CEOG Clinical and Experimental Obstetrics & Gynecology

Predictive value of biochemical marker ADAM-12 at first trimester of pregnancy for hypertension and intrauterine growth restriction

C. Kasimis[1], N. Evangelinakis[1], M. Rotas[1], M. Georgitsi[2], N. Pelekanos[2], D. Kassanos[1]

[1] 3rd Department of Obstertrics and Gynaecology, Medical School of Athens, General University Hospital "Attikon", Athens
[2] 4th Department of Internal Medicine, Medical School of Athens, General University Hospital "Attikon", Athens (Greece)

Summary

Purpose: Delineate whereas ADAM-12 levels at first trimester of pregnancy may be used as a marker for hypertension-preeclampsia (PE) and intrauterine growth restriction (IUGR). *Materials and Methods:* The present is a case control study. Serum ADAM-12 of women presenting for routine assessment of risk for chromosomal abnormalities at 11+0 to 13+6 weeks of gestation was measured. The study group comprised of 98 pregnancies that subsequently developed pregnancy-induced hypertension (PIH) or PE or small for gestational age fetuses (SGA), and were compared to 100 uncomplicated pregnancies. *Results:* There was no statistically significant difference of mean log multiple of the expected median (MoM) of ADAM12 between control group and the group that consisted of all women with complicated pregnancy (PE, PIH, and SGA). ADAM-12 levels in women who developed PE during pregnancy were significantly lower than in women of control group (mean log MoM: 0.109 *vs* 0.008, $p = 0.010$). Similarly, ADAM-12 levels in women who developed PE and/or PIH were significantly lower than in women of control group (mean log MoM: 0.066 *vs* 0.008, $p = 0.015$). There was no significant difference of ADAM12 levels between controls and pregnancies with SGA fetuses. *Conclusion:* Maternal serum levels of ADAM-12 at the first trimester are significantly lower in women who later develop PE when compared with women with uncomplicated pregnancies.

Key words: ADAM-12: Preeclampsia; Hypertension; Biochemical marker.

Introduction

Preeclampsia (PE) affects about 2% of pregnancies and remains a major cause of perinatal morbidity and mortality. The underlying pathophysiology for PE is thought to be impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries, and this hypothesis is confirmed by histological and Doppler studies of uterine arteries [1]. Hypertension during pregnancy is distinguished in pregnancy-induced hypertension (PIH) and pregnancy with hypertension accompanied with proteinuria that is called PE. PE is further distinguished in early (<34 weeks of gestation) and late PE (>34 weeks of gestation) [2].

Etiology of PE is multifactorial and remains basically unknown. Genetic predisposition, impaired placentation, immunological disorders, and pre-existing hypertension of the background of hypertension-PE. Over the last decade research has focused on identifying biochemical markers that could predict early on in pregnancy the onset of PE [3].

Biochemical marker ADAM-12 (a disintegrin and metalloproteinase) is associated with intrauterine growth restriction (IUGR), PE, and chromosomal abnormalities [4-6]. ADAM-12 is produced by the placenta and is a part of the ADAM proteins' group, which consists of more than 15 members. ADAM proteins play a key role in adhesion and apoptosis of cells.

In humans ADAM-12 is found in two forms. The soluble ADAM-12 (sADAM-12) is expressed in placenta, whereas ADAM-12-L is expressed in several different tissues. ADAM-12 exerts a proteolytic action on growth factors IGFBP-3 and IGFBP-5 and participates in regulation of action of insulin- growth factors, such as PAPP-A [7, 8]. Disintegration of IGFBP-3 stimulates growth of tissues and consequently non-disintegration of IGFBP-3 is associated with restriction of growth. Biochemical markers that have been associated with hypertension and PE are PAPP-A, β-hCG, placental growth factor (PlGF), PP13, and anti-angiogenic protein soluble FMS-like tyrosine kinase-1 [sFlt-1] [9].

In the first trimester, uterine artery Doppler pulsatility index [PI] alone has a sensitivity of around 20-30% for the detection of PE. This sensitivity is better for the prediction of early PE (40-50%), but this is improved by the addition of measurements of maternal serum biochemistry [10].

