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Screening for fetal growth restriction using fetal biometry combined with maternal biomarkers

Francesca Gaccioli, PhD; Irving L. M. H. Aye, PhD; Ulla Sovio, PhD; D. Stephen Charnock-Jones, PhD; Gordon C. S. Smith, MD, PhD

“Fetal growth restriction” and “small for gestational age”: differences between 2 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 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 (GA) and those with birthweight (BW) <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 (eg, <2500 g, <10th percentile, or <3rd percentile).

Although SGA and FGR are sometimes used interchangeably, the 2 terms

Fetal growth restriction is a major determinant of perinatal morbidity and mortality. Screening for fetal growth restriction 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 United States and United Kingdom, 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 fetal growth restriction 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 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 that are small through fetal growth restriction. As abnormal placentation is thought to be the major cause of fetal growth restriction, 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 uteroplacental circulation. However, another promising approach is to combine ultrasonic suspicion of small-for-gestational-age infant with a blood test indicating placental dysfunction. Thus far, much of the research on maternal serum biomarkers for fetal growth restriction 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. 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 fetal growth restriction. The development of “omic” technologies presents a huge opportunity to identify novel biomarkers for fetal growth restriction. The hope is that when such markers are measured alongside ultrasonic fetal biometry, the combination would have strong predictive power for fetal growth restriction 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 to achieve clinically effective population-based screening.

Key words: A-disintegrin and metalloprotease 12, alpha fetoprotein, biomarker, fetal biometry, fetal death, human chorionic gonadotropin, human placental lactogen, inhibin, models, placenta, placental growth factor, placental protein 13, prediction, pregnancy-associated plasma protein-A, randomized controlled trial, review, screening, small for gestational age, soluble endoglin, soluble fms-like tyrosine kinase-1, stillbirth, study design, ultrasound

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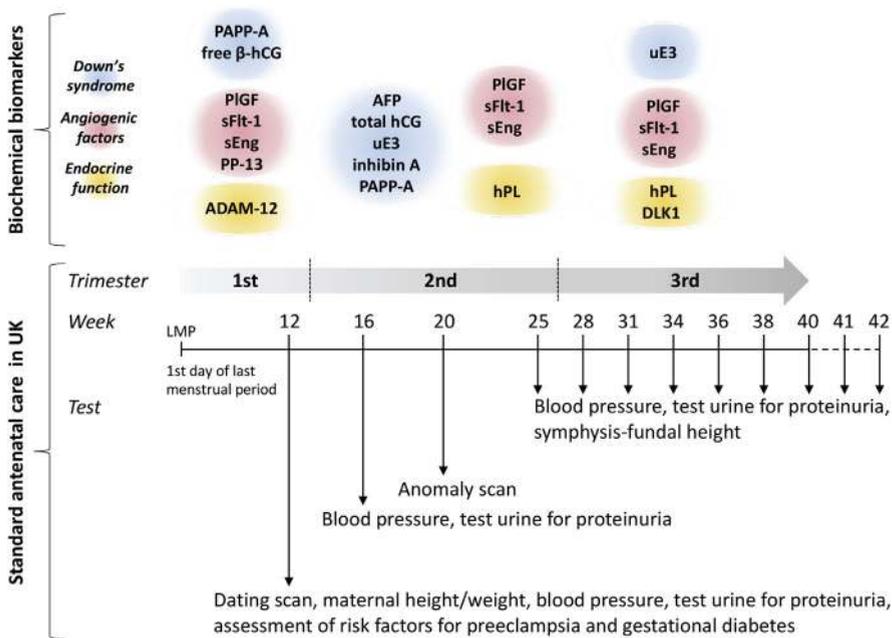
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are distinct, as many SGA infants are constitutionally small and healthy. Hence, clinical research on screening for FGR has to address 2 main issues: (1) the sensitive and specific detection of SGA fetuses, and (2) the ability to discriminate between FGR and healthy SGA. The

causes of FGR can be broadly categorized into maternal (eg, pregnancy-associated hypertensive diseases, autoimmune disease, poor nutrition, substance abuse, and teratogen exposure),⁴⁻⁶ fetal (eg, multiple gestations, infections, genetic and structural disorders),^{7,8} or placental.

FIGURE 1
Standard antenatal care in United Kingdom (UK) and biochemical markers measured throughout pregnancy



Measurement of pregnancy biomarkers in relation to UK antenatal care schedule. Biomarkers measured in clinical and research settings during pregnancy are plotted on a time scale representing standard antenatal care for nulliparous women in UK, which includes 10 routine midwife visits and additional visits for women delivering >40 weeks of gestation.

