DOI: 10.1111/1471-0528.12172
www.bjog.org

Systematic review

Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis

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Accepted 19 December 2012. Published Online 11 February 2013.

Background Several biomarkers for predicting intrauterine growth restriction (IUGR) have been proposed in recent years. However, the predictive performance of these biomarkers has not been systematically evaluated.

Objective To determine the predictive accuracy of novel biomarkers for IUGR in women with singleton gestations.

Search strategy Electronic databases, reference list checking and conference proceedings.

Selection criteria Observational studies that evaluated the accuracy of novel biomarkers proposed for predicting IUGR.

Data collection and analysis Data were extracted on characteristics, quality and predictive accuracy from each study to construct 2 × 2 tables. Summary receiver operating characteristic curves, sensitivities, specificities and likelihood ratios (LRs) were generated.

Main results A total of 53 studies, including 39 974 women and evaluating 37 novel biomarkers, fulfilled the inclusion criteria. Overall, the predictive accuracy of angiogenic factors for IUGR was minimal (median pooled positive and negative LRs of 1.7, range 1.0–19.8; and 0.8, range 0.0–1.0, respectively). Two small case–control studies reported high predictive values for placental growth factor and angiopoietin-2 only when IUGR was defined as birthweight centile with clinical or pathological evidence of fetal growth restriction. Biomarkers related to endothelial function/oxidative stress, placental protein/hormone, and others such as serum levels of vitamin D, urinary albumin : creatinine ratio, thyroid function tests and metabolomic profile had low predictive accuracy.

Conclusions None of the novel biomarkers evaluated in this review are sufficiently accurate to recommend their use as predictors of IUGR in routine clinical practice. However, the use of biomarkers in combination with biophysical parameters and maternal characteristics could be more useful and merits further research.

Keywords Biomarker, intrauterine growth restriction, meta-analysis, prediction, systematic review.

Please cite this paper as: Conde-Agudelo A, Papageorghiou A, Kennedy S, Villar J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. BJOG 2013;120:681–694.

Introduction

Intrauterine growth restriction (IUGR) is defined as a failure of the fetus to achieve its optimal growth potential[1] and constitutes a major clinical and public health problem, mainly in the developing world.[2] It is considered a heterogeneous syndrome associated with hypertensive disorders of pregnancy, smoking, infection, undernutrition and unexplained factors.[3] IUGR fetuses are at greater risk of perinatal death, birth hypoxia, neonatal complications, impaired neurodevelopment and manifestations of the metabolic syndrome in adult life such as type 2 diabetes, coronary heart disease and hypertension.[4–8]

Although no preventive interventions are available at present, being able to predict IUGR reliably would be valuable because: (i) it would identify fetuses that require early referral to secondary care and closer surveillance (ii) identifying at-risk fetuses would allow specific preventive interventions to be tested (iii) studies of predictors of IUGR could improve our understanding of the biological and

pathological mechanisms that cause fetal growth restriction, leading potentially to better interventions (iv) identifying low-risk fetuses would avoid the use of unnecessary interventions, and (v) accurate prediction, and prevention, of IUGR could be an early stage in a public health strategy that aims to avoid the adult consequences of fetal growth restriction.

In the last decade, several biomarkers have been proposed as predictors of IUGR.[9–11] Some have been assessed systematically, including five serum metabolites for prenatal screening of aneuploidy and open neural tube defects, as individual[12] or combined[13] biomarkers. However, to our knowledge, the predictive performance of other biomarkers for IUGR has not been adequately evaluated in a systematic manner.

Therefore, the objective of this systematic review and meta-analysis was to identify, and determine the accuracy of, biomarkers proposed from the year 2000 onwards for the prediction of IUGR in women with singleton gestations.

Methods

The systematic review was conducted following a prospectively prepared protocol and reported using the checklist recommended by the STARD initiative for reporting systematic reviews of diagnostic test accuracy.[14]

Literature search

An initial search was performed in PubMed, Embase, Cinahl, Lilacs and Medion (all from inception to 31 July 2012), and Google Scholar using a combination of keywords and text words related to *biomarkers* ('biomarker', 'marker'), *prediction* ('prediction', 'screening', 'diagnosis', 'accuracy', 'sensitivity', 'specificity', 'likelihood ratio'), and *IUGR* ('intrauterine growth restriction', 'intrauterine growth retardation', 'fetal growth restriction', 'fetal growth retardation', 'impaired fetal growth', 'small for gestational age', 'small for date', 'small for gestation'). In the initial search, we chose those biomarkers proposed from the year 2000 onwards for the prediction of IUGR. A further computerised search was conducted using keywords and text words for each of the biomarkers identified in the initial search and keywords and text words for IUGR described previously. Congress proceedings of international society meetings of maternal and fetal, and reproductive medicine, as well as international meetings on fetal growth, reference lists of identified studies, textbooks, previously published systematic reviews, and review articles were also searched. In addition, we contacted investigators involved in the field to locate unpublished studies. Language restrictions were not applied.

Study selection

Studies were included if: (i) they were cohort, cross-sectional or case–control studies that evaluated the accuracy of biomarkers for predicting IUGR or small-for-gestational-age (SGA) infants in women with singleton gestations at any level of risk (ii) the biological samples were collected before the clinical onset of IUGR and, if possible, before 30 weeks of gestation; and (iii) they allowed construction of 2 × 2 tables of accuracy. Studies were excluded if: (i) they were case series or reports, editorials, comments or reviews without original data (ii) biomarker data were reported only as mean or median values (iii) they did not report accuracy test estimates or reported insufficient data to construct a 2 × 2 table; or (iv) biomarkers were evaluated in women with suspected IUGR/SGA fetuses, pre-eclampsia, diabetes or multiple gestation. If a study based its results on mixed pregnancies (singleton and multiple), it was not considered for inclusion in the review unless singleton pregnancy data were extractable separately,

We excluded five serum biomarkers used in screening for aneuploidy and open neural tube defects (human chorionic gonadotrophin, unconjugated estriol, inhibin A, pregnancy-associated plasma protein A and alphafetoprotein) because a comprehensive systematic review published in 2008 found that those biomarkers had low predictive accuracy for SGA.[12] These findings have been confirmed in large studies published after the meta-analysis.[15–20] Uterine artery Doppler ultrasonography was also excluded because it is not considered a biomarker. Genetic biomarkers were not included in this review because they require a different methodological approach and meta-analytic techniques.

One reviewer (AC-A) screened titles and abstracts of all identified citations and selected potentially eligible studies. Then, all full-text articles were assessed by the same reviewer for inclusion and data extraction, and a 10% sample of the papers was examined by a second independent reviewer (JV). We resolved any disagreements by discussion and consensus. For multiple or duplicate publication of the same data set, we included only the most recent or complete study.

Reference standard

Acceptable reference standards for IUGR are traditionally based on birthweight below the tenth, fifth or third centile for gestational age or birthweight at least two standard deviation (SD) units less than the mean for gestational age regardless of birthweight reference or standard used. SGA is commonly defined as a birthweight below the tenth centile for gestational age. In the majority of situations the term refers to size rather than growth. Most clinicians and researchers define IUGR as being the same as SGA, which combines both constitutionally small and pathological fetuses.

Based on these principles, we anticipated that a key methodological issue of our review would be the definition of IUGR used in the included studies. The choice of an

appropriate reference standard is very important because estimates of predictive performance of biomarkers are based on the assumption that the test is being compared to a reference standard that is 100% sensitive and specific. However, no reference standard for the diagnosis of IUGR is 100% sensitive or 100% specific.

