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Second trimester two-step trisomy 18 screening using maternal serum markers

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Trisomy 21 maternal serum marker screening has led to screening for other anomalies, including trisomy 18. Trisomy 18 is generally prenatally diagnosed because of major morphological defects. However, in up to 30% of cases ultrasound signs are unclear, and in most cases diagnosis is performed late in pregnancy. Of the different maternal serum markers, PAPP-A is now considered as the best for trisomy 18 screening. However, pregnancy-associated plasma protein A (PAPP-A) is of value in first trimester screening for trisomy 21, but not in the second trimester. We therefore propose a two-step screening strategy. Based on 45 trisomy 18 cases, we confirm the values of alpha-fetoprotein (AFP) (median 0.61 MoM), free β-human chorionic gonadotrophin (β-hCG) (median 0.24 MoM) and of PAPP-A (median 0.08 MoM). In the first step, a 0.5 MoM cut-off for AFP or for free β-hCG resulted in detection of 37/45 trisomy 18 cases (82%) with a 10% false-positive rate. The second step consisted of the measurement of PAPP-A for all these false-positive cases. Using a PAPP-A cut-off of 0.5 MoM, all the 37 trisomy 18 cases were detected, but now with a 0.1–0.2% false-positive rate. Amniocentesis was only offered to these few patients. This two-step second trimester screening will be of value for patients who have not been included in first trimester screening based on nuchal translucency (NT) measurement combined with the first trimester markers, PAPP-A and free β-hCG. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 18; PAPP-A; biochemical screening; free β-hCG; AFP; prenatal screening

INTRODUCTION

Trisomy 21 maternal serum screening is now widely used and large prospective studies have led to improvements in the risk calculation model proposed by Wald *et al*. (1988). This has given rise to screening for other aneuploidies, particularly trisomy 18 (Canick *et al*., 1990; Palomaki *et al*., 1995; Sancken *et al*., 1999) and screening for pre-eclampsia based on high hCG levels (Muller *et al*., 1996). However, all such screening involves additional amniocentesis and its attendant risk. Different strategies have been proposed for trisomy 18 screening, using maternal serum markers alone (with a cut-off) or combined with the risk due to maternal age (risk calculation) (Palomaki *et al*., 1995; Benn *et al*., 1999; Kenndy *et al*., 2000) or combined with ultrasound signs (Brown *et al*., 1999; Sullivan *et al*., 1999). However, the cut-off strategy has a poor sensitivity/specificity ratio, which is improved by combination with the risk due to maternal age, and the third strategy has the disadvantage that second trimester ultrasound signs are absent in up to 30% of trisomy 18 cases (Feuchtbaum *et al*., 2000). Spencer has proposed a two-step strategy based on maternal serum markers (Spencer *et al*., 1999). We confirm here the efficacy of this approach.

PATIENTS AND METHODS

The trisomy 18 population consisted of women who presented through the trisomy 21 maternal serum screening programme in 15 accredited laboratories. Trisomy 18 was detected prenatally by karyotyping in all 45 cases. Trisomy 18 was free (no translocation) and homogeneous (no mosaic) in all cases. Second trimester ultrasound findings were recorded. One trisomy 18 case presented a small omphalocele (2×1.5 cm) and no case was associated with spina bifida. There were no trisomy 18 cases associated with large omphalocele or large open neural tube defect, which would have been detected at the first trimester ultrasonography screening at 12–14 weeks performed in France.

All serum samples were shipped frozen to the Hôpital Ambroise Paré for repeat assay (Perkin

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Elmer, Turku, Finland, kits) of three markers: alpha-fetoprotein (AFP), free β-human chorionic gonadotrophin (β-hCG) (dual kit) and pregnancy-associated plasma protein A (PAPP-A). Normal values (in multiples of median, MoM) were defined for each marker based on thousands of cases. Gestational age was dated by first trimester ultrasonography in all cases. False-positive rates were determined based on second trimester prospective trisomy 21 screening, using AFP and free β-hCG (15 000 cases), and on first trimester trisomy 21 screening using PAPP-A and free β-hCG (6000 cases).

RESULTS

All 45 trisomy 18 cases were prenatally detected. Amniocentesis was performed because of maternal age of 38 years or over in 12 cases (mean maternal age = 35 years), high risk of Down syndrome ($>1/250$) in four cases, fetal death in one case, and abnormal ultrasound signs in the 28 other cases.

Ultrasound signs were observed during the first trimester in four cases [one case of multiple malformations and three cases of abnormally high nuchal translucency (NT)], during the second trimester in 17 cases (isolated growth retardation in two cases and multiple malformations in 15 cases), and during the third trimester in seven cases (isolated growth retardation in three cases and multiple malformations not seen at the second trimester in four cases).The MoM values for AFP, free β-hCG and PAPP-A are shown in Figure 1. The respective standard deviations of $\log_{10}$ values of MoM, after trimming of outliers, were 0.183, 0.401 and 0.370. The only significant correlation ($p<0.05$) between MoM values of markers was for PAPP-A and free β-hCG (0.34).

