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Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation

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KEYWORDS: Bayes' theorem; first-trimester screening; mean arterial pressure; placental growth factor; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; survival model; uterine artery Doppler

ABSTRACT

Objective *To examine the diagnostic accuracy of a previously developed model for prediction of pre-eclampsia (PE) by a combination of maternal factors and biomarkers at 11–13 weeks' gestation.*

Methods *This was a prospective first-trimester multicenter study of screening for PE in 8775 singleton pregnancies. A previously published algorithm was used for the calculation of patient-specific risk of PE in each individual. The detection rates (DRs) and false-positive rates (FPRs) for delivery with PE < 32, < 37 and ≥ 37 weeks were estimated and compared with those for the dataset used for development of the algorithm.*

Results *In the study population, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE < 32, < 37 and ≥ 37 weeks, respectively. With combined screening by maternal factors, mean arterial pressure, uterine artery pulsatility index and serum placental growth factor, the DR was 100% (95% CI, 80–100%) for PE < 32 weeks, 75% (95% CI, 62–85%) for PE < 37 weeks and 43% (95% CI, 35–50%) for PE ≥ 37 weeks, at a 10% FPR. These DRs were similar to the estimated rates for the dataset used for development of the model: 89% (95% CI, 79–96%) for PE < 32 weeks, 75% (95% CI, 70–80%) for PE < 37 weeks and 47% (95% CI, 44–51%) for PE ≥ 37 weeks.*

Conclusion *Assessment of a combination of maternal factors and biomarkers at 11–13 weeks provides effective first-trimester screening for preterm PE. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Effective screening for preterm pre-eclampsia (PE) can be provided at 11–13 weeks' gestation by assessment of a combination of maternal characteristics and medical history (maternal factors) with multiples of the median (MoM) values of mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) and pregnancy-associated plasma protein-A (PAPP-A). In a previous study, we used data from prospective screening in 35 948 singleton pregnancies at 11–13 weeks to develop an algorithm for the calculation of patient-specific risk of PE[1]. Bayes' theorem was used to combine the *a-priori* risk from maternal factors[2] with various combinations of MAP, UtA-PI, PAPP-A and PlGF[1]. In pregnancies with PE, the deviation from normal for each biomarker was inversely related to the gestational age at delivery and, consequently, the performance of screening was better for early than late PE. The performance of each biomarker in combination with maternal factors was superior to that of screening by maternal factors alone. Similarly, the performance of screening by a combination of two or more biomarkers was superior to that by

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individual biomarkers. The only exception was serum PAPP-A, which did not provide significant improvement to any combination of biomarkers that included serum PlGF. With combined screening by maternal factors, MAP, UtA-PI and PlGF, the detection rate (DR) of delivery with PE < 32, < 37 and ≥ 37 weeks was 89%, 75% and 47%, respectively, at a false-positive rate (FPR) of 10%[1]. A limitation of the study is that the performance of screening by a model derived and tested using the same dataset may be overestimated.

The objective of this study was to determine the accuracy of the previously reported first-trimester screening model for PE[1] in a prospective, non-intervention, multicenter study including 8775 singleton pregnancies. We hypothesize that the performance of screening would be similar to that estimated from the original model[1].

METHODS

Study design and participants

This was a prospective, non-intervention, multicenter study in singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation in women booking for routine pregnancy care at: King's College Hospital, London, UK; Medway Maritime Hospital, Gillingham, UK; Homerton University Hospital, London, UK; North Middlesex University Hospital, London, UK; Southend University Hospital, Essex, UK; Lewisham University Hospital, London, UK; Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain; Hospital Universitario San Cecilio, Granada, Spain; Hospiten Sur, Tenerife, Spain; Centre Hospitalier Universitaire Brugmann, Brussels, Belgium; Attikon University Hospital, Athens, Greece; and Ospedale Maggiore Policlinico, Milan, Italy. The women were screened between February and September 2015 and gave written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee in the UK and the Ethics Committee of each participating hospital in other countries. The Standards for Reporting Diagnostic Accuracy Studies (STARD)[3] was adhered to.

Eligibility criteria for study inclusion were maternal age ≥ 18 years, no serious mental illness or learning difficulty, singleton pregnancy with live fetus demonstrated on 11–13-week ultrasound scan and subsequent delivery of a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. Multiple pregnancies, those with aneuploidy or major fetal abnormality and those ending in termination or miscarriage were excluded.