Since fetal size alone cannot distinguish pathological fetuses from constitutional SGA fetuses, the use of additional, *integrated* indicators of fetal and placental health has been proposed to enhance the accuracy of defining true IUGR.[21] The indicators proposed include amniotic fluid volume, biophysical profile, uterine and umbilical artery Doppler ultrasound, and placental pathology among others. Although an optimal scheme of combining birthweight with indicators of fetal and placental health remains to be determined, we have taken this integrated approach into account to define IUGR in assessing the methodological quality of the studies. Our aim is to identify IUGR subgroups that can be related to specific biomarkers.

Study quality assessment

The methodological quality of the included studies was assessed by at least one reviewer using a modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.[22] We evaluated five items believed to be important for the quality of studies evaluating the predictive accuracy of biomarkers for IUGR. Each item was scored as 'yes', 'no' or 'unclear'. The items evaluated and their interpretation, were as follows:

1 *Adequate study design*—'yes': pregnant women consecutively or randomly selected and prospective cohort design; 'no': convenience sampling (arbitrary recruitment or nonconsecutive recruitment) or case–control/retrospective design.
2 *Adequate selection criteria*—'yes': inclusion of 'idiopathic' SGA neonates (not associated with conditions such as pre-eclampsia, congenital or chromosomal anomalies, infection, chronic hypertension, diabetes, thrombophilia, lupus erythematosus or substance abuse, among others); 'no': inclusion of SGA neonates associated with any of these conditions.
3 *Appropriate reference standard*—'yes': birthweight below fifth centile or birthweight below tenth centile with additional clinical or pathological evidence of fetal growth restriction, e.g. abnormal umbilical or uterine artery Doppler, oligohydramnios, or abnormal placental pathology; 'no': birthweight at fifth centile or above without additional clinical or pathological evidence of fetal growth restriction.
4 *Adequate description of the test*—'yes': inclusion in the report of a detailed description of the execution of the test including assay used, manufacturer of assay, and gestational age at which the sample was collected; 'no': absence of this information in the report.
5 *Blinding*—'yes': masking of laboratory technicians to pregnancy outcomes and clinicians to the test results; 'no': unmasking of laboratory technicians to pregnancy outcomes or clinicians to the test results.If there was insufficient information available to make a judgment about these items, then they were scored as 'unclear'. We did not calculate a summary quality score for each study because the interpretation of such summary scores is problematic and potentially misleading.[23]

Data extraction

Data were extracted from each article by means of a standardised and pilot-tested data collection form. The following information was extracted from each article: study characteristics (design, prospective or retrospective data collection, recruitment of women, blinding of test results, completeness of follow up and reporting of withdrawals); participants (inclusion and exclusion criteria, sample size, demographic characteristics, and country and date of publication); description of the biomarker test (gestational age at sampling, frequency of test, sampling site, analytical method used and cut-off level); reference standard used (IUGR definition); and use of preventive/therapeutic interventions for IUGR during pregnancy.

For each study, for all cut-off values defining abnormality, and for several IUGR categories (birthweight below tenth centile, birthweight below fifth centile, birthweight below tenth or fifth centile with additional clinical or pathological evidence of fetal growth restriction, and IUGR requiring delivery before 34 weeks of gestation), we extracted numbers of true-positive, false-positive, true-negative, and false-negative results. When predictive accuracy data were not available, we recalculated them from the reported results including scatter-plot graphs. In studies where serial biomarker samples were collected, we extracted data separately for each gestational period. Ten authors were contacted in an attempt to obtain additional data.

Statistical analysis

Data extracted from each study were arranged in 2×2 contingency tables. When these tables contained cells for which the value was 0, we added 0.5 to each cell to allow calculations to be performed.[24] Sensitivity and specificity were calculated for each biomarker and for all reported cut-off values and outcomes. For each biomarker we planned to plot sensitivities and specificities in receiver operating characteristic (ROC) plots according to gestational age at testing (<20 and ≥ 20 weeks of gestation) and IUGR definition (below tenth centile and below fifth centile). We then constructed summary ROC curves regardless of cut-offs used by means of a bivariate random-effects

approach[25] and calculated area under the summary ROC curves with their corresponding 95% confidence intervals (CIs).[26] This measure allows for comparison of the predictive accuracy of the test for different outcomes. Two-sided $P < 0.05$ was considered to be statistically significant.

Meta-analyses were performed using subgroups of studies with similar characteristics such as gestational age at testing and outcome measures to minimise clinical heterogeneity. Pooled estimates of sensitivity and specificity with 95% CIs were calculated using a bivariate, random-effects, meta-regression model.[25] Thereafter, we derived likelihood ratios (LRs) with 95% CIs from the pooled sensitivities and specificities for each outcome reported.[27] LRs indicate by how much a given test result raises or lowers the probability of having the condition and so allow interpretation of the results for use in clinical practice.[28] It has been suggested that LRs >10 for a positive test result and LRs <0.1 for a negative test result provide convincing predictive evidence. Moderate prediction can be achieved with LRs values of 5–10 and 0.1–0.2 whereas those <5 and >0.2 provide only minimal prediction.[28]

For each biomarker, we planned to calculate the post-test probability of IUGR by using LRs generated from meta-analyses or individual studies for positive and negative biomarker test results at a range of different pretest probabilities of IUGR (5%, 10% and 15%).[28]

Heterogeneity of the results among studies was investigated through visual examination of forest plots of sensitivities and specificities, and ROC plots. In addition, the quantity I^2 was used to assess statistical heterogeneity. I^2 values $\geq 50\%$ indicated a substantial level of heterogeneity.[29] We explored potential sources of heterogeneity by performing meta-regression analysis of subgroups defined a priori (study design, sample size, study quality, IUGR definition used and gestational age at testing).[30] Publication and related biases were assessed visually by examining the symmetry of funnel plots and statistically by using the Egger's regression test.[31] $P < 0.1$ indicated significant asymmetry.

The bivariate models were fitted using the NLMIXED procedure (SAS 9.1 for Windows; SAS Institute, Inc., Cary, NC, USA). Summary ROC curves were constructed using the RevMan (Review Manager) 5.1.7. The remaining analyses were performed using SPSS version 15.0 (SPSS Inc, Chicago, IL, USA).

Results

Figure 1 summarises the processes used to identify and select studies. The searches produced 5383 citations, of which 215 were considered relevant. In all, 162 studies were excluded, the main reasons being the lack of data to construct 2 × 2 tables (43%) and not being a test accuracy study (25%). Fifty-three studies including 39 974 women, evaluating a total of 37 novel biomarkers, met the inclusion criteria.[32–84] Box 1 lists novel biomarkers for predicting IUGR that were identified in this review.

