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ORIGINAL ARTICLE

Fetal fraction of cell free DNA in screening for hypertensive disorders at 11–13 weeks

Ioakeim Sapantzoglou[a], Margarita Gallardo Arozena[a], Vlad Dragoi[a], Ranjit Akolekar[b], Kypros H. Nicolaides[a] and Argyro Syngelaki[a]

[a]Harris Birthright Research Centre for Fetal Medicine, Fetal Medicine Research Institute, King's College Hospital, London, UK; [b]Fetal Medicine Unit, Medway Maritime Hospital, Gillingham, UK

ABSTRACT

Objective: To investigate whether first-trimester maternal plasma fetal fraction is altered in women that subsequently develop preeclampsia (PE) or gestational hypertension (GH) and to examine its potential value in improving the performance of screening for PE and GH by maternal factors and maternal serum pregnancy associated plasma protein-A (PAPP-A), mean arterial pressure (MAP) and uterine artery pulsatility index (UtA-PI).

Methods: The study population of 10,131 pregnancies undergoing cell free fetal DNA testing at 11–13 weeks' gestation included 91 (0.9%) cases with preterm-PE, 222 (2.2%) cases with term-PE, 360 (3.6%) with GH and 9,458 (93.4%) cases unaffected by hypertensive disorders. Maternal plasma fetal fraction levels were expressed as multiples of the median (MoM) after adjustment for maternal factors and crown-rump length. The performance of screening for preterm-PE, term PE and GH by maternal factors and MoM values of fetal fraction, PAPP-A, UtA-PI and MAP was evaluated by receiver operating characteristic (ROC) curves.

Results: The median fetal fraction MoM was significantly lower in the preterm-PE (0.825; IQR 0.689–1.115 MoM, $p < .001$), term-PE (0.946; IQR 0.728–1.211 MoM, $p = .028$) and GH (0.928; IQR 0.711–1.182 MoM, $p < .001$) groups than in the unaffected group (1.002; IQR 0.785–1.251 MoM). However, the performance of screening for PE or GH by maternal factors alone or by maternal factors and PAPP-A, UtA-PI and MAP was not significantly improved by the addition of fetal fraction.

Conclusions: First trimester maternal plasma fetal fraction is not useful in screening for hypertensive disorders of pregnancy.

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Fetal fraction; cell free DNA; preeclampsia; hypertensive disorders; first trimester

Introduction

In 1999, Lo and colleagues reported that the median fetal DNA concentration in maternal serum was increased in women with established preeclampsia (PE) compared to controls [1]. Subsequently, several other studies have confirmed this finding [2–5] which has been attributed to accelerated apoptosis of trophoblastic cells resulting from placental ischemia [1] and reduced clearance of the cell free DNA from the maternal circulation in women with PE [6]. There is also some evidence that cell free DNA and fetal fraction are altered in women who subsequently develop PE from the first trimester of pregnancy [7–13]. A recent large prospective study on 5,582 women at 12–20 weeks reported that fetal fraction was significantly reduced in women with PE compared to the unaffected pregannncies [11]. Moreover, two other large prospective studies demonstrated that cell free fetal DNA and fetal fraction examined at 11–14 weeks were inversely related to uterine artery pulsatility index (UtA-PI) and mean arterial pressure (MAP) and had significant positive association with maternal serum pregnancy associated plasma protein-A (PAPP-A) [14,15]. Furthermore, the study of Rolnik et al, showed that the risk of PE calculated by a combination of maternal factors, UtA-PI, MAP and maternal serum PAPP-A and placental growth factor (PLGF) [16], was inversely related to fetal fraction [15].

The aim of this study is to investigate first, whether first-trimester maternal plasma fetal fraction is altered in women that subsequently develop PE or gestational hypertension (GH) and second, its potential value in improving the performance of screening for PE and

CONTACT Kypros H. Nicolaides kypros@fetalmedicine.com Harris Birthright Research Centre for Fetal Medicine, Fetal Medicine Research Institute, King's College Hospital, Denmark Hill, London, SE5 8BB, UK

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GH by maternal factors and maternal serum PAPP-A, MAP and UtA-PI.