Test methods

The index test, or the test for which the accuracy was being evaluated, was the previously reported algorithm for first-trimester assessment of risk for PE by maternal factors and various combinations of MAP, UtA-PI, PAPP-A and PlGF[1]. Maternal factors were recorded[2] and MAP was measured by a validated automated device following a standardized protocol[4]. Transabdominal color Doppler ultrasound was performed to measure the left and right UtA-PI and the average value was recorded[5]. Serum PAPP-A and PlGF concentrations were measured using an automated device (PAPP-A and PlGF 1-2-3™ kits, DELFIA® Xpress random access platform; PerkinElmer Inc., Wallac Oy, Turku, Finland). All operators performing Doppler assessment had received the appropriate Certificate of Competence from The Fetal Medicine Foundation. Measured values of MAP, UtA-PI, PAPP-A and PlGF were expressed as MoM, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$-transformed value, including maternal factors, in the prior model[6–9].

The index test was carried out prospectively in consecutive singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation; gestational age was determined from the measurement of fetal crown–rump length[10]. The results from screening were not made available to the patients or their physicians.

The target condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[11]. PE was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 hours apart, developing after 20 weeks of gestation in previously normotensive women. Hypertension was defined as proteinuria of ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above) developing after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or presence of hypertension at booking visit before 20 weeks' gestation, in the absence of trophoblastic disease).

Data on pregnancy outcome were collected from the hospital maternity records of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE.

Statistical analysis

The previously described algorithm[1] was used for calculation of patient-specific risk of delivery with PE < 32, < 37 and ≥ 37 weeks' gestation. The prespecified analyses for performance of screening by maternal factors and any combinations of maternal factors with MAP, UtA-PI, PAPP-A and PlGF were estimation of areas under the receiver–operating characteristics curve (AUC) and DR, with 95% CI, at FPRs of 5% and 10%. The statistical software package R was used for data analyses[12].

RESULTS

Participants

During the study period, 9041 pregnancies met the inclusion criteria and underwent screening for PE. A

Table 1 Characteristics of women with normal singleton pregnancy and of those who developed pre-eclampsia (PE) with delivery < 32 weeks, < 37 weeks or ≥ 37 weeks

		PE with delivery at:		
Characteristic	*Normal (n = 8536)*	*< 32 weeks (n = 17)*	*< 37 weeks (n = 59)*	*≥ 37 weeks (n = 180)*
Maternal age (years)	31.5 (27.3–35.0)	29.8 (26.7–34.6)	30.6 (26.0–34.7)	31.2 (27.8–34.8)
Maternal weight (kg)	66.2 (58.8–76.9)	72.6 (65.6–86.0)	69.8 (63.0–87.8)	75.0 (64.9–84.0)
Maternal height (cm)	165 (160–169)	164 (161–166)	164 (160–169)	164 (159–168)
Body mass index (kg/m^2)	24.5 (21.9–28.2)	27.3 (23.9–31.8)	27.1 (23.6–31.8)	27.8 (23.9–31.5)
Gestational age (weeks)	12.7 (12.3–13.1)	12.6 (12.3–12.7)	12.7 (12.4–13.0)	12.7 (12.3–13.2)
Racial origin				
Caucasian	6716 (78.7)	8 (47.1)	38 (64.4)	129 (71.7)
Afro-Caribbean	1040 (12.2)	8 (47.1)	14 (23.7)	36 (20.0)
East Asian	153 (1.8)	0 (0.0)	0 (0.0)	1 (0.6)
South Asian	447 (5.2)	0 (0.0)	3 (5.1)	12 (6.7)
Mixed	180 (2.1)	1 (5.9)	4 (6.8)	2 (1.1)
Medical history				
Chronic hypertension	75 (0.9)	3 (17.7)	9 (15.3)	16 (8.9)
Diabetes mellitus	63 (0.7)	2 (11.8)	3 (5.1)	2 (1.1)
APS/SLE	32 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Cigarette smoker	717 (8.4)	1 (5.9)	4 (6.8)	11 (6.1)
Family history of PE	434 (5.1)	1 (5.9)	7 (11.9)	17 (9.4)
Mode of conception				
Spontaneous	8254 (96.7)	17 (100)	57 (96.6)	173 (96.1)
In-vitro fertilization	218 (2.6)	0 (0.0)	2 (3.4)	7 (3.9)
Ovulation drugs	64 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Parity				
Nulliparous	3972 (46.5)	11 (64.7)	36 (61.0)	119 (66.1)
Parous				
No previous PE	4396 (51.5)	4 (23.5)	17 (28.8)	46 (25.6)
Previous PE	168 (2.0)	2 (11.8)	6 (10.2)	15 (8.3)
Interpregnancy interval (years)	2.7 (1.6–4.6)	5.4 (4.3–7.2)	4.1 (2.4–6.8)	3.4 (2.0–5.4)

