Screening for Fetal Growth Restriction using Fetal Biometry Combined with Maternal Biomarkers

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Abstract

Fetal growth restriction (FGR) is a major determinant of perinatal morbidity and mortality. Screening for FGR is a key element of prenatal care but it is recognized to be problematic. Screening using clinical risk assessment and targeting ultrasound to high risk women is the standard of care in the USA and UK, but the approach is known to have low sensitivity. Systematic reviews of randomized controlled trials do not demonstrate any benefit from universal ultrasound screening for FGR in the third trimester, but the evidence base is not strong. Implementation of universal ultrasound screening in low risk women in France failed to reduce the risk of complications among small for gestational age (SGA) infants but did appear to cause iatrogenic harm to false positives. One strategy to making progress is to improve screening by developing more sensitive and specific tests with the key goal of differentiating between healthy small fetuses and those which are small through FGR. As abnormal placentation is thought to be the major cause of FGR, one approach is to combine fetal biometry with an indicator of placental dysfunction. In the past, these indicators were generally ultrasonic measurements, such as Doppler flow velocimetry of the utero-placental circulation. However, another promising approach is to combine ultrasonic suspicion of an SGA infant with a blood test indicating placental dysfunction. Thus far, much of the research on maternal serum biomarkers for FGR has involved the secondary analysis of tests performed for other indications, such as fetal aneuploidies. An exemplar of this is pregnancy associated plasma protein A (PAPP-A). This blood test is performed primarily to assess the risk of Down syndrome, but women with low first trimester levels are now serially scanned in later pregnancy due to associations with placental causes of stillbirth, including FGR. The development of "omic" technologies presents a huge opportunity to identify novel biomarkers for FGR. The hope is that when such markers are measured alongside ultrasonic

fetal biometry, the combination would have strong predictive power for FGR and its related complications. However, a series of important methodological considerations in assessing the diagnostic effectiveness of new tests will have to be addressed. The challenge thereafter will be to identify novel disease-modifying interventions, which are the essential partner to an effective screening test in order to achieve clinically effective population based screening.

Key words:

Small for gestational age; Ultrasound; Placenta; Biomarker; Screening; Stillbirth; Review; fetal biometry; placental growth factor; PAPP-A; hCG; sFLT1; sEndoglin; PP13; Adam12; alpha fetoprotein; inhibin; human placental lactogen; SGA; models; prediction; fetal death; RCT; study design

Fetal growth restriction and small for gestational age: differences between two commonly used terms and clinical implications

Fetal growth restriction (FGR) is defined as the failure of the fetus to reach its genetically determined growth potential. FGR is a major determinant of perinatal and childhood morbidity and mortality, and is also associated with the risk of chronic diseases in later life.[1-3] An obstacle to the study of FGR is that there are no gold standard definition and diagnostic criteria for this condition. The size of the fetus or newborn is quantified with reference to the normal range for gestational age and those with birthweight (BW) less than the 10th percentile are called small for gestational age (SGA). Inaccurately, the small size of the baby often becomes synonymous with FGR, and different thresholds for these measurements are used to "define" a FGR infant (e.g. <2500 g, <10th percentile, or <3th percentile).

Although SGA and FGR are sometimes used interchangeably, the two terms are distinct, as many SGA infants are constitutionally small and healthy. Hence, clinical research on screening for FGR has to address two main issues: (i) the sensitive and specific detection of SGA fetuses, and (ii) the ability to discriminate between FGR and healthy SGA. The causes of FGR can be broadly categorized into maternal (e.g. pregnancy-associated hypertensive diseases, autoimmune disease, poor nutrition, substance abuse and teratogen exposure),[4-6] fetal (e.g. multiple gestations, infections, genetic and structural disorders)[7, 8] or placental. It is thought that placental dysfunction accounts for the majority of FGR cases.[9] Hence, one the most promising approaches to screening for FGR is to combine ultrasonic fetal biometry with measurement of biomarkers of abnormal placentation in the mother's blood.

Current status of screening with fetal biometry

In many countries, including the UK and USA, ultrasound scanning after the 20 week anomaly scan is only performed on the basis of clinical indications as universal ultrasound is not supported by the most recent Cochrane review.[10] It is worth noting that the evidence base can be described as an absence of evidence rather than compelling high-quality evidence of the absence of clinical effectiveness of screening. This is due to a number of problems with the 13 studies analyzed in the systematic review, including limited statistical power and lack of an effective interventional strategy.[11] Nevertheless, the current approach to screening for FGR is to assess the women for pre-existing risk factors, acquired complications of pregnancy and clinical examination (e.g. symphysis-fundal height [SFH] measurements) (Fig. 1). Women identified as high risk using these methods are then selected for ultrasonographic assessment. Screening for FGR is just one element of the universal ultrasound.[12] Other elements include macrosomia, late presentation of fetal anomalies, abnormalities of amniotic fluid volume and diagnosis of undetected malpresentation.

Ultrasonic markers of FGR

Fetal biometry and Doppler flow velocimetry are the primary methods used currently to diagnose FGR. The use of ultrasound markers of FGR is discussed in detail elsewhere in this issue, and will be only briefly summarized here. An estimated fetal weight (EFW) is derived from ultrasonic measurements of head size, abdominal circumference and femur length, and an EFW centile is calculated using a reference standard.[13, 14] While a single measurement of fetal size and the EFW<10th centile cut-off appears to be insufficient to discriminate growth restricted and healthy small fetuses, serial fetal biometry reveals the growth trajectory of the fetus, and this helps differentiate between healthy SGA and FGR.[15, 16] Doppler flow

velocimetry provides information on the resistance to blood flow in the feto-placental unit and it features in several proposed FGR definitions.[17] High resistance patterns of flow in the uterine and umbilical arteries in early and mid-pregnancy have been associated with an increased risk of preeclampsia, FGR and stillbirth.[18-22] Other measurements associated with adverse pregnancy outcomes are middle cerebral artery (MCA) and ductus venosus flow resistance, and cerebroplacental ratio (CPR) (reviewed elsewhere).[17, 18, 23]

Biochemical biomarkers for FGR

Abnormal placentation leads to inadequate remodeling of maternal spiral arteries, altered uteroplacental blood perfusion and impaired materno-fetal exchange of nutrients, gases and waste products. These defects, collectively referred as placental insufficiency, are thought to be underlying mechanisms of placentally-related complications including FGR, preeclampsia and stillbirth. Hence, biochemical markers reflective of placental insufficiency become attractive tools to identify women at risk of these adverse pregnancy outcomes (Figure 1, Table 1).

First trimester screening

It is increasingly recognized that placental dysfunction leading to disease in the second half of pregnancy has its origins in the first trimester of pregnancy.[24] Studies of associations have been facilitated by the secondary analysis of first trimester biomarkers derived from the placenta, which were evaluated in screening studies for aneuploidies.

Down syndrome markers: PAPP-A and fβ-hCG

Low maternal circulating levels of pregnancy associated plasma protein A (PAPP-A) and high concentrations of the free beta subunit of human chorionic gonadotropin (fβ-hCG) are both

associated with the risk of Down syndrome.[25] PAPP-A determines the availability of the insulin-like growth factors (IGFs), key pregnancy growth hormones, as it is a protease that acts on IGF binding proteins. A causal role for PAPP-A in controlling fetal growth has been established in the PAPP-A knock out mouse.[26] In women, low serum concentrations of PAPP-A in the first trimester are associated with an increased risk of FGR, preterm delivery, preeclampsia and stillbirth.[27-33] The last of these associations is particularly strong for stillbirth associated with placental dysfunction (preeclampsia, FGR and abruption).[34] In the UK, and many other countries, low first trimester PAPP-A levels are an indication for late pregnancy ultrasonic assessment of fetal growth.[35, 36] Human chorionic gonadotrophin (hCG) is predominantly produced by the placental syncytiotrophoblast cells.[37] hCG is a glycoprotein composed of an α-subunit (common to luteinizing hormone, follicle stimulating hormone and thyroid stimulating hormone) and a β-subunit (unique to hCG). In first trimester Down syndrome screening the fβ-hCG is measured.[25] In general, extremes of fβ-hCG in the first trimester are less strongly associated with adverse outcome than low PAPP-A.[27, 30-33]

Other placental markers: PlGF, sFLT1, sENG, PP-13 and ADAM-12

While many of the largest studies of first trimester markers have focused on secondary analysis of Down syndrome screening research, other investigators have focused on measuring proteins on the basis of a known role in placentation. Angiogenic factors play a key role in the extensive vasculature remodeling of the uterus during pregnancy. The placenta itself produces several factors with pro- or anti-angiogenic activity and regulation of their expression and secretion is necessary for optimal placentation, maternal adaptation to pregnancy and, consequently, fetal development and growth. Preeclampsia-like changes were induced in pregnant rats by adenoviral mediated expression of soluble fms-like tyrosine

kinase-1 (sFLT1)[38] or soluble endoglin (sENG) alone or in combination with sFLT1.[39] Placental growth factor (PlGF) is a pro-angiogenic factor highly expressed in placenta throughout all stages of pregnancy. It is readily detectable in maternal circulation where it may have direct effects on endothelial maintenance and well-being. Consistent with this role, low first trimester levels of this factor have been shown to be associated with an increased risk of later adverse perinatal outcome, including preeclampsia and SGA.[31, 40, 41] The results are variable for anti-angiogenic factors. High maternal levels of sENG in the first trimester were associated with preeclampsia and SGA.[41] However, results are less consistent for sFLT1.[40, 42, 43] A large scale study employing correction of analyte levels using multiples of the median actually demonstrated that low sFLT1 levels were associated with an increased risk of SGA, preterm birth and stillbirth[40] whereas data in later pregnancy indicate the opposite association (see below). These findings indicate that the commonly used sFLT1:PlGF ratio should be interpreted cautiously in the first trimester. Finally, increased attention has been paid to longitudinal changes of these factors during pregnancy, but the results are inconclusive.[41, 44, 45]

