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Short Report

Inhibin A is a maternal serum marker for Down's syndrome early in the first trimester

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Inhibin A is a maternal serum marker for fetal Down's syndrome (DS) in the second trimester. We examined whether inhibin A could be used early in the first trimester. Maternal serum concentrations of inhibin A were determined in 81 controls and 27 cases of fetal trisomy 21 in gestational week 5–11. The log MoM (*M*ultiple *o*f the *M*edian of normal pregnancies) inhibin A concentration in DS pregnancies increased with gestational age ($p = 0.001$) from a mean log MoM (standard deviation) of −0.1754 (0.3712) ($n = 11$) in week 7–8 to a mean log MoM (standard deviation) of 0.1842 (0.2145) ($n = 12$) in week 9–11. This corresponded to an increase in inhibin median MoM from 0.67 to 1.53. When inhibin A was used together with pregnancy-associated plasma protein-A, free β-human chorionic gonadotrophin and nuchal translucency as DS markers, the estimated detection rates were 81.4 and 82.6% in weeks 7–8 and 9–11, respectively, for false-positive rates of 0.9 and 1.0%. The performance of the latter combination early in the first trimester is nearly as good as that of integrated first- and second-trimester screening, with the further advantage that the risk can be reported to the pregnant woman in first trimester.

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Maternal serum screening for chromosomal abnormalities has moved into first trimester with the combined first-trimester serological and nuchal translucency screening [pregnancy-associated plasma protein-A (PAPP-A) + free βhuman chononic gonadotrophin (βhC; G) + nuchal translucency (NT) + age] (1). However, integrated screening [PAPP-A and NT in first trimester followed by the quadruple test [alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE3) and inhibin A] in second trimester] (2, 3) is currently the most effective screening method. The integrated test has the disadvantage that the risk is reported to the pregnant woman in second trimester. If more efficient first-trimester serological markers can be developed, it may be possible to rely completely on first-trimester markers without reducing screening performance.

Inhibin A is a dimeric glycoprotein of predominantly placental origin (4, 5). It belongs to the transforming growth factor-β superfamily (6) and is characterized by its ability to inhibit follicle-stimulating hormone secretion (7). Inhibin A has, if quantified with modern high-specificity assays (8, 9), been shown to be a second-trimester maternal serum marker for fetal Down's syndrome (DS), adding approximately 5% to the detection rate (DR) of the triple test (10–16).

We investigated whether inhibin A may be used as a maternal serum marker for fetal DS in early first trimester. Previous studies (10, 17–20) in the 10–14-week gestational age window have indicated that the effect of including inhibin A in a first-trimester screening program is insignificant (18), despite inhibin A MoM values ranging from 1.19 (19) to 2.46 in DS pregnancies (20). We analysed the distribution of inhibin A in week 5–11 and estimated the performance of normal serological first-trimester screening with inhibin A included. Furthermore, we estimated the

screening performance of early first-trimester serological screening with inhibin A in combination with NT determined in week 11–14.

Materials and methods

Maternal serum samples from gestational week 5–11 were obtained through maternal serum-screening programmes for syphilis and DS at Statens Serum Institut, Copenhagen. Serum samples from 81 women with a normal pregnancy outcome and 27 with a trisomy 21 fetus were identified through the Danish Central Cytogenetic Registry as part of quality control activities. Eighteen DS pregnancies were diagnosed in second trimester and nine at birth. All diagnoses were verified by karyotyping. All samples were stored at −20 °C from arrival at Statens Serum Institut to the time of analysis.

Inhibin A in serum was quantified using an enzyme-amplified enzyme immunoassay ultrasensitive Inhibin A dimer assay kit (Product code MCA 950KZZ, Serotec, Oxford, UK). Maternal serum concentrations of PAPP-A and β-hCG determined by a combined PAPP-A and β-hCG TrIFMA assay (21) were available for some of the samples.