Methods

Study population

The data for this study were derived from prospective screening for trisomies 21,18 and 13 in singleton pregnancies by a combination of maternal age, fetal nuchal translucency thickness, fetal heart rate and serum free β-hCG and PAPP-A [17] at 11^{+0}–13^{+6} weeks' gestation in women booking for routine pregnancy care at King's College Hospital, London and Medway Maritime Hospital, Gillingham from October 2013 to August 2019. The estimated risks for trisomy 21 and trisomies 18 or 13 were calculated at the time of screening and the higher of the two was considered in the stratification of the population. Women with high-risk (≥1 in 100) were offered the options of chorionic villus sampling, cfDNA testing or no further testing; women with an intermediate risk (1 in 1001 to 1 in 1000) were offered the options of cfDNA testing or no further testing and those with a low risk (< 1 in 1000) were reassured that fetal trisomies were unlikely and no further testing was necessary.

During the visit, we recorded maternal characteristics and medical history and performed transabdominal ultrasound examination to first, determine gestational age from the measurement of the fetal crown-rump length (CRL), second, diagnose any major fetal abnormalities [18], third, measure fetal nuchal translucency thickness as part of screening for aneuploidies [17] and fourth, measure the right and left UtA-PI by color Doppler and calculate the mean PI [19]. We also measured the weight and height and mean arterial pressure (MAP) by validated automated devices using a standardized protocol [20].

Women with an intermediate- or high-risk for fetal trisomies 21 or 18 and 13 who chose to have the option of cfDNA testing provided written informed consent and maternal blood (20 mL) was sent *via* courier to the USA for cfDNA testing (Harmony® prenatal test, Roche/Ariosa Diagnostics, Inc., San Jose, CA, USA). Chromosome-selective sequencing and fetal fraction optimized risk of trisomy evaluation were used to assay non-polymorphic and polymorphic loci, where fetal alleles differ from maternal alleles, enabling simultaneous determination of chromosome proportion and fetal fraction [21]. The method provides accurate and reproducible fetal fraction measurements and has been equally informative across different populations [22].

The inclusion criteria for this study were singleton pregnancy undergoing first-trimester combined screening for aneuploidy and subsequently delivering a phenotypically normal live birth or stillbirth at ≥24 weeks' gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities, those ending in termination, miscarriage or fetal death before 24 weeks and those with unknown outcome because the pregnancy was lost to follow-up.

The implementation of contingent screening was approved by the National Research Ethics Committee (REC reference 13/LO/0885).

Outcome measures

Outcome measures were preterm-PE, term-PE and GH. Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with preexisting or pregnancy associated hypertension were examined to determine if the condition was PE or GH, as defined by the American College of Obstetricians and Gynecologists (ACOG) [23]. According to this definition, diagnosis of PE requires the presence of new onset hypertension (blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic) at ≥ 20weeks' gestation and either proteinuria (≥300 mg/24h or protein to creatinine ratio >30 mg/mmol or ≥2 + on dipstick testing) or evidence of renal dysfunction (serum creatinine >97 μmol/L), hepatic dysfunction (transaminases ≥65 IU/L) or hematological dysfunction (platelet count <100,000/μL). Diagnosis of GH requires the presence of new onset hypertension (blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic) at ≥20 weeks' gestation in the absence of accompanied proteinuria or other organ dysfunction [23].