Small for gestational age (SGA) are considered the fetuses whose growth and weight is beneath the 10th percentile adjusted for the gestational age. SGA fetuses are distinguished by those that are constitutionally small due

Revised manuscript accepted for publication September 18, 2014

to genetic predisposition, those whose growth is restricted, have abnormal Doppler flow in the vessels, and are called IUGR fetuses. In IUGR fetuses there is usually abnormal flow in umbilical artery (increased resistance) and abnormal Doppler flow in middle cerebral artery (low resistance-low PI- redistribution) and later in the ductus venosus when hypoxia has established absent or reversed flow. The first step is the distinction of true IUGR fetuses, associated with signs of abnormal feto-placental Doppler flow from constitutionally SGA fetuses that have a normal Doppler measurements and perinatal outcome.

IUGR is associated with a poorer perinatal outcome, including impaired Doppler cerebro-placental ratio and an estimated fetal weight (EFW) below the 3rd centile. Once the diagnosis is established, differentiating into early- and late-onset IUGR is useful and distinguishes two quite different clinical situations concerning perinatal outcome and onset of PE [11-15].

The aim of the present study is to delineate where the biochemical marker ADAM-12, measured in maternal serum during the first trimester of pregnancy, could be used as an early predictor for hypertension-PE and IUGR.

Materials and Methods

The present is a case control study. Screening was performed in pregnant women that presented in the Fetal – Maternal unit of the 3rd Department of Obstetrics & Gynaecology of Attikon University Hospital for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency thickness and PAPP-A and free β-hCG at 11^{+0} to 13^{+6} weeks of gestation. Gestational age was determined by sonographic measurement of fetal crown–rump length (CRL).

The authors obtained a thorough medical history which included several maternal characteristics and subsequently blood was drawn. The serum harvested from whole blood was stored at -80 C for future biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study which was approved by Ethical committee of "Attikon" University Hospital.

The study population comprised of 98 pregnancies that subsequently developed PIH or PE or SGA fetuses – neonates, and 100 uncomplicated pregnancies, that delivered appropriate for gestational age and phenotypically normal neonates, as control group.

All women are of Greek origin and none of them had history of hypertension. In those pregnancies that were complicated by SGA neonates (SGA group), PE (PE group) and PE and/or PIH (PE and/or PIH group) maternal serum concentration of ADAM-12 was measured at 11^{+0} – 13^{+6} weeks of gestation. SGA group included pregnancies in which birth weight of neonates was below the 10th percentile adjusted for gestational age. Serum ADAM-12 was measured by a quantitative enzyme–linked immunoassay technique using two kits (human ADAM 12–immunoassay, Lot 306455, Lot 312925). Each sample was diluted twice and results of ADAM-12 are expressed in ng/ml.

Statistical analysis

Median ADAM-12 concentrations were obtained by the antilogarithm to the predicted ADAM12 values as obtained from linear regression analysis of the logarithm of ADAM-12 by gestational days in the control group. Afterwards all ADAM-12 measures were expressed as multiple of the expected median (MoM) in the control group. The logarithm of MoM was compared between the study groups.

Categorical variables are presented as absolute and relative frequencies. Quantitative variables are presented with mean and standard deviation (SD) or with median and interquartile range (IQR). Chi square and Fishers's exact tests were used for the comparison of proportions between the study groups. When the normality assumption was satisfied, the Student's *t*-test was used for the comparison of means of continuous variables between two groups and the Mann-Whitney test when the distribution was not normal. All *p* values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS (version 19.0).

Table 1. — *Demographics and clinical characteristics of the control and pathological groups.*

	Control n=100 n (%)	Pathological n=98 n (%)	*p*
Maternal age, mean (SD)	35.1 (5.0)	35.4 (6.5)	0.715‡
Racial origin			
Greek	100 (100)	98 (100)	-
Other	0 (0)	0 (0)	
BMI (kg/m²), mean (SD)	26 (3)	26.1 (4.7)	0.844‡
Smoking			
No	93 (93)	91 (92.9)	0.969*
Yes	7 (7)	7 (7.1)	
Diabetes			
No	100 (100)	90 (91.8)	0.003**
Yes	0 (0)	8 (8.2)	
Hypertension			
No	98 (100)	98 (100)	-
Yes	0 (0)	0 (0)	
Conception			
Spontaneous	100 (100)	95 (96.9)	0.119**
IVF	0 (0)	3 (3.1)	
Previous pregnancies			
0	76 (76)	78 (79.6)	0.542**
1	22 (22)	20 (20.4)	
2	2 (2)	0 (0)	
Gestational age (weeks), median (IQR)	12 (11.0-12.2)	12 (11.0-12.1)	0.354◊
Birth weight, median (IQR)	3200 (3000-3385)	2100 (1950-2500)	<0.001◊
Gestational age at birth (weeks), median (IQR)	39.1 (39-40)	38 (37-38)	<0.001◊
SGA			
No	100 (100)	23 (23.5)	<0.001
Yes	0 (0)	75 (76.5)	