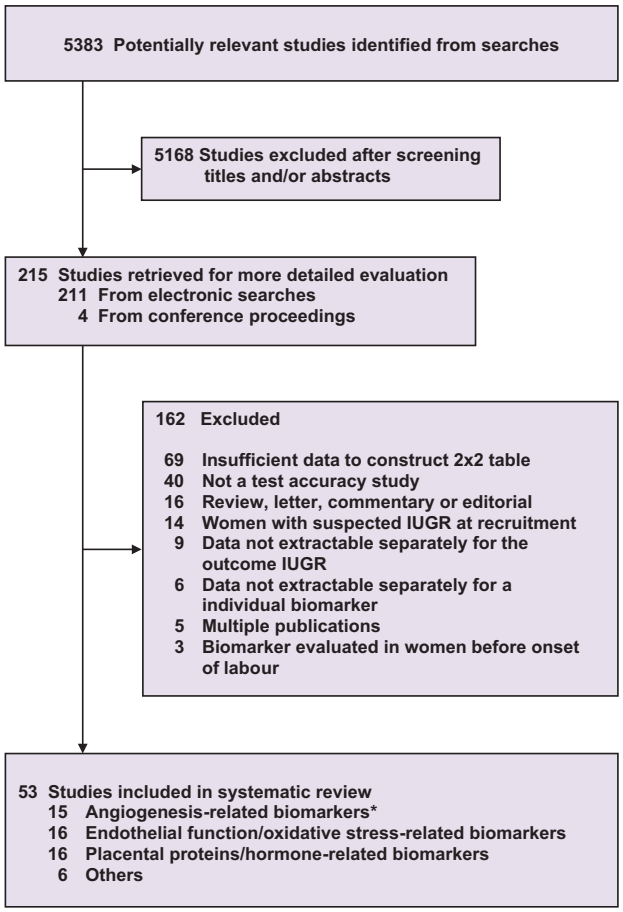

Figure 1. IUGR, intrauterine growth restriction. *One study reported on both angiogenesis- and placental proteins-related biomarkers.

Table S1 details the individual characteristics of the included studies (see Supplementary material). Thirty studies (57%) were performed in European countries and ten (19%) in North America. Only seven studies (13%) were conducted in developing countries. There were 31 case–control and 22 cohort studies. The sample size in the cohort studies ranged from 63[38] to 6016[62] (median, 485) women. The number of case participants enrolled in case–control studies ranged from 8[77] to 296[67] and the corresponding number of controls ranged from 8[77] to 3592.[82] Seventeen case–control studies had ≤ 40 IUGR cases. In cohort studies, the incidence rates for IUGR below the tenth centile ranged from 2.7 to 17.5% (median, 8.7%); the range for IUGR below the fifth centile was 4.9 to 11.2% (median, 5.1%). Two cohort studies evaluated biomarkers in populations at high risk for IUGR.[38,40] Biomarkers in the following biological samples were included in the

Box 1 Novel biomarkers for predicting IUGR identified in the literature

1 Angiogenesis-related biomarkers
- Placental growth factor
- Soluble fms-like tyrosine kinase-1
- Soluble endoglin
- Vascular endothelial growth factor
- Angiopoietin

2 Endothelial function/oxidative stress-related biomarkers
- Homocysteine
- Leptin
- Asymmetric dimethylarginine
- Soluble vascular cell adhesion molecule-1
- Soluble intercellular adhesion molecule-1
- Isoprostanes
- 8-oxo-7,8-dihydro-2′-deoxyguanosine
- Fibronectin
- Lactate dehydrogenase
- Pentraxin 3
- Interferon-γ
- Interleukin-1 receptor antagonist
- Interleukin-12
- Eotaxin
- Regulated on activation, normal T-cell expressed and secreted (RANTES)
- C-reactive protein
- Folate

3 Placental proteins/hormone-related biomarkers
- Insulin-like growth factor binding protein-1 and -3
- A disintegrin and metalloprotease-12
- Placental protein-13
- Activin A
- Placental growth hormone
- Pregnancy-specific β-1-glycoprotein
- Annexin A5
- Hepatocyte growth factor

4 Others
- Urinary albumin:creatinine ratio
- Vitamin D
- Thyroid function tests (thyroid-stimulating hormone, free thyroxine, free triiodothyronine)
- Metabolomics
- Genetic biomarkers

review: serum or plasma (46 studies), amniotic fluid (four studies) and urine (three studies). Forty-five studies evaluated biomarkers at $\leq$22 weeks and the remaining eight at 23–35 weeks of gestation. Four studies evaluated biomarkers serially in two gestational periods. The definition of IUGR used birthweight below tenth centile in 27 studies; below fifth centile in 18 studies; below third centile in three studies; below tenth, fifth and third centiles in one study; below tenth and third centiles in one study; $\leq$2 SD of the mean in two studies; and was unreported in one study.

The quality assessment of the included studies is shown in Figure 2. Only three studies (6%) fulfilled all five criteria, 12 (23%) fulfilled four criteria and the remaining 37 (71%) fulfilled three or fewer criteria. The most common shortcomings were in study design and the reference standard used. Only six studies used an appropriate reference standard that included birthweight centile with additional clinical or pathological evidence of fetal growth restriction.[38,39,45,48,53,60] Moreover, information on blinding and selection criteria was unclear in 30 and 25 studies, respectively.

Angiogenesis-related biomarkers

Thirteen studies reported data on placental growth factor (PlGF),[32–35,37,38,40–46] three on soluble fms-like tyrosine kinase-1,[38,40,43] two on soluble endoglin,[41,43] and one each on vascular endothelial growth factor[36] and angiopoietin-2.[39] Figure 3 shows the summary ROC curves of PlGF for predicting IUGR. Overall, PlGF had a low predictive accuracy for IUGR (area under the summary ROC curve 0.66, 95% CI 0.44–0.87; Figure 3A). Summary ROC curves of PlGF for predicting IUGR according to gestational age at testing and birthweight centile are depicted in Figure 3B. The greatest area under the summary ROC curve was for PlGF measured at $\geq$20 weeks of gestation for predicting IUGR (0.68, 95% CI 0.29–1.00) followed by PlGF for predicting IUGR below the tenth centile (0.67, 95% CI 0.58–0.76) and fifth centile (0.64, 95% CI 0.03–1.00), and PlGF measured at <20 weeks of gestation (0.58, 95% CI 0.38–0.79), although the differences were not statistically significant. Overall, the predictive accuracy of angiogenic factors for IUGR was minimal (median pooled positive and negative LRs of 1.7, range 1.0–19.8, and 0.8, range 0.0–1.0, respectively; Table 1). One small case–control study (nine cases/79 controls), which used a definition of IUGR based on birthweight centile and additional clinical (abnormal Doppler ultrasound or oligohydramnios) and pathological (abnormal placental pathology) evidence of fetal growth restriction, reported a sensitivity of 100% and a specificity of 95% for PlGF (positive and negative LRs of 19.8 and 0.0, respectively).[45] Another, similar, small case–control study (21 cases/23 controls) using a definition of IUGR based on birthweight below the tenth centile with abnormal umbilical artery Doppler ultrasound reported a sensitivity of 92% and a specificity of 78% for angiopoietin-2 (positive and negative LRs of 4.3 and 0.1, respectively).[39]

Endothelial function/oxidative stress-related biomarkers

Five studies evaluated homocysteine,[47–51] two each 8-oxo-7,8 dihydro-2-deoxyguanosine (8-OHdG)[56,57] and isoprostanes,[55,56] and nine several other inflammatory biomarkers, including leptin,[52] asymmetric dimethylarginine,[53] endothelial cell adhesion molecules,[54] fibronectin,[58] lactate dehydrogenase,[59] pentraxin 3,[60] cytokines,[61] C-reactive protein[62] and folate.[51] Homocysteine, 8-OHdG and isoprostanes were tested at <20 weeks of gestation in all included

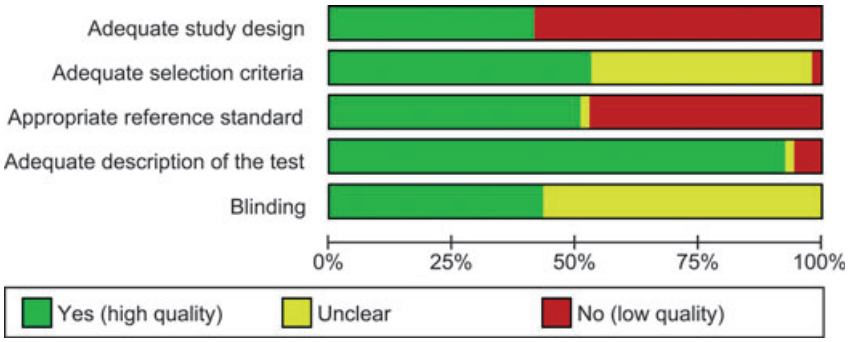

Figure 2. Methodological quality of studies included in the systematic review.

studies. Overall, the predictive accuracy of endothelial function/oxidative stress-related biomarkers for IUGR was minimal (median positive and negative LRs of 2.0, range 0.8–19.2, and 0.8, range 0.0–1.1, respectively; Table 2). Two studies showed high positive LRs, one for endothelial cell adhesion molecules[54] (9.0–19.2) and the other for fibronectin[58] (13.3), but at the expense of poor negative LRs (0.5–0.9).

