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First-Trimester Screening for Trisomy 21 with Adjustment for Biochemical Results of Previous Pregnancies

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Key Words
First-trimester screening · Trisomy 21 · Free β-hCG · PAPP-A · Nuchal translucency

Abstract
Objective: To investigate the effect of associations in serum free β-hCG and PAPP-A between successive pregnancies on the performance of screening for trisomy 21 at 11–13 weeks' gestation. ***Methods:*** In 8,499 women with two consecutive pregnancies, including 49 women with fetal trisomy 21 in the second pregnancy, the correlation in serum free β-hCG multiples of the median (MoM) and PAPP-A MoM between pregnancies was determined, and the effects of correcting for the correlation on the performance of screening was estimated. ***Results:*** There were significant associations between pregnancies in free β-hCG MoM (r = 0.4435) and PAPP-A MoM (r = 0.4796). In screening by maternal age and biochemistry at a risk cutoff of 1 in 100, in the second pregnancies the false-positive rate was 35.5% for those with screen-positive results in the first pregnancy, and this was reduced to 17.1% after adjustment for the results of the first pregnancy. Similarly, in women with screen-negative results in the first pregnancy, adjustment for the results improved the detection rate in the second pregnancy from 66.7 to 81.2%. ***Conclusions:*** In screening for trisomy 21, adjustment for the biochemical findings in a previous pregnancy has major effects on individual patient-specific risks, increases the detection rate and reduces the false-positive rate.

Introduction

Effective screening for fetal trisomy 21 is provided at 11–13 weeks' gestation by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and PAPP-A with a detection rate of about 90% at a false-positive rate of 5% [1]. In trisomy 21 pregnancies, maternal serum free β-hCG is about twice as high and PAPP-A is reduced to about half compared with values in chromosomally normal pregnancies. In the development of risk algorithms for combined screening, the estimation of accurate patient-specific risks necessitates adjustments in the measured free β-hCG and PAPP-A to take into account their association with gestational age, maternal weight, racial origin, smoking status and method of conception [2].

Free β-hCG and PAPP-A are produced by the placenta and, consequently, their serum concentration should be pregnancy specific. However, there must be additional maternal factors that affect the serum levels of these pla-

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cental products because there is a significant association between levels in consecutive pregnancies of the same women. Spencer [3] examined 1,002 women with two normal singleton pregnancies and reported that the correlation coefficients in serum free β-hCG multiple of the median (MoM) and PAPP-A MoM between the first and the second pregnancy were 0.398 and 0.437, respectively, whereas there was no significant association in the measurements of fetal NT. The author concluded that a consequence of this association in maternal serum markers is that women who have an increased risk for trisomy 21 in a first pregnancy are two to three times more likely to be also at an increased risk in a subsequent pregnancy, but it was suggested that any method of allowing for this association to be taken into account was unlikely to improve the overall performance of population screening.

The aim of this study in 8,499 women with two consecutive pregnancies, including 49 with fetal trisomy 21 in the second pregnancy, is to investigate further the correlation in serum free β-hCG and PAPP-A between pregnancies and the effects of such correlation both on patient-specific risks for trisomy 21 and the overall performance of population screening.

Methods

This was a prospective screening study for trisomy 21 in singleton pregnancies by a combination of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A in a one-stop clinic for first-trimester assessment of risk (OSCAR) at King's College Hospital and the Fetal Medicine Centre, London, UK [1]. The visits, which are held at 11^{+0}–13^{+6} weeks' gestation, included the recording of maternal demographic characteristics and previous obstetric and medical history, the measurement of maternal weight and height and calculation of the body mass index, as well as an ultrasound examination for the measurement of the fetal crown-rump length (CRL) to determine gestational age [4], measurement of the fetal NT thickness as part of screening for aneuploidies [5] and examination of the fetal anatomy for the diagnosis of major fetal defects [6]. Serum PAPP-A and free β-hCG were measured by automated machines that provide reproducible results within 30 min (Kryptor system; Brahms AG, Berlin, Germany or Delfia Express System; Perkin Elmer, Waltham, Mass., USA).

Maternal demographic characteristics, ultrasononographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became available. A search of the database was done to identify all women with first-trimester combined screening performed in two consecutive singleton pregnancies between July 1999 and September 2009.

