Developing Novel Tests to Screen for Fetal Growth Restriction

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Abstract
Fetal growth restriction (FGR) is a major determinant of global morbidity and mortality. There is an unmet need for methods to stratify the pregnant population on the basis of FGR risk. Despite evolutionary divergence in mammalian reproduction, studies of genetically modified mice have identified biomarkers which have been validated in women, and a systematic screen for genes which control fetal growth in animals could help identify novel clinical biomarkers. Current approaches to biomarker identification using human samples include both targeted and discovery approaches (omics). Application of omic methods to the placenta and maternal blood has yielded promising results, but comes with logistical, experimental and analytical challenges, and all studies are limited by the lack of a gold standard for disease.
Fetal Growth and Public Health

Fetal growth restriction (FGR), broadly defined as failure of the fetus to achieve its genetically determined growth potential, is a major determinant of the global burden of morbidity and mortality. It is a major cause of stillbirth and, through its association with spontaneous and medically indicated preterm birth, neonatal death[1]. A large body of work in animals indicates that impairment of fetal growth leads to life-long predisposition to important determinants of later ill health[2], including cardiovascular and metabolic abnormalities. This research was stimulated by epidemiological associations between low birth weight (LBW) and the risk of diverse conditions of adult life, including ischemic heart disease, stroke, hypertension and type 2 diabetes[3]. Common determinants of FGR and lifelong ill health are also evidenced by the association between FGR and the later risk of disease in the mother of the FGR infant[4], and even the grandparents of the child[5]. Given the above, one might expect that the clinical approach to screening for FGR and interventions to prevent its diverse detrimental associations might be highly sophisticated. However, in the UK and USA, population based screening is focused on measuring the size of the mother’s “bump” with a tape measure and the primary disease modifying intervention remains early delivery of the baby, which can itself be associated with mortality or lifelong morbidity[6]. The aims of this review are to identify the factors impeding development of enhanced methods of screening for FGR and to propose key elements in the approach to future research.

Defining the Outcome

A significant challenge in the study of FGR is that the condition has no gold standard, i.e. as the genetically determined growth potential of the fetus is unknown, it is impossible unambiguously to classify all pregnancies in terms of having achieved or not achieved that potential [6]. In effect, all diagnostic criteria employed are proxies of a condition that is impossible to define (Figure 1 and Table 1). The birth weight of the baby is one of the fundamental diagnostic criteria for FGR. The size is, as a minimum, related to the gestational age and quantified with reference to the distribution of birth
weights at that stage of pregnancy. However, even this introduces complexity. Relative weight is expressed as a centile for gestational age and small for gestational age (SGA) is usually defined as <10th percentile. Setting any diagnostic threshold on a continuous scale will result in misclassification of affected and unaffected individuals. For example, a baby might have birth weight within the “normal” range (10th to 90th) but have been affected by FGR as the true – and unknown – genetically determined centile was the 95th. Other complexities include whether the reference distribution should be from a random sample of pregnant women or only of healthy women[7], and whether the centile should be adjusted for parental characteristics[8], issues which are discussed in detail elsewhere[6]. Furthermore, infants born at a given week of gestation are a selected sample of all infants that were alive and in utero at the start of that week. Infants born at, for example, 28 weeks of gestational age (wkGA) are, by definition, pathological. Obstetricians assess estimated fetal weights (EFW) at 28wkGA using the distribution of all EFW in the whole population, delivered and undelivered. However, paediatricians quantify the percentile of birth weight at 28wkGA by comparing a given value with the distribution of all observed birth weights at 28wkGA, i.e. defining normal on the basis of a distribution derived from a pathological group.