Data exist from a number of other proteins. Low maternal first trimester levels of placental protein 13 (PP-13), another protein regulating placental vascular development, have been reported in pregnancies complicated by SGA,[31, 46] but results are, again, inconsistent.[47, 48] Similarly, A-disintegrin and metalloprotease 12 (ADAM12), a protease with similar function to PAPP-A, was reduced between 11 and 14 weeks in mothers who subsequently delivered small infants (BW<5th or <10th centile).[31, 49, 50]

Second and third trimester

The screening efficacy of tests performed in the second and third trimester was assessed for two main reasons: (i) the availability of data collected during screening studies for the identification of aneuploidies and birth defects during the second trimester; (ii) the idea that measurements performed in the third trimester may have better predictive ability due to being temporally closer to the onset of disease.[51]

Down syndrome and anomaly screening: AFP, total hCG, uE3 and inhibin A

The second trimester quadruple screening is performed at 15-22 weeks of gestation (wkGA) and includes measurements of alpha fetoprotein (AFP), hCG (intact and/or its β subunit), unconjugated estriol (uE3) and inhibin A.[52] The factors measured may also provide information on placental permeability (AFP) and endocrine activity (hCG, uE3 and inhibin A). Hence many studies have addressed the ability of these proteins to predict placentally-related pregnancy complications.

Elevated maternal serum levels of AFP are associated with SGA (BW<5th centile) with or without preterm delivery[53, 54] and stillbirth due to reduced BW (<5th centile).[55] The combination of low PAPP-A in the first trimester and high AFP in the second trimester is particularly strongly predictive of severe FGR.[56 57] In the FASTER trial, fβ-hCG alone (≥2.0 MoM) was not associated with any adverse outcome studied. In contrast, maternal circulating AFP (≥2.0 MoM), Inhibin A (≥2.0 MoM) and uE3 (≤0.5 MoM) were significantly associated with an increased risk of delivering an SGA infant.[58] The risk was particularly high when measurements were combined. In other studies, women with an elevation of hCG alone (threshold starting at >2.5 MoM) or in combination with high AFP had an increased risk of SGA.[54, 59] Low levels of unconjugated estriol (uE3) alone or in combination with AFP and/or hCG were associated with SGA (BW<5th percentile).[54, 60]

Other placental markers: sFLT1, PlGF, ENG, hPL, uE3

A number of large scale studies have analyzed biomarkers in the second and third trimester with the aim of identifying useful candidates for FGR screening. The SCOPE study recruited >5000 low risk women and has reported that maternal levels of sFLT1 and PAPP-A were decreased in pregnancies with SGA without maternal hypertension, whereas PAPP-A and PlGF were decreased in pregnancies with SGA and maternal hypertension.[61] Multicenter studies conducted by Prof Kypros Nicolaides (King's College Hospital, London, UK) have assessed the predictive associations of a number of circulating biomarkers at different stages in the second and third trimesters. At 19-24wkGA, maternal PlGF was lower and AFP were higher in women who subsequently delivered an SGA infant preterm. Term SGA was associated with lower maternal levels of PlGF, sFLT1 and PAPP-A.[53] When measured at 30-34wkGA, low PlGF and high sFLT1 were associated with delivery of an SGA infant[62] and the associations were stronger for preterm SGA. At 35–37wkGA, PlGF and sFLT1 concentrations in the lowest and highest 5th centile, respectively, were associated with SGA.[63] Moreover, extremes of the ratio have also been associated with the subsequent risk of intra-uterine fetal death.[64]

Mothers with a reduced rate of decrease of the anti-angiogenic factor sENG between the first and second trimesters had a higher risk of adverse pregnancy outcome, including SGA (BW<10th centile).[65] Moreover, as mentioned above, maternal plasma sENG concentrations remained elevated throughout the second and third trimester in patients destined to deliver an SGA neonate.[41] Maternal serum levels of the hormones human placental lactogen (hPL) and uE3 were shown to be reduced in pregnancies with SGA fetuses in the second and third trimesters.[66, 67]

Strength of prediction

A systematic review evaluated the strength of association between a wide range of biomarkers and utero-placental Doppler and different causes of stillbirth. The review concluded that only high resistance patterns of uterine Doppler in the second trimester and low PAPP-A in the first trimester had associations which were in the clinically useful range, with positive likelihood ratios of 5 to 15.[68] Moreover, these strong associations were only observed for the sub-types of stillbirth attributed to placental dysfunction. However, the review did not report the combination of ultrasound and biomarkers. The same authors also evaluated 53 studies and 37 potential biomarkers for FGR screening, and suggested that none of the proposed maternal circulating factors had a high predictive accuracy.[69] Their conclusion was that combining biomarkers with biophysical measurements and maternal characteristics could be a more effective strategy. Table 2 summarizes the strongest associations identified in the 2 meta-analyses.

Combined assessment using ultrasonic biometry and biomarkers

An international, prospective, multicenter observational study determined the prediction of SGA achieved by the combination of ultrasonic fetal biometry and maternal serum PlGF in women who were identified as clinically small for dates between 24-37wkGA. While the study demonstrated that median PlGF concentrations were lower in SGA pregnancies[70] these authors reported that the combination of EFW and of PlGF had only modest test performance. However, it should be borne in mind that many SGA infants are healthy, i.e. they are constitutionally small. It would not be expected that PlGF, a test for pathology, would be strongly associated with the delivery of a healthy small infant. One of the studies from the King's group reported that placental biomarkers measured at 35-37 weeks performed poorly as a screening test for perinatal morbidity.[63] An important feature of that study was that the

results of ultrasound scans, performed at the same time as the biomarker, were reported and would have influenced clinical care. Both of these studies raise important questions about the methodological approach to future studies attempting to develop novel screening tests for FGR, and these are discussed below.

We performed a prospective study of unselected nulliparous women, the Pregnancy Outcome Prediction (POP) study, which combined the use of ultrasound and biochemical markers, where the results of both were blinded.[71, 72] The analysis of this study is on-going. However, we have published a preliminary report that the combination of ultrasonic EFW<10th percentile and an elevated sFLT1:PlGF ratio at 36wkGA was strongly predictive for late FGR (BW<10th centile plus perinatal morbidity and/or preeclampsia), with a positive likelihood ratio of 17.5 and a sensitivity and specificity of 38% and 98%, respectively.[73]

Future directions

Despite years of research, screening for FGR remains clinical. Implementation of *ad hoc* screening using ultrasound appears to cause more harm than good.[11] This probably reflects the fact that, currently, the primary intervention to manage FGR is delivery of the infant. In the event of a false positive diagnosis in the preterm or early term weeks of gestational age, the effect will be to cause harm through the associations between earlier delivery and neonatal morbidity. The lack of progress could lead to the perception that the task being attempted is futile. However, there are a number of issues about the approach to this problem which have been relatively neglected. We believe that addressing some of the issues outlined below may help accelerate research into clinically useful tools for screening and intervention (Figure 3).

Identifying populations to screen

One of the most important screening parameters is the positive predictive value (PPV) of the test. For the individual woman, this might, indeed, be her primary interest: how likely is it that she will experience an adverse event. The PPV is determined by two factors: the prior odds of disease and the positive likelihood ratio of the test. Much of the research on screening has focused on the latter. However, if a woman has a very low prior risk of an outcome, even a highly predictive test may result in a low absolute risk that she experiences disease. This issue suggests that screening efforts might initially focus on women who are high risk and nulliparous women. In the latter group the key marker of risk in pregnancy – previous pregnancy outcome – is necessarily absent. Screening studies that include a high proportion of parous women with previous normal pregnancies will tend to yield results with low PPVs.

High quality studies of diagnostic effectiveness

With the increasing awareness of the importance of Evidence-Based Medicine, it is universally recognized that new interventions, such as novel drugs, must be evaluated, wherever possible, by a double blind, randomized controlled trial (RCT). This is due to the biases that result from patients and their caregivers being aware of allocation to the novel treatment. The equivalent approach in studies of diagnostic effectiveness is to blind the results of the new test. If a research study of screening reveals the test result, it ceases to be purely a research study and becomes an *ad hoc* screening program. Such an approach may arise from a perception that it would be unethical to conceal the result of a screening test and is based on the assumption that revealing the result of the test would produce a net benefit. This is true for some situations, such as diagnosis of major placenta praevia by ultrasound scan.

