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ADAM 12 as a first-trimester maternal serum marker in screening for Down syndrome

Jennie Laigaard[1], Kevin Spencer[2]*, Michael Christiansen[1], Nicholas J. Cowans[2], Severin Olesen Larsen[1], Bent Norgaard Pedersen[1] and Ulla M. Wewer[3]

[1]*Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark*
[2]*Prenatal Screening Unit, Clinical Biochemistry Department, Harold Wood Hospital, Romford, UK*
[3]*Institute of Molecular Pathology, University of Copenhagen, Denmark*

Background   A Disintegrin And Metalloprotease 12 (ADAM 12) is a glycoprotein synthesised by placenta and it has been shown to be a potential first-trimester maternal serum marker for Down syndrome (DS) in two small series. Here we analyse further, the potential of ADAM 12 as a marker for DS in a large collection of first-trimester serum samples.

Materials and Methods   The concentration of ADAM 12 was determined in 10–14-week pregnancy sera from 218 DS pregnancies and 389 gestational age-matched control pregnancies, which had been collected as part of routine prospective first-trimester screening programs (DS = 105) or as part of previous research studies (DS = 113). ADAM 12 was measured using a semi-automated time resolved immunofluorometric assay and median values for normal pregnancies were established by polynomial regression. These medians were then used to determine population distribution parameters for DS and normal pregnancy groups. Correlation with previously established PAPP-A and free β-hCG multiple of the medians (MoMs) and delta nuchal translucency (NT) were determined and used to model the performance of first-trimester screening with ADAM 12 in combination with other first-trimester markers at various time periods across the first trimester. The benefits of a contingent testing model incorporating early measurement of PAPP-A and ADAM 12 were also explored.

Results   The maternal serum concentration of ADAM 12 was significantly reduced ($p = 0.0049$) with an overall median MoM of 0.79 in the DS cases and a $\log_{10}$ MoM SD of 0.3734 in the DS cases and 0.3353 in the controls. There was a significant correlation of ADAM 12 MoM in DS cases with gestational age ($r = 0.375$) and the median MoM increased from 0.50 at 10–11 weeks to 1.38 at 13 weeks. ADAM 12 was correlated with maternal weight (r(controls) $= 0.283$), PAPP-A (r(controls) $= 0.324$, r(DS) $= 0.251$) but less so with free β-hCG (r(controls) $= 0.062$, r(DS) $= 0.049$) and delta NT (r(controls) $= 0.110$, r(DS) $= 0.151$). ADAM 12 was significantly ($p = 0.026$) lower in smokers (0.87 *vs* 1.00) and elevated in Afro-Caribbean women compared to Caucasian women (1.34 *vs* 1.00).

Population modelling using parameters from this and an earlier study showed that a combination of ADAM 12 and PAPP-A measured at 8–9 weeks and combined with NT and free β-hCG measured at 12 weeks could achieve a detection rate of 97% at a 5% false-positive rate or 89% at a 1% false-positive rate. PAPP-A and ADAM 12 alone at 8–9 weeks could identify 91% of cases at a 5% false-positive rate. Using this as part of a contingent-screening model to select an intermediate risk group of women for NT and free β-hCG at 11–12 weeks would enable the detection of 92% of cases with a 1% false-positive rate at a cost of providing NT and free β-hCG for 6% of women with 94% of women having completed screening by the 10th week of pregnancy.

Conclusion   ADAM 12 in early first trimester is a very efficient marker of DS. In combination with existing markers, it offers enhanced screening efficiency in a two-stage sequential first-trimester screening program or in a contingent-screening model, which may have benefits in health economies where universal access to high quality ultrasound is difficult. More data on early first-trimester cases with DS are required to establish more secure population parameters by which to assess further the validity of these models. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: prenatal screening; PAPP-A; free β-hCG; growth factors; ADAM 12; aneuploidy; trisomy 21

INTRODUCTION

ADAM 12 is the secreted form of A Disintegrin And Metalloprotease 12, a glycoprotein synthesised by the placenta and secreted throughout pregnancy (Gilpin *et al.*, 1998; Shi *et al.*, 2000). ADAM12 has a proteolytic function against insulin-like growth factor binding protein, IGFBP-3 and IGFBP-5 (Loechel *et al.*, 2000) and regulates the bioavailability and action of IGF-II and IGF-I (Rosenfeld and Roberts, 1999; Lamson *et al.*, 1993).