The definition of FGR may also be supported by associated complications, such as stillbirth or preterm preeclampsia. Antenatal imaging can also be used to support the diagnosis. In FGR the fetus often becomes progressively smaller with advancing gestational age. Serial measurements can be used to quantify “growth velocity” and this feature is the most discriminating ultrasonic predictor of morbidity in SGA fetuses near term[9]. Doppler flow velocimetry of the utero-placental and fetal circulations is also employed in classification. However, as imaging is used as a diagnostic test, there is the potential for circular arguments if it is also used to define the outcome. As placental dysfunction is thought to have a key role in the aetiology of many cases of FGR it might be anticipated that placental histopathological examination might be used as a gold standard. However, many cases of very well
defined FGR lack histopathological abnormalities in the placenta and a significant proportion of completely healthy pregnancies exhibit placental abnormalities[10].

Although these points might seem rather technical, the use of poorly performing proxies for FGR is one of the major obstacles to progress. Equating SGA with FGR is widespread because it is convenient but, as most SGA infants are constitutionally small, these studies are biased to generate null associations with markers of pathology because most of the “cases” are healthy. Employing better performing and more sophisticated proxies for FGR requires detailed assessment which will usually involve a prospective study[11]. This is problematic as the relative rarity of true FGR means a large sample size is required and large prospective studies with detailed assessment are expensive. One potential solution for this problem is to use stricter percentile thresholds. A much smaller proportion of severe SGA infants (<3rd percentile) are healthy. Although, by definition, rarer than SGA it retains the attraction of being easy to define. However, large scale, prospective studies, encompassing diverse populations across different healthcare systems will be required to overcome the key obstacles to better understanding of the causes, diagnosis and care for pregnancies complicated by FGR (See Outstanding Questions).

**Identifying Novel Predictors**

**Studying Maternal Blood**

The most obvious approach to identifying novel biomarkers for FGR is to analyse maternal blood samples. Many studies have reported associations with candidate biomarkers. However, at present, there are none which are currently clinically recommended consistently across international guidelines[6]. The lack of tests may reflect weaknesses in the evidence base. Many studies of biomarkers for FGR were secondary analyses of assays performed to address other research questions, in particular developing screening tests for Down’s syndrome, such as pregnancy associated plasma protein A[12]. Other studies have reported targeted assessment of known
candidates[13]. However, the pathophysiology of FGR is incompletely understood. It is impossible to assess the predictive utility of the key elements of a pathway involved in the pathogenesis of FGR when these pathways are incompletely characterised.

One approach is to use omic methods (Figure 2), which can be applied to both tissue and blood samples. As these methods assess potential markers across multiple domains of biological activity (e.g. endocrine, inflammatory, angiogenic) they are an effective way of trying to identify new tests in a research area where underlying disease mechanisms are unknown. However, omic methods present multiple challenges. First, they are frequently expensive and this limits the total number of cases and controls that can be studied. Second, they typically report hundreds, thousands or tens of thousands of observations on each sample. Very large numbers of observations on small numbers of cases and controls leads to the problem of false discovery. An important statistical element of this is to use methods which take into account the number of hypothesis tests. A consequence of this is that these methods may only be able to detect very strong and consistent associations, because these qualities would result in a P value sufficiently low to be robust to methods of false discovery correction. In the absence of individual clearly validated signals researchers frequently apply high dimensional modelling methods to these datasets, such as partial least squares discriminant analysis (PLS-DA). However, multivariable statistical models are prone to over-fitting in contexts of small numbers of cases and controls and large numbers of potential predictors[14].

The problem of false discovery can be addressed in the experimental design, by performing multiple temporally separated assays. If a measurement made on a single sample yields an extreme result through the play of chance, the next measurement will usually be closer to the average through the statistical phenomenon of regression to the mean. However, in the case of real signals, these are frequently observed to become progressively stronger in the interval preceding the clinical manifestation of disease (Figure 3). A metabolomic analyses of serial blood samples obtained at 12,
20, 28 and 36 weeks of gestational age identified four potentially novel metabolite predictors of FGR[15]. The statistical approach to selecting candidates was to use a mixed linear model fitted to the first three values to generate a P value which was lower if markers were consistently different across all three measurements. All four associations were stronger at 36 weeks than at the earlier weeks of gestation, the opposite of what would be predicted by regression to the mean and consistent with true signals. The success of the approach was confirmed by external validation in a demographically dissimilar cohort[15]. Biomarkers can also be identified by studying samples obtained at the time of disease presentation. This approach was used to identify a pattern of changes in maternal RNA-seq profile which was strongly associated with preterm, severe preeclampsia [16].