ADAM12, A-disintegrin and metalloprotease 12; AFP, alpha fetoprotein; DLK1, delta-like 1 homolog; hCG, human chorionic gonadotropin; hPL, human placental lactogen; PAPP-A, pregnancy-associated plasma protein A; PIGF, placental growth factor; PP, placental protein; sENG, soluble endoglin; sFLT1, soluble fms-like tyrosine kinase-1; uE3, unconjugated estriol

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It is thought that placental dysfunction accounts for the majority of FGR cases.⁹ Hence, one of 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 United Kingdom and United States, 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 preexisting risk factors, acquired complications of pregnancy, and clinical examination (eg, symphysis-fundal height measurements) (Figure 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 fetoplacental 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 and ductus venosus flow resistance, and cerebroplacental ratio (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 to as placental insufficiency, are thought to be underlying mechanisms of placently-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 and 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

TABLE 1
Maternal circulating biochemical markers for predicting fetal growth restriction

Biomarker	Main function	Changes in maternal circulating levels frequently associated with FGR		
		1st Trimester	2nd Trimester	3rd Trimester
Down syndrome biomarkers				
PAPP-A	<ul style="list-style-type: none"> Protease activity toward IGFBPs Decrease of IGF bioavailability and signaling 	↓	↓	
hCG	<ul style="list-style-type: none"> Maintenance of progesterone secretion from corpus luteum 	↓	↑	
AFP	<ul style="list-style-type: none"> Protein of fetal origin with similar function to albumin in adult Carrier-molecule for several ligands (bilirubin, steroids, and fatty acids) 		↑	
uE3	<ul style="list-style-type: none"> Estrogen agonist 		↓	↓
inhibin A	<ul style="list-style-type: none"> Negative feedback on pituitary follicle-stimulating hormone secretion Preventing ovulation during pregnancy 		↑	
Angiogenic factors				
PlGF	<ul style="list-style-type: none"> Member of VEGF family Proangiogenic factor 	↓	↓	↓
sFLT1	<ul style="list-style-type: none"> Decrease of PlGF and VEGF bioavailability and signaling 	↑, ↓	↓, =	↑
sFLT1:PlGF ratio		↑	↑	↑
sENG	<ul style="list-style-type: none"> Decrease of TGF-β1 and TGF-β3 bioavailability and signaling 	↑	↑	↑
PP-13	<ul style="list-style-type: none"> Promoting trophoblast invasion and spiral artery remodeling 	↓, =		
Hormonal factors				
ADAM12	<ul style="list-style-type: none"> Protease activity toward IGFBPs Decrease of IGF bioavailability and signaling 	↓		
hPL	<ul style="list-style-type: none"> Induction of maternal insulin resistance and lipolysis Induction of mammary glands development and milk production 		↓	↓
DLK1	<ul style="list-style-type: none"> Adipose tissue homeostasis Maternal metabolic adaptation to pregnancy 			↓

ADAM12, A-disintegrin and metalloprotease 12; AFP, alpha fetoprotein; DLK1, delta-like 1 homolog; FGR, fetal growth restriction; hCG, human chorionic gonadotropin; hPL, human placental lactogen; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; PAPP-A, pregnancy-associated plasma protein A; PlGF, placental growth factor; PP, placental protein; sENG, soluble endoglin; sFLT1, soluble fms-like tyrosine kinase-1; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; uE3, unconjugated estriol.

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of first-trimester biomarkers derived from the placenta, which were evaluated in screening studies for aneuploidies.

Down syndrome markers: PAPP-A and β -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 (β -hCG) are

both associated with the risk of Down syndrome.²⁵ PAPP-A determines the availability of the insulin-like growth factors, key pregnancy growth hormones, as it is a protease that acts on insulin-like growth factor binding proteins. A causal role for PAPP-A in controlling fetal growth has been established in the PAPP-A knockout 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 United Kingdom, 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 $f\beta$ -hCG is measured.²⁵ In general, extremes of $f\beta$ -hCG in the first trimester are less strongly associated with adverse outcome than low PAPP-A.^{27,30-33}