In small series of maternal serum samples, ADAM 12 has been shown to be reduced in the first trimester in Down syndrome (DS) (Laigaard *et al.*, 2003, 2006) and trisomy 18 pregnancies (Laigaard *et al.*, 2005b) as well as in women later developing preeclampsia (Laigaard

*Correspondence to: Kevin Spencer, Prenatal Screening Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, UK. E-mail: KevinSpencer1@aol.com

et al., 2005a). The discriminatory efficiency of ADAM 12 appears to decrease with advancing gestation and it has been suggested, based on a very small number of samples, that ADAM 12 is not useful in gestational week 11–13 (Laigaard *et al*., 2006), at a time when serum screening is often performed in conjunction with nuchal translucency (NT) as part of the combined screening test (Spencer *et al*., 2003a).

In order to develop further our understanding of ADAM 12 and its potential value as a first-trimester marker of DS, we undertook a large-scale study of cases and controls collected as part of a first-trimester combined ultrasound and biochemical screening program for chromosomal anomalies (OSCAR) (Spencer *et al*., 2003a).

MATERIALS AND METHODS

All women booked for maternity care at Harold Wood Hospital, Essex (between June 1998 and December 2003), King George Hospital, Goodmayes (between July 2001 and December 2003), Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2003), William Harvey Hospital, Ashford (between July 2002 and December 2003) and Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2003) were offered screening for DS by a combination of fetal NT and maternal serum free β-hCG and pregnancy associated plasma protein-A (PAPP-A) at 11^{+0} to 13^{+6} weeks. The maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyser (Brahms AG, Berlin) and the performance of this assay has been described previously (Spencer *et al*., 1999). An ultrasound examination was carried out to measure the fetal NT and crown-rump length (CRL) and to diagnose any major structural defects. All scans were carried out by sonographers who had obtained the Fetal Medicine Foundation certificate of competence in the 11–14 weeks scan (www.fetalmedicine.com). Patient specific risks were calculated by a multivariate approach using population parameters established in large-scale retrospective studies and prospective studies (Snijders *et al*., 1998; Spencer *et al*., 1999) and the maternal age and gestational related risk of DS at the time of screening (Snijders *et al*., 1999). Women with a risk of $>1:300$

were offered invasive testing to determine the fetal karyotype. Data on pregnancy outcome were obtained from the cytogenetics laboratories, the National Down's Syndrome Cytogenetic Register, and the maternity units in which the fetus was delivered. From amongst this population of 45 257 singleton pregnancies screened some 105 cases with DS were ascertained. Of the DS cases, 96 were identified by the combined screening program and nine were live-born cases missed by the program. As a control group, 389 samples from across the gestational ranges 10–14 weeks were selected. All samples had been analysed for routine biochemical markers within 1 h of sample collection and then stored at 4 °C prior to archive storage at −20 °C or −40 °C at the end of the day. The cases with DS were further supplemented with a series of 113 samples collected as part of previous research studies (Spencer *et al*., 2002). Table 1 shows the distribution of cases and controls by gestational week. In all cases, gestational age at sample collection (median controls = 85 days; median cases = 88 days) was determined by CRL measurement (median controls = 56.9 mm; median cases = 62.0 mm). Samples had on average been frozen and thawed as aliquots twice (range 1–3). The control group had a median maternal age of 29.5 years while that for the group of cases was 37.0 years. The maternal weight of the two groups was similar (median controls = 65.0 kg; median cases was 63.5 kg). The proportion of smokers in the controls was 17% in the controls and 10% amongst the cases. The group was predominantly of Caucasian origin (85% in the controls and 83% in the cases). All samples were collected as part of projects approved by relevant local ethics committees or were obtained as part of routine screening services for which patients gave written consent for excess diagnostic material to be used for research purposes.