‡Student's *t*-test; *Chi-square test; **Fisher's exact test; ◊Mann-Whitney test.

Results

ADAM-12 was measured in 100 normal pregnancies and 98 complicated pregnancies. Demographics and clinical characteristics for both groups are shown in Table 1. The two groups were similar in terms of maternal age, BMI, smoking, conception, number of previous pregnancies, and gestational age at the time of ADAM-12 measurement. Eight women

Table 2. — *Demographics and clinical characteristics of the control, PE , and PE and/or PIH groups.*

		Control n=100 A n (%)	SGA n=75 B n (%)	PE and/or PIH n=43 C n (%)	PE n=15 D n (%)	A *vs.* B *p*	A *vs.* C *p*	A *vs.* D *p*
Maternal age, mean (SD)		35.1 (5.0)	35.8 (6.9)	35.3 (5.9)	36.1 (6.6)	0.437‡	0.835	0.490
Racial origin	Greek	100 (100)	75 (100)	43 (100)	15 (100)	-	-	-
	Other	0 (0)	0 (0)	0 (0)	0 (0)			
BMI (kg/m^2), mean (SD)		26 (3)	25.8 (4.7)	26.9 (4.2)	26.7 (3.8)	0.778‡	0.158	0.371
Smoking	No	93 (93)	68 (90.7)	43 (100)	15 (100)	0.573*	0.102**	0.592**
	Yes	7 (7)	7 (9.3)	0 (0)	0 (0)			
Diabetes	No	100 (100)	67 (89.3)	43 (100)	15 (100)	0.001**	-	-
	Yes	0 (0)	8 (10.7)	0 (0)	0 (0)			
Hypertension	No	98 (100)	75 (100)	43 (100)	15 (100)	-	-	-
	Yes	0 (0)	0 (0)	0 (0)	0 (0)			
Conception	Spontaneous	100 (100)	72 (96)	40 (93)	12 (80)	0.077**	0.026**	0.002**
	IVF	0 (0)	3 (4)	3 (7)	3 (20)			
Previous pregnancies	0	76 (76)	61 (81.3)	31 (72.1)	15 (100)	0.496**	0.564**	0.079**
	1	22 (22)	14 (18.7)	12 (27.9)	0 (0)			
	2	2 (2)	0 (0)	0 (0)	0 (0)			
Gestational age (weeks), median (IQR)		12 (11.0-12.2)	12 (11.1-12.1)	11.7 (11-12)	11 (11-12)	0.490◊	0.242◊	0.356◊
Birth weight, median (IQR)		3200 (3000-3385)	2000 (1923-2350)	2790 (1995-3100)	2440 (1995-3424)	<0.001◊	<0.001◊	<0.001◊
Gestational age at birth (weeks), median (IQR)		39.1 (39-40)	38 (36-38)	38 (36-38)	38 (36-39)	<0.001◊	<0.001◊	<0.001◊
SGA	No	100 (100)		23 (53.5)	4 (26.7)	-	<0.001*	<0.001**
	Yes	0 (0)		20 (46.5)	11 (73.3)			

‡Student's t-test; *Chi-square test; **Fisher's exact test; ◊Mann-Whitney test.

Table 3. — *Mean log MoM of ADAM-12 in the study groups.*

Group	Mean (SD)	log MoM Minimum	Maximum	*p**
Control	0.008 (0.155)	-0.313	0.351	
Pathological	-0.012 (0.232)	-0.505	0.502	0.470
PE	-0.109 (0.191)	-0.283	0.127	0.010
PE and/or PIH	-0.066 (0.184)	-0.359	0.188	0.015
SGA	0.025 (0.234)	-0.505	0.502	0.576

**p* value *vs.* control group.

(8.2%) in the complicated group had diabetes. Birth weight and gestational age at birth were significantly lower in that group. Seventy-five infants (76.5%) in the complicated group were classified as SGA. In that group 43 (43.9%) women had PE and/or PIH and 15 (15.3%) women had only PE.