Other placental markers: PIGF, 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 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 proangiogenic or antiangiogenic 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 (PIGF) is a proangiogenic 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 antiangiogenic 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 (MoMs) 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:PIGF 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 11-14 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 2 main reasons: (1) the availability of data collected during screening studies for the identification of aneuploidies and birth defects during the second trimester; and (2) 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 and includes measurements of alpha fetoprotein (AFP), hCG (intact and/or its β -subunit), unconjugated estriol (uE3), and inhibin A.⁵² These factors may also provide information on placental permeability (AFP) and endocrine activity (hCG, uE3, and inhibin A). Hence, many studies have addressed their ability

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, $f\beta$ -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 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 uE3 alone or in combination with AFP and/or hCG were associated with SGA (BW <5th percentile).^{54,60}

Other placental markers: sFLT1, PIGF, sENG, 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 reported that maternal levels of sFLT1 and PAPP-A were decreased in pregnancies with SGA without maternal hypertension, whereas PAPP-A and PIGF were decreased in pregnancies with SGA and maternal hypertension.⁶¹ Multi-center studies conducted by Prof Kypros Nicolaides have assessed the predictive associations of a number of circulating biomarkers at different stages in the second and third trimesters. At 19-24 weeks of gestation, maternal PIGF was lower and AFP was higher in women who subsequently delivered a SGA infant preterm. Term SGA was associated with lower maternal levels of PIGF, sFLT1, and PAPP-A.⁵³ When measured at 30-34 weeks low PIGF and high sFLT1 were associated with delivery of a SGA infant⁶² and the associations were stronger for preterm SGA. At 35-37 weeks PIGF 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 intrauterine fetal death.⁶⁴

Mothers with a reduced rate of decrease of the antiangiogenic 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 a SGA neonate.⁴¹ Maternal serum levels of the hormones human placental lactogen 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 uteroplacental 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 in the clinically useful range, with positive likelihood ratios of 5-15.⁶⁸ Moreover, these strong associations were only observed for the subtypes 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 PIGF in women who were identified as clinically small for dates between 24-37 weeks of gestation. While the study demonstrated that median PIGF concentrations were lower in SGA pregnancies,⁷⁰ these authors reported that the combination of EFW and of PIGF had only modest test performance. However, it should be borne in mind that many SGA infants are healthy, ie, they are constitutionally small. It would not be expected that PIGF, a test for pathology, would be strongly associated with the delivery of a healthy small infant. A study by Valino et al⁶³ reported that placental biomarkers measured at 35-37 weeks performed poorly as a screening test for perinatal morbidity. 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 ongoing. However, we have published a preliminary report that the combination of ultrasonic EFW <10th percentile and an elevated sFLT1:PIGF ratio at 36 weeks of gestation 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 that 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 2](#)).

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 2 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 high-risk and nulliparous women. In the latter group the key marker of risk in pregnancy, ie 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

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 syndrome biomarkers						
PAPP-A	Stillbirth	3.3 (1.8–6.0)	0.9 (0.8–1.0)	15 (8–26)	95 (95–96)	21,158 ^{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)	186,802 ^{60,100–106}
uE3	Stillbirth	4.0 (3.0–5.3)	0.9 (0.8–0.9)	15 (11–20)	96 (96–96)	58,417 ^{58,60,107}
Inhibin A	Stillbirth	6.1 (4.0–9.3)	0.8 (0.8–0.9)	19 (12–27)	97 (97–97)	33,145 ⁵⁸
PAPP-A + maternal characteristics	Stillbirth	3.5 (2.8–4.4)	0.7 (0.6–0.8)	35 (28–43)	90 (90–90)	33,452 ¹⁰⁸
+ US markers	SGA-related stillbirth	4.4 (3.2–5.9)	0.6 (0.5–0.8)	44 (31–57)	90 (90–90)	33,365 ¹⁰⁸
Inhibin A + maternal characteristics	Stillbirth	4.1 (2.8–6.0)	0.8 (0.8–0.9)	20 (14–29)	95 (95–95)	35,253 ¹⁰⁹
Angiogenic factors						
PIGF	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:PIGF 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, and 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