In order to better assess how ADAM 12 changed in normal pregnancy into the second trimester, a series of 362 second trimester samples collected between the 14th and 19th week of gestation were also assessed (Christiansen *et al*., 2006).

ADAM 12 was measured by a semi-automated time resolved immunofluorometric assay using the AutoDelfia platform (PerkinElmer, Turku, Finland) as previously described (Laigaard *et al*., 2005a). Briefly

Table 1—Distribution of cases and controls by gestational age and study

Gestational week	Down			Controls	
	Laigaard *et al*., 2003	This study research series	This study routine series	Laigaard *et al*., 2003	This study
7	3	0	0	1	0
8	3	0	0	26	0
9	7	0	0	52	0
10	2	0	3	36	52
11	3	23	17	21	117
12	0	60	49	12	116
13	0	30	36	6	104

recombinant human ADAM 12 was used for standardisation. A previously described (Gilpin *et al.*, 1998) monoclonal capture antibody (6E6) was coated onto the surface of microtitre plates after pre-washing the coated plates; 50 uL of sample and 50 uL of AutoDelfia multibuffer were added to each well followed by a 2-h room temperature incubation and four cycles of wash with multibuffer. The detection antibody was a previously described (Gilpin *et al.*, 1998) monoclonal (8F8) which was biotinylated (Laigaard *et al.*, 2005a). The biotinylated detection antibody was added to each well and incubated for 1 h at room temperature followed by a further four cycles of wash. Europium labelled streptavidin was added to each well and incubated for 1 h at room temperature followed by three wash cycles and the addition of enhancement solution and counting of immunofluoresence after 10 min. The analytical performance of this assay has been previously described (Laigaard *et al.*, 2005a).

Statistical analysis of data was performed using Microsoft Excel 2000 and Analyse-It (Smart Software, Leeds, UK), a statistical software add-in. To correct for biochemical marker variations with gestational age, each value was converted to a multiple of the median (MoM) for unaffected pregnancies at the same gestational age using either previously established relationships for PAPP-A and free β-hCG (Ong *et al.*, 2000) or as established for ADAM 12 in this study. Regression analysis was carried out to derive the relationship between ADAM 12 and gestational age. Correction of each MoM for maternal weight was performed as previously described (Spencer *et al.*, 2003b) and as described for ADAM 12. Goodness of fit to a Gaussian distribution of $\log_{10}$ ADAM 12 MoM in the control group and the Down group was confirmed using normal probability plots and the Shapiro-Wilk test. NT was normalised for gestational age by conversion to delta NT as previously described (Spencer *et al.*, 2003c). Marker correlations were determined using Pearson's correlation coefficient.

The performance of various marker combinations as potential screening procedures was examined using standard statistical modelling techniques (Royston and Thompson, 1992). Using the observed population parameters for ADAM 12 and those for PAPP-A and free β-hCG from Spencer *et al.* (2003d) and for delta NT from Spencer *et al.* (2003c), a series of random MoM or delta NT values were selected from the distributions in unaffected and affected pregnancies. These values were then used to calculate likelihood ratios for the combinations. The likelihood ratios were then used together with the age related risk of DS at 12 weeks of gestation (Snijders *et al.*, 1999) to calculate the expected detection rate of affected pregnancies at various false-positive rates, in a population with the maternal age distribution of pregnancies in England and Wales for an age standardised population with a mean age of 27.0 (SD 5.5) using the methodology and parameters described in Cuckle *et al.*, 2004.