Statistical Analysis

In each pregnancy, the risk for trisomy 21 was calculated by multiplying the maternal age-related odds, adjusted according to the gestational age at the time of screening [7], with the likelihood ratios for fetal NT and maternal serum free β-hCG and PAPP-A. The measured NT was transformed into a likelihood ratio for trisomy 21 using the mixture model of NT distributions [8]. The measured free β-hCG and PAPP-A were converted into MoM for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception and machine for the assays, and likelihood ratios for trisomy 21 were derived from the bivariate gaussian distributions for log MoM values in trisomy 21 and in unaffected pregnancies [2].

In each woman in her second pregnancy two risks for trisomy 21 were calculated. Firstly, risks derived from maternal age, and the measurements of fetal NT for CRL and serum free β-hCG and PAPP-A in the second pregnancy (unadjusted). Secondly, risks derived from the above measurements after adjustment for serum free β-hCG and PAPP-A by using the distribution of second-pregnancy log MoM conditional on the first-pregnancy log MoM. The adjusted risk that results is thus conditional on the first- and the second-pregnancy biochemistry. The distribution theory and parameter estimates used are outlined in Appendix I.

Crude detection rates and false-positive rates for the second pregnancy were calculated by taking the proportions with risks above a given risk threshold and stratified according to the first-pregnancy risk group. To demonstrate the association between first-pregnancy biochemistry and second-pregnancy screening performance, the data were stratified into tertiles defined by the first-pregnancy biochemistry likelihood ratio. Standardised detection rates were computed by obtaining the likelihood ratios for biochemistry and NT, as appropriate, in trisomy 21 pregnancies in the sample, and then applying these to each year of maternal age from 12 to 50 to estimate the age-specific false-positive rates. These were then weighted according to the maternal age distribution of trisomy 21 pregnancies in England and Wales in 2000–2002 [9]. Similarly, standardised false-positive rates were computed by obtaining the likelihood ratios for biochemistry and NT, as appropriate, in unaffected pregnancies in the sample, and then applying these to each year of maternal age from 12 to 50 to estimate the age-specific detection rates. These were then weighted according to the maternal age distribution of trisomy 21 pregnancies in England and Wales in 2000–2002. Confidence intervals were obtained by bootstrapping. Estimates and 95% confidence intervals, with no adjustment for multiplicity, are presented.

Results

The search of the database identified a total of 8,499 cases in which the first pregnancy resulted in the birth of a euploid or phenotypically normal neonate. In the second pregnancies, 49 fetuses or neonates had trisomy 21 and 8,455 were normal. The observed number of cases with trisomy 21 was consistent with the expected number of 45.9 (95% prediction interval 32.7–59.2) derived from the maternal age and gestational age distribution of the sample. The characteristics of the first and second pregnancies are summarized in table 1.

The correlations for free β-hCG MoM and PAPP-A MoM between the first and second pregnancies are shown in table 2. There was no evidence to suggest that the cor-

Table 1. Characteristics of the first and second pregnancies in the study population (n = 8,499)

Characteristic	Result
Racial origin, n	
Caucasian	7,414 (87.2%)
African-Caribbean	554 (6.5%)
South Asian	303 (3.6%)
East Asian	135 (1.6%)
Mixed	93 (1.1%)
Cigarette smoker, n	
First pregnancy	321 (3.8%)
Second pregnancy	267 (3.1%)
Spontaneous conception, n	
First pregnancy	7,895 (92.9%)
Second pregnancy	8,192 (96.4%)
Maternal age, years	
First pregnancy	
Mean ± SD	32.81 ± 4.471
Median (IQR)	33.2 (30.4–35.9)
Range	15.7–48.7
Second pregnancy	
Mean ± SD	34.93 ± 4.520
Median (IQR)	35.4 (32.5–38.0)
Range	17.6–51.3
Difference	
Mean ± SD	2.116 ± 8.971
Median (IQR)	2.0 (1.6–2.5)
Range	0.3–23.6
Maternal weight, kg	
First pregnancy	
Mean ± SD	65.11 ± 11.480
Median (IQR)	63.6 (57.5–70.0)
Range	38–143
Second pregnancy	
Mean ± SD	66.10 ± 12.289
Median (IQR)	64.0 (58.0–72.0)
Range	39–148
Difference	
Mean ± SD	0.984 ± 18.254
Median (IQR)	0.8 (–1.4 to 3.3)
Range	–19 to 37
Gestational age, days	
First pregnancy	
Mean ± SD	88.67 ± 4.157
Median (IQR)	89 (86–91)
Range	79–99
Second pregnancy	
Mean ± SD	88.38 ± 3.928
Median (IQR)	88 (86–91)
Range	68–99
Difference	
Mean ± SD	–0.289 ± 13.494
Median (IQR)	0 (–4 to 3)
Range	–23 to 19
Serum PAPP-A MoM	
First pregnancy	
Mean ± SD	1.138 ± 0.601
Median (IQR)	1.020 (0.718–1.440)
Range	0.003–5.951
Second pregnancy	
Mean ± SD	1.126 ± 0.602
Median (IQR)	1.002 (0.698–1.413)
Range	0.059–6.439
$\log_{10}$ PAPP-A MoM	
First pregnancy	
Mean ± SD	–0.001 ± 0.230
Median (IQR)	0.009 (–0.144 to 0.158)
Range	–2.602 to 0.775
Second pregnancy	
Mean ± SD	–0.006 ± 0.228
Median (IQR)	0.001 (–0.156 to 0.150)
Range	–1.226 to 0.809
Serum free β-hCG MoM	
First pregnancy	
Mean ± SD	1.202 ± 0.819
Median (IQR)	0.990 (0.678–1.474)
Range	0.096–11.940
Second pregnancy	
Mean ± SD	1.194 ± 0.8116
Median (IQR)	0.981 (0.671–1.467)
Range	0.083–9.404
$\log_{10}$ free β-hCG MoM	
First pregnancy	
Mean ± SD	0.004 ± 0.251
Median (IQR)	–0.004 (–0.169 to 0.168)
Range	–1.019 to 1.077
Second pregnancy	
Mean ± SD	0.000 ± 0.254
Median (IQR)	–0.008 (–0.173 to 0.166)
Range	–1.079 to 0.973
Fetal NT, mm	
First pregnancy	
Mean ± SD	1.692 ± 0.524
Median (IQR)	1.600 (1.400–1.900)
Range	0.600–13.000
Second pregnancy	
Mean ± SD	1.797 ± 0.513
Median (IQR)	1.800 (1.500–2.000)
Range	0.000–12.000