*Studying the Placenta*

The placenta is thought to play a key, if only partially understood, role in the pathogenesis of FGR[1]. Hence, one approach to the identification of novel predictors of FGR is to study the placenta from FGR cases and controls to determine whether placental associations with FGR might identify likely candidate biomarkers. Preeclampsia is an exemplar of this approach. Expression microarray studies of the placenta indicated that cases of preeclampsia had higher levels of mRNA encoding the soluble fms-like tyrosine kinase receptor-1 (sFlt-1)[17]. The placental levels of the mRNA were reflected by higher levels of sFlt-1 protein in the blood of women both prior to preeclampsia and when presenting with symptoms or signs of the disease. Assay of sFlt-1 is now an element of ruling out the disease in women in whom it is clinically suspected and this has been implemented into clinical guidelines in the UK. The sFlt-1:placental growth factor (PIGF) ratio has also been found to be highly predictive for FGR complicated by preeclampsia, both term and preterm[18]. More recently, maternal serum levels of follistatin-like 3 protein (FSTL3) were found to be predictive of FGR, with a 4-fold risk of FGR among women with values >97th percentile, and this candidate was identified through placental RNA-seq of FGR cases and matched controls [19].
Animal Studies

Candidate biomarkers for FGR may also be identified by animal studies. For example, a series of experiments on genetically manipulated mice indicated that the source of the massive rise in maternal circulating concentrations of delta-like homolog 1 (DLK1) that occurs in pregnancy is the fetus, not the placenta or the mother[20]. The mouse studies demonstrated a relationship between fetal size and maternal DLK1 levels. Remarkably, in human pregnancy, maternal DLK1 levels were also associated with SGA birth weight. Moreover, when SGA infants were phenotyped on the basis of serial ultrasonography, the association was specific for FGR and was particularly strong for cases of FGR associated with high resistance patterns of umbilical arterial blood flow[20]. Systematic studies of null mutant mice have demonstrated associations between a wide range of null mutants and defects of placentation [21]. Analysis of the different placental phenotypes associated with poor growth has indicated a number of patterns, specifically, reduced placental size, reduced complexity of the fetal vascular tree, increased barrier to diffusion, increased development of acquired lesions (e.g. fibrosis) and abnormalities of differentiation, and many of these have parallels in the human [22]. A systematic search for key pathways involved in controlling fetal growth in genetically modified mice could help identify candidate biomarkers for human FGR.

Mechanisms Underlying FGR

Mechanistic understanding of many cases of FGR is still limited. Given the major differences in reproductive biology between rodents and humans and the problems with performing experimental studies in pregnant women, this is likely to continue to be a problem. However, genome wide association studies (GWAS) have provided some important insights into the control of birth weight. These studies involve disentangling indirect effects of the maternal genotype which influence fetal growth through an altered intra-uterine environment and direct effects of the maternal genotype which influence fetal growth by fetal inheritance of growth-controlling genes [23]. Genetic variation in insulin sensitivity is an example of this. Insulin resistance in the mother tends to promote increased
birth weight through maternal hyperglycaemia whereas insulin resistance in the fetus tends to be associated with reduced birth weight through decreased promotion of fetal growth through insulin receptor signalling [24]. However, these issues relate more to physiological variation in growth and it has been observed that there is a dissociation between common weight determining genes and birth weight in FGR, presumably reflecting FGR-associated attenuation of the genetically determined growth potential [25]. Interestingly, GWAS of maternal and offspring pairs have suggested that the association between birth weight and the risk of disease in later life in both the mother and child is likely to be explained by common genetic determinants of birth weight and cardio-metabolic disease [26]. Epidemiological studies had yielded the same conclusion, based on the relationship between birth weight and both maternal and grandparental risk of ischaemic heart disease [5]. Genetic determinants of fetal growth will interact with environmental factors, such as pollutants and psychosocial stress[27, 28] and studying these interactions is likely to be a major area for future research.