Statistical analysis

Descriptive data were presented as medians and interquartile ranges for continuous variables and as numbers and percentages for categorical variables. Comparison between the outcome groups was done by χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney-U test for continuous variables. The distribution of fetal fraction was logarithmically transformed to obtain a symmetric distribution of residuals with approximately constant standard deviation. This was assessed by inspecting histograms and probability plots. In the normal group of pregnancies, after excluding cases with preterm birth <37 week's

gestation, pregnancies resulting in the delivery of small (<10th percentile) or large (≥90th percentile) for gestational age neonates, stillbirth, or pregnancies complicated by PE, GH or gestational diabetes mellitus, logistic regression analysis with backward stepwise elimination was used to determine which of the factors among fetal CRL, maternal age, weight, height, racial origin, smoking status, parity and method of conception were significant predictors of $\log_{10}$ fetal fraction. The distributions of $\log_{10}$ fetal fraction, expressed as multiple of the median (MoM) in preterm-PE, term-PE, GH and unaffected groups were determined and the Mann–Whitney *U*-test was used to calculate the significance of differences in the median values between the outcome groups. The *a priori* risk for preterm-PE based on maternal characteristics and obstetric history was determined using multivariate logistic regression analysis. Values of UtA-PI, MAP and PAPP-A were expressed as MoM after adjustment for maternal characteristics and history as previously described [24–26]. Subsequently, multivariable logistic regression analysis was used to determine whether the log transformed *a priori* risk (logit) for preterm-PE based on maternal factors and the $\log_{10}$ MoM value of each of the biomarkers had significant contribution in predicting preterm-PE, term-PE and GH. The variables which provided a significant contribution in the multivariable analysis were used to determine the patient-specific risk of the outcome measures using the equation odds/(1 + odds), where odds = e^Y and Y was estimated from the coefficients of variables in the logistic regression analysis. The distribution of patient-specific risks was used to determine the performance of screening by receiver–operating characteristics (ROC) curve analysis. The statistical software package SPSS 25.0 (SPSS Inc., Chicago, Ill., USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

During the study period, cfDNA testing was performed in 10,917 singleton pregnancies with a live fetus at 11^{+0} to 13^{+6} weeks' gestation. We excluded 786 (7.2%) cases because they had missing outcome data ($n = 153$) or the pregnancies resulted in miscarriage before 24 weeks' gestation, termination or had major fetal defects ($n = 633$). In the remaining 10,131 pregnancies included in the study, there were 313 (3.1%) cases with PE including 91 (0.9%) cases with preterm-PE and 222 (2.2%) cases with term-PE, 360 (3.6%) with GH and 9,458 (93.4%) cases unaffected by PE or GH. The maternal characteristics of each of the outcome groups are presented in Table 1.

Multivariable regression analysis in the normal group ($n = 6,616$) of pregnancies demonstrated that for the $\log_{10}$ fetal fraction significant independent contributions were provided by fetal CRL, maternal age, weight and height, Black, East Asian and mixed racial origin and conception by *in-vitro* fertilization (Table S1 in the Supplementary Appendix).

The median fetal fraction MoM was significantly lower in the preterm-PE (0.825; IQR 0.689–1.115 MoM, $p < .001$), term-PE (0.946; IQR 0.728–1.211 MoM, $p = .028$) and GH (0.928; IQR 0.711–1.182 MoM, $p < .001$) groups than in the unaffected group (1.002; IQR 0.785–1.251 MoM) (Table 1).

Prediction of hypertensive disorders and performance of screening

Logistic regression analysis demonstrated that in the prediction of preterm-PE based on maternal factors there were significant contributions from maternal weight (OR 1.029; 95% CI 1.018–1.040, $p < .001$), South Asian racial origin (OR 3.457; 95% CI 1.778–6.719, $p < .001$), history of chronic hypertension (OR 5.000; 95% CI 2.614–9.567, $p < .001$), type 1 diabetes mellitus (OR 10.159; 95% CI 3.338–30.915, $p < .001$), history of PE in a previous pregnancy (OR 4.453; 95% CI 2.349–8.439, $p < .001$) and no history of PE in a previous pregnancy (OR 0.566; 95% CI 0.355–0.902, $p < .001$).