However, given that screening for SGA in France actually seemed to result in net harm[11] and given that routine ultrasound screening has not been shown to be safe and effective,[10] it could equally be argued that revealing the result of an unproven screening test and intervening on the basis of the result is unethical. Similarly, there are several studies evaluating the combination of ultrasound and biomarkers where the ultrasound scan result is revealed but the biochemical tests are not. In these cases, the ability of the biochemical test to predict the adverse outcome may be underestimated due to interventions initiated by an abnormal ultrasound result. These issues underline the value of studies where all new elements of the approach to screening are conducted blind, wherever possible. These examples indicate that blinding of test results in studies diagnostic effectiveness is justified. Otherwise, it is very hard to see how progress on screening using new diagnostic tests in pregnancy can be achieved.

Classification of SGA

SGA is defined on the basis of an arbitrary threshold of birth weight percentile. It follows that many infants born SGA were healthy. Using unqualified SGA as an outcome may, therefore, lead to weaker associations with an effective screening test than would have been obtained if the analysis was focused on cases of SGA where the baby was small due to FGR. A number of studies of candidate biomarkers have shown stronger associations with preterm compared to term SGA. These results might indicate that FGR is more common at preterm gestational ages. However, another interpretation is that the population of SGA infants delivered preterm is enriched with cases of true FGR compared to SGA births at term. Similarly, the association between a true test of placental dysfunction and SGA is likely to be stronger when the outcome of SGA is combined with an indicator of maternal or perinatal morbidity, such as preeclampsia or asphyxia, respectively. Therefore, studies which simply report the screening

statistics for all SGA without reference to whether there was evidence supporting a pathological cause may underestimate the screening performance of an informative test.

Assessment of new biomarkers may also be facilitated by phenotyping SGA using prenatal ultrasound. We have recently shown an association between SGA and low maternal plasma levels of the non-canonical NOTCH1 ligand delta-like 1 homolog (DLK1).[74] Using genetically modified mice, it was demonstrated that during pregnancy maternal circulating DLK1 is mostly of fetal origin. This protein appears to provide a link between fetal demand and maternal metabolic adaptation to pregnancy. Altered maternal levels of DLK1 were recently measured in pregnancies complicated with preeclampsia.[75] In our cohort, low DLK1 levels were associated with SGA only in presence of one or more ultrasonic indicators of FGR, and levels were not different in SGA without such markers (Figure 2). Interestingly, in every case of SGA with a high resistance pattern of umbilical artery Doppler, maternal serum DLK1 was lower than in the matched control. This also suggests that biomarkers might help define subgroups of causes of FGR. Recent studies in human placenta have correlations between methylation in the DLK1 domain and birth weight.[76]

Developing multi-parameter models

It is possible in the future that a single highly informative marker for FGR, or a subtype of FGR, might be identified on the basis of mechanistic understanding of the cause of the disease. However, in the meantime, it is more likely that screening tests for FGR will include multiple measurements, which are derived from both imaging procedures and measurement of biomarkers. Several studies adopting this approach have been described above.[61-63, 73] Development of screening models based on a multi-parameter assessment raises several challenges. These include measuring and scaling the given parameter to generate consistent

associations in different centers, combining multiple measures into a single predictive model, and accounting for interactions between parameters. Approaching this task has a number of common pitfalls, such as over-fitting statistical models leading to over optimistic prediction. These issues necessitate the use of statistical methods that account for optimism[77] and underline the value of studies which assess the development and validation of the novel test in separate groups of patients.[78, 79] These issues are particularly important when using "omic" methods which might yield hundreds or thousands of data points per patient. These methods carry a high risk of generating over-optimistic prediction and they require a rigorous methodological approach. Nevertheless, next generation sequencing (NGS) allows for unbiased interrogation of genomes or transcriptomes in clinical specimens, providing unprecedented opportunities to accelerate the discovery of novel biomarkers in FGR screening. A successful application of the NGS techniques to the biomarkers discovery field has been maternal circulating cell-free fetal DNA (cff-DNA) measurement for Down syndrome and trisomy 18 screening.[80, 81] Elevated maternal cff-DNA levels have also been measured in pregnancies complicated by FGR compared to normal pregnancies,[82] but more recent data were not always consistent with these initial findings.[83, 84]

Given the key role of the placenta in the etiology of FGR, an alternative approach is to study the differences in the placental transcriptome comparing cases of FGR and controls. This may allow identification of placental pathways altered in FGR and potential biomarkers for the condition although, so far, this approach has been extensively utilized in relation to preeclampsia.[85-87] Development of biomarkers would follow either by measurement of differentially expressed RNA molecules (mRNAs, miRNAs and lncRNAs) or by measurement of the proteins encoded by the differentially expressed genes. The exemplar of this approach is

the identification of the role of sFLT1 in preeclampsia: microarray studies identified up-regulation of *FLT1* as one of the key changes in the preeclamptic placenta and this was paralleled by elevated levels of sFLT1 protein in the maternal circulation.[38] Prof Stephen Tong's group has used the alternative approach of measuring mRNAs in the mother's plasma and they have identified a number of placental transcripts measured in maternal blood at 26-30wkGA which were associated with the subsequent risk of term FGR.[88, 89] Proteomic and metabolomic technologies offer theoretical advantages, because proteins and metabolites are potentially more closely linked to the phenotype under investigation than mRNA. One example of a validated proteomics study of FGR identified apolipoproteins CII and CIII in maternal serum of mothers with FGR compared to gestational age matched controls.[90] Despite the vast opportunities of "omic" research, there are still many challenges, including the difficulty related to handling large and highly complex datasets. To date, most "omic" studies assessing pregnancy disorders display several limitations: small sample sizes, lack of predictive ability, and the absence of validation experiments. A set of guidelines published by the Institute of Medicine[91] may help standardisation of future "omic" research.

Identifying novel biomarkers

It is apparent from the summary above that many biomarkers identified for FGR were originally developed as predictive tests for other conditions, such as Down syndrome and preeclampsia. A PubMed search yields approximately 3 times the number of citations for genetic array studies of the placenta in preeclampsia compared with FGR. Moreover, given the issues of phenotyping discussed above, application of "omic" methods to the placenta in cases of SGA will require detailed phenotyping of the case. Hence, a further approach to

improving screening for FGR would be to increase the efforts to identify the biological pathways involved in the placenta in optimally phenotyped cases of FGR.

Meaningful clinical outcomes

An extension of the issues relating to phenotyping is the identification of clinically important outcomes when studying FGR. Studies which focus on SGA have a limited potential as this would not be a likely primary outcome in trials of screening and intervention until disease modifying therapies are available. The most serious adverse outcome of FGR is intra-uterine fetal death. However, this outcome affects about 4 per 1000 pregnancies. Even the highest estimates only indicate that 50% of stillbirths might be due to FGR.[92] Other studies suggest that the proportion may be lower.[93] In order to be powered to study stillbirth related to FGR, tens of thousands of women or, in the case of a very strongly predictive test, many thousands of women would need to be recruited. Given the expense involved, many studies use non-lethal proxies of stillbirth due to FGR. While it is likely that true FGR has common features whether the baby survives or dies, the use of weak proxies will tend to obscure associations. One commonly used proxy is caesarean section for fetal distress, which is particularly problematic when it is employed in situations where the novel test result has been revealed. If the attending clinician is aware that a scan has identified a baby as suspected FGR, this could lead to an association with antepartum or intrapartum caesarean section for fetal distress even where the new test is not informative. There are a number of possible approaches to developing meaningful clinical outcomes. One might be to combine an assessment of the infant's birth weight with anthropometric measures which support a diagnosis of FGR. This would allow some degree of separation between healthy SGA and FGR. However, this assumes that such measures are reproducible and will correlate with clinically meaningful

outcomes. One approach we have used is to combine SGA birth weight with clinical complications which are consistent with FGR. These include signs of fetal asphyxia or compromise, such as depressed 5 minute Apgar score, the presence of metabolic acidosis in cord blood gases obtained at delivery and admission to the neonatal intensive care unit.[16] The last of these could reflect asphyxia or it could reflect other complications of FGR, such as hypoglycaemia. Finally, the co-existence of SGA and maternal preeclampsia is suggestive that the baby's small size is more likely to be due to placental dysfunction and this could also have utility in differentiating between healthy and pathological SGA infants.

The future for RCTs of screening and intervention

Given the capacity for screening to cause harm, any future program of screening and intervention will need to be evaluated by an RCT. We have previously discussed some of the issues around the design of such studies.[51, 94] If the primary outcome is clinically important it is likely that any trial will have to be very large (>10,000 women). Sample size can be reduced by randomizing screen positive women to revealing the result plus intervention versus concealing the result. It should also be self-evident that a trial of screening will only improve outcomes if the screening test is coupled to an effective intervention. At present, the primary disease modifying intervention is to deliver the baby. Given the strong associations between preterm birth and perinatal morbidity and mortality, we have suggested that this provides a rationale for focusing initial efforts on screening for FGR at term. However, mechanistic understanding of the causes of FGR could lead to the development of novel therapeutic approaches, such as re-purposing of existing drugs[95] and gene therapy.[96]

References

1. BARKER DJ, OSMOND C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1986;1:1077-81.

2. HALES CN, BARKER DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 1992;35:595-601.

3. COOPER C, FALL C, EGGER P, HOBBS R, EASTELL R, BARKER D. Growth in infancy and bone mass in later life. Ann Rheum Dis 1997;56:17-21.