Deep sequencing may help yield insights into the mechanisms that prevent the fetus achieving its genetically determined growth potential. DNA-seq applied to whole placental biopsies demonstrated that the genomic landscape of the placenta is unlike any other healthy human tissue studied, being more akin to a cancer; biopsies were clonal expansion from a single cell, had high frequencies of somatic mutations, and frequently exhibited copy number variations[29]. Analysis of the syncytiotrophoblast obtained using laser capture microdissection (LCM) demonstrated that this quality was explained by the trophoblast, not the fetal component of the placental villi. Metagenomic analysis of human placental DNA-seq demonstrated strong evidence that Streptococcus agalactiae (Group B streptococcus, GBS) invades the placenta prior to the onset of labour and this was more frequently observed in severely SGA infants[30]. Metagenomic analysis of human placental RNA-seq demonstrated that placentas from women with preeclampsia were more likely to have expression of genes encoded by human herpes virus 6 (HHV6)[31]. Further studies confirmed that fetal inheritance
of chromosomally incorporated HHV6 from the mother or father resulted in mRNA expression in the placenta and an increased risk of preeclampsia.

**Cervical Cancer as an Exemplar for Screening and Prevention**

Cervical cancer is an interesting exemplar for the progression from screening to prevention in Women’s Health in the 20th century. The association between cervical cytology and the risk of cervical cancer led to screening using the PAP smear. This programme saved thousands of women’s lives before the mechanism of cervical cancer development, namely, human papilloma virus (HPV) infection, was identified[32]. Determining the mechanistic link provided the route to preventing the disease at its origin through mass HPV vaccination. Key lessons from this example are, first, that screening and intervention prevented deaths prior to mechanistic understanding of disease and, second, that the first clues to mechanism arose from careful analysis of human clinical samples (see for example [33]).

**Intervention**

*Fetal Monitoring and Delivery*

Screening is only clinically and economically justified when there is an intervention which mitigates the risk in women who screen positive. Ultimately, the primary disease modifying intervention in FGR is to deliver the fetus before the spontaneous onset of labour to prevent intra-uterine fetal death (IUFD). A diagnosis of FGR would also influence the use of fetal monitoring to inform the timing of this intervention, i.e. a screening programme could consist of identifying pregnancies at high risk of FGR and targeting them with enhanced monitoring with the aim of optimising early delivery[6]. Moreover, FGR fetuses are also at increased risk of intrapartum asphyxia and diagnosis of FGR can be used to inform enhanced fetal assessment during labour and, in extreme cases, the need for pre-labour caesarean.
False Positives

While the relatively crude nature of the intervention may dampen enthusiasm for screening for FGR, this should be seen in the context of the current approach. An increasingly large number of women have early delivery to reduce the risk of stillbirth, but the ratio of induced early deliveries to stillbirths prevented is high [34]. Hence, the use of poorly performing methods of screening labels large numbers of women as high risk who would have had a normal outcome in the absence of intervention. In obstetric care, false positives have multiple adverse effects, including creating unnecessary anxiety, additional costs, and neonatal and long term morbidity due to unnecessary preterm term or early term delivery[34, 35]. Hence, development of better screening tests could also potentially have benefits through ruling out disease, thereby preventing medicalisation of healthy women and targeting resources to the women at highest risk of complications. A key statistic in screening studies is the positive predictive value (PPV), as it indicates the ratio of true to false positives. Choosing a threshold of PPV will depend on the clinical context. At extreme preterm gestational ages very high PPVs may be required (>80%) given the potential for severe iatrogenic harm through medically indicated preterm birth. In contrast, when screening near term, induction of labour is more benign and intervention may be considered appropriate with much lower PPVs, e.g. 20-30%.

