pulsatility index and AC nor growth rate in normally grown fetuses. In severe fetal growth restriction, Doppler changes are related to absolute fetal AC size, not growth rate.

1. Introduction

Fetal growth restriction is most commonly secondary to utero-placental insufficiency, which leads in a stepwise, though not always predictable, manner to fetal hypoxia, acidemia and, if delivery does not take place, intrauterine demise [1]. Doppler blood flow velocimetry allows non-invasive assessment of the vascular impedance in the fetal circulation thereby inferring the degree of fetal hypoxia [2,3]. Fetuses exhibiting absent or reversed fetal end-diastolic frequencies in the umbilical artery have reduced placental vascularization and cross-sectional diameter of the terminal vili compared with unaffected pregnancies [4]. Meta-analysis of Doppler ultrasonography of the umbilical artery in high-risk pregnancies revealed a significant reduction in the number of antenatal admissions, induction of labour, Caesarean sections for fetal distress and perinatal deaths in the Doppler-monitored group [5].

Fetal hypoxia as a result of placental insufficiency results in compensatory haemodynamic changes [6,7] with blood flow redirected from peripheral circulations towards essential vascular beds, such as those perfusing the brain [8,9]. The
redistribution of organ blood flow during fetal hypoxia can be measured directly in experimental preparations, such as fetal lambs, either via injected labelled microspheres [10] or implanted flow probes in the carotid and femoral circulations [11].

Abnormalities in Doppler flow velocimetry affect different vessels in a sequential manner with a reduction in middle cerebral artery (MCA) impedance followed by increase in umbilical artery impedance and the development of absent end diastolic flow. Later changes include reverse umbilical artery flow and ductus venosus flow abnormalities [12,13]. The relationship between changes in Doppler indices and progressive stepwise fetal deterioration has been described [14,15] and led to attempts at categorizing the severity of fetal growth restriction [15,16].

Although these Doppler changes are well described, the relationship between Doppler changes and fetal size and growth rate is not known. This type of analysis is difficult to achieve for several reasons. First, few pregnancies will have longitudinal Doppler and growth measurements at similar gestational ages to allow direct comparison and analysis between cases. Second, as Doppler impedance indices change with advancing gestational age, the relationship between abnormalities of Doppler indices and the severity of fetal growth restriction is likely to be gestational age dependent. Finally, most statistical techniques will be biased by the undue weighting in those cases where there are multiple observations.

Functional linear discriminant analysis (FLDA) is an established technique in engineering [17]. FLDA has only recently been applied to the statistical analysis of longitudinal biomedical datasets in the context of early pregnancy growth [18,19] and the relationship between umbilical artery Doppler and fetal size in gastrochisis [20]. FLDA maximizes the ratio of the between-class relative to the within-class variation and is useful where only fragments of the curves are observed. The technique is therefore ideal for Doppler measurements, which are repeated during pregnancy at varying visit times from patient to patient. This study used FLDA to analyse longitudinal data, adjusted to remove the effect of gestational age, to investigate the relationship between fetal arterial and venous Doppler impedance with fetal size and fetal growth rate in human pregnancy complicated with severe early onset fetal growth restriction.

2. Material and methods

Between January 2003 and December 2010, pregnancies with severe early onset fetal growth restriction were examined at the time of referral to a specialist fetal medicine unit. Data were collected prospectively on those with an estimated fetal weight less than 500 g (hence generally considered below a 'viable' weight for delivery) at 24 weeks gestation and umbilical artery PI > 95th percentile. In none was there confirmed or suspected genetic, chromosomal or infective aetiology. All had gestational age assigned from a crown–rump length measurement performed at 10–14 weeks.

Forty cases comprising 200 scans were included in the study. The data were recorded on a separate database with restricted access on the hospital server, and all anonymized prior to analysis. Serial umbilical artery and MCA Doppler and abdominal circumference (AC) measurements were performed and recorded on a fetal medicine database (Astraia GmBH, Munich, Germany) as part of routine clinical analysis.