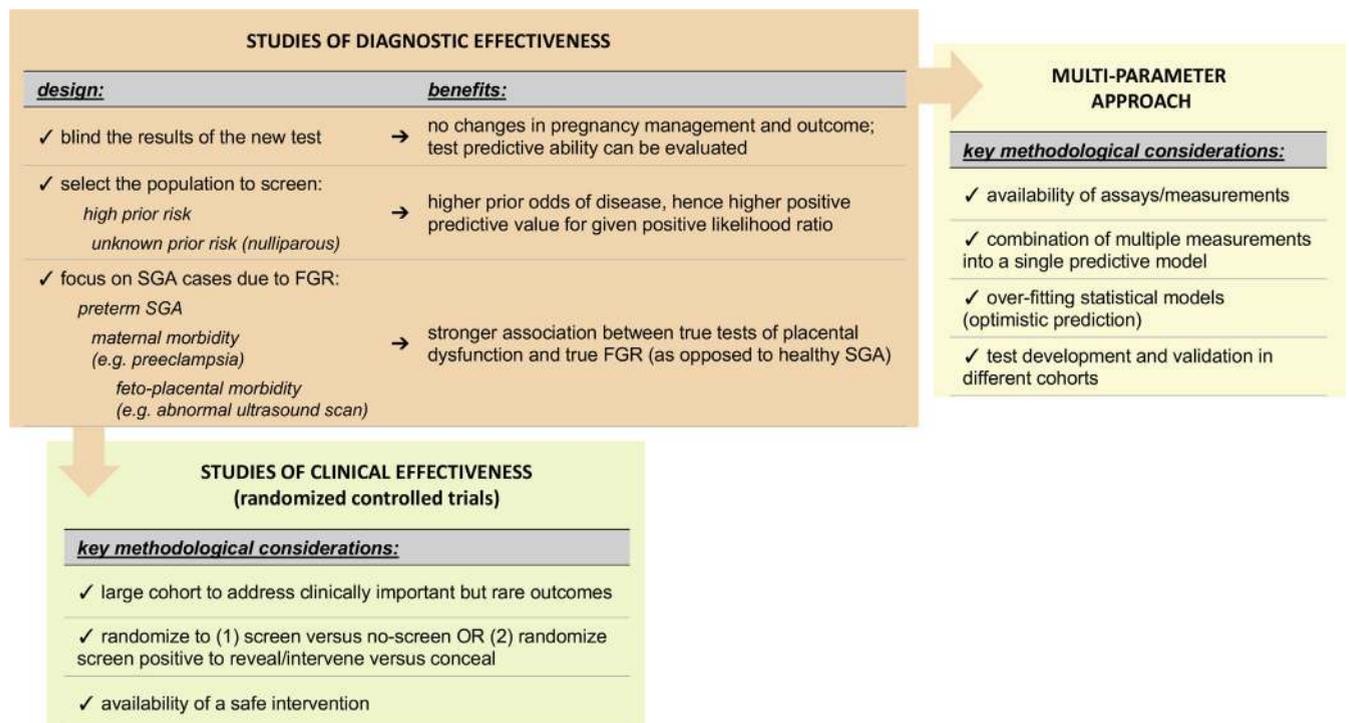
Based on Conde-Agudelo et al.^{68,69}

ADAM12, A-disintegrin and metalloprotease 12; AFP, alpha fetoprotein; BW, birthweight; CI, confidence interval; FGR, fetal growth restriction; hCG, human chorionic gonadotropin; IFN, interferon; IGFBP, insulin-like growth factor binding protein; IL-1Ra, interleukin-1 receptor antagonist; LR, likelihood ratio; PAPP-A, pregnancy-associated plasma protein A; PIGF, placental growth factor; PP, placental protein; sENG, soluble endoglin; sFLT1, soluble fms-like tyrosine kinase-1; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; UA, umbilical artery; uE3, unconjugated estriol; US, ultrasound; UT, uterine artery; VEGF, vascular endothelial growth factor.

Gaccioli. Screening for fetal growth disorders. Am J Obstet Gynecol 2017.

FIGURE 2

Summary of key points to improve fetal growth restriction screening



FGR, fetal growth restriction; SGA, small for gestational age.

Gaccioli. Screening for fetal growth disorders. *Am J Obstet Gynecol* 2017.

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 previa by ultrasound scan. However, given that screening for SGA in France actually seemed to result in net harm¹¹ 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 of diagnostic effectiveness is justified. Otherwise, it is very hard to see how progress on screening using new diagnostic tests in pregnancy can be achieved.