To enhance the modelling of gestational age–dependant detection rates, we incorporated ADAM 12 data from Down cases and controls collected between 8 and 11 weeks from a previous study (Laigaard *et al.*, 2003)

to allow an analysis of the temporal relationship of ADAM 12 across the first trimester. Table 1 summarises the origin and gestation week distributions of the cases and controls from the various sample groups. We utilised the temporal relationship for free β-hCG and PAPP-A as described in previous studies (Spencer *et al.*, 2002, 2003d). Using the measured parameters for ADAM 12, the correlations established in this study and the published parameters for free β-hCG and PAPP-A (Spencer *et al.*, 2002, 2003d) we modelled the expected detection rate at various gestations across the first trimester using various marker combinations incorporating ADAM 12.

The performance of contingent, stepwise screening using the combination of PAPP-A and ADAM 12 in week 8–9 to select the pregnancies that may benefit from NT screening in combination with free βhCG in week 11–13, was assessed as previously described for PAPP-A and βhCG (Christiansen and Larsen, 2002). The precise calculations were performed as a Monte Carlo simulation where initially 100 000 *a priori* risk values were established for cases with DS and for normal cases using age dependent risk values (Cuckle *et al.*, 1987) and a standard age distribution for birth giving mothers (van der Veen *et al.*, 1997). These risk values were then combined with the same number of simulated normally distributed marker values for DS and normal cases allowing correlation between all markers involved. Risk values were subsequently calculated combining likelihood ratio values with the *a priori* risk values, and it was determined in step 1 for each case whether a positive result (risk $> 1:65$) leading to immediate amniocentesis/CVS or a negative result (risk $< 1:1000$) leading to no further action was reported. For a number of cases, intermediate results ($1:1000 <$ risk $< 1:65$) were calculated leading to further screening in step 2 taking account also of the marker values found for step 1. In the final step, a risk of $1:400$ for giving birth to a DS child was considered the cutoff for invasive diagnostic procedures.

RESULTS

In normal pregnancies, we found that ADAM 12 concentrations declined with gestation through the first trimester to reach a trough in the early second trimester from which ADAM 12 then began to increase. The best fit to the data was described by a third order polynomial of the form Median ADAM 12 ug/L $=$ $0.0009 \times GA3 - 0.2206 \times GA2 + 14.271 \times GA - 82.755$ with GA being gestational age in days weighted by the number of cases in each gestational week (Figure 1).