The median for each gestational week was estimated as the mean $\log_{10}$ concentration of inhibin A in normal pregnancies. This empiric median was used to express all concentrations, both in normal pregnancies and in affected pregnancies as $\log_{10}$ MoMs (*M*ultiples *o*f the *M*edian). Compatibility with the normal distribution was assessed by normal plots. Performance estimates of combinations of screening markers were produced using a published S-PLUS algorithm (22). The *a priori* risks based on maternal age and distributional and correlation parameters of nuchal translucency, PAPP-A, and β-hCG were from the literature (23), and we used a standardized age distribution of maternal age among pregnant women for the calculation (24). It was assumed that the nuchal translucency measurements did not correlate with other markers.

Results

The inhibin A maternal serum concentrations in normal pregnancies are shown in Fig. 1 as a function of gestational age. The concentration increased from week 5, peaked at week 9 and decreased slightly from week 9–11.

The maternal serum concentrations of inhibin A in DS pregnancies, expressed as multiples of the medians (MoMs) of the normal samples, are shown in Fig. 2. In both controls and trisomy, 21 pregnancies the log MoM distributions were compatible with the normal distribution as judged from normal plots (not shown). The MoM values increased in DS pregnancies significantly with gestational age from week 6 ($p = 0.001$). It is seen that the MoM values of trisomy 21 pregnancies are lower than 1 in week 7–8, whereas they are greater than 1 in week

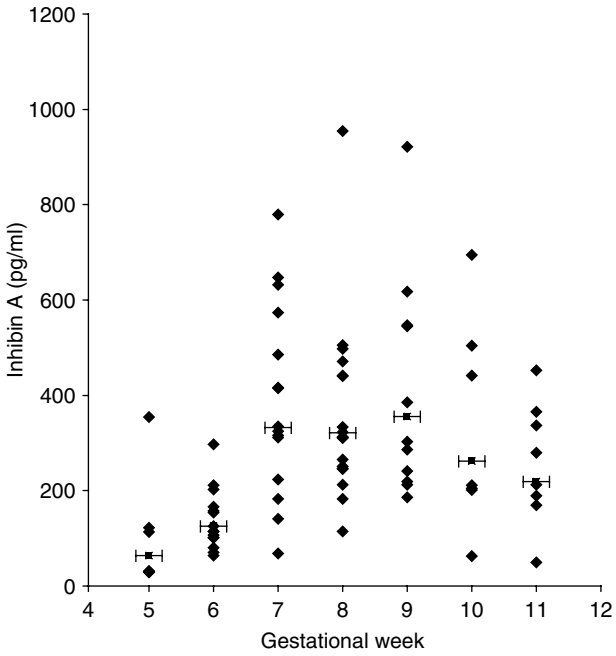

Fig. 1. Inhibin A serum concentrations in normal pregnancies. Bars denote empiric medians.

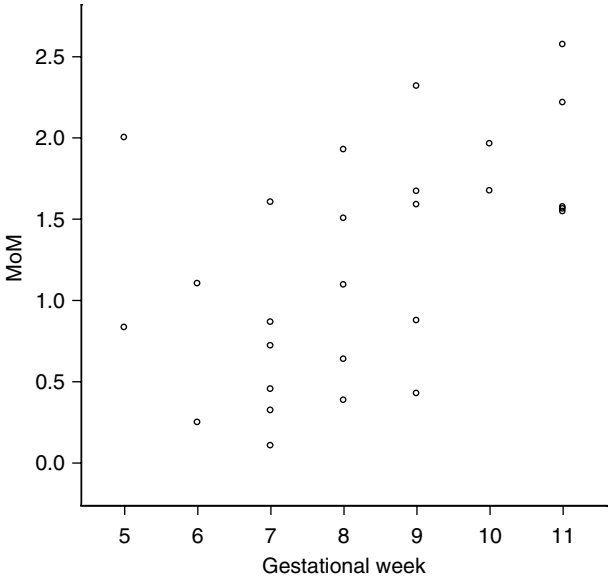

Fig. 2. Inhibin A MoM (*M*ultiple *o*f the *M*edian of normal pregnancies) values in DS pregnancies through gestation. From week 6, there was a significant increase with gestation.

