Prenatal screening in twin pregnancies

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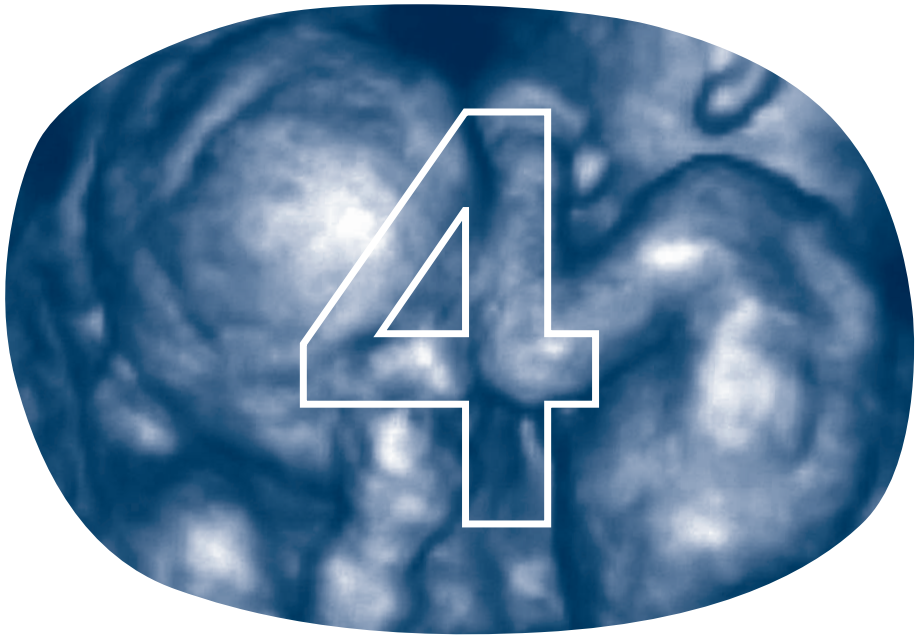

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ADAM12s as a first trimester screening marker of trisomy

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Abstract

. . . Objective
To evaluate the potential of maternal serum A Disintegrin And Metalloprotease 12s (ADAM12s) as an additional marker for the combined test in the Dutch first trimester national Down syndrome (DS) screening program.

. . . Methods
Serum samples were collected between 2004 and 2007 as part of the national screening program. A total of 218 singleton cases of trisomy 21 (DS), 62 trisomy 18 (Edwards syndrome) and 29 trisomy 13 (Patau syndrome) were identified. All cases were matched with controls for gestation, maternal weight and maternal age. The serum concentration of ADAM12s was determined 'blind' to outcome and expressed in multiples of the gestation-specific median for controls (MoM).

. . . Results
The median ADAM12s was 1.00 MoM in controls, and in the DS cases at 8, 9, 10, 11, 12, 13 weeks it was 0.45 (n=3), 0.73 (22), 0.74 (53), 0.85 (37), 0.92 (71), 1.06 (32) MoM, respectively. The median for trisomy 18 was 0.85 MoM and for trisomy 13 0.63 MoM.

. . . Conclusions
The ADAM12s MoM values were clearly reduced in early first trimester for all trisomies. However, the screening performance for DS did not greatly improve adding ADAM12s. ADAM12s could be an additional biochemical marker for first trimester screening for trisomies other than DS.

Introduction

In the Netherlands, all pregnant women are informed of the possibility of having a screening test for Down syndrome (DS) with the first trimester combined test as policy of choice. This test combines maternal age with maternal serum concentrations of pregnancy associated plasma protein-A (PAPP-A), free β subunit of human chorionic gonadotrophin (free β-hCG) and fetal nuchal translucency (NT) measurement by ultrasound. The performance of the Dutch screening program has been described previously [1].

A Disintegrin And Metalloprotease 12s (ADAM12s), the short and secreted spliceform of ADAM12 is a placenta-derived glycoprotein produced by trophoblasts, which is involved in growth and differentiation [2]. Previous studies have shown reduced serum ADAM12s levels in the first trimester of pregnancies with trisomy 21 and in cases with trisomy 18 and other rare aneuploidies [3-6]. ADAM12s is described to improve the test performance of the first trimester combined test [7].

In this study we determined first trimester ADAM12s levels in maternal serum of trisomy 21, 18 and 13 affected pregnancies and evaluated the potential of ADAM12s as an additional marker for the first trimester combined test in the current national screening program. We studied two large series including a wide range of first trimester gestational ages since other published series have indicated that the marker is more discriminatory at early gestations.