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups were by chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test for continuous variables. APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus.

Table 2 Performance of screening for delivery with pre-eclampsia (PE) < 32, < 37 or ≥ 37 weeks' gestation in validation dataset of 8775 singleton pregnancies using previously developed algorithm based on maternal factors and combinations of biomarkers

	PE with delivery < 32 weeks (n = 17)			*PE with delivery < 37 weeks (n = 59)*			*PE with delivery ≥ 37 weeks (n = 180)*		
		DR (%) at:			*DR (%) at:*			*DR (%) at:*	
Screening method	*AUC*	*FPR = 5%*	*FPR = 10%*	*AUC*	*FPR = 5%*	*FPR = 10%*	*AUC*	*FPR = 5%*	*FPR = 10%*
Maternal factors	0.8045	41 (18–67)	53 (28–77)	0.7583	29 (18–42)	41 (28–54)	0.7449	18 (13–25)	37 (30–45)
Maternal factors plus:									
MAP	0.9071	59 (33–82)	71 (44–90)	0.8243	36 (24–49)	47 (34–61)	0.7789	26 (20–33)	37 (30–45)
UtA-PI	0.9309	71 (44–90)	82 (57–96)	0.8537	47 (34–61)	61 (47–73)	0.7539	22 (16–29)	39 (32–47)
PAPP-A	0.8546	47 (23–72)	59 (33–82)	0.7825	37 (25–51)	47 (34–61)	0.7504	21 (15–28)	37 (30–44)
PlGF	0.9506	65 (38–86)	88 (64–99)	0.8722	49 (36–63)	63 (49–75)	0.7578	20 (14–27)	39 (32–46)
MAP, UtA-PI	0.9667	82 (57–96)	94 (71–100)	0.8958	53 (39–66)	71 (58–82)	0.7875	27 (20–34)	41 (34–49)
MAP, PAPP-A	0.9133	65 (38–86)	76 (50–93)	0.8342	41 (28–54)	49 (36–63)	0.7827	28 (21–35)	40 (33–48)
MAP, PlGF	0.9674	76 (50–93)	88 (64–99)	0.8985	53 (39–66)	69 (56–81)	0.7870	29 (22–36)	43 (36–51)
UtA-PI, PAPP-A	0.9339	71 (44–90)	82 (57–96)	0.8583	49 (36–63)	66 (53–78)	0.7571	24 (18–31)	40 (33–48)
UtA-PI, PlGF	0.9772	82 (57–96)	100 (80–100)	0.9000	61 (47–73)	75 (62–85)	0.7619	22 (16–29)	39 (32–47)
PlGF, PAPP-A	0.9510	65 (38–86)	88 (64–99)	0.8741	51 (37–64)	66 (53–78)	0.7589	20 (14–27)	39 (32–47)
MAP, UtA-PI, PAPP-A	0.9644	88 (64–99)	94 (71–100)	0.8956	61 (47–73)	69 (56–81)	0.7892	29 (22–36)	42 (35–50)
MAP, PAPP-A, PlGF	0.9672	76 (50–93)	88 (64–99)	0.8998	54 (41–67)	69 (56–81)	0.7882	29 (22–36)	43 (36–51)
MAP, UtA-PI, PlGF	0.9870	94 (71–100)	100 (80–100)	0.9242	66 (53–78)	75 (62–85)	0.7916	32 (25–39)	43 (35–50)
UtA-PI, PAPP-A, PlGF	0.9769	82 (57–96)	100 (80–100)	0.9004	61 (47–73)	75 (62–85)	0.7626	23 (17–30)	38 (31–46)
MAP, UtA-PI, PAPP-A, PlGF	0.9865	94 (71–100)	100 (80–100)	0.9241	66 (53–78)	80 (67–89)	0.7923	31 (24–38)	43 (35–50)