9–11. The mean inhibin A log MoM value in trisomy 21 cases in week 7–8 (n = 11) was −0.1754, with an SD of 0.3712. The normal controls had a mean log MoM of 0.0061, with an SD of 0.2377. In week 9–11, the mean log MoM value in trisomy 21 pregnancies (n = 12) was 0.1842 with an SD of 0.2115, and in the controls, the mean log MoM was 0.0393 with an SD of 0.2807. In week 7–8, the difference between log MoM inhibin A in normal and trisomy 21 pregnancies did not reach statistical significance ($p = 0.089$), whereas this difference was significant in week 9–11 ($p = 0.046$). The log MoM inhibin A values in DS pregnancies were significantly lower in week 7–8, as compared to week 9–11 ($p = 0.003$).

In normal pregnancies, there was a significant positive correlation ($r = 0.67$, $p < 0.001$) between the log MoM values of PAPP-A and inhibin A from week 5 to week 11. There was no correlation between PAPP-A and inhibin A in DS pregnancies. In neither normal pregnancy nor in DS pregnancy was there any significant correlation between inhibin A and free β-hCG.

The performance of inhibin A as a maternal serum marker for DS in different combinations with other first-trimester markers in the two gestational age windows, 7–8 weeks and 9–11 weeks, is illustrated in the Receiver-Operator-Characteristic (ROC) curves in Fig 3(a, b). The ROC curves show relationship between a chosen sensitivity of a test and the corresponding specificity.

The DRs of different combinations of inhibin A for the risk cut-offs 1 : 100, 1 : 250 and 1 : 400 (meaning that the pregnancy is considered screen positive, if the risk is greater than risk cut-off) are given in Table 1 using a standardized age distribution of pregnant women (see *Materials and methods* for details).

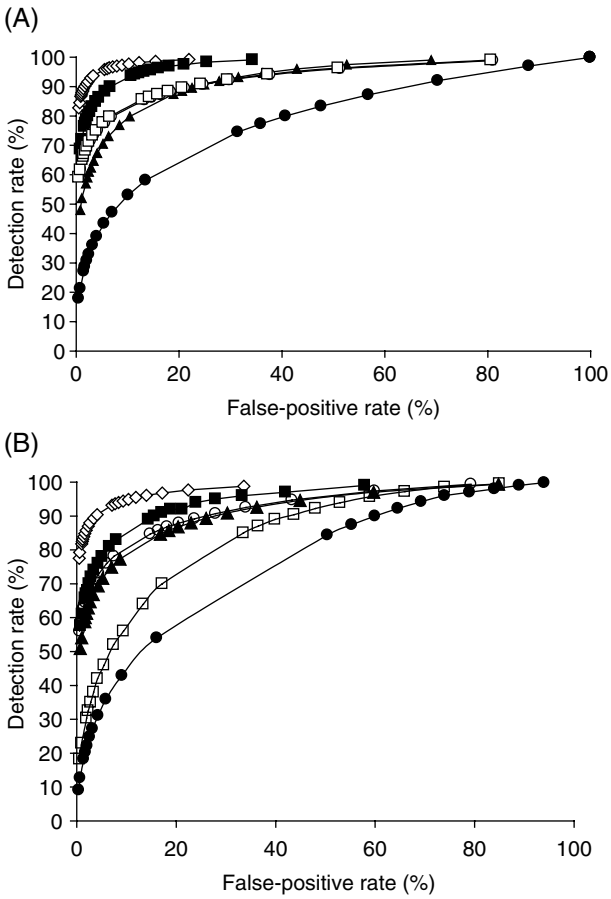

Fig. 3. Receiver–operator characteristics curves (depicting sensitivity as a function of the screen-positive rate) for different screening strategies in (a) week 7–8 and (b) week 9–11. Inhibin A + age (●), inhibin A + age + β-human chorionic gonadotrophin (β-hCG) (▲), inhibin A + age + PAPP-A (○), inhibin A + age + NT (□), inhibin A + age + PAPP-A + β-hCG (■) and inhibin A + age + PAPP-A + β-hCG + NT (◇).

Discussion

The inhibin A concentration in maternal serum is reduced in week 7–8 and increased in week 9–11 in DS pregnancies compared with normal pregnancies. This finding is comparable to the findings for SP1 (25, 26), proMBP (27), hCG (28) and PAPP-A (29), but for these markers, the transitions between reduced and elevated/normal in DS pregnancies occur in week 10–12, 10–12, 7–8 and 14–15, respectively. The transition has not been explained, but it seems to be a general mechanism.