Methods

Serum samples were collected between 2004 and 2007 as part of the first trimester screening program in the Netherlands in two centers, the National Institute for Public Health and the Environment (RIVM) and the VU university medical center (VUMC) Amsterdam.
In the screening program blood was taken at 8-13 (RIVM) or 9-13 (VUMC) completed weeks of gestation and immediately tested for serum PAPP-A and free β-hCG using commercially available kits on either a DelfiaXpress (VUMC) or AutoDelfia (RIVM) analyzer (PerkinElmer, Turku, Finland). The agreement between these two assay platforms has been described elsewhere [8]. Unused material was frozen and stored at -20°C for research purposes. Pregnancy outcome was evaluated by questionnaires and collected through self-reporting of the participating women.

During the study period a total of 218 singleton cases of trisomy 21 (DS), 62 trisomy 18 (Edwards syndrome) and 29 trisomy 13 (Patau syndrome) were identified and specimens were retrieved from storage. Sera of the cases were matched with at least seven sera from singleton controls of exactly the same gestational age and as accurate as possible for

sample date (+/- 6 months), maternal weight (within 5-10 kg) and maternal age (years) at sampling. A total of 2466 control samples were studied. The samples had been exposed to a maximum of two freeze/thaw cycles before ADAM12s analysis. Serum ADAM12s was measured blinded for clinical outcome, using a semi-automatically performed time-resolved immunofluorometic assay (AutoDelfia) (PerkinElmer, Turku, Finland). Interassay coefficient of variation (CV) for the ADAM12s assay was below 5% at all levels.
The gestational age at sample date was indicated by the requesting health professional based on either a dating scan or first day of last menstrual period. All samples were accompanied by a form containing information on maternal age, gestation, maternal weight, insulin-dependent diabetes mellitus, NT and crown-rump length.
All markers were expressed as multiple of the median (MoM) for unaffected singleton pregnancies. Log-quadratic regression of the median concentration on median gestation for each completed week of gestation in controls, weighted for the number each week, was used. All MoM values were adjusted for maternal weight using inverse regression.

Results

Patient characteristics of cases and controls are shown in Table 1. Possible confounding variables, ethnicity and smoking status were similar in cases and controls. Because of the matching procedure maternal age, gestational age and maternal weight were also similar. In controls ADAM12s concentrations ranged from 19-1096 µg/L; median concentrations were 195, 237, 302, 349, 415, 475 µg/L in week 8, 9, 10, 11, 12, 13, respectively. ADAM12s concentrations in trisomy 21 cases ranged from 65-917 µg/L. Table 2 shows that the median ADAM12s MoM level in trisomy 21 pregnancies was reduced at before 10 weeks of gestation but the extent of reduction diminished as pregnancy progressed until at 13 weeks the median was above 1.0 MoM. Table 2 also shows the corresponding median PAPP-A and free β-hCG MoM values.

Table 1 Cases and controls baseline characteristics

	Cases (n 309)	Controls (n 2466)	p-value
Median maternal age (years)	37.2 ± 3.8	36.8 ± 3.2	0.15
Median gestational age at sample (days)	82 (range 59-97)	82 (range 59-97)	0.86
Median maternal weight (kg)	67 (range 48-114)	66 (range 42-109)	0.46
Ethnicity (%)			
> Caucasian	95.8	95.3	0.78
> Non-Caucasian	4.2	4.7	
Smoking (%)			
> no	91.9	93.7	0.22
> yes	8.1	6.3	

Table 2 Median maternal serum ADAM12s, PAPP-A and free β-hCG MoM levels in trisomy 21 affected pregnancies according to gestation and the controls; SD* (log10MoM) shown in parentheses

Week	N	ADAM12s	PAPP-A	Free β-hCG
8	3	0.45**(***)	0.46 (***)	0.95 (***)
9	22	0.73 (0.19)	0.51 (0.29)	1.38 (0.26)
10	53	0.74 (0.15)	0.38 (0.28)	1.61 (0.26)
11	37	0.85 (0.19)	0.48 (0.33)	1.53 (0.30)
12	71	0.92 (0.14)	0.53 (0.29)	1.92 (0.28)
13	32	1.06 (0.15)	0.52 (0.23)	2.30 (0.24)
Controls	2466	1.00 (0.16)	1.00 (0.26)	1.00 (0.26)