The median maternal serum AFP (0.61 MoM; range 0.22–2.26), free β-hCG (0.24 MoM; range 0.04–7.5) and PAPP-A (0.08 MoM; range 0.01–1.13) values were significantly lower ($p<0.0001$) than in unaffected pregnancies. In five cases the calculated risk for Down syndrome was $>1/250$, due to the association of high hCG values and low AFP values in three cases, and due to isolated low AFP values in two cases (Ortho Clinical Software, New York, USA).

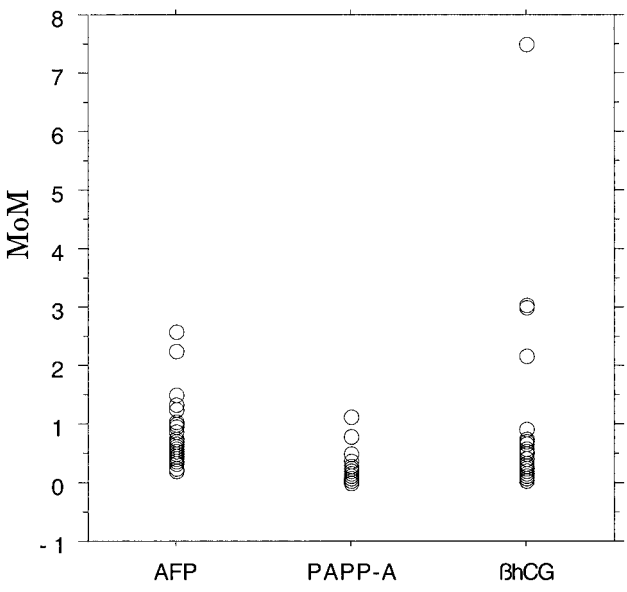

Figure 1—Values (MoM) of AFP, PAPP-A and free β-hCG in the 45 cases of trisomy 18

PAPP-A is the best marker, all values but one (1.13) being below 0.5 MoM. However, because PAPP-A is ineffective for trisomy 21 screening during the second trimester, there is no reason to test all sera for PAPP-A. Therefore, a two-step study was analysed for trisomy 18 screening. Three strategies, all based on AFP and free β-hCG measurement, were initially tested in the first step (Table 1). In the first of these three strategies, a cut-off of 0.75 for AFP was combined with 0.55 for free β-hCG. This allowed detection of 16/45 trisomy 18 cases. The second strategy used a free β-hCG cut-off <0.5 MoM regardless of AFP value. This allowed detection of 34/45 cases. In the third strategy, use was made of a cut-off for free β-hCG <0.5 MoM or for AFP <0.5 MoM. This gave a detection rate of 37/45 cases. However, this first step gave a high false-positive rate, ranging from 3% to 10%.

A second step must therefore be added. If PAPP-A had been measured in all cases selected by any of the three strategies of the first step, a 0.5 MoM PAPP-A cut-off would have allowed detection of all of the trisomy 18 cases selected by the first step. Therefore, the best compromise would be to use the following two-step strategy: a first step (third strategy) based on AFP or free β-hCG <0.5 MoM and a second step in which PAPP-A is measured in all false-positive cases (9.29%). Using a PAPP-A cut-off of 0.5 MoM, the trisomy 18 detection rate would be 82% (37/45 cases), but now with a false-positive rate below 0.2%.

When the observed statistical parameters were used in the mathematical model of a population with the maternal age distribution of pregnancies, the estimated detection rate combining free β-hCG, PAPP-A and

Table 1—Trisomy 18 (45 cases) detection rate based on a two-step strategy

	Trisomy 18 screened-positive	False-positive rate (%)
First step		
Strategy 1: AFP < 0.75 MoM and free β-hCG < 0.55 MoM	16/45	3.01
Strategy 2: free β-hCG < 0.50 MoM	34/45	8.27
Strategy 3: AFP < 0.50 MoM or free β-hCG < 0.50 MoM	37/45	9.29
Second step PAPP-A cut-off 0.5 MoM		
First step strategy 1	16/45	< 0.2
First step strategy 2	34/45	< 0.2
First step strategy 3	37/45	< 0.2

maternal age at a fixed false-positive rate of 5% was 91%.

Maternal serum marker values did not differ between cases detected during the first, second and third trimesters. They also did not depend on the nature of the fetal malformation [isolated intrauterine growth retardation (IUGR) versus multiple malformations] or on maternal age.

In conclusion, second trimester maternal serum trisomy 18 screening can be performed in addition to trisomy 21 screening with an additional marker (PAPP-A) in 10% of cases, leading to a 82% trisomy 18 detection rate for only an additional 0.1% amniocentesis rate.