The results of multivariable logistic regression analyses for the prediction of preterm-PE, term PE and GH by maternal factors and biomarkers is presented in Table S2 in the Supplementary Appendix. In the prediction of preterm-PE, significant contribution was provided by the maternal factor-derived *a-priori* risk and MoM values of PAPP-A, UtA-PI, MAP and fetal fraction ($R^2 = 0.268$; $p < .0001$). In the prediction of term-PE, significant contribution was provided by the maternal factor-derived *a-priori* risk and MoM values of PAPP-A and MAP, but not from MoM values of UtA-PI and fetal fraction ($R^2 = 0.106$; $p < .0001$). In the prediction of GH, significant contribution was provided by the maternal factor-derived *a-priori* risk and MoM values of PAPP-A and MAP, but not from MoM values of UtA-PI and fetal fraction ($R^2 = 0.113$; $p < .0001$).

The performance of screening for preterm-PE is shown in Figure 1 and Table 2. The DR for preterm-PE, at 10% FPR, increased from 48.4% when using maternal factors alone to 72.5% with the addition of all biomarkers ($p < .001$). In the prediction of preterm-PE, the

Table 1. Characteristics of the study population.

Characteristic	No PE or GH ($n = 9,458$)	Preterm-PE ($n = 91$)	Term-PE ($n = 222$)	GH ($n = 360$)
Gestational age at screening (weeks)	12.9 (12.4–13.3)	12.9 (12.6–13.1)	12.9 (12.5–13.3)	12.7 (12.3–13.1)
Age (years)	35.1 (31.2–38.5)	34.5 (31.6–38.4)	35.1 (30.9–39.1)	36.1 (31.8–39.4)
Weight (Kg)	68.0 (60.1–79.0)	77.0 (68–91.3)	73.0 (64.0–87.3)	74.0 (64.5–87.5)
Height (cm)	165 (160–169)	166 (161–169)	166 (160–169)	165 (160–170)
Racial origin				
White	7,117 (75.2)	53 (58.2)	153 (68.9)	249 (69.2)
Black	1246 (13.2)	22 (24.2)	47 (21.2)	69 (19.2)
South Asian	536 (5.7)	11 (12.1)	11 (5.0)	17 (4.7)
East Asian	341 (3.6)	0	6 (2.7)	11 (3.1)
Mixed	218 (2.3)	5 (5.5)	5 (2.3)	14 (3.9)
Conception				
Natural	9,057 (95.8)	85 (93.4)	201 (90.5)	340 (94.4)
Assisted by use of ovulation drugs	31 (0.3)	2 (2.2)	0	4 (1.1)
In vitro fertilization	370 (3.9)	4 (4.4)	21 (9.5)	16 (4.4)
Cigarette smoker	566 (6.0)	3 (3.3)	14 (6.3)	19 (5.3)
Mother had preeclampsia	364 (3.8)	7 (7.7)	20 (9.0)	18 (5.0)
Medical history				
Chronic hypertension	172 (1.8)	15 (16.5)	27 (12.2)	0
Systemic lupus erythematosus / Antiphospholipid syndrome	30 (0.3)	0	0	1 (0.3)
Diabetes mellitus	115 (1.2)	6 (6.6)	2 (0.9)	12 (3.3)
Obstetrical history				
Nulliparous	4,176 (44.2)	41 (45.1)	125 (56.3)	200 (55.6)
Multiparous without preeclampsia	5,072 (53.6)	33 (36.3)	66 (29.7)	128 (35.6)
Multiparous with preeclampsia	210 (2.2)	17 (18.7)	31 (14.0)	3 (8.9)
First trimester screening markers				
Pregnancy associated plasma protein-A (MoM)	0.681 (0.451–1.046)	0.563 (0.340–0.790)	0.634 (0.386–0.935)	0.613 (0.403–0.964)
Mean arterial pressure (MoM)	1.011 (0.959–1.062)	1.057 (1.002–1.107)	1.046 (0.995–1.114)	1.072 (1.009–1.136)
Uterine artery pulsatility index (MoM)	1.053 (0.845–1.269)	1.424 (1.136–1.658)	1.093 (0.853–1.336)	1.058 (0.837–1.309)
Fetal fraction (MoM)	1.002 (0.785–1.251)	0.825 (0.689–1.115)	0.946 (0.728–1.211)	0.928 (0.711–1.182)