Eleven women undergoing at least two ultrasound scans after 24 weeks whose pregnancies were considered healthy (fetal AC was greater than 10th percentile but less than 90th for gestation) and where umbilical artery Doppler PI was recorded were randomly chosen and added to the database for analysis.

The Research Ethics Committee advised that analysis and publication of routinely collected, fully anonymized obstetric growth measurements entered into an ultrasound software system did not require approval.

3. Data and statistical analysis

AC and the change of AC with time between observations (the first differential of AC with time: dAC/dt) were considered with respect to the Doppler indices: umbilical artery pulsatility index (UA-PI), MCA-PI and ductus venosus pulsatility index (DV-PI) in the cases of severe fetal growth restriction. In the case of the normally grown fetuses, the Doppler data were limited to umbilical artery PI, as MCA and DV-PI were not routinely collected without another clinical indication. We transformed all Doppler PI data to z-scores for a given gestation, defined as the difference between the observed and expected value expressed in standard deviations.

For the Doppler indices, scatter plots were superimposed on reference charts [21], and expressed as the mean, fifth and 95th percentile. z-Scores were then calculated with respect to the expected Doppler values in normal pregnancies after log- or square-transformation of the indices. The change in AC over time (dAC/dt) was calculated as the difference in AC between two consecutive scans divided by the number of days between these two scans. In the analyses, the calculated dAC/dt values were used as data points available at the gestational age in the middle between the two time points at which the two consecutive scans were performed. For both AC and dAC/dt, z-scores were calculated with respect to their expected size for gestation in normal pregnancies derived from the AC charts [22].

To derive the relationships between size and the Doppler indices in severe growth-restricted fetuses, we first applied a cross-sectional approach on the corresponding z-scores. For the majority of women, data from multiple scans were available. All data, neglecting possible co-dependencies between data obtained from the same woman at multiple time points during pregnancy, were included.

To correct for co-dependency, a similar cross-sectional approach was followed, but in which data from only one randomly chosen scan per woman were included for the calculation of the correlation coefficients. This procedure was repeated a sufficient number of times, each time with a different combination of data points randomly selected from the subjects. The disadvantage of this approach is data loss. To be able to include all available data points while controlling for co-dependency between scans of the same subject, FLDA was applied to the dataset. This enabled calculation of a longitudinal curve for each considered relationship between Doppler pulsatility index and size [17].

As the FLDA technique is sensitive to the most extreme points on the x-axis (i.e. z-score AC or z-score dAC/dt), the portion of the FLDA curves that is most reliable was first defined using the concept of robust bivariate boxplots [23]. The 50% convex hull, defined as the largest hull containing not more than 50% of the data and which can be considered as a two-dimensional extension of the interquartile range of the univariate boxplot, was determined. Subsequently, a robust bivariate centroid was derived from the inner 50% of
the data. Finally, a cut-off at each end of the FLDA curves was determined, encompassing 95% of the data points.

Each FLDA-derived longitudinal curve within the 95% range was converted into an ‘FLDA coefficient’, defined as a correlation coefficient derived from the individual FLDA curves of the subjects. First, applying the FLDA, spline curves were fitted through all data points belonging to each individual subject. The median number of data points per subject was calculated and subsequently data points were randomly selected from each individual curve, resulting in times data points with the number of subjects in the dataset. Finally, the Spearman correlation coefficient was calculated for these data points. The random selection of data points from each curve was repeated 1000 times due to the variance on the correlation coefficient when different data points along the curves are chosen. The median correlation coefficients and -values are reported.

4. Results

The untransformed original data points for all cases are shown in figure 1. Fetal Doppler PI indices and AC values were transformed to z-scores by normalizing for gestation, with a z-score of 0 representing the mean value. The mean and median z-scores for AC were \(-2.56\) and \(-2.52\); \(d(AC)/dt\) \(-3.99\) and \(-3.1\); umbilical PI 2.32 and 2.46, and MCA-PI \(-2.28\) and \(-2.38\), respectively. The z-scores of UA-PI and MCA-PI were then plotted against the z-scores of AC and \(d(AC)/dt\) (figure 2).