Placental protein/hormone-related biomarkers

Three studies provided data on a disintegrin and metalloprotease (ADAM)-12,[65–67] four on placental protein 13 (PP-13),[68–71] three on pregnancy-specific β-1-glycoprotein,[33,74,75] two on hepatocyte growth factor,[77,78] and one each on insulin-like growth factor binding proteins 1[63] and 3,[64] activin A,[72] placental growth hormone[73] and annexin A5.[76] ADAM-12 and PP-13 were assessed at <20 weeks of gestation in all included studies. In general, these biomarkers had a low predictive accuracy for IUGR (pooled sensitivities between 12 and 77%, median 34%; specificities 47–95%, median 87%; positive LRs 1.0–3.6, median 2.2; and negative LRs 0.3–1.1, median 0.8) (Table 2).

Other biomarkers

Two studies evaluated serum levels of vitamin D,[80,81] two evaluated metabolomic profile,[83,84] and one each urinary albumin : creatinine ratio[79] and thyroid function tests.[82] Overall, these biomarkers had low positive LRs (0.8–3.9) and high negative LRs (0.3–1.0; Table 2).

As a consequence of the low predictive accuracy of all novel biomarkers identified and evaluated in this study, no further calculation of post-test probabilities of IUGR was performed. There was a substantial level of heterogeneity ($I^2 \geq 50\%$) among studies in 10 of 19 meta-analyses performed. Meta-regression analysis showed that heterogeneity was mainly explained by study design, IUGR definition used, and gestational age at testing. In general, studies with a case–control design, that used birthweight below the tenth centile to define IUGR and that tested biomarkers at $\geq$20 weeks of gestation tended to overestimate the accuracy of the predictive tests. All funnel plots showed no asymmetry, either visually or statistically ($P > 0.10$ for all, by Egger test).

Discussion

Main findings

This systematic review shows that, at the present time, there is no clinically useful biomarker for predicting IUGR in women with a singleton gestation. Overall, none of the 37 novel biomarkers evaluated in our review showed a high predictive accuracy for IUGR. Subgroup analyses according to birthweight centile used to define IUGR and gestational age at testing did not improve predictive accuracy. Nevertheless, two small case–control studies reported better predictive values for two angiogenic factors when a definition of IUGR based on both birthweight centile and additional clinical or pathological evidence of fetal growth restriction was used.[39,45] It was noteworthy that only 13% of the studies included in the review were conducted in developing countries despite IUGR being a major public health problem in such countries.

The poor performance of the biomarkers evaluated in this review could be explained by the following. (i) The multifactorial nature of the IUGR syndrome. There are many causes of IUGR including maternal, fetal and placental factors. Therefore, given the pathophysiological heterogeneity of the condition, the limited clinical utility of any individual biomarker for predicting IUGR is understandable. (ii) The use of an inadequate definition of IUGR in the included studies. Only six of the 53 studies included in the review used a definition for IUGR that went beyond birthweight for gestational age. It is well known that not all small fetuses are growth restricted because some are just constitutionally small. Thereby, it is possible that many fetuses defined as growth-restricted in these studies were actually healthy, constitutionally small but not growth-restricted. (iii) Differences in predictive accuracy of

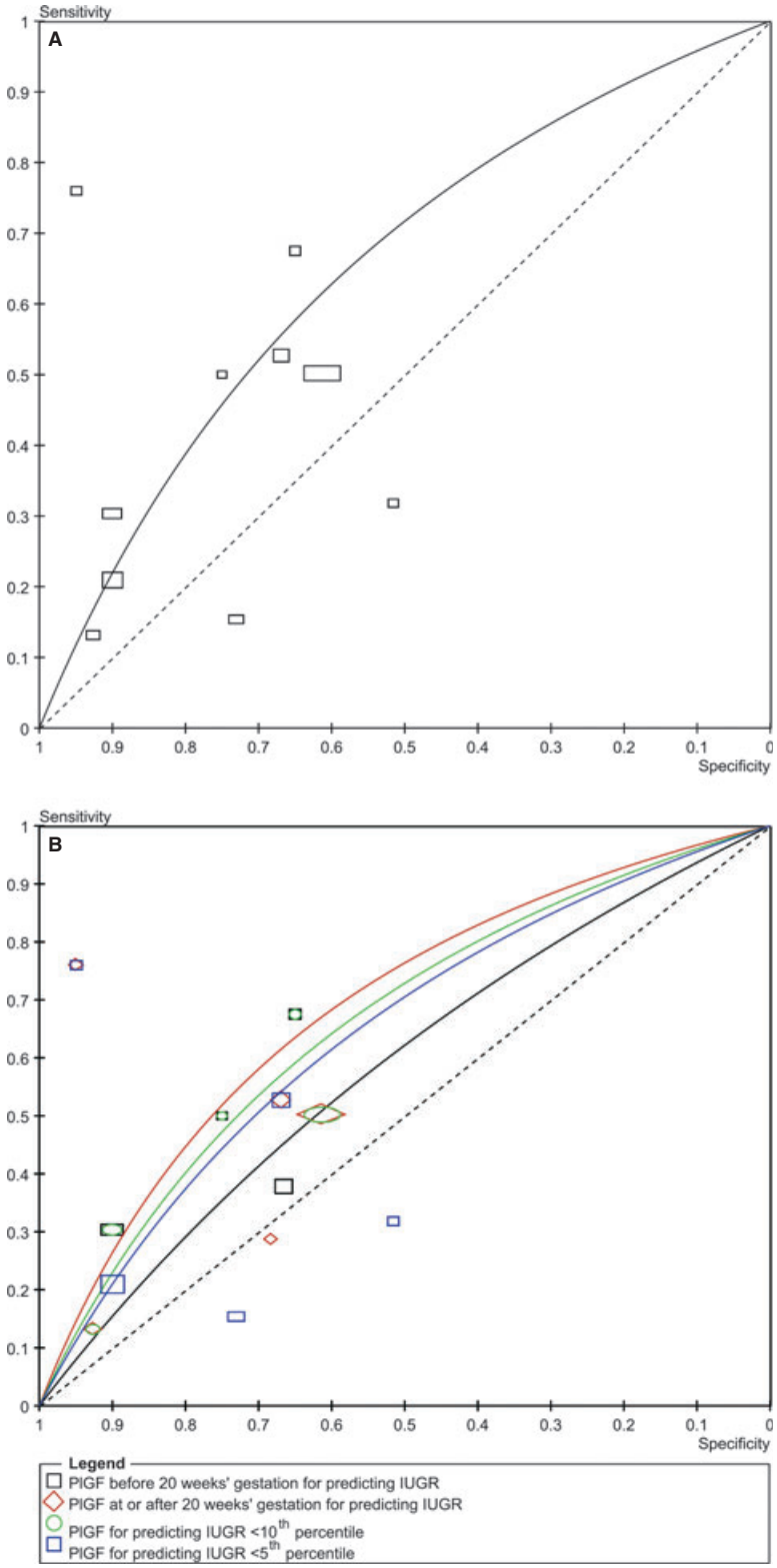

Figure 3. Summary ROC curves of PlGF to predict IUGR: (A) all studies (B) according to gestational age at testing and birthweight centile. Area of each circle, rectangle, and diamond is proportional to study's sample size.