Values in parentheses are 95% CI. AUC, area under receiver–operating characteristics curve; DR, detection rate; FPR, false–positive rate; MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

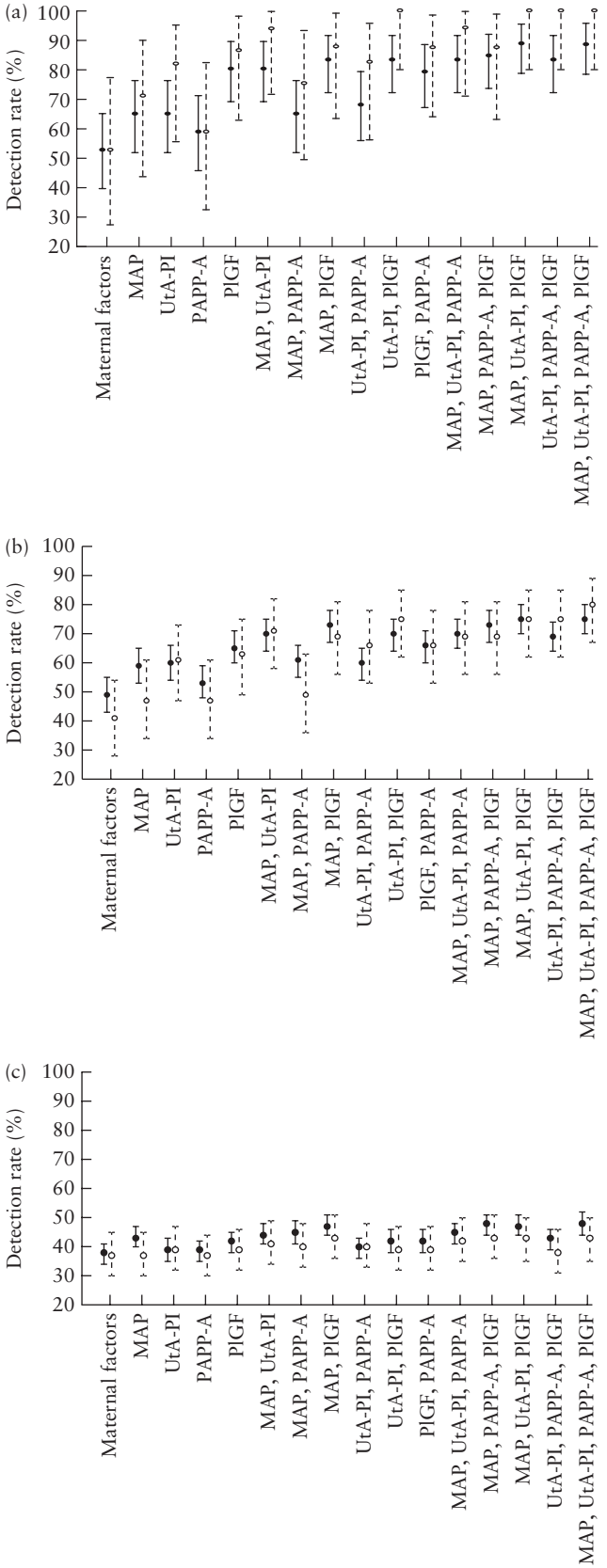

Figure 1 Detection rate (95% CI), at 10% false-positive rate, of pre-eclampsia delivering at: (a) < 32 weeks; (b) < 37 weeks; or (c) ≥ 37 weeks of gestation, with screening by maternal factors and combinations of biomarkers in current dataset (○) and in dataset used for development of screening algorithm[1] (●). MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

total of 266 (2.9%) cases were excluded because they had a major fetal defect ($n = 33$), the pregnancy resulted in termination ($n = 39$) or miscarriage ($n = 88$) or there was no follow up ($n = 106$).

In the study population of 8775 pregnancies, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE < 32, < 37 and ≥ 37 weeks, respectively. Baseline demographic and clinical characteristics of the participants are shown in Table 1. In total, 12 maternity hospitals in five different countries were involved in patient recruitment, 127 doctors participated in the measurement of UtA-PI and 152 doctors or nurses were involved in the measurement of MAP.