The number of scans included were for investigating the relationships between umbilical PI, MCA-PI and DV-PI in relation to AC were respectively 180, 122 and 55. For umbilical PI, MCA-PI and DV in relation to \(d(AC)/dt\) were 187, 127 and 57, respectively. There were slightly more scans for the latter group as some scans were not accompanied by biometry data, where this was within a week of the previous scan; however, \(d(AC)/dt\) could be calculated from the AC before and the AC after this Doppler measurement.

The relationships between Doppler indices and AC and change in AC over time expressed as z-scores were then analysed using FLDA. For each relationship, the individual FLDA curves were converted to an FLDA coefficient (figure 3a–c).

For the 11 cases where there was normal fetal growth (AC greater than 10th and less than 90th percentile), there was no relationship between umbilical artery PI z-score and

Figure 1. (a–d) Scatter plots of measured parameters versus gestational age. (a) Abdominal circumference versus gestational age (circles represent normally grown fetuses; crosses represent growth restricted fetuses). The reference chart [22] is represented by its mean (solid line) and fifth and 95th percentile (dashed lines). (b) Umbilical artery pulsatility index versus gestational age (circles represent normally grown fetuses; crosses represent growth restricted fetuses). The reference chart [21] is represented by its mean (solid line) and fifth and 95th percentile (dashed lines). (c) Middle cerebral artery pulsatility index versus gestational age for growth restricted fetuses. The reference chart [21] is represented by its mean (solid line) and fifth and 95th percentile (dashed lines). (d) Ductus venosus pulsatility index versus gestational age for growth restricted fetuses. The reference chart [21] is represented by its mean (solid line) and fifth and 95th percentile (dashed lines). (Online version in colour.)
either $z$-score AC or $z$-score $d(AC)/dt$. In severe growth restriction, a significant relationship was found, expressed as $z$-scores, between UA-PI and AC (correlation coefficient $-0.36; p$-value $4.4 \times 10^{-7}$), MCA-PI and AC (correlation coefficient $0.70; p$-value $1.1 \times 10^{-17}$) and DV-PI and AC (correlation coefficient $-0.50; p$-value $8.1 \times 10^{-4}$).

The relationships between umbilical artery, MCA and ductus venosus PI $z$-scores and $d(AC)/dt$ $z$-scores were not significant (respectively: correlation coefficient $-0.03; p$-value 0.467; correlation coefficient 0.21; $p$-value 0.086; correlation coefficient 0.34; $p$-value 0.150).

5. Discussion
In fetal growth restriction, Doppler impedance is related to the absolute fetal abdominal size deviation from normal, not its growth rate. There is a strong and highly significant
negative correlation between the \( z \)-score of umbilical artery and \( z \)-score AC; similarly for the \( z \)-score of the ductus venosus PI and AC, and a positive correlation between \( z \)-score of the MCA pulsatility index with \( z \)-score AC. There is no correlation with slowing fetal abdominal growth rate, namely the first differential with respect to time of AC (\( z \)-score \( \frac{d(AC)}{dt} \)), and any of the Doppler parameters studied. In the normally grown babies, no relationship exists between umbilical artery Doppler impedance and \( z \)-score AC or AC growth rate.

The relationships described are biologically plausible in that an increase in umbilical and ductus venosus PI is seen in growth-restricted fetuses [12,13]. The converse is true for MCA-PI; the fetal brain responds to hypoxia by reducing cerebral arterial impedance: a phenomenon known as ‘brain sparing’ or ‘cerebral redistribution’ [8–11].

Previously, birth weight \( z \)-scores have been shown to be the strongest predictor of adverse neonatal outcomes in pregnancies complicated by severe placental insufficiency [24]. Although previous studies have attempted to classify growth-restricted fetuses in relation to different Doppler patterns [15,16], delivery timing is primarily driven by abnormal fetal heart rate (cardiotocography) recordings in combination with fetal Doppler abnormalities and fetal biometric parameters [25].