Table 1. Predictive accuracy of angiogenesis-related biomarkers in serum for intrauterine growth restriction

Biomarker	Outcome	No of studies	No of women	Pooled sensitivity,% (95% CI)	Pooled specificity,% (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	I^2 (%)
PlGF	IUGR*	10[32–35,37,42–46]	5709	38 (35–42)	71 (70–72)	1.3 (1.2–1.5)	0.9 (0.8–0.9)	83
	IUGR <10th centile	5[32,34,35,37,46]	3917	46 (41–51)	68 (66–69)	1.4 (1.2–1.6)	0.8 (0.7–0.9)	74
	IUGR <5th centile	5[33,42–45]	1792	33 (29–37)	80 (78–82)	1.7 (1.4–2.0)	0.8 (0.8–0.9)	88
	IUGR* (<20 weeks at testing)	7[32,33,35,42–44,46]	2451	30 (26–34)	82 (80–83)	1.6 (1.4–1.9)	0.9 (0.8–0.9)	80
	IUGR* ($\geq$20 weeks at testing)	5[33, 34, 37, 43, 45]	3739	49 (44–53)	64 (63–66)	1.4 (1.2–1.5)	0.8 (0.7–0.9)	85
	IUGR with additional evidence of fetal growth restriction**	1[45]	88	100 (70–100)	95 (88–98)	19.8 (7.6–51.3)	0.0 (0.0–0.3)	NA
	IUGR <5th centile in women with abnormal uterine artery Doppler	2[38,40]	124	65 (43–82)	67 (58–76)	2.0 (1.3–3.0)	0.5 (0.3–1.0)	0
PlGF slope	IUGR <10th centile	1[41]	346	59 (51–67)	50 (43–57)	1.2 (1.0–1.5)	0.8 (0.6–1.0)	NA
sFlt-1	IUGR <5th centile	1[43]	447	48 (40–56)	67 (61–72)	1.4 (1.1–1.8)	0.8 (0.7–0.9)	NA
	IUGR <5th centile in women with abnormal uterine artery Doppler	2[38,40]	124	75 (53–89)	60 (50–69)	1.9 (1.3–2.6)	0.4 (0.2–0.9)	57
Δ sFlt-1	IUGR <10th centile	1[41]	346	48 (40–56)	54 (47–60)	1.0 (0.8–1.3)	1.0 (0.8–1.2)	NA
sFlt-1/PlGF	IUGR <5th centile	1[43]	411	48 (40–56)	67 (61–72)	1.4 (1.1–1.8)	0.8 (0.7–0.9)	NA
	IUGR <5th centile in women with abnormal uterine artery Doppler	2[38,40]	124	75 (53–89)	57 (47–66)	1.7 (1.2–2.4)	0.4 (0.2–1.0)	63
VEGF	IUGR $\leq$2 SD mean birthweight	1[36]	49	56 (27–81)	88 (74–95)	4.4 (1.6–12.2)	0.5 (0.2–1.1)	NA
Endoglin	IUGR <5th centile	1[43]	355	61 (52–69)	67 (60–73)	1.8 (1.4–2.3)	0.6 (0.5–0.7)	NA
Δ endoglin	IUGR <10th centile	1[41]	346	19 (14–27)	92 (88–95)	2.4 (1.4–4.3)	0.9 (0.8–1.0)	NA
Angiopoietin	IUGR <10th centile	1[39]	44	71 (50–86)	78 (58–90)	3.3 (1.5–7.5)	0.4 (0.2–0.7)	NA
	IUGR with additional evidence of fetal growth restriction**	1[39]	36	92 (67–99)	78 (58–90)	4.3 (1.9–9.4)	0.1 (0.0–0.7)	NA

NA, not applicable; sFlt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; SD, standard deviation; VEGF, vascular endothelial growth factor.
*Regardless of the IUGR definition used.

Table 2. Predictive accuracy of endothelial function/oxidative stress-related and placental proteins/hormone-related biomarkers, and other biomarkers for intrauterine growth restriction

Biomarker	Biological sample	Outcome	No of studies	No of women	Pooled sensitivity,% (95% CI)	Pooled specificity,% (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	I^2 (%)
Endothelial function/oxidative stress-related biomarkers									
Homocysteine	Blood	IUGR*	5[47–51]	9007	27 (23–31)	84 (84–85)	1.7 (1.5–2.0)	0.9 (0.8–0.9)	89
		IUGR <10th centile	2[48,50]	2088	26 (19–33)	89 (87–90)	2.3 (1.7–3.1)	0.8 (0.8–0.9)	0
		IUGR <5th centile	2[49,51]	6170	29 (25–34)	81 (80–82)	1.6 (1.3–1.9)	0.9 (0.8–0.9)	97
Leptin	Blood	IUGR <10th centile	1[52]	139	63 (41–81)	72 (63–79)	2.2 (1.4–3.5)	0.5 (0.3–0.9)	NA
Asymmetric dimethylarginine	Blood	IUGR <10th centile	1[53]	43	75 (47–91)	55 (38–71)	1.7 (1.0–2.8)	0.5 (0.2–1.3)	NA
sVCAM-1	Blood	IUGR <10th centile	1[54]	1404	16 (7–30)	98 (97–99)	9.0 (3.9–20.7)	0.9 (0.7–1.0)	NA
sICAM-1	Blood	IUGR <10th centile	1[54]	1404	42 (28–58)	98 (97–99)	19.2 (11.5–32.1)	0.6 (0.5–0.8)	NA
F2-Isoprostane	Amniotic fluid	IUGR <3rd centile	1[55]	114	100 (84–100)	72 (63–80)	3.6 (2.6–5.0)	0.0 (0.0–0.3)	NA
8-OHdG	Urine	IUGR <10th centile	2[56,57]	597	38 (28–48)	78 (74–81)	1.7 (1.3–2.4)	0.8 (0.7–0.9)	3
Isoprostane	Urine	IUGR <10th centile	1[56]	508	16 (7–32)	80 (76–83)	0.8 (0.3–1.8)	1.1 (0.9–1.2)	NA
Fibronectin	Blood	IUGR <10th centile	1[58]	130	57 (33–79)	96 (90–98)	13.3 (5.0–35.0)	0.5 (0.2–0.8)	NA
LDH	Amniotic fluid	IUGR <10th centile	1[59]	93	88 (53–98)	82 (73–89)	5.0 (2.9–8.4)	0.2 (0.0–1.0)	NA
Pentraxin 3	Blood	IUGR with additional evidence of fetal growth restriction**	1[60]	72	50 (25–75)	57 (44–68)	1.2 (0.6–2.2)	0.9 (0.5–1.6)	NA
Cytokines	Blood	IUGR <10th centile	1[61]	128					
Interferon γ					35 (24–48)	87 (78–93)	2.8 (1.4–5.6)	0.7 (0.6–0.9)	NA
Interleukin-1ra					54 (42–67)	82 (71–89)	3.0 (1.7–5.1)	0.6 (0.4–0.8)	NA
Interleukin-12					65 (52–76)	66 (55–76)	1.9 (1.3–2.8)	0.5 (0.4–0.8)	NA
Eotaxin					30 (20–43)	85 (74–91)	1.9 (1.0–3.8)	0.8 (0.7–1.0)	NA
RANTES					68 (56–79)	70 (59–80)	2.3 (1.6–3.5)	0.5 (0.3–0.7)	NA
C-reactive protein	Blood	IUGR <5th centile	1[62]	5969	5 (3–8)	97 (97–98)	1.8 (1.1–3.1)	1.0 (0.9–1.0)	NA
Folate	Blood	IUGR <5th centile	1[51]	5774	30 (25–35)	80 (79–81)	1.5 (1.2–1.8)	0.9 (0.8–1.0)	NA
Placental proteins/hormone-related biomarkers									
IGFBP-1	Blood	IUGR <10th centile	1[63]	172	24 (12–43)	91 (85–95)	2.7 (1.1–6.5)	0.8 (0.7–1.0)	NA
IGFBP-3	Amniotic fluid	IUGR <10th centile	1[64]	162	69 (54–81)	61 (52–69)	1.8 (1.3–2.4)	0.5 (0.3–0.8)	NA
ADAM-12	Blood	IUGR <5th centile	3[65–67]	1947	12 (10–14)	95 (93–96)	2.2 (1.6–3.1)	0.9 (0.9–1.0)	43
PP-13	Blood	IUGR*	4[68–71]	4456	22 (17–26)	91 (90–91)	2.3 (1.8–2.8)	0.9 (0.8–0.9)	0
		IUGR <5th centile	3[68,69,71]	3854	34 (27–42)	91 (90–92)	3.6 (2.8–4.7)	0.7 (0.6–0.8)	0
		IUGR <10th centile	2[70,71]	3066	12 (8–17)	94 (93–95)	2.0 (1.4–3.0)	0.9 (0.9–1.0)	0
Activin A	Blood	IUGR <5th centile	1[72]	635	50 (42–59)	47 (43–51)	1.0 (0.8–1.1)	1.1 (0.9–1.3)	NA
PGH	Blood	IUGR <5th centile	1[73]	180	47 (35–59)	58 (49–66)	1.1 (0.8–1.6)	0.9 (0.7–1.2)	NA
SP1	Blood	IUGR*	3[33,74,75]	944	32 (26–38)	87 (85–90)	2.5 (1.9–3.3)	0.8 (0.7–0.9)	0
Annexin A5	Amniotic fluid	IUGR <10th centile	1[76]	145	67 (42–85)	67 (59–74)	2.0 (1.3–3.1)	0.5 (0.2–1.0)	NA