Using the median curve derived above, the overall control population showed a median ADAM 12 MoM of 0.99. When the distribution of $\log_{10}$ MoM ADAM 12 was examined in the first-trimester control case, the mean $\log_{10}$ ADAM 12 was -0.002 with a standard deviation of 0.3485. In the Down cases the overall median ADAM 12 MoM was 0.79, with a median of 0.65 in the cases from routine screening and 0.86 in the research study series. When the distribution of $\log_{10}$
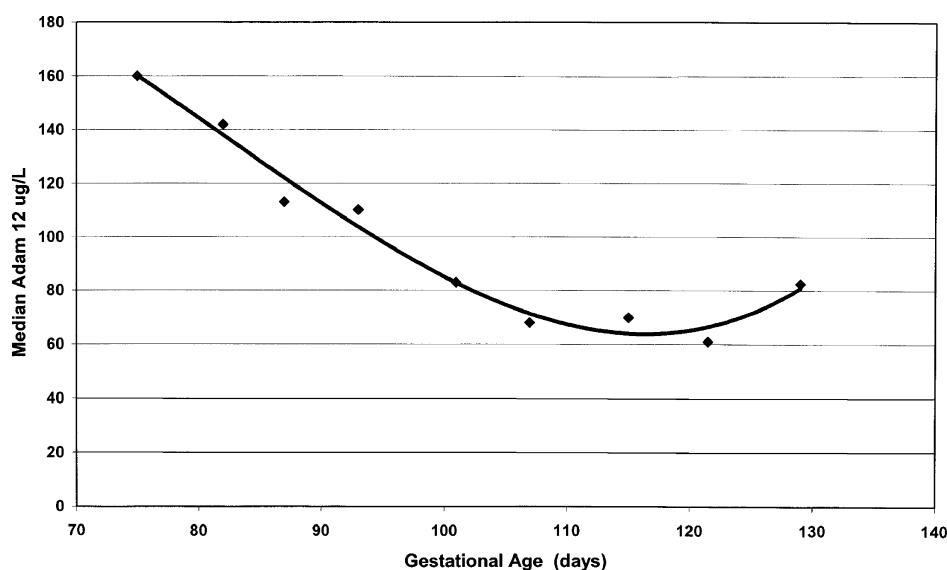
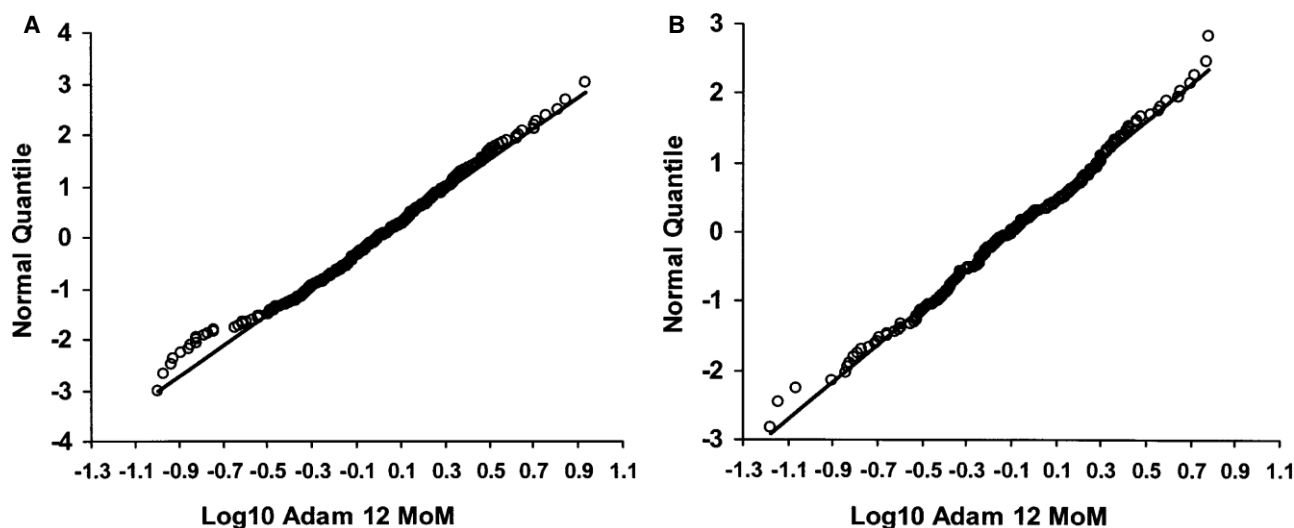Figure 1—Variation of ADAM 12 with gestational age

Figure 2—(a) Normal probability plot for Log_{10} ADAM 12 MoM in unaffected pregnancies. (b) Normal probability plot for Log_{10} ADAM 12 MoM in Down pregnancies

MoM ADAM 12 was examined in the Down cases the mean $\log_{10}$ ADAM 12 was −0.095 with a standard deviation of 0.3694. ADAM 12 MoM fitted reasonably well to a Gaussian distribution after $\log_{10}$ transformation (Figure 2). When the $\log_{10}$ ADAM 12 MoM in the control group and the Down group were compared using *t*-tests of unequal variance the mean in the Down group was significantly lower ($p = 0.0049$) than in the control group.