Therapeutics

Ideally, research might lead to mechanistic understanding which would yield disease-modifying therapies other than delivery. One approach is to use in vitro models to assess the effects of currently licensed drugs on, for example, placental pathways which may be involved in the pathogenesis of the disease. One issue that commonly affects these studies is the potential for “off target” effects with supra-therapeutic concentrations of drugs, i.e. a drug shows a potentially attractive property in an in vitro system but the effect is achieved at concentrations which are well in excess of what can be achieved or tolerated in clinical practice. Moreover, the experience with novel approaches to therapeutics in pregnancy would tend to lead to a cautious approach. A large body of work indicated
that oxidative stress was associated with the pathogenesis of preeclampsia. However, a carefully conducted double blind placebo controlled trial demonstrated that antioxidant therapy in the form of vitamin C and E had no effect on the risk of preeclampsia but was associated with an increased risk of stillbirth and low birth weight[36]. This side effect may be explained by the ubiquitous role of reactive oxygen species in cell signalling[37]. A similarly large body of evidence indicated a key role for nitric oxide in the control of placental vascular smooth muscle relaxation and animal studies suggested that FGR might be treated by sildenafil citrate, which amplifies the effect of nitric oxide through inhibiting breakdown of cyclic guanosine monophosphate phosphodiesterase type 5 [38]. However, a carefully conducted double blind placebo controlled trial demonstrated no beneficial effect of sildenafil on outcome in early onset severe FGR [39]. Moreover, another trial demonstrated increased rates of neonatal death in the sildenafil group caused by persistent pulmonary hypertension [40], most likely related to the important role for nitric oxide in mediating pulmonary vasodilation in the transition from the fetal to neonatal circulation[41]. Finally, two of the major disasters for Pharma in the 20th century involved unanticipated problems of novel therapeutics in pregnancy, thalidomide and diethylstilboestrol (DES). The DES case is of particular concern in relation to developing novel therapeutics, as the adverse effects of the drug were often manifested many decades after exposure of the infant and there were also adverse effects on the next generation [42], i.e. the grandchildren of the woman who took the drug in pregnancy. The same lifelong importance of FGR in determining health raises the concern that adverse effects of trying to enhance fetal growth could manifest themselves decades after treatment and could even result in transgenerational adverse effects. Hence, the role for novel therapeutics is, for the moment, confined to cases where the short term outcome is so bleak that the potential for long term harm could be regarded as justifiable [43].

**Concluding Remarks**

Better methods of stratifying women for their risk of FGR and for ruling the condition in or out has the potential to result in improved experience and outcomes of pregnancy (see Clinician’s Corner).
Women could avoid unnecessary intervention caused by being wrongly labelled as high risk and adverse events could be prevented by targeting intervention to women with high absolute risks of disease. Given the lack of clear mechanistic understanding of the determinants of normal and abnormal fetal growth, a strong case can be made for investment in approaches which make no assumptions about the likely deterministic pathways for disease.
Clinician's Corner

- Fetal growth restriction has no gold standard diagnosis. Equating FGR and SGA will result in diagnosis of FGR in many healthy infants. Placental pathology does not provide a gold standard for diagnosis.

- Existing methods of screening for fetal growth restriction have been used for decades and are known to perform poorly.

- Low sensitivity means that some FGR infants die in utero where death could have been prevented by medically indicated delivery, whereas low specificity means that many women are classified as being at risk but would have had a normal outcome in the absence of intervention.

- Combining ultrasonic assessment of size and growth with the sFLT1:PIGF ratio increases positive predictive value over purely ultrasonic methods of detection but is relatively specific for cases of FGR where there is coexistent preeclampsia.

- Omic analyses, both of the placenta and the mother’s blood, have the potential to identify biomarkers for other pathophysiological pathways which lead to FGR. The combination of ultrasound and biomarkers may generate an approach to screening which combines high sensitivity and specificity.
Highlights

- Human placenta has a unique genomic architecture, with trophoblast within a given biopsy representing clonal expansion, and showing cancer-like levels of mutation and frequent copy number variation
- Streptococcus agalactiae invades the uterus prior to labour in 5-10% of women
- Preeclampsia has a characteristic pattern of circulating RNA in maternal plasma and provides an indirect assessment of placental dysfunction and fetal development
- Maternal blood has a metabolic signature of placental dysfunction which predicts FGR
- Maternal genotype can impact growth indirectly through altering the intra-uterine environment and directly by fetal inheritance of growth determining genes, but insulin resistance has opposing effects depending on maternal or fetal genotype
- GWAS studies demonstrate common genetic determinants of low birth weight and cardio-metabolic disease in adulthood
Outstanding questions

- What is the best performing method for characterising a fetus that has failed to achieve its genetically determined growth potential?