Table 2. (Continued)

Biomarker	Biological sample	Outcome	No of studies	No of women	Pooled sensitivity,% (95% CI)	Pooled specificity,% (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	I^2 (%)
HGF	Blood	IUGR*	2[77,78]	46	77 (53–90)	72 (54–85)	2.8 (1.5–5.3)	0.3 (0.1–0.8)	0
Others									
Albumin:creatinin ratio	Urine	IUGR <10th centile	1[79]	257	78 (45–94)	59 (53–65)	1.9 (1.3–2.8)	0.4 (0.1–1.3)	NA
Vitamin D	Blood	IUGR*	2[80,81]	1562	51 (45–57)	54 (51–57)	1.1 (1.0–1.3)	0.9 (0.8–1.0)	0
Thyroid function	Blood	IUGR <5th centile	1[82]	3804					
THS					3 (1–6)	98 (97–98)	1.1 (0.5–2.6)	1.0 (1.0–1.0)	NA
Free T_4					5 (3–8)	98 (97–98)	1.9 (1.0–3.6)	1.0 (0.9–1.0)	NA
Free T_3					2 (1–5)	98 (97–98)	0.8 (0.3–2.1)	1.0 (1.0–1.0)	NA
Metabolomic profile	Blood	IUGR <10th centile	2[83,84]	156	74 (63–82)	81 (71–88)	3.9 (2.4–6.3)	0.3 (0.2–0.5)	0

ADAM, a disintegrin and metalloprotease; HGF, hepatocyte growth factor; IGFBP, insulin-like growth factor binding protein; LDH, lactate dehydrogenase; NA, not applicable; PP-13, placental protein 13; PGH, placental growth hormone; RANTES, regulated on activation, normal T-cell expressed and secreted; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; 8-OHdG, 8-oxo-7,8 dihydro-2-deoxyguanosine; SP1, pregnancy-specific β-1-glycoprotein; TSH, thyroid-stimulating hormone; T_3, triiodothyronine T_4, thyroxine.
*Regardless of the IUGR definition used.

biomarkers according to the gestational age at which the sample is collected. For example, we found that PlGF had a lower sensitivity and a higher specificity when measured at <20 weeks than at $\geq$20 weeks. (iv) Other factors, such as differences in storage time of the samples, frequency of sampling, methods of analysis (sensitivity and specificity of the assays, techniques used, thawed and refrozen of the samples), cut-off values used for defining abnormality, ethnicity, and statistical analysis used.

Strengths and limitations

This systematic review used a similar methodology to that used in our previous review on novel biomarkers for the prediction of spontaneous preterm birth.[85] The strengths of our review lie in its compliance with stringent criteria for performing a rigorous systematic review of predictive test accuracy. These included the use of a prospective protocol designed to address a research question; extensive and continually updated literature searches without language restrictions; strict assessment of the quality of the studies; the use of contemporary statistical methods, recently recommended for meta-analyses of diagnostic and predictive tests; the performance of subgroup analyses according to gestational age at testing and IUGR definition; the exploration of potential sources of heterogeneity; the quantitative way of summarising the evidence, and the assessment of a wide range of biomarkers instead of only a few.

Some potential limitations of our review must be considered. First, the reliability of the results of a meta-analysis is limited by the methodological quality of the studies included in the review. In our review, less than one-third of included studies met at least four predefined quality criteria. Thirty-one of the 53 studies included in the review had a case–control design. Studies evaluating tests in a diseased population and a separate control group overestimate the diagnostic performance compared with studies that use a clinical population.[86] In addition, case–control studies do not permit accurate estimation of the screening effectiveness in the general population. The issue of an appropriate reference standard has been discussed above. Second, there was substantial heterogeneity among individual studies and results in about half the meta-analyses performed. We explored the sources of heterogeneity as thoroughly as possible and only partial explanations were provided by the study design, definition of IUGR used and gestational age at testing. Third, information on selection criteria and blinding was omitted or could not be determined in about half the studies included. Poor reporting of selection criteria is relevant because neonates with IUGR associated with congenital or chromosomal anomalies or conditions such as pre-eclampsia, diabetes or infection could have been included in the study. Unmask-

ing of clinicians to test results most likely resulted in women with abnormal test results being followed up more carefully or receiving therapy, with both events biasing evaluation of any biomarker's predictive accuracy. Fourth, 69 studies were excluded because they did not report sufficient information to construct a 2 × 2 table resulting in a potential loss of relevant data. The great majority of these studies found there were no statistically significant differences in mean or median concentrations of novel biomarkers between women with an IUGR neonate and those with a non-IUGR neonate. Fifth, the number of studies and IUGR cases available for analysis of most novel biomarkers is still too small for us to draw conclusions. Finally, several studies using the same biomarker to predict IUGR differed substantially in the threshold selected to distinguish normal from abnormal results. Taking into account all of these methodological issues, results must be interpreted with caution.