In the control population, there was a significant negative correlation of ADAM 12 MoM with maternal weight ($r = 0.283$) and a suitable reciprocal weight correction formula was derived to correct for this (Corrected MoM = Measured MoM/((50.00*1/weight) +0.2272). When corrected for maternal weight the overall median for the cases was still 0.79 with the median for the routine screening series being 0.67 and

research study series 0.87. This difference was not statistically significant ($p = 0.059$). Weight corrected SD's were 0.3353 in the controls and 0.3734 for T21 cases. Correlation analysis was performed after exclusion of outliers outside of ±3 SD. Correlation of weight corrected $\log_{10}$ ADAM 12 MoM with $\log_{10}$ PAPP-A MoM in the control series was 0.324 and 0.251 in the Down cases. Correlation of weight corrected $\log_{10}$ ADAM 12 MoM with $\log_{10}$ free β-hCG MoM in the control series was 0.062 and 0.049 in the Down cases. Correlation of weight corrected $\log_{10}$ ADAM 12 MoM with delta NT in the control series was 0.110 and 0.151 in the Down series.

In the control group, the median MoM ADAM 12 was significantly lower in women who smoked compared with non-smokers (0.87 *vs* 1.00; $p = 0.026$). In the control group, there was an apparent higher median
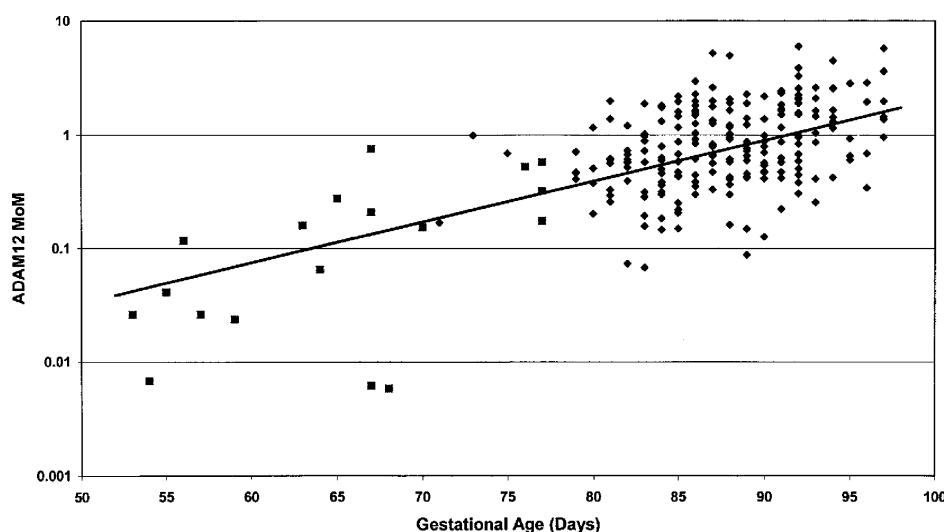
Figure 3—ADAM 12 MoM in cases with Down syndrome in the first trimester from the new study (♦) and from the original Laigaard *et al.*, 2003 study (■). The solid line is the regression line

weight corrected MoM of 1.34 for ADAM 12 in the group of Afro-Caribbean women ($n = 19$) compared with 0.96 in the group of Asian women ($n = 38$) and 1.00 in the Caucasian group ($n = 318$).

When the median ADAM 12 MoM was examined in the DS group with gestational age a significant association was found (Figure 3). The correlation coefficient between $\log_{10}$ ADAM 12 MoM and gestational days was 0.6329. When analysed by completed gestational week the median MoM was 0.59 at 10 weeks ($n = 3$), 0.49 at 11 weeks ($n = 39$), 0.74 at 12 weeks ($n = 108$) and 1.38 at 13 weeks ($n = 64$). Overall, taking the group within the 10th and 11th completed week, the median ADAM 12 MoM in cases was 0.50 with a $\log_{10}$ SD after maternal weight correction of 0.325 in cases and 0.290 in controls. In the 12th and 13th completed week, the median ADAM 12 MoM in cases was 0.93 with a $\log_{10}$ SD of 0.360 in cases and 0.361 in controls. From the

previously published series (Laigaard *et al.*, 2003), the median in the 15 cases at 7–10 weeks of gestation was 0.06 with a $\log_{10}$ SD of 0.6256 in cases and 0.2929 in the 115 controls. The median regression line was Log_{10} MoM $= 0.03599 \times$ Gestational Day $- 3.27112$.