4. OUNSTED M, MOAR VA, SCOTT A. Risk factors associated with small-for-dates and large-for-dates infants. Br J Obstet Gynaecol 1985;92:226-32.

5. SMYTH A, OLIVEIRA GH, LAHR BD, BAILEY KR, NORBY SM, GAROVIC VD. A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. Clin J Am Soc Nephrol 2010;5:2060-8.

6. American College of Obstetricians and Gynecologists, Practice bulletin no. 134: fetal growth restriction. Obstet Gynecol 2013;121:1122-33.

7. DESAI M, TER KUILE FO, NOSTEN F, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis 2007;7:93-104.

8. HENDRIX N, BERGHELLA V. Non-placental causes of intrauterine growth restriction. Semin Perinatol 2008;32:161-5.

9. MIFSUD W, SEBIRE NJ. Placental pathology in early-onset and late-onset fetal growth restriction. Fetal Diagn Ther 2014;36:117-28.

10. BRICKER L, MEDLEY N, PRATT JJ. Routine ultrasound in late pregnancy (after 24 weeks' gestation). Cochrane Database Syst Rev 2015:CD001451.

11. MONIER I, BLONDEL B, EGO A, KAMINISKI M, GOFFINET F, ZEITLIN J. Poor effectiveness of antenatal detection of fetal growth restriction and consequences for obstetric management and neonatal outcomes: a French national study. BJOG 2015;122:518-27.

12. Committee on Practice Bulletins, Obstetrics and American Institute of Ultrasound in Medicine. Practice Bulletin No. 175: Ultrasound in Pregnancy. Obstet Gynecol 2016;128:e241-e56.

13. HADLOCK FP, HARRIST RB, SHARMAN RS, DETER RL, PARK SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol 1985;151:333-7.

14. HADLOCK FP, HARRIST RB, MARTINEZ-POYER J. In utero analysis of fetal growth: a sonographic weight standard. Radiology 1991;181:129-33.

15. ALTMAN DG, HYTTEN FE. Intrauterine growth retardation: let's be clear about it. Br J Obstet Gynaecol 1989;96:1127-32.

16. Sovio U, White IR, Dacey A, Pasupathy D, Smith GC. Screening for fetal growth restriction with universal third trimester ultrasonography in nulliparous women in the Pregnancy Outcome Prediction (POP) study: a prospective cohort study. Lancet 2015;386:2089-97.

17. Dall'Asta A, Brunelli V, Prefumo F, Frusca T, Lees CC. Early onset fetal growth restriction. Matern Health Neonatol Perinatol 2017;3:2.

18. Kalache KD, Duckelmann AM. Doppler in obstetrics: beyond the umbilical artery. Clin Obstet Gynecol 2012;55:288-95.

19. Yu CK, Smith GC, Papageorghiou AT, Cacho AM, Nicolaides KH, Fetal Medicine Foundation Second Trimester Screening G. An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. Am J Obstet Gynecol 2005;193:429-36.

20. Smith GC, Yu CK, Papageorghiou AT, Cacho AM, Nicolaides KH, Fetal Medicine Foundation Second Trimester Screening G. Maternal uterine artery Doppler flow velocimetry and the risk of stillbirth. Obstet Gynecol 2007;109:144-51.

21. Brar HS, Platt LD. Reverse end-diastolic flow velocity on umbilical artery velocimetry in high-risk pregnancies: an ominous finding with adverse pregnancy outcome. Am J Obstet Gynecol 1988;159:559-61.

22. Gordijn SJ, Beune IM, Thilaganathan B, et al. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol 2016;48:333-9.

23. Schreurs CA, de Boer MA, Heymans MW, et al. Prognostic accuracy of cerebroplacental ratio and middle cerebral artery Doppler for adverse perinatal outcomes: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2017.

24. Smith GC. First-trimester determination of complications of late pregnancy. JAMA 2010;303:561-2.

25. Spencer K, Macri JN, Aitken DA, Connor JM. Free β-hCG as first-trimester marker for fetal trisomy. The Lancet 1992;339:1480.

26. Conover CA, Bale LK, Overgaard MT, et al. Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. Development 2004;131:1187-94.

27. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab 2002;87:1762-7.

28. D'ANTONIO F, RIJO C, THILAGANATHAN B, et al. Association between first-trimester maternal serum pregnancy-associated plasma protein-A and obstetric complications. Prenat Diagn 2013;33:839-47.

29. KHALIL A, SODRE D, SYNGELAKI A, AKOLEKAR R, NICOLAIDES KH. Maternal hemodynamics at 11-13 weeks of gestation in pregnancies delivering small for gestational age neonates. Fetal Diagn Ther 2012;32:231-8.

30. ONG CY, LIAO AW, SPENCER K, MUNIM S, NICOLAIDES KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. BJOG 2000;107:1265-70.

31. KARAGIANNIS G, AKOLEKAR R, SARQUIS R, WRIGHT D, NICOLAIDES KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther 2011;29:148-54.

32. DUGOFF L, HOBBINS JC, MALONE FD, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol 2004;191:1446-51.

33. KRANTZ D, GOETZL L, SIMPSON JL, et al. Association of extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. Am J Obstet Gynecol 2004;191:1452-8.

34. SMITH GC, CROSSLEY JA, AITKEN DA, et al. First-trimester placentation and the risk of antepartum stillbirth. JAMA 2004;292:2249-54.

35. Saving Babies' Lives - A care bundle for reducing stillbirth. NHS England 2016.

36. Small-for-Gestational-Age Fetus, Investigation and Management *Green-top Guideline No 31*: Royal College of Obstetricians and Gynaecologists, 2013.

37. HOSHINA M, BOOTHBY M, BOIME I. Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. J Cell Biol 1982;93:190-8.

38. MAYNARD SE, MIN JY, MERCHAN J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649-58.

39. VENKATESHA S, TOPORSIAN M, LAM C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006;12:642-9.

40. SMITH GC, CROSSLEY JA, AITKEN DA, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. Obstet Gynecol 2007;109:1316-24.

41. ROMERO R, NIEN JK, ESPINOZA J, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med 2008;21:9-23.

42. BERGEN NE, BOUWLAND-BOTH MI, STEEGERS-THEUNISSEN RP, et al. Early pregnancy maternal and fetal angiogenic factors and fetal and childhood growth: the Generation R Study. Hum Reprod 2015;30:1302-13.

43. JACOBS M, NASSAR N, ROBERTS CL, HADFIELD R, MORRIS JM, ASHTON AW. Levels of soluble fms-like tyrosine kinase one in first trimester and outcomes of pregnancy: a systematic review. Reprod Biol Endocrinol 2011;9:77.

44. TRIUNFO S, CROVETTO F, RODRIGUEZ-SUREDA V, et al. Changes in uterine artery Doppler velocimetry and circulating angiogenic factors in the first half of pregnancies delivering a small-for-gestational-age neonate. Ultrasound Obstet Gynecol 2017;49:357-63.

45. ASVOLD BO, VATTEN LJ, ROMUNDSTAD PR, JENUM PA, KARUMANCHI SA, ESKILD A. Angiogenic factors in maternal circulation and the risk of severe fetal growth restriction. Am J Epidemiol 2011;173:630-9.

46. SCHNEUER FJ, NASSAR N, KHAMBALIA AZ, et al. First trimester screening of maternal placental protein 13 for predicting preeclampsia and small for gestational age: in-house study and systematic review. Placenta 2012;33:735-40.

47. SERAVALLI V, GRIMPEL YI, MEIRI H, BLITZER M, BASCHAT AA. Relationship between first-trimester serum placental protein-13 and maternal characteristics, placental Doppler studies and pregnancy outcome. J Perinat Med 2016;44:543-9.

48. COWANS NJ, SPENCER K, MEIRI H. First-trimester maternal placental protein 13 levels in pregnancies resulting in adverse outcomes. Prenat Diagn 2008;28:121-5.

49. YU N, CUI H, CHEN X, CHANG Y. First trimester maternal serum analytes and second trimester uterine artery Doppler in the prediction of preeclampsia and fetal growth restriction. Taiwan J Obstet Gynecol 2017;56:358-61.

50. COWANS NJ, SPENCER K. First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. Prenat Diagn 2007;27:264-71.

51. SMITH GC. Researching new methods of screening for adverse pregnancy outcome: lessons from pre-eclampsia. PLoS Med 2012;9:e1001274.

52. AITKEN DA, WALLACE EM, CROSSLEY JA, et al. Dimeric inhibin A as a marker for Down's syndrome in early pregnancy. N Engl J Med 1996;334:1231-6.

53. LESMES C, GALLO DM, GONZALEZ R, POON LC, NICOLAIDES KH. Prediction of small-for-gestational-age neonates: screening by maternal serum biochemical markers at 19-24 weeks. Ultrasound Obstet Gynecol 2015;46:341-9.

54. ODIBO AO, SEHDEV HM, STAMILIO DM, MACONES GA. Evaluating the thresholds of abnormal second trimester multiple marker screening tests associated with intra-uterine growth restriction. Am J Perinatol 2006;23:363-7.