Data are *n* (%), median (interquartile range). MoM: Multiple of normal median; PE: preeclampsia; GH: gestational hypertension.

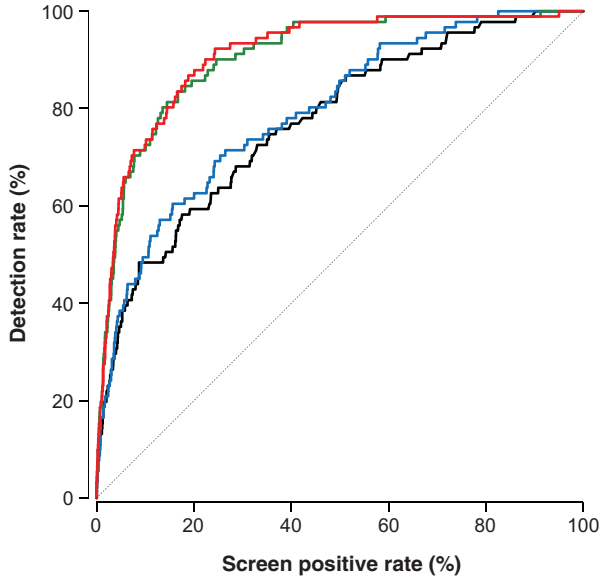

Figure 1. Receiver operating characteristic curves for the prediction of preterm preeclampsia by maternal factors (black), fetal fraction (blue), uterine artery pulsatility index, mean arterial pressure and pregnancy associated plasma protein-A (green), uterine artery pulsatility index, mean arterial pressure, pregnancy associated plasma protein-A and fetal fraction (red).

AUROC for maternal factors with fetal fraction was not significantly different than the AUROC for maternal factors alone ($p = .526$). Similarly, the AUROC for maternal factors with fetal fraction, UtA-PI, MAP, and PAPP-A was not significantly different than the AUROC for maternal factors with UtA-PI, MAP, and PAPP-A ($p = .850$).

Discussion

Main findings of the study

The main findings of this study are: first, the fetal fraction at 11–13 week's gestation is lower in pregnancies that subsequently develop preterm-PE, term-PE and GH, compared to unaffected pregnancies, second, fetal fraction can provide significant contribution in the prediction of preterm-PE when combined with the *a-priori* risk from maternal factors and PAPP-A, UtA-PI, MAP but not in the prediction of term-PE or GH, and third, the performance of screening for preterm-PE by UtA-PI, MAP, and PAPP-A is only marginally improved by the addition of fetal fraction.

Table 2. Performance of screening for preterm preeclampsia by maternal factors and biomarkers.

		Detection rate for fixed FPR	
Screening test	AUROC (95% CI)	10%	20%
Maternal factors	0.773 (0.723–0.823)	48.4	59.3
Plus fetal fraction	0.795 (0.749–0.842)	49.5	62.6
Plus UtA-PI, MAP, PAPP-A	0.907 (0.877–0.936)	71.4	85.7
Plus UtA-PI, MAP, PAPP-A, fetal fraction	0.911 (0.883–0.940)	72.5	86.8

AUROC: area under the roc curve; CI: confidence interval; FPR: false positive rate; PAPP-A: pregnancy-associated plasma protein-A; UtA-PI: uterine artery pulsatility index; MAP: mean arterial pressure.