- What are the major pathways leading to FGR and can deep clinical phenotyping of cases identify major subgroups of FGR infants? If so, are tests specific to the different phenotypes or are there commonalities between groups which would allow screening with high sensitivity?

- Does serial assessment through pregnancy enhance the ability to identify better performing tests and can this be translated into longitudinal screening of pregnant women?

- Are there features of the delivered placenta which allow better refinement of the diagnosis of FGR? Why are pathological features of the placenta commonly seen in pregnancies with a normal outcome?

- Is it possible to develop disease modifying therapies that improve outcomes? How can the risks of remote effects of interventions be mitigated?

- Can we safely target medically indicated delivery at term to those at highest risk of stillbirth? How can we rule out placental disease in cases where the fetus is not small for gestational age?
**Glossary** (450 words)

**Delta-like homolog 1 (DLK1):** a fetal protein which circulates in the mother’s blood and affects maternal fat metabolism.

**Doppler PI & RI:** pulsatility index and resistance index, respectively. Both of these values are derived from the imaged waveform generated by Doppler ultrasound, where the Y axis is flow velocity and the X axis is time.

**Estimated fetal weight (EFW):** an estimate of fetal mass (in g or kg) which is generated by biometric measurements of fetal structures in mm (typically head, abdomen and femur) using a multiple linear regression equation. Median error of estimates = 5 to 10% of actual birth weight.

**Fetal growth restriction (FGR):** is defined as failure of the fetus to achieve its genetically determined growth potential.

**Follistatin-like 3 (FSTL3):** a secreted glycoprotein which antagonizes members of the transforming growth factor (TGF)-beta family. Elevated placental expression of elevated maternal serum levels are associated with FGR.

**Gestational age (GA):** a period of time in weeks equivalent to the interval from the first day of the last menstrual period until the day in question. The actual post conception age of the fetus = gestational age minus two weeks.

**Genome wide association study (GWAS):** comparison of the frequency of large numbers of single nucleotide polymorphisms across the whole genome in cases versus controls.

**Human herpes virus 6 (HHV6 A or B):** a double stranded DNA virus and an almost ubiquitous infection in childhood. However, HHV6 is incorporated into a telomeric region of a chromosome in 0.7% of people and is inherited in a Mendelian fashion. Transcriptional activity of inherited virus has been proven in the human placenta and is associated with an increased risk of preeclampsia.

**Human papilloma virus (HPV):** virus which is causally involved in majority of cases of cervical intra-epithelial neoplasia and invasive cancer.
**Intra-uterine fetal death (IUFD):** death of the infant prior to birth, generally called “stillbirth” when the death occurs at a gestational age where the baby was potentially viable.

**Laser capture microdissection (LCM):** allows analysis of specific microscopic elements, e.g. trophoblast layer of placental villi distinct from the fetal core of the villi.

**Low birth weight (LBW):** defined as birth weight <2500g.

**Nuclear magnetic resonance (NMR):** used in metabolomics, it is the major alternative method to mass spectrometry (MS, preceded by either gas or liquid chromatography, GC-MS and LC-MS, respectively). NMR has the advantages of generating absolute concentrations and being suitable for large scale use. However, it is less sensitive (i.e. higher limits of detection).

**Positive predictive value (PPV):** proportion of women with a positive screening test who would experience the disease in the absence of intervention.