55. SMITH GC, SHAH I, WHITE IR, PELL JP, CROSSLEY JA, DOBBIE R. Maternal and biochemical predictors of antepartum stillbirth among nulliparous women in relation to gestational age of fetal death. BJOG 2007;114:705-14.

56. SMITH GC, SHAH I, CROSSLEY JA, et al. Pregnancy-associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. Obstet Gynecol 2006;107:161-6.

57. PROCTOR LK, TOAL M, KEATING S, et al. Placental size and the prediction of severe early-onset intrauterine growth restriction in women with low pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2009;34:274-82.

58. DUGOFF L, HOBBINS JC, MALONE FD, et al. Quad screen as a predictor of adverse pregnancy outcome. Obstet Gynecol 2005;106:260-7.

59. BENN PA, HORNE D, BRIGANTI S, RODIS JF, CLIVE JM. Elevated second-trimester maternal serum hCG alone or in combination with elevated alpha-fetoprotein. Obstet Gynecol 1996;87:217-22.

60. YARON Y, CHERRY M, KRAMER RL, et al. Second-trimester maternal serum marker screening: maternal serum alpha-fetoprotein, beta-human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome. Am J Obstet Gynecol 1999;181:968-74.

61. MCCOWAN LM, THOMPSON JM, TAYLOR RS, et al. Prediction of Small for Gestational Age Infants in Healthy Nulliparous Women Using Clinical and Ultrasound Risk Factors Combined with Early Pregnancy Biomarkers. PLoS One 2017;12:e0169311.

62. VALINO N, GIUNTA G, GALLO DM, AKOLEKAR R, NICOLAIDES KH. Biophysical and biochemical markers at 30-34 weeks' gestation in the prediction of adverse perinatal outcome. Ultrasound Obstet Gynecol 2016;47:194-202.

63. VALINO N, GIUNTA G, GALLO DM, AKOLEKAR R, NICOLAIDES KH. Biophysical and biochemical markers at 35-37 weeks' gestation in the prediction of adverse perinatal outcome. Ultrasound Obstet Gynecol 2016;47:203-9.

64. CHAIWORAPONGSA T, ROMERO R, EREZ O, et al. A low angiogenic index-1 (placental growth factor/soluble vascular endothelial growth factor receptor-1 ratio) at 24-28 weeks of gestation is a biomarker to identify the patient at risk for subsequent fetal death. Am J Obstet Gynecol 2017.

65. EREZ O, ROMERO R, ESPINOZA J, et al. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. J Matern Fetal Neonatal Med 2008;21:279-87.

66. MARKESTAD T, BERGSJO P, AAKVAAG A, et al. Prediction of fetal growth based on maternal serum concentrations of human chorionic gonadotropin, human placental lactogen and estriol. Acta Obstet Gynecol Scand Suppl 1997;165:50-5.

67. STEFANIDIS K, SOLOMOU E, MOUZAKIOTI E, STEFOS T, FARMAKIDES G. Comparison of somatomedin-C (SMC/IGF-I), human placental lactogen and Doppler velocimetry between appropriate and small-for-gestational-age pregnancies. Clin Exp Obstet Gynecol 1998;25:20-2.

68. CONDE-AGUDELO A, BIRD S, KENNEDY SH, VILLAR J, PAPAGEORGHIOU AT. First- and second-trimester tests to predict stillbirth in unselected pregnant women: a systematic review and meta-analysis. BJOG 2015;122:41-55.

69. CONDE-AGUDELO A, PAPAGEORGHIOU AT, KENNEDY SH, VILLAR J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. BJOG 2013;120:681-94.

70. GRIFFIN M, SEED PT, WEBSTER L, et al. Diagnostic accuracy of placental growth factor and ultrasound parameters to predict the small-for-gestational-age infant in women presenting with reduced symphysis-fundus height. Ultrasound Obstet Gynecol 2015;46:182-90.

71. PASUPATHY D, DACEY A, COOK E, CHARNOCK-JONES DS, WHITE IR, SMITH GC. Study protocol. A prospective cohort study of unselected primiparous women: the pregnancy outcome prediction study. BMC Pregnancy Childbirth 2008;8:51.

72. GACCIOLI F, LAGER S, SOVIO U, CHARNOCK-JONES DS, SMITH GCS. The pregnancy outcome prediction (POP) study: Investigating the relationship between serial prenatal ultrasonography, biomarkers, placental phenotype and adverse pregnancy outcomes. Placenta 2017;59:S17-S25.

73. SOVIO U, GACCIOLI F, HUND M, CHARNOCK-JONES DS, SMITH GCS. 155: Screening for late fetal growth restriction using ultrasound and the sFlt-1:PlGF ratio. American Journal of Obstetrics & Gynecology 2017;216:S104.

74. CLEATON MA, DENT CL, HOWARD M, et al. Fetus-derived DLK1 is required for maternal metabolic adaptations to pregnancy and is associated with fetal growth restriction. Nat Genet 2016;48:1473-80.

75. SCHREY S, WURST U, EBERT T, et al. The adipokine preadipocyte factor-1 is downregulated in preeclampsia and expressed in placenta. Cytokine 2015;75:338-43.

76. PRATS-PUIG A, CARRERAS-BADOSA G, BASSOLS J, et al. The placental imprinted DLK1-DIO3 domain: a new link to prenatal and postnatal growth in humans. Am J Obstet Gynecol 2017;217:350 e1-50 e13.

77. SMITH GC, SEAMAN SR, WOOD AM, ROYSTON P, WHITE IR. Correcting for optimistic prediction in small data sets. Am J Epidemiol 2014;180:318-24.

78. STEYERBERG EW, VERGOUWE Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. Eur Heart J 2014;35:1925-31.

79. PAVLOU M, AMBLER G, SEAMAN SR, et al. How to develop a more accurate risk prediction model when there are few events. BMJ 2015;351:h3868.

80. CHIU RW, AKOLEKAR R, ZHENG YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401.

81. BIANCHI DW, PARKER RL, WENTWORTH J, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med 2014;370:799-808.

82. ILLANES S, PARRA M, SERRA R, et al. Increased free fetal DNA levels in early pregnancy plasma of women who subsequently develop preeclampsia and intrauterine growth restriction. Prenat Diagn 2009;29:1118-22.

83. STEIN W, MULLER S, GUTENSOHN K, EMONS G, LEGLER T. Cell-free fetal DNA and adverse outcome in low risk pregnancies. Eur J Obstet Gynecol Reprod Biol 2013;166:10-3.

84. POON LC, MUSCI T, SONG K, SYNGELAKI A, NICOLAIDES KH. Maternal plasma cell-free fetal and maternal DNA at 11-13 weeks' gestation: relation to fetal and maternal characteristics and pregnancy outcomes. Fetal Diagn Ther 2013;33:215-23.

85. KLEINROUWELER CE, VAN UITERT M, MOERLAND PD, RIS-STALPERS C, VAN DER POST JA, AFINK GB. Differentially expressed genes in the pre-eclamptic placenta: a systematic review and meta-analysis. PLoS One 2013;8:e68991.

86. BREW O, SULLIVAN MH, WOODMAN A. Comparison of Normal and Pre-Eclamptic Placental Gene Expression: A Systematic Review with Meta-Analysis. PLoS One 2016;11:e0161504.

87. SHEIKH AM, SMALL HY, CURRIE G, DELLES C. Systematic Review of Micro-RNA Expression in Pre-Eclampsia Identifies a Number of Common Pathways Associated with the Disease. PLoS One 2016;11:e0160808.

88. WHITEHEAD CL, MCNAMARA H, WALKER SP, et al. Identifying late-onset fetal growth restriction by measuring circulating placental RNA in the maternal blood at 28 weeks' gestation. Am J Obstet Gynecol 2016;214:521 e1-8.

89. WHITEHEAD CL, WALKER SP, MENDIS S, LAPPAS M, TONG S. Quantifying mRNA coding growth genes in the maternal circulation to detect fetal growth restriction. Am J Obstet Gynecol 2013;209:133 e1-9.

90. WOLTER M, ROWER C, KOY C, RATH W, PECKS U, GLOCKER MO. Proteoform profiling of peripheral blood serum proteins from pregnant women provides a molecular IUGR signature. J Proteomics 2016;149:44-52.

91. In: Micheel CM, Nass SJ, Omenn GS, eds. *Evolution of Translational Omics: Lessons Learned and the Path Forward*. Washington (DC), 2012.

92. SMITH GC, FRETTS RC. Stillbirth. Lancet 2007;370:1715-25.

93. MORAITIS AA, WOOD AM, FLEMING M, SMITH GC. Birth weight percentile and the risk of term perinatal death. Obstet Gynecol 2014;124:274-83.

94. FLENADY V, WOJCIESZEK AM, MIDDLETON P, et al. Stillbirths: recall to action in high-income countries. Lancet 2016;387:691-702.

95. GANZEVOORT W, ALFIREVIC Z, VON DADELSZEN P, et al. STRIDER: Sildenafil Therapy In Dismal prognosis Early-onset intrauterine growth Restriction--a protocol for a systematic review with individual participant data and aggregate data meta-analysis and trial sequential analysis. Syst Rev 2014;3:23.