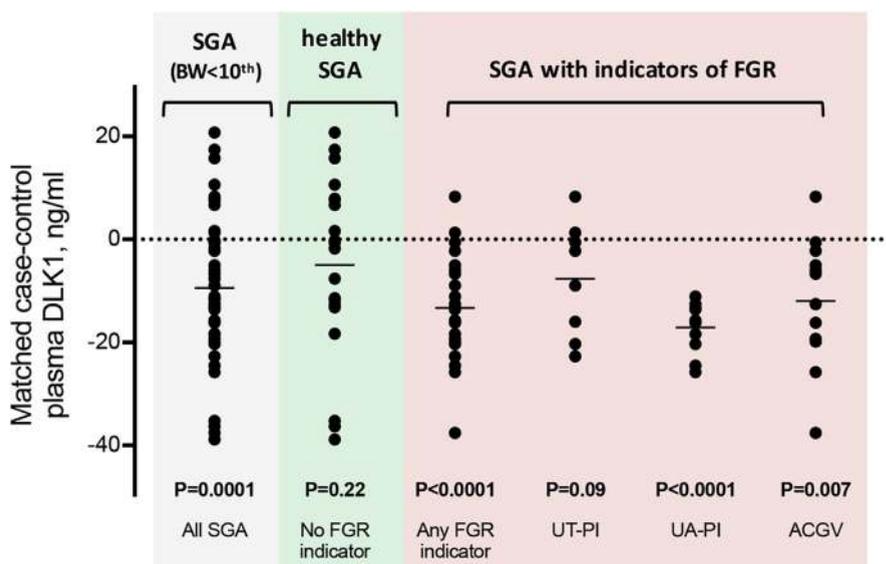
Classification of SGA. SGA is defined on the basis of an arbitrary threshold of BW percentile. It follows that many infants born SGA were healthy. Using unqualified SGA as an outcome may, therefore, lead to weaker associations with an effective screening test than would have been obtained if the analysis 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 that 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 noncanonical NOTCH1 ligand delta-like 1 homolog (DLK1).⁷⁴ Using

FIGURE 3

Low maternal delta-like 1 homolog (DLK1) levels are associated with fetal growth restriction (FGR)



Maternal circulating DLK1 and fetal growth. Scatterplot of differences in maternal plasma concentrations of DLK1. DLK1 concentration was measured in maternal plasma from pregnancies with small for gestational age (SGA) (birthweight [BW] < 10th centile) and normally grown infants ($n = 43$ matched pairs; matching was based on maternal age, body mass index, smoking status, fetal sex, and mode of delivery). FGR indicators are: uterine artery (UT)-pulsatility index (PI) in 10th decile at 20 weeks of gestational age ($n = 8$ pairs); umbilical artery (UA)-PI in 10th decile at 36 weeks ($n = 10$ pairs); abdominal circumference growth velocity (ACGV) in 1st decile at 20-36 weeks ($n = 12$ pairs). Horizontal bars represent means of differences. Modified from Cleaton et al.⁷⁴

Gaccioli. Screening for fetal growth disorders. *Am J Obstet Gynecol* 2017.

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 ≥ 1 ultrasonic indicators of FGR, and levels were not different in SGA without such markers (Figure 3). 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

demonstrated a correlation between methylation in the DLK1 domain and BW.⁷⁶

Developing multiparameter 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 multiparameter 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 overfitting statistical models leading to overly optimistic prediction. These issues necessitate the use of statistical methods that account for optimism⁷⁷ and underline the value of studies that 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 that might yield hundreds or thousands of data points per patient. These methods carry a high risk of generating overly optimistic prediction and they require a rigorous methodological approach. Nevertheless, next-generation sequencing 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 next-generation sequencing techniques to the biomarkers discovery field has been maternal circulating cell-free fetal DNA measurement for Down syndrome and trisomy 18 screening.^{80,81} Elevated maternal cell-free fetal 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 (messenger RNAs [mRNAs], microRNAs [miRNAs], and long non-coding RNAs [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 26-30 weeks of gestation, 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 data sets. 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 Medicine⁹¹ may help standardization 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 patient. Hence, a further approach to improving screening for FGR would be to increase the efforts to identify the biological pathways underlying placental

dysfunction 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 that 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 intrauterine fetal death. However, this outcome affects about 4 per 1000 pregnancies. Even the highest estimates indicate that 50% of stillbirths might be due to FGR.⁹² Other studies suggest that the proportion may be lower.⁹³ 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 nonlethal 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 cesarean delivery 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 cesarean delivery 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 BW with anthropometric measures that 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 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 hypoglycemia. Finally, the coexistence 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 (Figure 3).^{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 vs 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 repurposing of existing drugs⁹⁵ and gene therapy.⁹⁶ ■

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