**Proximity Extension Assay (PEA):** a proteomic method that uses pairs of antibodies for a given protein. Each antibody is coupled to an oligonucleotide. In the presence of the protein, binding of the antibodies brings the two oligonucleotides into close proximity and they hybridize. Relative protein concentrations are estimated by quantifying the hybridized oligonucleotides (either PCR or DNA-seq).

**Placenta growth factor (PlGF):** Pro-angiogenic growth factor which is bound and inactivated in maternal circulation by sFlt-1.

**Partial least squares discriminant analysis (PLS-DA):** One of a number of statistical methods applied to high dimensional omics data sets which aims to identify patterns within multiple numerical measurements in relation to a binary outcome (e.g. case and control).

**Soluble fms-like tyrosine kinase receptor 1 (sFlt-1):** An anti-angiogenic protein released by the placenta in response to hypoxia and high maternal circulating levels associated with increased risk of preeclampsia.

**Small for gestational age (SGA):** a fetus or new born where the estimated or actual weight falls into the smallest 10% for a given population at that stage in gestation.
Figure legends

**Figure 1. Data used to support or rule out the diagnosis of fetal growth restriction.** See Table 1 for details.

**Figure 2. Application of omic methods to studying fetal growth restriction.** The list of samples and methods are representative of those most commonly studied and neither is exhaustive. Neither are all possible combinations of sample type and method necessarily technically feasible. For example, at present, a minority of the omic methods could be applied to the study of single cells.

**Figure 3. Simulated data to demonstrate the utility of serial measurements in differentiating signal from noise in omic studies.** A large number of measurements were made at two time points in a group of cases and controls. The plots are the ratio of mean value in cases to the mean value in controls. The median and inter-quartile range of the ratios are represented by the bars. Blue circles reflect an extreme value in the analysis of time point #1 which occurred through the play of chance. The statistical property of regression to the mean explains the tendency for the next measurement at time point #2 to be closer to the median. The red circles reflect true signals. As time point #2 is closer to disease onset than time point #1, the true signal is further from the median at the second time point compared with the first.
Figure 1

- Preeclampsia
- Preterm birth
- Morbidity/mortality
- Anthropometry
- Gross and microscopic pathology
- Infant: placental weight ratio
- Infant

Outcome

- Maternal
- Doppler
- Uteroplacental
- Fetal

- Shape
- Placenta
- Fetal biometry
- Relative size (percentile)
- Velocity (change in percentile)
- Proportionality
Figure 2

Blood
- Serum
- Plasma
- Extracellular vesicles
- Leukocytes

Tissue
- Biopsy
- LCM
- Single cell

Nucleic acids
- DNA
  - Mutation/CNV
  - Methylation
  - Chromatin state/modification
  - Metagenomics
- RNA
  - mRNA
  - Long non-coding RNA
  - Small RNAs