96. KRISHNAN T, DAVID AL. Placenta-directed gene therapy for fetal growth restriction. Semin Fetal Neonatal Med 2017; http://dx.doi.org/10.1016/j.siny.2017.04.005.

97. YARON Y, HEIFETZ S, OCHSHORN Y, LEHAVI O, ORR-URTREGER A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn 2002;22:778-82.

98. MARTTALA J, PEUHKURINEN S, LAITINEN P, GISSLER M, NIEMINEN P, RYYNANEN M. Low maternal PAPP-A is associated with small-for-gestational age newborns and stillbirths. Acta Obstet Gynecol Scand 2010;89:1226-8.

99. LEPAGE N, CHITAYAT D, KINGDOM J, HUANG T. Association between second-trimester isolated high maternal serum maternal serum human chorionic gonadotropin levels and obstetric complications in singleton and twin pregnancies. Am J Obstet Gynecol 2003;188:1354-9.

100. BURTON BK. Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum alpha-fetoprotein. Obstet Gynecol 1988;72:709-13.

101. WALLER DK, LUSTIG LS, CUNNINGHAM GC, GOLBUS MS, HOOK EB. Second-trimester maternal serum alpha-fetoprotein levels and the risk of subsequent fetal death. N Engl J Med 1991;325:6-10.

102. ROOP AP, BOUGHMAN JA, BLITZER MG. Study of the relationship between elevated maternal serum alpha-fetoprotein and adverse pregnancy outcome. Md Med J 1991;40:779-84.

103. PEJTSIK B, RAPPAI G, PINTER J, KELEMEN A. [Correlation between elevated maternal serum alpha-fetoprotein, certain pregnancy complications and fetal death]. Orv Hetil 1992;133:2621-4.

104. MAHER JE, DAVIS RO, GOLDENBERG RL, BOOTS LR, DUBARD MB. Unexplained elevation in maternal serum alpha-fetoprotein and subsequent fetal loss. Obstet Gynecol 1994;83:138-41.

105. KRAUSE TG, CHRISTENS P, WOHLFAHRT J, et al. Second-trimester maternal serum alpha-fetoprotein and risk of adverse pregnancy outcome(1). Obstet Gynecol 2001;97:277-82.

106. ANFUSO S, SONCINI E, BONELLI P, PIANTELLI G, GRAMELLINI D. Second-trimester maternal serum alpha-fetoprotein elevation and its association with adverse maternal/fetal outcome: ten years experience. Acta Biomed 2007;78:214-9.

107. DURIC K, SKRABLIN S, LESIN J, KALAFATIC D, KUVACIC I, SUCHANEK E. Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy. Eur J Obstet Gynecol Reprod Biol 2003;110:12-5.

108. AKOLEKAR R, BOWER S, FLACK N, BILARDO CM, NICOLAIDES KH. Prediction of miscarriage and stillbirth at 11-13 weeks and the contribution of chorionic villus sampling. Prenat Diagn 2011;31:38-45.

109. DUGOFF L, CUCKLE HS, HOBBINS JC, et al. Prediction of patient-specific risk for fetal loss using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers. Am J Obstet Gynecol 2008;199:290 e1-6.

110. TJOA ML, VAN VUGT JM, MULDERS MA, SCHUTGENS RB, OUDEJANS CB, VAN WIJK IJ. Plasma placenta growth factor levels in midtrimester pregnancies. Obstet Gynecol 2001;98:600-7.

111. BERSINGER NA, ODEGARD RA. Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. Acta Obstet Gynecol Scand 2004;83:37-45.

112. KRAUSS T, PAUER HU, AUGUSTIN HG. Prospective analysis of placenta growth factor (PlGF) concentrations in the plasma of women with normal pregnancy and pregnancies complicated by preeclampsia. Hypertens Pregnancy 2004;23:101-11.

113. THADHANI R, MUTTER WP, WOLF M, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. J Clin Endocrinol Metab 2004;89:770-5.

114. ESPINOZA J, ROMERO R, NIEN JK, et al. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. Am J Obstet Gynecol 2007;196:326 e1-13.

115. POON LC, ZARAGOZA E, AKOLEKAR R, ANAGNOSTOPOULOS E, NICOLAIDES KH. Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11(+0) to 13(+6) weeks of gestation. Prenat Diagn 2008;28:1110-5.

116. VANDENBERGHE G, MENSINK I, TWISK JW, BLANKENSTEIN MA, HEIJBOER AC, VAN VUGT JM. First trimester screening for intra-uterine growth restriction and early-onset pre-eclampsia. Prenat Diagn 2011;31:955-61.

117. BENTON SJ, HU Y, XIE F, et al. Can placental growth factor in maternal circulation identify fetuses with placental intrauterine growth restriction? Am J Obstet Gynecol 2012;206:163 e1-7.

118. BOUCOIRAN I, THISSIER-LEVY S, WU Y, et al. 762: Risk for preeclampsia and intrauterine growth restriction: effective value of PlGF, Sflt-1 and Inhibin A in singleton and multiple pregnancies. American Journal of Obstetrics & Gynecology;206:S336-S37.

119. STEPAN H, UNVERSUCHT A, WESSEL N, FABER R. Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion. Hypertension 2007;49:818-24.

120. DIAB AE, EL-BEHERY MM, EBRAHIEM MA, SHEHATA AE. Angiogenic factors for the prediction of pre-eclampsia in women with abnormal midtrimester uterine artery Doppler velocimetry. Int J Gynaecol Obstet 2008;102:146-51.

121. BERSINGER NA, ODEGARD RA. Serum levels of macrophage colony stimulating, vascular endothelial, and placenta growth factor in relation to later clinical onset of pre-eclampsia and a small-for-gestational age birth. Am J Reprod Immunol 2005;54:77-83.

122. WANG Y, TASEVSKI V, WALLACE EM, GALLERY ED, MORRIS JM. Reduced maternal serum concentrations of angiopoietin-2 in the first trimester precede intrauterine growth restriction associated with placental insufficiency. BJOG 2007;114:1427-31.

123. PIHL K, LARSEN T, KREBS L, CHRISTIANSEN M. First trimester maternal serum PAPP-A, beta-hCG and ADAM12 in prediction of small-for-gestational-age fetuses. Prenat Diagn 2008;28:1131-5.

124. POON LC, CHELEMEN T, GRANVILLANO O, PANDEVA I, NICOLAIDES KH. First-trimester maternal serum a disintegrin and metalloprotease 12 (ADAM12) and adverse pregnancy outcome. Obstet Gynecol 2008;112:1082-90.

125. BEWLEY S, CHARD T, GRUDZINSKAS G, COOPER D, CAMPBELL S. Early prediction of uteroplacental complications of pregnancy using Doppler ultrasound, placental function tests and combination testing. Ultrasound Obstet Gynecol 1992;2:333-7.

126. CHAFETZ I, KUHNREICH I, SAMMAR M, et al. First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. Am J Obstet Gynecol 2007;197:35 e1-7.

127. THAN NG, ROMERO R, MEIRI H, et al. PP13, maternal ABO blood groups and the risk assessment of pregnancy complications. PLoS One 2011;6:e21564.

128. FRANCO-SENA AB, GOLDANI MZ, TAVARES DO CARMO M, VELASQUEZ-MELENDEZ G, KAC G. Low leptin concentration in the first gestational trimester is associated with being born small for gestational age: prospective study in Rio de Janeiro, Brazil. Neonatology 2010;97:291-8.

129. WANG Z, XIONG G, ZHU Y. The predictive value of plasma fibronectin concentration on fetal growth retardation at earlier stage of the third trimester. J Tongji Med Univ 2001;21:253-5.

130. D'ANNA R, BAVIERA G, CORRADO F, IENTILE R, GRANESE D, STELLA NC. Plasma homocysteine in early and late pregnancies complicated with preeclampsia and isolated intrauterine growth restriction. Acta Obstet Gynecol Scand 2004;83:155-8.

131. DODDS L, FELL DB, DOOLEY KC, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem 2008;54:326-34.

132. KRAUSS T, EMONS G, KUHN W, AUGUSTIN HG. Predictive value of routine circulating soluble endothelial cell adhesion molecule measurements during pregnancy. Clin Chem 2002;48:1418-25.

133. GEORGIOU HM, THIO YS, RUSSELL C, et al. Association between maternal serum cytokine profiles at 7-10 weeks' gestation and birthweight in small for gestational age infants. Am J Obstet Gynecol 2011;204:415 e1-15 e12.

Table 1. Maternal circulating biochemical markers for predicting FGR.