Comparison with results of previous studies

Our study is the first to examine the potential value of fetal fraction in the prediction of PE when combined with maternal factors and maternal serum PAPP-A, MAP and UtA-PI. However, seven previous studies have examined the levels of fetal fraction in women who subsequently developed hypertensive disorders in pregnancy [9–13,15,27]. Five previous studies, examining between 240 and 5,582 pregnancies at 10–20 weeks' gestation [9–13], reported that fetal fraction was significantly lower in women who subsequently developed PE but in three of these studies which examined this association in relation to maternal factors, this difference was not observed after adjusting for maternal characteristics and gestational age [9,11,12]. A large cohort study investigating whether fetal fraction >95th percentile was associated with adverse pregnancy outcomes in 2,033 pregnancies found that there was no significant association between high fetal fraction and hypertensive disorders of pregnancy [27]. A recent study on 4,713 singleton pregnancies undergoing screening for PE by a combination of maternal factors and biophysical and biochemical markers at 11–13 weeks' gestation demonstrated that fetal fraction was inversely related to the risk for PE <34 weeks and <37 weeks and there was an inverse association with MAP and Ut-API and a positive association with PAPP-A and PLGF [15].

Strengths and limitations

The strengths of our study include, first, the large population examined, second, the use of multivariable regression analysis to determine the factors across maternal characteristics and gestational age that provided significant contribution in the prediction of log transformed fetal fraction, and third, the use of multivariable regression analysis to determine the variables which had significant contribution in predicting preterm-PE, term-PE and GH among the *a priori* risk for preterm-PE based on maternal factors and the MoM values of the UtA-PI, MAP, PAPP-A and fetal fraction. A limitation of our study relates to the inclusion criteria of the recruited population which included only women with an intermediate or high risk for fetal trisomies 21 or 18 and 13, rather than an unselected population. As such, we cannot be certain as to whether our results can be generalized and applied to the overall pregnant population.

Implications for clinical practice

Effective screening for PE can be provided by a combination of maternal factors, MAP, UtA-PI and serum PAPP-A and PLGF at 11–13 weeks' gestation [16]. The major benefit of such early identification of high-risk pregnancies for PE is that prophylactic use of low-dose aspirin can significantly reduce the prevalence of the disease [28]. While cell free DNA testing has been a highly successful screening test for chromosomal abnormalities, it is unlikely that the use of fetal fraction could improve the early screening for PE.

Conclusion

In summary, in women with an intermediate or high risk for fetal trisomies, fetal fraction is lower in women who subsequently develop PE but it does not add any value in improving the performance of screening forPE achieved by screening with maternal factors and MAP, UtA-PI and serum PAPP-A.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Lo YM, Leung TN, Tein MS, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. Clin Chem. 1999;45(2):184–188.

[2] Smid M, Vassallo A, Lagona F, et al. Quantitative analysis of fetal DNA in maternal plasma in pathological conditions associated with placental abnormalities. Ann NY Acad Sci. 2001;945:132–137.

[3] Zhong XY, Laivuori H, Livingston JC, et al. Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclampsia. Am J Obstet Gynecol. 2001;184(3):414–419.

[4] Farina A, Sekizawa A, Rizzo N, et al. Cell free fetal DNA (SRY locus) concentration in maternal plasma is directly correlated to the time elapsed from the onset of preeclampsia to the collection of blood. Prenat Diagn. 2004;24(4):293–297.

[5] Alberry MS, Maddocks DG, Hadi MA, et al. Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. Am J Obstet Gynecol. 2009;200:98 e1–6.

[6] Lau TW, Leung TN, Chan LY, et al. Fetal DNA clearance from maternal plasma is impaired in preeclampsia. Clin Chem. 2002;48(12):2141–2146.

[7] Kim SY, Kim HJ, Park SY, et al. Early prediction of hypertensive disorders of pregnancy using cell-free fetal DNA, cell-free total DNA, and biochemical markers. Fetal Diagn Ther. 2016;40(4):255–262.