Once fetal growth restriction is diagnosed, obstetric management is pivoted between preterm delivery with its known sequelae, or allowing fetal intrauterine maturation and risk of demise in a potentially hypoxic environment. While it is recognized that the severely growth-restricted fetus less than 30 weeks of gestation has a significant reduction in perinatal mortality for each week it remains in utero, striking the balance between delivery and surveillance poses a critical obstetric challenge as iatrogenic preterm delivery is associated with neurodevelopmental delay and cerebral palsy [26].

An advance in designing clinical protocols for both monitoring and delivery timing would be to define mathematical relationships between arterial and venous Doppler impedance changes with fetal size and growth rate in fetal growth restriction. These changes, though inferred, have not previously been characterized. A barrier to doing this was that the Doppler changes occur at different gestational ages, and Doppler ranges differ with gestation. By normalizing all data for gestation using \( z \)-scores and using all data points as is possible with FLDA, the problems of this type of analysis are to a large extent overcome.

It is important for this analysis not to disregard data points for all given ultrasound examinations but at the same time to acknowledge that for a given case they are not independent. Disregarding data points and choosing, for example, the results of the first or last examination per fetus has previously been performed in the assessment of fetal growth and Doppler indices to remove the bias of multiple data points for a given case or cases [12,21]. However, this practice is wasteful of data and weakens statistical power. An alternative approach, namely to assume that data points from multiple examinations of the same fetus [27] are independent is methodologically flawed. This problem of co-dependence is overcome by using FLDA. This technique treats each subject’s longitudinal measurements as a separate curve; it is these curves that are then combined preventing bias from single cases, for example, with multiple observed points.

A potential criticism of this study is that it refers to a highly selected group of severely growth-restricted fetuses and the relationships that we describe may therefore not be generalizable to a population affected with less severe growth restriction. This was necessary and intentional in the study design to determine the ‘proof of principle’ of whether a mathematical relationship existed between size, growth rate and Doppler changes. Such an analysis depends on describing the relationship between values for AC size, AC growth rate and Doppler changes that are most deviated from normal. Further, given our aim to describe the principle of Doppler relationships with fetal abdominal size and growth rate, and the highly selected fetuses studied, we have not attempted to correlate clinical outcome with Doppler findings. Several well powered observational studies describe perinatal outcome in relation to fetal Doppler abnormalities [28].

Further, the number of fetuses with normal growth that we present is small. These are not controls, but were selected to investigate whether there was any relationship between umbilical PI, AC size and growth where fetal growth is within the normal range in a healthy pregnancy. For this reason, only umbilical artery Doppler PI data were available as MCA and DV-PI were not routinely measured unless another clinical indication was present, which would then have led to their exclusion.

The strength of this study is that we report a relatively complete Doppler and growth dataset of a highly defined group of severely growth-restricted babies, each with a median of four scans per case. The rarity of the severity of growth restriction considered necessitates data collection over a relatively long timescale, however, in this period, the protocol for ultrasound surveillance did not change in the unit.

The data are best interpreted as establishing that a relationship between fetal arterial and venous Doppler impedance and absolute deviation in fetal size as measured by AC but not growth rate exists in potentially hypoxic fetuses. It is somewhat reassuring in this context that most clinical protocols for intensive fetal monitoring are based on an ultrasound assessment of fetal size deviation (e.g. third or fifth percentile for AC or estimated fetal weight) rather than on reduced growth velocity per se. Novel as these findings are in themselves, they primarily describe fetal pathophysiological responses in severe growth restriction. Any clinical monitoring protocol would need to be validated with respect to outcome without automatic extrapolation from this population of fetuses. Nevertheless, this study could represent a useful reference point for further investigation to determine when and how to follow up in this population of fetuses that is particularly vulnerable to morbidity and mortality prior to and after delivery.

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