Table 1 and 2 present the demographics and clinical characteristics for SGA, for women with PE, and women with PE and/or PIH. The SGA group, the PE group, and the PE and/or PIH group were similar when compared to the control group in terms of maternal age, BMI, smoking, number of previous pregnancies, and gestational age when ADAM-12 was measured. Diabetes was more frequent in the SGA group. IVF was more frequent in the PE group and the PE and/or PIH group and as expected the birth weight and gestational age at birth were significantly lower in the aforementioned groups and the SGA group as compared to the controls.

Mean log MoM of ADAM12 for all study groups are described in Table 3. There was no statistically significant difference of mean log MoM of ADAM-12 between control group and the group that consisted of all women with complicated pregnancy (regardless if it was PE, PIH or SGA). On the contrary, ADAM-12 levels in women who developed PE during pregnancy had a mean log MoM of -0.109, which was significantly lower than the mean log MoM of 0.008 for ADAM12 levels observed in samples from women of the control group ($p = 0.010$). Similarly, ADAM-12 levels in women who developed PE and/or PIH during pregnancy had a mean log MoM of -0.066, which was significantly lower than the mean log MoM of 0.008 for ADAM12 levels observed in samples from women with normal pregnancies ($p = 0.015$) (Figure 1). Furthermore, no significant difference was found in mean log MoM of ADAM-12 when compared between pregnancies with SGA infants and controls.

Discussion

ADAM-12 is a protease with important role in muscle development and neurogenesis [16]. The gene of ADAM-12 produces two different transcripts: one long form attached to

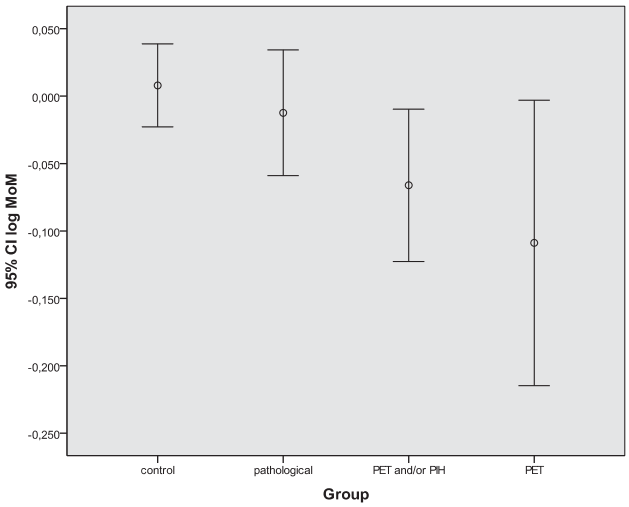

Figure 1. — Error bars of log MoM of ADAM-12 in the study groups.

the membrane, and one shorter that is soluble. Concentration of ADAM-12 is studied in many different conditions during pregnancy and is found to be altered when pathology is involved. Specifically, maternal serum concentrations of ADAM-12 are lower in pregnancies of fetuses with trisomies, such as 18 and 21 [17]. Similar results are reported, also, in pregnancies with other aneuploidies and SGA fetuses [18].

ADAM-12 is a biological marker that has been studied in chromosomally abnormal and IUGR fetuses, and less extensively in PE. The present study shows that women that eventually developed PE and/or hypertension during pregnancy had statistically significantly lower levels of ADAM-12 during the first trimester. The same statistical significance was revealed when women only with PE were studied and compared to the control group. No statistical significance in ADAM-12 serum levels was revealed among women with SGA neonates and the control group. The present results seem to be in accordance to the conclusions presented by other research groups, such as Laigard *et al.*, el-Sharbiny *et al.* [4-7]. In particular, Laigaard *et al.* reported significantly lower concentrations of ADAM-12 in the first trimester in a population of 160 women with PE compared to 324 healthy women with uncomplicated pregnancies [4]. Spencer *et al.* reported that ADAM-12 can be used as a marker for chromosomal abnormalities [17].