There is growing interest in the use of combinations of tests because no single biomarker has yet been shown to meet all the requirements of a clinically useful predictive test for IUGR in women with a singleton gestation. Some recent studies have reported on the accuracy of the combination of tests for predicting SGA.[13,87,88] A systematic review by Hui et al.[13] reported low predictive values for combinations of serum biomarkers used in prenatal screening for aneuploidy and open neural tube defects. Karagiannis et al.[87] developed a model for predicting SGA below the fifth centile based on combinations of maternal characteristics and biophysical and biochemical markers. At a fixed specificity of 90%, this model yielded a sensitivity of 47% for all SGA, 73% for preterm SGA, and 46% for term SGA. Papastefanou et al.[88] reported that a combination of maternal characteristics and first-trimester ultrasound parameters and biochemical markers had a sensitivity of 55% for predicting SGA up to the fifth centile at a 20% screen positive rate.

Conclusion

In conclusion, none of the novel biomarkers evaluated in this review are sufficiently accurate to recommend their use as a predictor of IUGR in routine clinical practice. However, the use of biomarkers in combination with biophysical parameters and maternal characteristics could be more useful and merits further research. Before further, large-scale, prospective, longitudinal studies are conducted to establish the real potential of novel biomarkers as individual predictive tests for IUGR, stronger evidence is needed linking potential biomarkers with specific subgroups of the IUGR syndrome. In addition, future studies should involve populations from developing countries where the condition is more prevalent.

Disclosure of interest

None.

Contribution to authorship

All authors conceived and designed the study. AC-A extracted and analysed the data, interpreted the results, and drafted the manuscript. The other authors (ATP, SHK and JV) critically revised the draft. The final version of the paper was approved by all authors.

Details of ethics approval

No ethics approval was required.

Funding

Departmental funds were used to support the study.

Acknowledgements

ATP and SHK are supported by the Oxford Partnership Comprehensive Biomedical Research Centre with funding from the Department of Health NIHR Biomedical Research Centres funding scheme.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of studies included in the systematic review. ■

References

1 ACOG Practice Bulletin. Intrauterine growth restriction. Number 12, January 2000. Clinical management guidelines for obstetrician-gynecologists. *Int J Gynaecol Obstet* 2001;72:85–96.
2 de Onis M, Blössner M, Villar J. Levels and patterns of intrauterine growth retardation in developing countries. *Eur J Clin Nutr* 1998;52 (Suppl 1):S5–15.
3 Villar J, Carroli G, Wojdyla D, Abalos E, Giordano D, Ba'aqeel H, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? *Am J Obstet Gynecol* 2006;194:921–31.
4 Kady SM, Gardosi J. Perinatal mortality and fetal growth restriction. *Best Pract Res Clin Obstet Gynaecol* 2004;18:397–410.
5 Pallotto EK, Kilbride HW. Perinatal outcome and later implications of intrauterine growth restriction. *Clin Obstet Gynecol* 2006;49:257–69.
6 Leitner Y, Fattal-Valevski A, Geva R, Eshel R, Toledano-Alhadef H, Rotstein M, et al. Neurodevelopmental outcome of children with intrauterine growth retardation: a longitudinal, 10-year prospective study. *J Child Neurol* 2007;22:580–7.
7 Barker DJ. Adult consequences of fetal growth restriction. *Clin Obstet Gynecol* 2006;49:270–83.
8 Varvarigou AA. Intrauterine growth restriction as a potential risk factor for disease onset in adulthood. *J Pediatr Endocrinol Metab* 2010;23:215–24.
9 Tjoa ML, Oudejans CB, van Vugt JM, Blankenstein MA, van Wijk IJ. Markers for presymptomatic prediction of preeclampsia and intrauterine growth restriction. *Hypertens Pregnancy* 2004;23:171–89.

10 Breeze AC, Lees CC. Prediction and perinatal outcomes of fetal growth restriction. *Semin Fetal Neonatal Med* 2007;12:383–97.

11 Tuuli MG, Odibo AO. First- and second-trimester screening for preeclampsia and intrauterine growth restriction. *Clin Lab Med* 2010;30:727–46.

12 Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. *BMC Pregnancy Childbirth* 2008;8:33.

13 Hui D, Okun N, Murphy K, Kingdom J, Uleryk E, Shah PS. Combinations of maternal serum markers to predict preeclampsia, small for gestational age, and stillbirth: a systematic review. *J Obstet Gynaecol Can* 2012;34:142–53.

14 Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *BMJ* 2003;326:41–4.

15 Spencer K, Cowans NJ, Avgidou K, Molina F, Nicolaides KH. First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. *Ultrasound Obstet Gynecol* 2008;31:15–9.

16 Pihl K, Sørensen TL, Nørgaard-Pedersen B, Larsen SO, Nguyen TH, Krebs L, et al. First-trimester combined screening for Down syndrome: prediction of low birth weight, small for gestational age and pre-term delivery in a cohort of non-selected women. *Prenat Diagn* 2008;28:247–53.

17 Montanari L, Alfei A, Albonico G, Moratti R, Arossa A, Beneventi F, et al. The impact of first-trimester serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A on the diagnosis of fetal growth restriction and small for gestational age infant. *Fetal Diagn Ther* 2009;25:130–5.

18 Goetzinger KR, Singla A, Gerkowicz S, Dicke JM, Gray DL, Odibo AO. The efficiency of first-trimester serum analytes and maternal characteristics in predicting fetal growth disorders. *Am J Obstet Gynecol* 2009;201:412.e1–6.

19 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.

20 Kirkegaard I, Henriksen TB, Uldbjerg N. Early fetal growth, PAPP-A and free β-hCG in relation to risk of delivering a small-for-gestational age infant. *Ultrasound Obstet Gynecol* 2011;37:341–7.

21 Zhang J, Merialdi M, Platt LD, Kramer MS. Defining normal and abnormal fetal growth: promises and challenges. *Am J Obstet Gynecol* 2010;202:522–8.

22 Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003;3:25.

23 Whiting P, Harbord R, Kleijnen J. No role for quality scores in systematic reviews of diagnostic accuracy studies. *BMC Med Res Methodol* 2005;5:19.

24 Sankey S, Weisfiels L, Fine M, Kapoor W. An assessment of the use of the continuity correction for sparse data in meta-analysis. *Commun Stat Simul Comput* 1996;25:1031–56.

25 Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol* 2005;58:982–90.

26 Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. *Stat Med* 2002;21:1237–56.

27 Zwinderman AH, Bossuyt PM. We should not pool diagnostic likelihood ratios in systematic reviews. *Stat Med* 2008;27:687–97.

28 Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. *JAMA* 1994;271:703–7.

29 Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–60.

30 Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med* 2002;21:1525–37.

31 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analyses detected by a simple graphical test. *BMJ* 1997;315:629–34.

32 Tjoa ML, vanVugt JM, Mulders MA, Schutgens RB, Oudejans CB, van Wijk IJ. Plasma placenta growth factor levels in midtrimester pregnancies. *Obstet Gynecol* 2001;98:600–7.

33 Bersinger NA, Ødegård 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.

34 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.

35 Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, 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.

36 Bersinger NA, Ødegård 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.

37 Espinoza J, Romero R, Nien JK, Gomez R, Kusanovic JP, Gonçalves LF, 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.

38 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.

39 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.

40 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.

41 Erez O, Romero R, Espinoza J, Fu W, Todem D, Kusanovic JP, 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.

42 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.

43 Åsvold 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.

44 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.