The distribution of inhibin A values in DS pregnancies in early first trimester has not previously been studied systematically. However, in one study (10), a graphic depicts clearly reduced MoM values in a few DS pregnancies around week 7–8, whereas the mean MoM is reported to be 0.98 in pooled DS samples from week 7–11. Not a surprising finding if the maternal serum concentration of inhibin A in DS pregnancies is low prior to week 9 and elevated thereafter. Our findings are not compatible with a major recent study (18), where inhibin A MoM values were not increased in week 11–12, but only from week 12–14. From the SURUSS study in the UK (3), where serological results were used non-interventionally, an empiric MoM of inhibin A of 1.12 in completed 10 weeks has been reported together with evidence of a highly significant increase in inhibin A MoM in DS pregnancies from week 10 to week 15. In the SURUSS study, there is a discrepancy between the regressed MoMs and the empiric MoMs, leading to very high inhibin

Table 1. Nuchal translucency (NT) is determined in week 10–14, so screening modalities using NT cannot report a risk result prior to the NT screening

	Risk cut-off					
	1 : 400		1 : 250		1 : 100	
Markers	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)
Gestational week 7–8						
Inhibin A + age	52.6	10.1	42.8	5.4	26.8	1.5
Inhibin A + age + β-hCG	62.6	9.2	53.3	5.2	36.9	1.6
Inhibin A + age + PAPP-A	76.3	8.1	69.9	5.0	56.8	1.8
Inhibin A + Double test	81.7	6.5	76.4	4.3	64.7	1.6
Inhibin A+NT	77.7	5.2	73.5	3.2	65.4	1.2
Inhibin A + age + NT + PAPP-A	85.9	4.6	82.6	3.0	75.4	1.2
Inhibin A + Combined test	89.3	3.4	86.8	2.2	81.4	0.9
Gestational week 9–11						
Inhibin A + age	47.8	11.8	36.1	6.0	18.4	1.4
Inhibin A + age + β-hCG	63.8	13.3	51.5	7.3	30.2	2.0
Inhibin A + age + PAPP-A	80.8	7.3	75.6	4.7	63.9	1.9
Inhibin A + Double test	83.4	6.1	78.8	4.0	68.5	1.6
Inhibin A + NT	76.0	5.8	71.4	3.5	62.6	1.3
Inhibin A + age + NT + PAPP-A	86.8	3.5	84.2	2.2	78.7	0.9
Inhibin A + Combined test	90.4	3.3	88.0	2.2	82.6	1.0
Double test (PAPP-A + β-hCG)	76.0	9.6	68.8	6.1	53.0	2.3
Combined test (Double test + NT)	85.9	4.7	82.5	3.1	75.3	1.3

DR, detection rate; FPR, false-positive rate; hcG, human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein-A; NT, nuchal translucency.
Double test, first-trimester serological test. Combined test, first-trimester serological test (double test) + NT. Risk cut-off, the risk value separating screen positive pregnancies (risk > risk cut-off) from screen-negative women (risk < risk cut-off). Risk, risk to give birth to a Down's syndrome child.

A MoM values in second-trimester DS pregnancies and very low in first-trimester DS pregnancies. This discrepancy may be due to bias from sampling patients in different ways and the use of not optimally defined regressed medians. As inhibin A decreases from a plateau in week 9 (Fig. 1), it is difficult to use log-regression of inhibin A concentration values in week 9.

A significant correlation between PAPP-A and inhibin A has previously been noted in DS pregnancies (18), and the lack of correlation between inhibin A and β-hCG found here is compatible with some (10, 30), but not all studies (18).

The discriminatory power of β-hCG has been questioned in very early first trimester (31), so we have also calculated the performance if this parameter was not used. We found (Table 1) that the inclusion of β-hCG in the marker panel is not necessary to obtain a good screening performance.

The performance suggested from Table 1 is so good that inhibin A should be further examined in first-trimester screening studies, preferably in a prospective setting, where the clinical performance of inhibin A may be properly settled. Furthermore, in order to become an attractive analyte for large-scale screening, more user-friendly, and cheaper, assays with improved specificity (32) should be developed for semi-automated analytical platforms.

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