BIOMARKER	MAIN FUNCTION	Changes in maternal circulating levels frequently associated with FGR		
		1st trimester	2nd trimester	3rd trimester
Down's syndrome biomarkers				
pregnancy associated plasma protein A, **PAPP-A**	• Protease activity towards insulin-like growth factor binding proteins (IGFBPs) • Decrease of insulin-like growth factors bioavailability and signaling	↓	↓	
human chorionic gonadotrophin, **hCG**	• Maintenance of progesterone secretion from the corpus luteum	↓	↑	
alpha fetoprotein, **AFP**	• Protein of fetal origin with similar function to albumin in the adult • Carrier-molecule for several ligands (bilirubin, steroids, and fatty acids)		↑	
unconjugated estriol, **uE3**	• Estrogen agonist		↓	↓
inhibin A	• Negative feedback on pituitary follicle-stimulating hormone secretion, • Preventing ovulation during pregnancy		↑	
Angiogenic factors				
placental growth factor, **PlGF**	• Member of the vascular endothelial growth factor (VEGF) family • Pro-angiogenic factor	↓	↓	↓
soluble fms-like tyrosine kinase-1 **sFLT1**	• Decrease of plgf and VEGF bioavailability and signaling	↑ , ↓	↓ , =	↑
sFLT1:PlGF ratio		↑	↑	↑
soluble Endoglin, **sENG**	• Decrease of TGF-β1 and TGF-β3 bioavailability and signaling	↑	↑	↑
Placental protein 13 **PP-13**	• Promoting trophoblast invasion and spiral artery remodeling	↓ , =		
Hormonal factors				
A-disintegrin and metalloprotease 12, **ADAM12**	• Protease activity towards insulin-like growth factor binding proteins (IGFBPs) • Decrease of insulin-like growth factors bioavailability and signaling	↓		
human placental lactogen, **hPL**	• Induction of maternal insulin resistance and lipolysis • Induction of mammary glands development and milk production		↓	↓
delta-like 1 homolog, **DLK1**	• Adipose tissue homeostasis • Maternal metabolic adaptation to pregnancy			↓

Table 2. Predictive accuracy of maternal circulating biomarkers for stillbirth and fetal growth restriction.

BIOMARKER	Outcome	Positive LR (95% CI)	Negative LR (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Population (n)
Down's syndrome biomarkers						
PAPP-A	Stillbirth	3.3 (1.8–6.0)	0.9 (0.8–1.0)	15 (8–26)	95 (95–96)	21158[97, 98]
	Placental abruption and/or SGA-related stillbirth	14.1 (9.3–21.4)	0.3 (0.1–0.8)	70 (40–89)	95 (95–96)	7919[34]
hCG	Stillbirth	2.8 (1.9–4.3)	0.4 (0.2–1.0)	70 (40–89)	75 (73–77)	2406[99]
AFP	Stillbirth	4.0 (3.4–4.7)	0.9 (0.9–0.9)	9 (8–11)	98 (98–98)	186802[60, 100-106]
uE3	Stillbirth	4.0 (3.0–5.3)	0.9 (0.8–0.9)	15 (11–20)	96 (96–96)	58417[58, 60, 107]
inhibin A	Stillbirth	6.1 (4.0–9.3)	0.8 (0.8–0.9)	19 (12–27)	97 (97–97)	33145[58]
PAPP-A + mat. characteristics + US markers	Stillbirth	3.5 (2.8-4.4)	0.7 (0.6-0.8)	35 (28-43)	90 (90-90)	33452[108]
	SGA-related stillbirth	4.4 (3.2–5.9)	0.6 (0.5–0.8)	44 (31–57)	90 (90–90)	33365[108]
inhibin A + mat. characteristics	Stillbirth	4.1 (2.8–6.0)	0.8 (0.8–0.9)	20 (14–29)	95 (95–95)	35253[109]
Angiogenic factors						
PlGF	FGR	1.3 (1.2–1.5)	0.9 (0.8–0.9)	38 (35–42)	71 (70–72)	5709[45, 110-118]
	FGR with BW<5th centile and abnormal UT-Doppler	2.0 (1.3–3.0)	0.5 (0.3–1.0)	65 (43–82)	67 (58–76)	124[119, 120]
	FGR with placental pathology	19.8 (7.6–51.3)	0.0 (0.0–0.3)	100 (70–100)	95 (88–98)	88[117]
sFLT1	FGR with BW<5th centile and abnormal UT-Doppler	1.9 (1.3-2.6)	0.4 (0.2-0.9)	75 (53-89)	60 (50-69)	124[119, 120]
sFLT1:PlGF ratio	FGR with BW<5th centile and abnormal UT-Doppler	1.7 (1.2-2.4)	0.4 (0.2-1.0)	75 (53-89)	57 (47-66)	124[119, 120]
sENG	FGR with BW<5th centile	1.8 (1.4–2.3)	0.6 (0.5–0.7)	61 (52–69)	67 (60–7	355[45]
sENG slope	FGR with BW<10th centile	2.4 (1.4–4.3)	0.9 (0.8–1.0)	19 (14–27)	92 (88–95)	346[65]

VEGF	FGR with BW≤2SD	4.4 (1.6–12.2)	0.5 (0.2–1.1)	56 (27–81)	88 (74–95)	49[121]
Angiopoietin	FGR with BW<10th centile and UA-Doppler	4.3 (1.9–9.4)	0.1 (0.0–0.7)	92 (67–99)	78 (58–90)	36[122]
Hormonal factors, endothelial stress markers & cytokines						
ADAM12	FGR with BW<5th centile	2.2 (1.6–3.1)	0.9 (0.9–1.0)	12 (10–14)	95 (93–96)	1947[50, 123, 124]
IGFBP-1	FGR with BW<10th centile	2.7 (1.1–6.5)	0.8 (0.7–1.0)	24 (12–43)	91 (85–95)	172[125]
PP-13	FGR with BW<5th centile	3.6 (2.8–4.7)	0.7 (0.6–0.8)	34 (27–42)	91 (90–92)	3854[46, 126, 127]
Leptin	FGR with BW<10th centile	2.2 (1.4–3.5)	0.5 (0.3–0.9)	63 (41–81)	72 (63–79)	139[128]
Fibronectin	FGR with BW<10th centile	13.3 (5.0–35.0)	0.5 (0.2–0.8)	57 (33–79)	96 (90–98)	130[129]
Homocysteine	FGR with BW<10th centile	2.3 (1.7–3.1)	0.8 (0.8–0.9)	26 (19–33)	89 (87–90)	2088[130, 131]
sVCAM-1	FGR with BW<10th centile	9.0 (3.9–20.7)	0.9 (0.7–1.0)	16 (7–30)	98 (97–99)	1404[132]
sICAM-1	FGR with BW<10th centile	19.2 (11.5–32.1)	0.6 (0.5–0.8)	42 (28–58)	98 (97–99)	1404[132]
IFN-γ	FGR with BW<10th centile	2.8 (1.4–5.6)	0.7 (0.6–0.9)	35 (24–48)	87 (78–93)	128[133]
IL-1Ra	FGR with BW<10th centile	3.0 (1.7–5.1)	0.6 (0.4–0.8)	54 (42–67)	82 (71–89)	128[133]

Abbreviations: LR, likelihood ratio; CI, confidence interval; SGA, small for gestational age; FGR, fetal growth restriction; BW, birthweight; UT, uterine artery; UA, umbilical artery; SD, standard deviation; PAPP-A, pregnancy associated plasma protein A; hCG, human chorionic gonadotropin; AFP, alpha fetoprotein uE3, unconjugated estriol; mat., maternal; PlGF, placenta growth factor; sFLT1, soluble fms-like tyrosine kinase-1; sENG, soluble endoglin; VEGF, vascular endothelial growth factor; ADAM12, A-disintegrin and metalloprotease 12; IGFBP, insulin-like growth factor binding protein 1; PP-13, placental protein 13; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intercellular adhesion molecule; IFN-γ, interferon gamma; IL-1Ra, interleukin-1 receptor antagonist. Based on Conde-Agudelo et al (2013) and Conde-Agudelo et al (2015).[68, 69]

Figure legends

Figure 1. Standard antenatal care in UK and biochemical markers measured throughout pregnancy. Biomarkers measured in clinical and research settings during pregnancy are plotted on a time scale representing the standard antenatal care for nulliparous women in the UK, which includes 10 routine midwife visits and additional visits for women delivering after 40wkGA.

Figure 2. Low maternal DLK1 levels are associated with FGR. Scatterplot of differences in maternal plasma concentrations of DLK1. DLK1 concentration was measured in maternal plasma from pregnancies with SGA (BW<10th centile) and normally grown infants (n=43 matched pairs; matching was based on maternal age, BMI, smoking status, fetal sex and mode of delivery). FGR indicators are: UT-PI in the 10th decile at 20wkGA (n=8 pairs); UA-PI in the 10th decile at 36wkGA (n=10 pairs); ACGV in the 1st decile at 20–36wkGA (n=12 pairs). BMI indicates body mass index; UT-PI indicates uterine artery pulsatility index; UA-PI indicates umbilical artery pulsatility index; ACGV indicates abdominal circumference growth velocity. Horizontal bars represent the means of the differences. Modified from Cleaton et al, Nature Genetics 2016[74].

Figure 3. Summary of key points to improve FGR screening.