DISCUSSION

Trisomy 18 is a lethal chromosomal abnormality leading to fetal or neonatal death. Prenatal diagnosis of this defect is important to allow patients different management options such as termination of pregnancy (TOP) or preparation for delivery of an affected child. Prenatal diagnosis (karyotyping) is based on three criteria: advanced maternal age, abnormal ultrasound findings or abnormal maternal serum markers. Ultrasonography detects most cases of trisomy 18 because of the multitude of major structural fetal malformations (severe IUGR, choroid plexus cysts, cardiac abnormalities, abnormal positioning of fingers and clenched hand). Several studies report a prenatal detection rate of approximately 80% (Benacerraf *et al*., 1994; Grandjean *et al*., 1998). However, this high detection rate is not universal (Shields *et al*., 1998; Feuchtbaum *et al*., 2000). A genetic sonogram in the form of a scoring index was developed to optimise the detection of fetuses with aneuploidy (Benacerraf *et al*., 1994). This score allows a detection rate of 85% of fetuses with trisomy 18 for a 4% false-positive rate. Of the ultrasound signs, choroid plexus cyst examination was proposed in order to improve the sensitivity/specificity ratio. However, a meta-analysis of the 13 largest studies of fetuses with choroid plexus cysts showed that the incidence of trisomy 18 was only 0.27% among fetuses with isolated cysts (Gross *et al*., 1995).

Since the introduction of second trimester Down syndrome screening, protocols for the identification of trisomy 18-affected pregnancies have been developed. A positive trisomy 18 screen was defined as AFP up to 0.75 MoM, hCG or β-hCG up to 0.55 MoM and unconjugated oestriol (uE_3) up to 0.60 MoM. In summary, these give a 50–60% detection rate for an additional 0.5–1% false-positive rate (Canick *et al*., 1990; Spencer *et al*., 1993, 1999; Palomaki *et al*., 1995). Other protocols combine ultrasound findings and maternal serum markers. Feuchtbaum proposed ultrasound examination of maternal serum screen-positive patients (Feuchtbaum *et al*., 2000). This gave a 65% trisomy 18 detection rate, which the authors concluded was too low to avoid amniocentesis for all screened-positive patients. However, based on 30 trisomy 18-affected fetuses, Brumfield observed that 37% had a positive trisomy 18 screen and 70% had abnormalities detected by ultrasound (Brumfield *et al*., 2000). The authors concluded that ultrasound is more likely to be abnormal than maternal serum marker screening for trisomy 18-affected pregnancies. Based on a theoretical Bayesian model, Gratton *et al*. (1996) proposed that amniocentesis should not be performed when isolated choroid plexus cyst is associated with normal multiple marker screen results. This model has been prospectively tested (Brown *et al*., 1999; Sullivan *et al*., 1999). However, these protocols are based on few trisomy 18 cases and so no firm conclusion can be drawn. In an economic study evaluation, Vintzileos concluded that routine second trimester amniocentesis in patients at increased risk for fetal trisomy 18, due either to the presence of fetal choroid plexus cysts or to abnormal triple screening, is not justified from the cost/benefit point of view (Vintzileos *et al*., 1998).

Spencer therefore proposed another strategy (Spencer *et al*., 1999) based on maternal serum markers alone: AFP, free β-hCG and PAPP-A. He demonstrated for the first time that PAPP-A has the best discriminatory power. Because PAPP-A is not a second trimester maternal serum marker for trisomy 21 screening, he proposed a two-stage screening algorithm. The first step based on the combination of AFP, free β-hCG and maternal age screening would give a 2% false-positive rate for a 70% trisomy 18 detection rate. Analysis of PAPP-A levels as a secondary screen in these cases will diminish the false-positive rate to 0.1–0.2% with the same 70% detection rate.

The results of the present study are largely in agreement with published findings. The AFP median of 0.61 MoM compares with the published values of 0.71 (Spencer *et al*., 1993), 0.65 (Palomaki *et al*., 1995) and 0.66 (Spencer *et al*., 1999). The free β-hCG median of 0.24 compares with the literature values of 0.37 (Spencer *et al*., 1993) and 0.327 (Spencer *et al*., 1999). Few studies have been performed on PAPP-A in trisomy 18 cases during the second trimester. Bersinger *et al*. (1999) observed a median MoM of 0.1 and Spencer (Spencer *et al*., 1999) a median MoM of 0.108, values similar to the present 0.08 MoM. The standard deviation ($\log_{10}$ values) of free β-hCG and PAPP-A were similar to those observed during the first trimester by Tul *et al*. (1999).

Of the first three steps we proposed, that based on AFP < 0.5 MoM or free β-hCG < 0.5 MoM leads to an 80% trisomy 18 detection rate and a 10% false-positive rate. This strategy differed from that of Spencer in the first step, because we measured PAPP-A in 10% more cases, and gave a higher trisomy 18 detection rate. Because PAPP-A is now often used routinely in first trimester trisomy 21 screening, it will be easy to test an additional 10% of samples. This will allow an 80% trisomy 18 detection rate for a false-positive rate of just 0.1–0.2%. When maternal age is taken into account, the detection rate would be 91% for a 5% screened-positive rate. This second trimester two-step strategy would only be of value for patients who do not undergo first trimester screening (Tul *et al*., 1999).

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