45 Benton SJ, Hu Y, Xie F, Kupfer K, Lee SW, Magee LA, 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.

46 Boucoiran I, Thissier-Levy S, Wu Y, Wei SQ, Zhong-Cheng L, Delvin E, et al. Risk for preeclampsia and intrauterine growth restriction: effective value of PlGF, Sflt-1 and Inhibin Ain singleton and multiple pregnancies. *Am J Obstet Gynecol* 2012;206(Suppl):S336–7.

47 Murakami S, Matsubara N, Saitoh M, Miyakaw S, Shoji M, Kubo T. The relation between plasma homocysteine concentration and methylenetetrahydrofolate reductase gene polymorphism in pregnant women. *J Obstet Gynaecol Res* 2001;27:349–52.

48 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.

49 Onalan R, Onalan G, Gunenc Z, Karabulut E. Combining 2nd-trimester maternal serum homocysteine levels and uterine artery Doppler for prediction of preeclampsia and isolated intrauterine growth restriction. *Gynecol Obstet Invest* 2006;61:142–8.

50 Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. *Clin Chem* 2008;54:326–34.

51 Bergen NE, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H, et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancyoutcomes: the Generation R Study. *BJOG* 2012;119:739–51.

52 Franco-Sena AB, Goldani MZ, Tavares do Carmo MG, Velásquez-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.

53 Speer PD, Powers RW, Frank MP, Harger G, Markovic N, Roberts JM. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants. *Am J Obstet Gynecol* 2008;198:112.e1–7.

54 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.

55 Longini M, Perrone S, Kenanidis A, Vezzosi P, Marzocchi B, Petraglia F, et al. Isoprostanes in amniotic fluid: a predictive marker for fetal growth restriction in pregnancy. *Free Radic Biol Med* 2005;38:1537–41.

56 Stein TP, Scholl TO, Schluter MD, Leskiw MJ, Chen X, Spur BW, et al. Oxidative stress early in pregnancy and pregnancy outcome. *Free Rad Res* 2008;42:841–8.

57 Potdar N, Singh R, Mistry V, Evans MD, Farmer PB, Konje JC, et al. First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. *BJOG* 2009;116:637–42.

58 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.

59 Borna S, Abdollahi A, Mirzaei F. Predictive value of mid-trimester amniotic fluid high-sensitive C-reactive protein, ferritin, and lactate dehydrogenase for fetal growth restriction. *Indian J Pathol Microbiol* 2009;52:498–500.

60 Cetin I, Cozzi V, Papageorghiou AT, Maina V, Montanelli A, Garlanda C, et al. First trimester PTX3 levels in women who subsequently develop preeclampsia and fetal growth restriction. *Acta Obstet Gynecol Scand* 2009;88:846–9.

61 Georgiou HM, Thio YS, Russell C, Permezel M, Heng YJ, Lee S, 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.e1–12.

62 Ernst GD, de Jonge LL, Hofman A, Lindemans J, Russcher H, Steegers EA, et al. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. *Am J Obstet Gynecol* 2011;205:132e1–12.

63 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.

64 Murisier-Petetin G, Gremlich S, Damnon F, Reymondin D, Hohlfeld P, Gerber S. Amniotic fluid insulin-like growth factor binding protein 3 concentration as early indicator of fetal growth restriction. *Eur J Obstet Gynecol Reprod Biol* 2009;144:15–20.

65 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.

66 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.

67 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.

68 Chafetz I, Kuhnreich I, Sammar M, Tal Y, Gibor Y, Meiri H, et al. First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol* 2007;197:35.e1–7.

69 Than NG, Romero R, Meiri H, Erez O, Xu Y, Tarquini F, et al. PP13, maternal ABO blood groups and the risk assessment of pregnancy complications. *PLoS ONE* 2011;6:e21564.

70 Meints L, Cahill A, Huster K, Odibo L, McVean D, Odibo A. PP13, PAPP-A, first-trimester uterine-artery Doppler measurements, and maternal characteristics in the prediction of small for gestational age fetuses. *Am J Obstet Gynecol* 2012;206(Suppl):S167.

71 Schneuer FJ, Nassar N, Khambalia AZ, Tasevski V, Ashton AW, Morris JM, 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.

72 Ong CY, Liao AW, Munim S, Spencer K, Nicolaides KH. First-trimester maternal serum activin A in pre-eclampsia and fetal growth restriction. *J Matern Fetal Neonatal Med* 2004;15:176–80.

73 Sifakis S, Akolekar R, Kappou D, Mantas N, Nicolaides KH. Maternal serum placental growth hormone at 11-13 weeks' gestation in pregnancies delivering small for gestational age neonates. *J Matern Fetal Neonatal Med* 2012;25:1796–9.

74 Westergaard JG, Teisner B, Hau J, Grudzinskas JG. Placental protein measurements in complicated pregnancies I. Intrauterine growth retardation. *Br J Obstet Gynaecol* 1984;91:1216–23.

75 Pihl K, Larsen T, Laursen I, Krebs L, Christiansen M. First trimester maternal serum pregnancy-specific beta-1-glycoprotein (SP1) as a marker of adverse pregnancy outcome. *Prenat Diagn* 2009;29:1256–61.

76 Van Eerden P, Wu XX, Chazotte C, Rand JH. Annexin A5 levels in midtrimester amniotic fluid: association with intrauterine growth restriction. *Am J Obstet Gynecol* 2006;194:1371–6.

77 Clark DE, Salvig JD, Smith SK, Charnock-Jones DS. Hepatocyte growth factor levels during normal and intra-uterine growth-restricted pregnancies. *Placenta* 1998;19:671–3.

78 Tjoa ML, Mulders MA, van Vugt JM, Blankenstein MA, Oudejans CB, van Wijk IJ. Plasma hepatocyte growth factor as a marker for small-for-gestational age fetuses. *Eur J Obstet Gynecol Reprod Biol* 2003;110:20–5.

79 Baweja S, Kent A, Masterson R, Roberts S, McMahon LP. Prediction of pre-eclampsia in early pregnancy by estimating the spot urinary albumin:creatinine ratio using high-performance liquid chromatography. *BJOG* 2011;118:1126–32.

80 Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, et al. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *J Nutr* 2010;140:999–1006.

81 Ertl R, Yu CK, Samaha R, Akolekar R, Nicolaides KH. Maternal serum vitamin D at 11–13 weeks in pregnancies delivering small for gestational age neonates. *Fetal Diagn Ther* 2012;31:103–8.

82 Karagiannis G, Ashoor G, Maiz N, Jawdat F, Nicolaides KH. Maternal thyroid function at eleven to thirteen weeks of gestation and subsequent delivery of small for gestational age neonates. *Thyroid* 2011;21:1127–31.

83 Horgan RP, Broadhurst DI, Walsh SK, Dunn WB, Brown M, Roberts CT, et al. Metabolic profiling uncovers a phenotypic signature of small for gestational age in early pregnancy. *J Proteome Res* 2011;10:3660–73.

84 Heazell AE, Bernatavicius G, Warrander L, Brown MC, Dunn WB. A metabolomic approach identifies differences in maternal serum in third trimester pregnancies that end in poor perinatal outcome. *Reprod Sci* 2012;19:863–75.

85 Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG* 2011;118:1042–54.

86 Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, Van der Meulen JH, et al. Empirical evidence of design-related bias in studies of diagnostic tests. *JAMA* 1999;282:1061–6. Erratum in JAMA 2000;283:1963.

87 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.

88 Papastefanou I, Souka AP, Pilalis A, Eleftheriades M, Michalitsi V, Kassanos D. First trimester prediction of small- and large-for-gestation neonates by an integrated model incorporating ultrasound parameters, biochemical indices and maternal characteristics. *Acta Obstet Gynecol Scand* 2012;91:104–11.