* Standard deviation estimated from the 10-90th centile range divided by 2.563
** 0.39, 0.45 and 0.80 MoM
*** Too few to estimate

We observed a strong association between ADAM12s and PAPP-A. Correlation coefficients were calculated between logtransformed MoMs, after excluding outliers exceeding 3 standard deviations from the mean. There were too few DS cases at 8 weeks, but the correlation coefficient at 9 weeks was 0.69 ($p<0.0005$), 10 weeks 0.50 ($p<0.0005$), 11 weeks 0.54 ($p<0.001$), 12 weeks 0.40 ($p<0.0005$) and 13 weeks 0.29 ($p=0.11$). In the controls the r-value was 0.41 ($p<0.0001$). The corresponding r-values for ADAM12s and free β-hCG were lower: in DS cases at 9 weeks 0.05 ($P=0.82$), 10 weeks 0.31 ($p<0.05$), 11 weeks 0.34 ($p<0.05$), 12 weeks 0.13 ($p=0.27$) and 13 weeks 0.02 ($p=0.91$); and in controls 0.16 ($p<0.0001$).

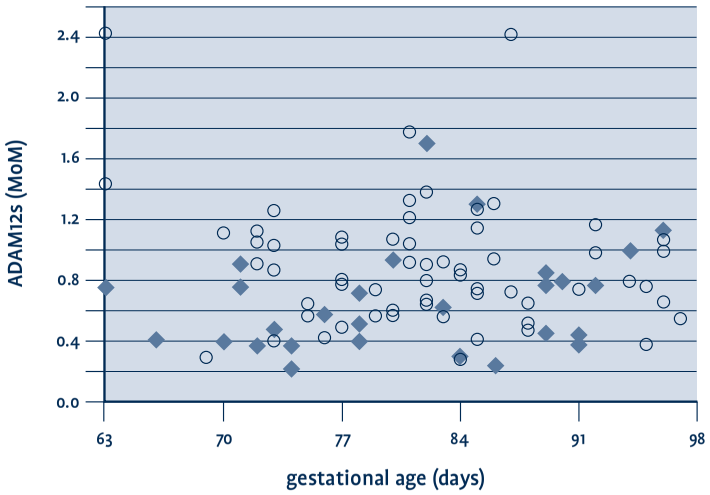
The median MoM of maternal serum ADAM12s in trisomy 18 affected pregnancies was 0.85 MoM and for trisomy 13 affected pregnancies 0.63 MoM (Table 3), a statistically significant difference ($P<0.005$, two-sided Wilcoxon Rank Sum Test). There is no obvious tendency for levels to change with gestation (Figure 1). An association between ADAM12s and PAPP-A was also present in both trisomy 18, with correlation coefficient 0.43 ($P<0.001$), and trisomy 13 with correlation coefficient 0.60 ($P<0.001$). The corresponding values for ADAM12s and free β-hCG were 0.19 ($P=0.17$) and 0.63 ($P<0.0005$), respectively for trisomy 18 and 13.

Table 3 Median maternal serum ADAM12s, PAPP-A and free β-hCG (MoM) and SD* (log10MoM) for trisomies 18 and 13 affected pregnancies

	ADAM12s		PAPP-A		Free β-hCG	
	Median	SD	Median	SD	Median	SD
Trisomy 18 (n 62)	0.85	0.17	0.20	0.44	0.18	0.28
Trisomy 13 (n 29)	0.63	0.22	0.24	0.38	0.44	0.32

*Standard deviation estimated from the 10-90th centile range divided by 2.563

Figure 1 Individual ADAM12s levels for trisomy 18 (○ dot) and trisomy 13 (◆ diamond) according to gestation



Discussion

ADAM12s was introduced as a promising marker for DS screening, but the promise has not been delivered yet. The initial study of Laigaard et al. (2003) showed decreased levels of ADAM12s, with a MoM value of 0.14 in eighteen early first trimester DS pregnancies [3]. ADAM12s seemed to be most powerful at discriminating between trisomy 21 and normal pregnancies early in pregnancy, before 10 weeks. Modelling has predicted that ADAM12s and PAPP-A at 8-9 weeks, combined with NT and free β-hCG at 12 weeks could achieve a detection rate of 97% at a 5% false positive rate [7]. However, the extreme reductions in ADAM12s found in trisomy 21 affected pregnancies seen by Laigaard et al. could not be confirmed to such an extent in more recent studies [9;10]. Our large study on a wide range of first trimester gestations was designed to provide sufficient data to determine the potential of ADAM12s for DS screening at different gestations.