Test results

The AUC and DR, at FPRs of 5% and 10%, of delivery with PE < 32, < 37 and ≥ 37 weeks' gestation with screening by maternal factors and biomarkers using the previously reported algorithm[1] are given in Table 2 and compared to previously reported values in Figure 1. The DRs in this validation dataset were similar to the estimated rates for the dataset used for development of the model.

The performance of screening for PE < 37 weeks was superior to that of PE ≥ 37 weeks. The best performance of screening was achieved by a combination of maternal factors, MAP, UtA-PI and PlGF and this was not improved significantly by addition of PAPP-A.

DISCUSSION

Main findings

This prospective multicenter validation study demonstrates the feasibility of incorporating first-trimester screening for PE into routine clinical practice. The findings demonstrate that the performance of screening for PE at 11–13 weeks by a combination of maternal factors and biomarkers is similar to that estimated from the original model[1]. The DR of screening by maternal factors, MAP, UtA-PI and PlGF, at 10% FPR, was 100% (95% CI, 80–100%) for PE < 32 weeks, 75% (95% CI, 62–85%) for PE < 37 weeks and 43% (95% CI, 35–50%) for PE ≥ 37 weeks; the estimated rates for the dataset used for development of the model were 89% (95% CI, 79–96%), 75% (95% CI, 70–80%) and 47% (95% CI, 44–51%), respectively[1].

Study limitations

The main limitation of the study relates to the low incidence of delivery with PE, with the inevitable wide CIs obtained for performance of screening. Nevertheless, the values obtained in the validation study are similar to those in the dataset of 35 948 pregnancies used for development of the algorithm.

Implications for practice

Screening and diagnosis of PE is traditionally based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second or third trimester of pregnancy. In a proposed new pyramid of pregnancy care[13], assessment of risk at 11–13 weeks' gestation aims to identify pregnancies at high risk of developing PE and, through pharmacological intervention with such medications as low-dose aspirin, to reduce the prevalence of these complications[14,15].

The findings of this validation study confirm that screening at 11–13 weeks identifies a high proportion of cases that will develop PE < 37 weeks, but the performance of screening at this stage for those that will develop PE ≥ 37 weeks is poor[1]. This is particularly important because the prophylactic use of low-dose aspirin is effective in the prevention of preterm PE rather than term PE[16]. We reported previously that effective prediction of PE ≥ 37 weeks requires screening at 35–36 weeks[17].

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REFERENCES

1. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11–13 weeks' gestation. *Am J Obstet Gynecol* 2016; **214**: 103.e1–12.
2. Wright D, Syngelaki A, Akolekar R, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal characteristics and medical history. *Am J Obstet Gynecol* 2015; **213**: 62.e1–10.
3. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, LijmerJG Moher D, Rennie D, de Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF, For the STARD Group. STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015; **351**: h5527.
4. Poon LC, Zymeri NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11–13 weeks' gestation. *Fetal Diagn Ther* 2012; **31**: 42–48.
5. Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2007; **30**: 742–749.
6. Wright A, Wright D, Ispas A, Poon LC, Nicolaides KH. Mean arterial pressure in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 698–706.
7. Tayyar A, Guerra L, Wright A, Wright D, Nicolaides KH. Uterine artery pulsatility index in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 689–697.
8. Wright D, Silva M, Papadopoulos S, Wright A, Nicolaides KH. Serum pregnancy associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **46**: 42–50.
9. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 591–598.
10. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
11. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX–XIV.
12. R Development Core Team. *R. A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. 2011; ISBN 3-900051-07-0, http://www.R-project.org/.
13. Nicolaides KH. Turning the pyramid of prenatal care. *Fetal Diagn Ther* 2011; **29**: 183–196.
14. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, Forest JC, Giguère Y. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet Gynecol* 2010; **116**: 402–414.
15. Roberge S, Nicolaides K, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 491–499.
16. Roberge S, Villa P, Nicolaides K, Giguère Y, Vainio M, Bakthi A, Ebrashy A, Bujold E. Early administration of low dose aspirin for the prevention of preterm and term pre-eclampsia: a systematic review and meta-analysis. *Fetal Diagn Ther* 2012; **31**: 141–146.
17. Andrietti S, Silva M, Wright A, Wright D, Nicolaides KH. Competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 35–37 weeks' gestation. *Ultrasound Obstet Gynecol* 2016; **48**: 72–79.