Some research groups published less promising results. Poon *et al.* report that measurements of ADAM-12 concentrations at first trimester do not predict SGA and PE [19].

Up-to-date studies indicate that ADAM-12 may have a role in cellular activity and development. ADAM-12-L, that is mainly located at the cellular membrane, as well as the soluble form (s-ADAM-12,. are supposed to be produced by trophoblasts. Some researchers support that ADAM-12 contains receptor areas, like integrins, and this is the manner in which they affect differentiation and cellular survival.

Placentas of preeclamptic women appear to express higher levels of adhesive substances, such as integrins. It is possible that decreased levels of ADAM-12 affect the time required for differentiation and development of the cells.

Previous studies indicate that ADAM-12 disintegrates IGFBP-3 and IGFBP- 5, and it is known that the function of IGF axis is crucial for the development of PE. IGFBP-3 appears to have inhibitory effect on the development of cells. In case of lower levels of ADAM-12, IGFBP-3 will not disintegrate and development of cells is inhibited [20]. Other researchers support that ADAM-12–L interacts with heparin–binding–epidermal growth factor, with placenta leukine aminopeptidase and with other growth factors [21].

Thus, in order to develop a complete screening tool for PE it is important to combine evaluation of risk factors (such as genetic predisposition, history of PE in previous pregnancy, etc.) along with biochemical markers (such as PAPP-A, inhibin, PLGF, ADAM-12) and Doppler measurements at first trimester. False positive results will be significantly decreased and early detection of high-risk women for developing PE will give the chance for early intervention and therapy [11].

Conclusion

Maternal serum levels of ADAM-12 are significantly lower during the first trimester in women who later develop PE during pregnancy when compared to the levels of women with normal pregnancies. Thus, ADAM-12 could be a useful prediction marker for PE, especially when used in combination with other biological markers or Doppler measurements. Maternal serum levels of ADAM-12 are non statistical significantly different during the first trimester in women who later had fetuses SGA when compared with levels of ADAM-12 in normal and uncomplicated pregnancies.

References

[1] Pignenborg R.,Anthony J., Daney D.A., Rees A., Tiltman A., Vercruysse L., Van Assche A.: "Placental bed spiral arteries in the hypertensive disorders of pregnancy". *BJOG*, 1991, *98*, 648.

[2] Livingston J.C., Haddad B., Gorski L.A., Neblett P., Ahokas R.A., Ramsey R., Sibai B.M.: "Placenta growth factor is not an early marker for the development of severe preeclampsia". *Am. J. Obstet. Gynecol.,* 2001, *184*, 1218.

[3] Al Jameil N., Aziz Khan F., Fareed Khan M.: "A brief overview of preeclamsia". *J. Clin. Med. Res.,* 2014, *6*, 1

[4] Laigaard J., Sorensen T., Placing S., Holck P., Frohlich C., Wojdemann K., *et al.*: "Reduction of the disintegrin and metalloprotease ADAM12 in preeclampsia". *Obstet. Gynecol.*, 2005, *106*, 144.

[5] El-Sherbiny W., Nasr A., Soliman A.: "Metalloprotease [ADAM 12-S] as a predictor of preeclampsia: correlation with severity, maternal complications, fetal outcome and Doppler parameters". *Hypertens. Pregnancy*, 2012, *4*, 442

[6] Laigard J., Cristiansen M., Frohlich C., Pedersen B.N., Ottesen B., Wewer U.M.: "The level of Adam 12 –S in maternal serum is an early first trimester marker of fetal trisomy 18 ". *Prenatal. Diagn., 2005, 25*, 45.

[7] Laigard J., Sorensen T., Frohlich C., Pedersen B.N., Christiansen M., Shiott K., *et al.*: "Adam 12: a novel first trimester maternal serum marker for Down syndrome". *Prenat. Diagn.*, 2003, *23*, 1086.

[8] Shi Z., Xu W., Loechel F., Wewer U.M,. Murphy L.J.: "ADAM-12, a disintegrin metalloprotease, interacts with insuline-like growth factor-binding protein-3". *J. Biol. Chem.*, 2000, *275*, 18574

[9] Smith G.C., Stenhouse E.J., Grosley J.A., Aitken D.A., Cameron A.D., Connor J.M.: "Early pregnancy levels of pregnancy – associated plasma protein and the risk of intrauterine growth restriction, premature birth, preeclamsia and stillbirths". *J. Clin. Endocrinol. Metab.*, 2002, *87*, 1762.