Table 4 summarises the results of all the published first trimester studies of ADAM12s in trisomy 21 affected pregnancies, according to gestational age. All of them show that levels are not different from unaffected pregnancies by 13 weeks of gestation. Furthermore, the lowest values were found before 10 weeks. Our study shows a gradual rise of ADAM12s MoMs in trisomy 21 in the early first trimester. Apparently the upward trend continues into the second trimester when a median of 1.36 MoM was recently reported [11;12].
ADAM12s has been shown to be reduced in cases with trisomy 18 and other rare

Table 4 Median ADAM12s (MoM) in trisomy 21 affected pregnancies according to gestational age in seven studies (number of cases in parentheses)

Study	n	Gestation (completed weeks)					
		6-8	9	10	11	12	13
Laigaard et al. 2003	18	0.03 (7)	0.18 (6)	0.34 (2)	0.32 (3)	-	-
Laigaard et al. 2006a	16	-	0.43 (1)	1.33 (5)	0.61 (9)	1.61 (1)	-
Laigaard et al. 2006b	214	-	-	0.59 (3)	0.49 (39)	0.74 (108)	1.38 (64)
Spencer et al. 2008b	10	0.59 (3)	0.60 (6)	1.34 (1)	-	-	-
Spencer et al. 2008a	46	-	-	-	0.91 (7)	0.90 (23)	1.03 (16)
Spencer et al. 2008	54	0.61 (13)	0.60 (13)	1.15 (8)	0.66 (11)	0.88 (6)	1.52 (3)
Current study	218	0.45 (3)	0.73 (22)	0.74 (53)	0.85 (37)	0.92 (71)	1.06 (32)

aneuploidies [4-6;13]. Laigaard et al. reported a median of 0.28 MoM in 10 trisomy 18 cases [4]. A second study from this group reported two further trisomy 18 cases, one with elevated levels and the other reduced [13]. Spencer et al. reported in 132 first trimester cases of trisomy 18 a median of 0.83 MoM and a median of 0.66 MoM in 60 first trimester trisomy 13 cases [5]. Our findings are remarkably close to these (0.85 MoM in 62 trisomy 18 cases and 0.63 MoM in 29 trisomy 13 cases).

Moreover, notice should be given to the fact that ADAM12s might also be beneficial if screening for adverse pregnancy outcome, other than fetal chromosomal anomalies. Previous studies have found that ADAM12s is decreased in cases developing preeclampsia later in pregnancy [14;15]. Recently, a reduction in first trimester ADAM12s levels has been found in cases later developing fetal growth restriction [16].
ADAM12s as screening marker for DS can only be used in the early first trimester of pregnancy, preferably before 10 weeks of gestation [7;17]. The results of the current study with large numbers of DS affected pregnancies support the results previously published. In the clinical setting of an OSCAR clinic in which serum withdrawal and NT are conducted at the same day ADAM12s will not be a valuable marker. In the Dutch screening program where serum and NT can be conducted separately, and thus serum can be taken earlier in pregnancy, ADAM12s might have some potential. We assessed this by modelling with the observed ADAM12s means, standard deviations and r-values in the current study and previously published meta-analysis parameters for the first trimester combined test

markers [18]. This predicted that the addition of ADAM12s at 9 weeks to the other markers at 11 weeks would increase the detection rate for 5% false-positive rate only from 87% to 88%.
The model assumes that in DS the correlation between ADAM12s and the other serum markers is the same as that observed when all are tested at 9 weeks. If PAPP-A testing was also brought forward to 9 weeks the predicted detection rate, based on the mean at that gestation estimated by Spencer et al. (2002) was 90% [19]. However, these results are based on modelling. Implementation in clinical practice would have far-reaching logistic and financial consequences.

Routine screening for trisomies other than DS in the near future is foreseeable and ADAM12s could be an additional biochemical marker for that purpose in the first trimester. Modelling with the parameters derived by Spencer and Nicolaides (2002) shows that the first trimester combined test has a predicted combined trisomy 18 and 13 detection rate for a 0.5% false-positive rate of 68%. With the addition of ADAM12s this increases to 70% [20].

. . . Acknowledgements

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