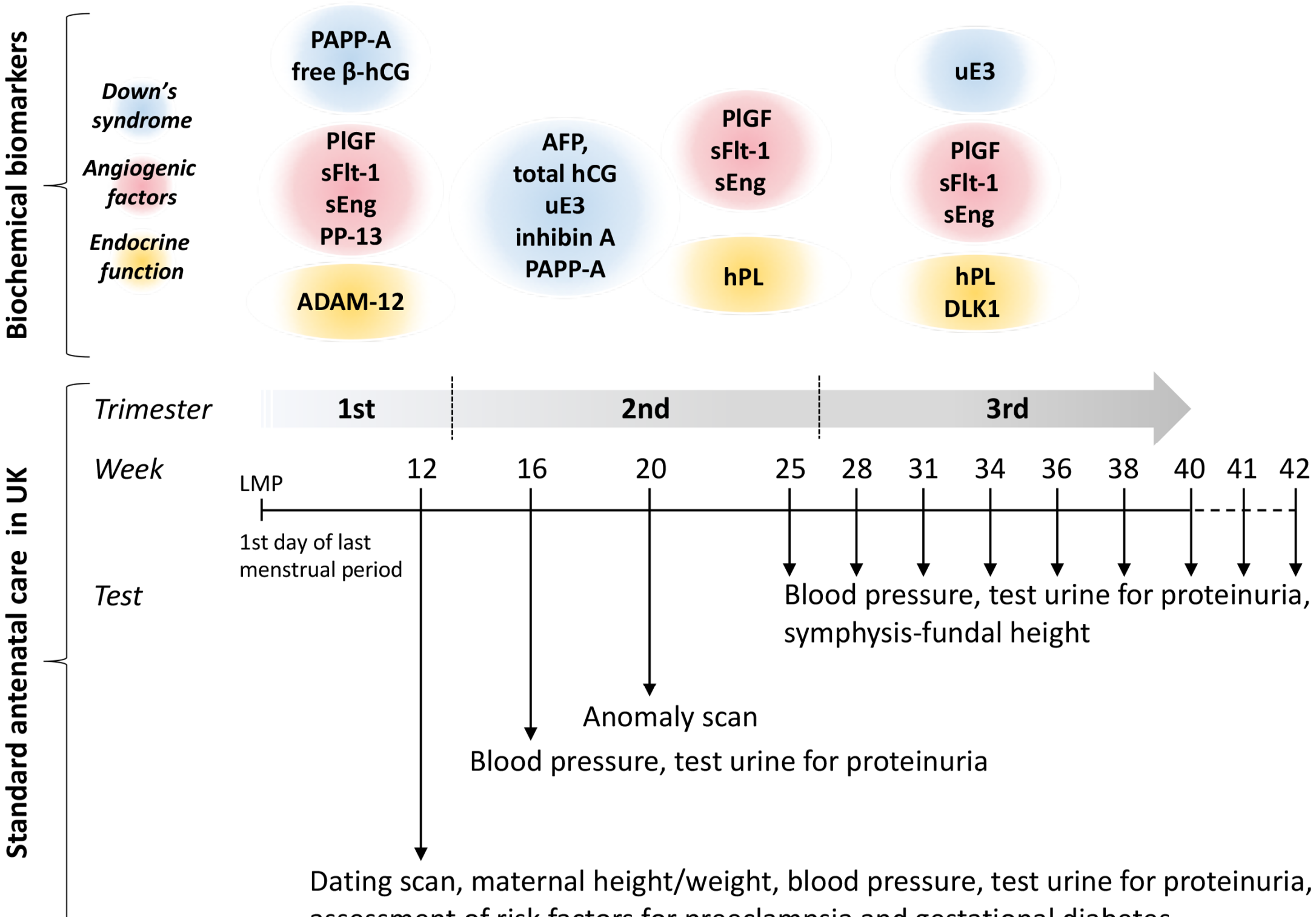
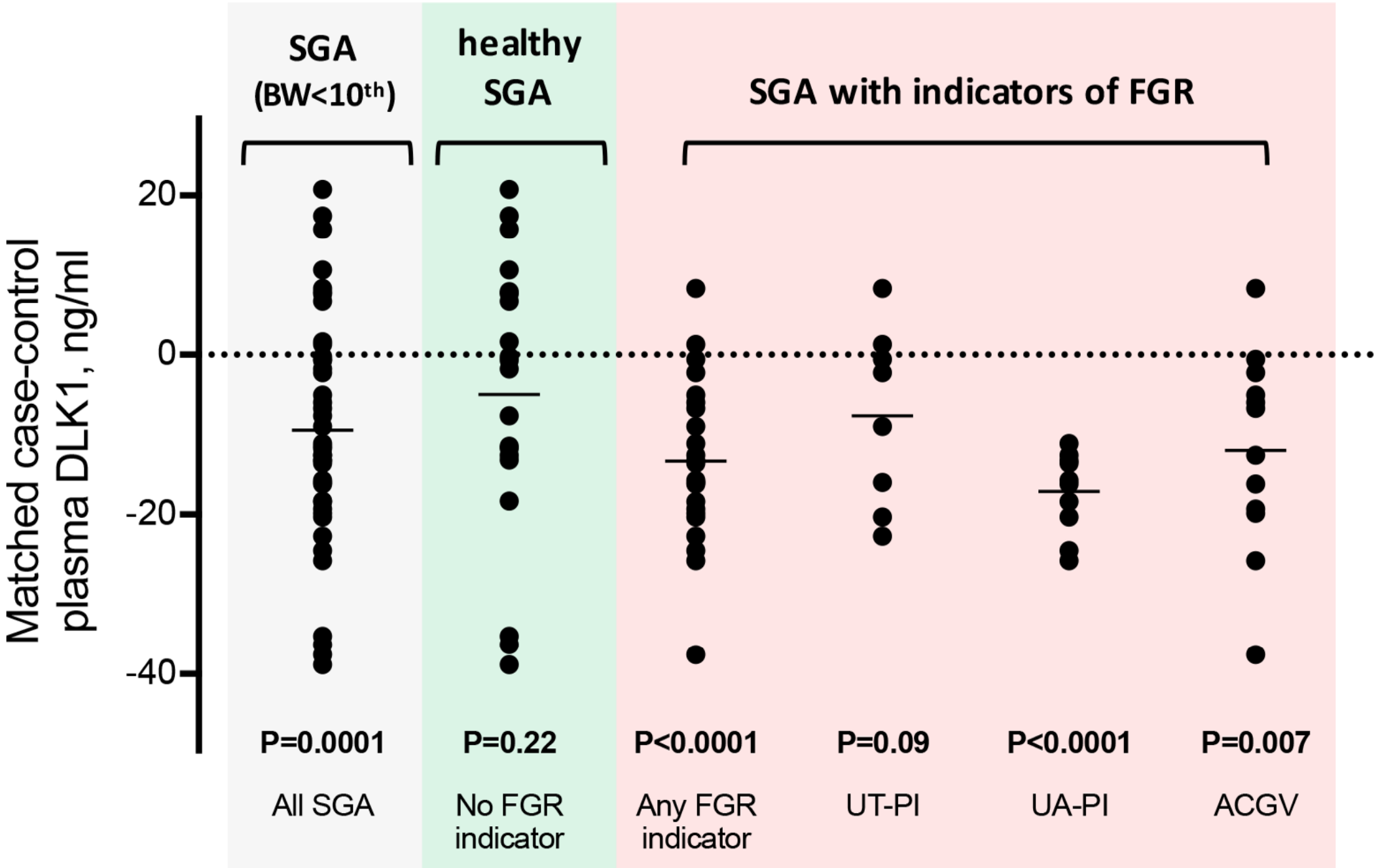

Biochemical biomarkers
Down's syndrome
Angiogenic factors
Endocrine function
PAPP-A
free β-hCG
PlGF
sFlt-1
sEng
PP-13
ADAM-12
AFP,
total hCG
uE3
inhibin A
PAPP-A
PlGF
sFlt-1
sEng
hPL
uE3
PlGF
sFlt-1
sEng
hPL
DLK1
Standard antenatal care in UK
Trimester
1st
2nd
3rd
Week
12
16
20
25
28
31
34
36
38
40
41
42
LMP
1st day of last menstrual period
Test
Blood pressure, test urine for proteinuria, symphysis-fundal height
Anomaly scan
Blood pressure, test urine for proteinuria
Dating scan, maternal height/weight, blood pressure, test urine for proteinuria,

Figure 2


SGA (BW<10th)
healthy SGA
SGA with indicators of FGR
Matched case-control plasma DLK1, ng/ml
20
0
-20
-40
P=0.0001
P=0.22
P<0.0001
P=0.09
P<0.0001
P=0.007
All SGA
No FGR indicator
Any FGR indicator
UT-PI
UA-PI
ACGV

STUDIES OF DIAGNOSTIC EFFECTIVENESS

		benefits:
the results of the new test	→	no changes in pregnancy management and outcome; test predictive ability can be evaluated
t the population to screen: *h prior risk* *nknown prior risk (nulliparous)*	→	higher prior odds of disease, hence higher positive predictive value for given positive likelihood ratio
on SGA cases due to FGR: *term SGA* *aternal morbidity* *.g. preeclampsia)* *feto-placental morbidity* *(e.g. abnormal ultrasound scan)*	→	stronger association between true tests of placental dysfunction and true FGR (as opposed to healthy SGA)

MULTI-PARAMETER APPROACH

key methodological considerati
✓ availability of assays/measurem
✓ combination of multiple measur into a single predictive model
✓ over-fitting statistical models (optimistic prediction)
✓ test development and validation different cohorts

STUDIES OF CLINICAL EFFECTIVENESS (randomized controlled trials)

methodological considerations:
arge cohort to address clinically important but rare outcomes
andomize to (1) screen versus no-screen OR (2) randomize een positive to reveal/intervene versus conceal
vailability of a safe intervention