The measured gestational age-specific parameters for ADAM 12 as described above and those for the other biochemical markers from (Spencer *et al.*, 2002, 2003d) used in the simulation exercise are summarised in Table 1. Table 3 shows the results of modelling detection rates at various false-positive rates when the biochemical markers are combined with maternal age in different gestational weeks and when also combined with NT measurement at 12 weeks of gestation.

The performance of contingent screening is outlined in Figure 4. In accordance with the performance data in Table 2, the large majority, 81.5% of DS cases, would have a risk $>1 : 65$ and consequently be offered
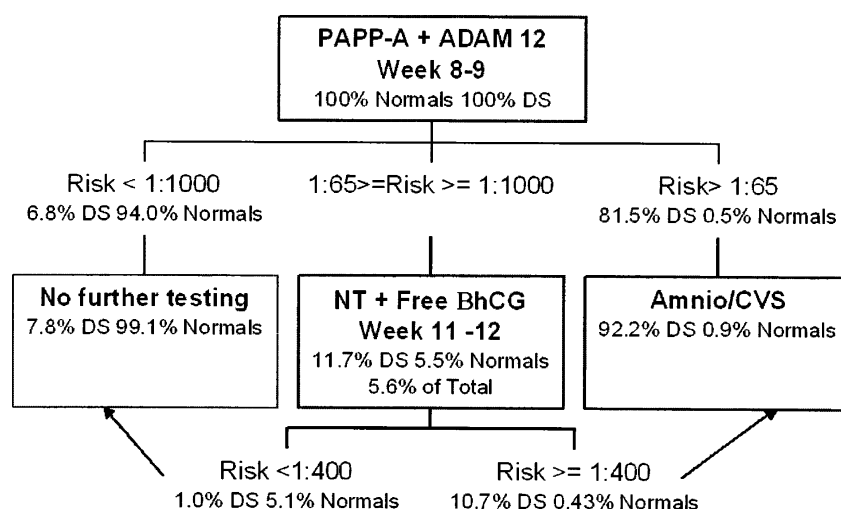

Figure 4—Contingent screening using PAPP-A and ADAM 12 measured at 8–9 weeks, with the selection of women with risks between $1 : 65$ and $1 : 1000$ for further testing at 11–12 weeks with NT and free β-hCG

Table 2—Summary of population parameters used in the simulation study

Period	Mean $\log_{10}$ MoM T21	SD T21	SD control
ADAM 12 8–9 weeks	−1.222	0.6256	0.2929
ADAM 12 10–11 weeks	−0.3010	0.3251	0.2900
ADAM 12 12–13 weeks	−0.0320	0.3603	0.3612
Free β-hCG 8–9 weeks	0.1535	0.2858	0.2618
Free β-hCG 10–11 weeks	0.2683	0.2858	0.2618
Free β-hCG 12–13 weeks	0.3857	0.2858	0.2618
PAPP-A 8–9 weeks	−0.4529	0.3027	0.2362
PAPP-A 10–11 weeks	−0.3369	0.3027	0.2362
PAPP-A 12–13 weeks	−0.2445	0.3027	0.2362
NT 12–13 weeks	0.3050	0.2350	0.1200

Correlation (r) values

Marker	T21	Controls
PAPP-A *vs* ADAM 12	0.2512	0.3240
Free β-hCG *vs* ADAM 12	0.0490	0.0624
PAPP-A *vs* Free β-hCG	0.1250	0.2178
PAPP-A *vs* NT	0	0
Free β-hCG *vs* NT	0	0
ADAM 12 *vs* NT	0.1510	0.1100

CVS without further—and later—screening, whereas 94% of the non-affected pregnancies would have a risk <1:1000 and consequently not be offered further screening as such screening would improve DR marginally. Only 5.6% of all pregnancies would be offered NT and free βhCG screening. The overall detection for DS would be 92% for a mere 0.9% false-positive rate. And more than 90% of all pregnant women would receive a final risk estimate before week 10.