[8] Sifakis S, Zaravinos A, Maiz N, et al. First-trimester maternal plasma cell-free fetal DNA and preeclampsia. Am J Obstet Gynecol. 2009;201(5):472.e1–472.e7.

[9] Rolnik DL, O'Gorman N, Fiolna M, et al. Maternal plasma cell-free DNA in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol. 2015;45(1):106–111.

[10] Krishna I, Badell M, Loucks TL, et al. Adverse perinatal outcomes are more frequent in pregnancies with a low fetal fraction result on noninvasive prenatal testing. Prenat Diagn. 2016;36(3):210–215.

[11] Suzumori N, Sekizawa A, Ebara T, et al. Fetal cell-free DNA fraction in maternal plasma for the prediction of hypertensive disorders of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2018;224:165–169.

[12] Bender WR, Koelper NC, Sammel MD, et al. Association of fetal fraction of cell-free DNA and hypertensive disorders of pregnancy. Amer J Perinatol. 2019;36(03):311–316.

[13] Gerson KD, Truong S, Haviland MJ, et al. Low fetal fraction of cell-free DNA predicts placental dysfunction and hypertensive disease in pregnancy. Pregnancy Hypertens. 2019;16:148–153.

[14] Poon LC, Musci T, Song K, et al. Maternal plasma cell-free fetal and maternal DNA at 11-13 weeks' gestation: relation to fetal and maternal characteristics and pregnancy outcomes. Fetal Diagn Ther. 2013;33(4):215–223.

[15] Rolnik DL, da Silva Costa F, Lee TJ, et al. Association between fetal fraction on cell-free DNA testing and first-trimester markers for pre-eclampsia. Ultrasound Obstet Gynecol. 2018;52(6):722–727.

[16] O'Gorman N, Wright D, Syngelaki A, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11–13 weeks gestation. Am J Obstet Gynecol. 2016;214(1):103.e1–103.e12.

[17] Kagan KO, Wright D, Baker A, et al. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol. 2008;31(6):618–624.

[18] Syngelaki A, Hammami A, Bower S, et al. Diagnosis of fetal non-chromosomal abnormalities on routine ultrasound examination at 11–13 weeks' gestation. Ultrasound Obstet Gynecol. 2019;54(4):468–476.

[19] Plasencia W, Maiz N, Bonino S, et al. Uterine artery Doppler at 11^{+0} to 13^{+6} weeks in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol. 2007;30(5):742–749.

[20] Poon LC, Zymeri NA, Zamprakou A, et al. Protocol for measurement of mean arterial pressure at 11–13 weeks' gestation. Fetal Diagn Ther. 2012;31(1):42–48.

[21] Sparks AB, Struble CA, Wang ET, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;206(4):319.e1–319.e9.

[22] Schmid M, White K, Stokowski R, et al. Accuracy and reproducibility of fetal-fraction measurement using relative quantitation at polymorphic loci with microarray. Ultrasound Obstet Gynecol. 2018;51(6):813–817.

[23] American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Gestational hypertension and preeclampsia. ACOG Practice Bulletin No. 202. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2019;133:e1–25.

[24] Tayyar A, Guerra L, Wright A, et al. Uterine artery pulsatility index in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol. 2015;45(6):689–697.

[25] Wright A, Wright D, Ispas CA, et al. Mean arterial pressure in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol. 2015;45(6):698–706.

[26] Wright D, Silva M, Papadopoulos S, et al. Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound Obstet Gynecol. 2015;46(1):42–50.

[27] Shook LL, Clapp MA, Roberts PS, et al. High fetal fraction on first trimester cell-free DNA aneuploidy screening and adverse pregnancy outcomes. Am J Perinatol. 2020;37(01):008–013.

[28] Rolnik DL, Wright D, Poon LC, et al. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. N Engl J Med. 2017;377(7):613–622.