[10] Khalil A., Cowans N.J., Spencer K., Goichman S., Meiri H., Harrington K.: "First-Trimester markers for the prediction of pre-eclampsia in women with a-priori high risk". *Ultrasound Obstet. Gynaecol.,* 2010, *35*, 671.

[11] Figueras F., Gratacos E.: "Update on the diagnosis and classification of fetal growth restriction and proposal of a stage-based management protocol". *Fetal Diagn. Ther.*, 2014, *36*, 86. doi: 10.1159/000357592. Epub 2014 Jan 23.

[12] GRIT study Group: "A randomized trial of timed delivery for the compromised preterm fetus: short – term outcomes and Bayesian interpretation". *BJOG*, 2003, *110*, 27.

[13] Martin A.M., Bindra R., Curcio P., Cicero S., Nicolaides K.H.: "Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation". *Ultrasound Obstet. Gynecol.*, 2001, *18*, 583

[14] Poon L.C., Musci T., Song K., Syngelaki A., Nicolaides K.H.: "Maternal plasma cell-free fetal and maternal DNA at 11-13 weeks' gestation: relation to fetal and maternal characteristics and pregnancy outcomes". *Fetal Diagn. Ther.*, 2013, *33*, 215

[15] Karagiannis G., Akolekar R., Sarquis R., Wright D., Nicolaides K.H.: "Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks". *Fetal Diagn. Ther.*, 2011, *29*, 148.

[16] Yang P., Baker K.A., Hagg T.: "A disintegrin and metalloprotease 21 (ADAM21) is associated with neurogenesis and axonal growth in developing and adult rodent CNS". *J. Comp. Neurol.,* 2005, *490*, 163.

[17] Spencer K., Cowans N.J.: "ADAM12 as a marker of trisomy 18 in the first and second trimester of pregnancy". *J. Matern. Fetal Neonatal Med.*, 2007, *20*, 645.

[18] Spencer K., Cowans N.J., Stamatopoulou A.: "Maternal serum ADAM12s as a marker of rare aneuploidies in the first or second trimester of pregnancy" *Prenat. Diagn.*, 2007, *27*, 1233.

[19] Poon L.C., Chelemen T., Granvillano O., Pandeva I., Nicolaides K.H.: "First trimester maternal serum a disintegrin and netalloprotease 12 [ADAM 12] and adverse pregnancy outcome". *Obstet. Gynecol.*, 2008, *112*, 1082.

[20] Landford K.S., Nicolaides K.H.,Jones J., Abbas A., Mc Gregor A.M., Miell J.P.: "Serum insulin-like growth factor-binding protein-3 [IGFBP -3] levels and IGFBP-3 protease activity in normal, abnormal and multiple human pregnancy". *J. Clin. Endocrinol. Metab.*, 1995, *80*, 21.

[21] Poon L.C., Maiz N., Valencia C., Plasencia W., Nicolaides K.H.: "First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia". *Ultrasound Obstet. Gynecol.,* 2009, *33*, 23.

[22] Coolman M., Timmermans S., de Groot C.J., Russcher H., Lindemans J., Hofman A., *et al.*: "Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcome". *Obstet. Gynecol.*, 2012, *119*, 1190. doi: 10.1097/AOG.0b013e318256187f.

[23] Rana S., Powe C.E., Salahuddin S., Verlohren S., Perschel F.H., Levine R.J., et al.: "Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia". *Circulation*, 2012, *125*, 911. doi: 10.1161/CIRCULATIONAHA.111.054361. Epub 2012 Jan 18.

[24] Figueras F., Eixarch E., Gratacos E., Gardosi J.: "Predictiveness of antenatal umbilical artery Doppler for adverse pregnancy outcome in small-for-gestational age babies according to customised birthweight centiles: population-based study". *BJOG*, 2008, *115*, 590.

Address reprint requests to:
C. KASIMIS, M.D.
3rd Dept. of Obstetrics and Gynecology
University of Athens, "Attikon" Hospital
Rimini 1, Chaidari,
Athens 12462 (Greece)
e-mail: h.kasimis@hotmail.com