Metabolites
- Mass spec
- NMR

Proteins
- Mass spec
- Ligand
- Aptamer
- Antibody (PEA)
<table>
<thead>
<tr>
<th>Birth weight</th>
<th>Definition</th>
<th>Label</th>
<th>Strengths</th>
<th>Weaknesses</th>
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<tbody>
<tr>
<td>Absolute threshold</td>
<td>&lt;2500g</td>
<td>Low birth weight (LBW)</td>
<td>Very simple to define</td>
<td>Includes many preterm births without FGR Sensitivity and specificity for FGR varies with gestational age (GA)</td>
</tr>
<tr>
<td>GA threshold</td>
<td>&lt;10th for GA</td>
<td>Small for gestational age (SGA)</td>
<td>Simple to define</td>
<td>Low specificity: includes many constitutionally small infants</td>
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<td></td>
<td>&lt;3rd for GA</td>
<td>Severe SGA</td>
<td>Simple to define</td>
<td>Lower sensitivity: excludes many FGR cases</td>
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<th>Definition</th>
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<th>Strengths</th>
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<tr>
<td>Uterine artery Doppler</td>
<td>Presence of notches Indices of resistance (PI or RI)</td>
<td>High resistance pattern thought to reflect impaired trophoblast invasion of the maternal uterine resistance vessels</td>
<td>Associated with FGR complicating preeclampsia</td>
<td>Not strongly associated with FGR at or near term</td>
</tr>
<tr>
<td>Umbilical artery Doppler</td>
<td>Pattern of end diastolic flow (absent or reversed) Indices of resistance (PI or RI)</td>
<td>High resistance pattern thought to reflect underdevelopment of the fetal vascular tree in placenta</td>
<td>Strongly associated with outcome in preterm FGR and proven useful in RCTs</td>
<td>Not strongly associated with FGR at or near term</td>
</tr>
<tr>
<td>Fetal growth velocity</td>
<td>Change in relative size of fetal biometry with advancing GA: EFW, AC</td>
<td>Progressive placental insufficiency results in progressively greater deviation from genetically determined growth potential</td>
<td>Direct assessment of actual fetal growth impairment as pregnancy advances</td>
<td>Requires serial ultrasound Biometry prone to measurement error (bias and noise) Reporting result may lead to change in clinical management which impacts on associations in research studies</td>
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<td></td>
<td>FGR more common in preterm PE, due to common associations with placental dysfunction Also feature of small proportion of PE at term</td>
<td>Requires detailed assessment of mother’s clinical record</td>
<td>Only applies to a sub-set of cases of FGR</td>
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<td>Pre-eclampsia</td>
<td>Coexisting SGA and PE strongly indicate FGR</td>
<td>Requires basic information from mother’s clinical record</td>
<td>By definition excludes term FGR and pathophysiology of preterm and term disease differs</td>
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<td>Preterm birth</td>
<td>Preterm infants very over-represented with cases of FGR</td>
<td>FGR shown to precede spontaneous preterm birth Medically indicated preterm delivery strongly associated with severest FGR</td>
<td>Requires basic information from mother’s clinical record</td>
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<td>Neonatal morbidity</td>
<td>FGR infant more likely to experience perinatal hypoxia/asphyxia</td>
<td>FGR infant has fewer stored energy reserves and may have mild hypoxia/acidosis prior to labour onset</td>
<td>Analysis of cord blood Apgar score Neonatal unit admission Nature of treatment (head cooling)</td>
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<td>Perinatal death</td>
<td>Antepartum and intrapartum IUFD and neonatal death associated with SGA and severe SGA</td>
<td>Same continuum as neonatal morbidity</td>
<td>Vital statistics and hospital records</td>
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<td></td>
<td>Very low sensitivity: majority of cases of FGR survive Most cases of perinatal death not due to FGR Post mortem changes may lead to SGA birth weight in normally grown IUFD infant</td>
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<table>
<thead>
<tr>
<th>Examination</th>
<th>Assessment</th>
<th>Associations</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>Morphology, weight, macroscopic appearance, histopathology</td>
<td>Peripheral insertion of umbilical cord Low placental weight Maternal vascular malperfusion Placental inflammation (villitis of unknown etiology)</td>
<td>Provides direct evidence of placental dysfunction</td>
<td>Abnormal findings are commonly seen in normal pregnancies Severe complications can be associated with completely normal placenta Potential for ascertainment bias if pathologist aware of...</td>
</tr>
</tbody>
</table>
| Infant | Assessment of fetal growth symmetry | Reduced size of abdomen to head and low ponderal index (weight/length^3) | Provides direct evidence of inadequate development of fetal energy stores during gestation | adverse outcome and does not see healthy controls
Expensive and time consuming

| Parameters change with gestational age
Detailed examination of all infants problematic at scale
Associations may be complex due to other determinants (genetics, maternal diabetes) |
References


25. Beaumont, R.N. et al. (2020) Common maternal and fetal genetic variants show expected polygenic effects on risk of small- or large-for-gestational-age (SGA or LGA), except in the smallest 3% of babies. PLoS Genet 16 (12), e1009191.


40. Pels, A. et al. (2020) Maternal Sildenafil vs Placebo in Pregnant Women With Severe Early-Onset Fetal Growth Restriction: A Randomized Clinical Trial. JAMA Netw Open 3 (6), e205323.