DISCUSSION

The present study is the first large-scale study of the performance of ADAM 12 as a first-trimester marker for DS and the results confirm the findings in the previously published small series (Laigaard *et al.*, 2003, 2006). It

is also confirmed that the difference between ADAM 12 levels in DS and control pregnancies decrease going from week 10–11 to week 12–13, but, surprisingly, even though the difference is negligible in week 12–13, the marker is still useful in combination with PAPP-A and free βhCG (Table 3). This is most likely a result of the significant correlation with PAPP-A (Table 2).

The previously expressed doubt about the suitability of ADAM 12 as a marker in the OSCAR setting (Laigaard *et al.*, 2006) does not seem to be well founded. However, ADAM 12 has the best discriminatory efficiency early in the first trimester (Table 2). In that respect, the marker behaves in a manner similar to PAPP-A (Spencer *et al.*, 2002, 2003d; Christiansen and Jaliashvili, 2003), ProMBP (Christiansen *et al.*, 1999, 2004) and SP1 (Qin *et al.*, 1997).

From the performance of ADAM 12 in combination with PAPP-A as the first step—performed in week 8–9—in a contingent screening model, it is seen that a very impressive overall screening performance can be obtained with less than 6% being offered NT screening and more than 90% of women having a final risk result before week 10. However, from a health economic point of view the benefit to detection of adding the second biochemistry test at 12 weeks (free βhCG) may be questionable.

Thus, one potential role for ADAM 12 may be in health economies where universal access to high quality ultrasound and NT measurement is difficult either due to lack of availability of trained sonographers or when cost is a major consideration. In this regard, a simple blood test at 8–9 weeks could be used in the Contingent type model along with PAPP-A. One perceived disadvantage to such a system may be that women in some health economies do not present this early for screening; however, in Europe there is a growing desire to introduce early biochemical screening which takes into account the additional detection brought about by early screening with PAPP-A, and which enables a combined risk and counselling to be provided at the time of the NT examination.

The molecular background for the reduced maternal serum level of ADAM 12 in DS pregnancies has not been established, so the clinical use of the marker should

Table 3—Simulated population detection rates (%) at various screen-positive rates using biochemistry at 8–9 weeks, 10–11 weeks or 12–13 weeks, and NT (when included) at 12 weeks

Screen-positive rate	5%			3%			1%		
Combination (weeks)	8–9	10–11	12–13	8–9	10–11	12–13	8–9	10–11	12–13
ADAM 12 & maternal age	90	48	43	87	42	32	63	24	19
Free β-hCG & maternal age	30	39	52	23	31	43	14	19	27
PAPP-A & maternal age	56	43	35	48	36	28	30	20	17
PAPP-A, Free β-hCG & age	64	61	64	55	52	56	39	37	40
PAPP-A, ADAM 12 & age	91	58	50	89	49	41	69	32	27
ADAM 12, Free β-hCG & age	90	57	62	87	49	53	66	34	38
Free β-hCG, ADAM 12, PAPP-A & age	92	70	74	88	63	66	71	47	51
Free β-hCG, PAPP-A, NT & age	86	85	86	82	80	82	72	71	73
PAPP-A, ADAM 12, NT & age	97	84	80	95	80	76	87	70	66
Free β-hCG, ADAM 12, NT & age	96	84	85	94	80	81	87	71	72
Free β-hCG, PAPP-A, ADAM 12, NT & age	97	89	90	95	86	86	89	78	78

be performed with care and it is imperative that the marker is only used in a setting with full follow-up and continuous monitoring of screening parameters.

Further studies should be directed at documenting the retrospective and prospective efficiency of the markers, particularly in conjunction with other markers in the early first trimester.

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