Table 2. Correlations in free β-hCG MoM and PAPP-A MoM between the first unaffected pregnancies and second trisomy 21 and unaffected pregnancies (overall and by time interval between pregnancies)

Pregnancy	Free β-hCG MoM		PAPP-A MoM	
	n	r (95% confidence limit)	n	r (95% confidence limit)
Overall				
Trisomy 21	49	0.4969 (0.2508–0.6827)	49	0.4884 (0.2402–0.6767)
Unaffected	8,443	0.4435 (0.4262–0.4604)	8,431	0.4796 (0.4630–0.4959)
Time interval				
<1 year	264	0.3448 (0.2338–0.4469)	260	0.4511 (0.3486–0.5430)
1–2 years	4,151	0.4471 (0.4224–0.4711)	4,147	0.4798 (0.4560–0.5029)
2–3 years	3,202	0.4590 (0.4312–0.4859)	3,199	0.4948 (0.4682–0.5206)
3–4 years	700	0.4043 (0.3404–0.4645)	699	0.4364 (0.3744–0.4946)
4–5 years	110	0.3800 (0.2075–0.5296)	110	0.5252 (0.3749–0.6487)
>5 years	16	0.5508 (0.0758–0.8221)	16	0.3168 (–0.2122 to 0.7023)

In the unaffected group, we excluded 7 gross outliers in free β-hCG MoM and 19 gross outliers in PAPP-A MoM.

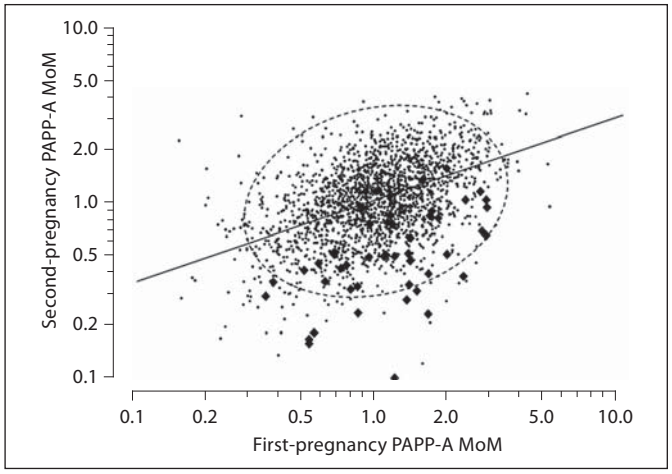

Fig. 1. Scatter diagram showing the relationship between the first unaffected pregnancy and second trisomy 21 (black diamonds) and unaffected (small dots) pregnancies with regression lines and 95% contours (interrupted line) for unaffected pregnancies. PAPP-A MoM values are shown. For the purposes of the display, random samples of 2,000 unaffected pregnancies are shown.

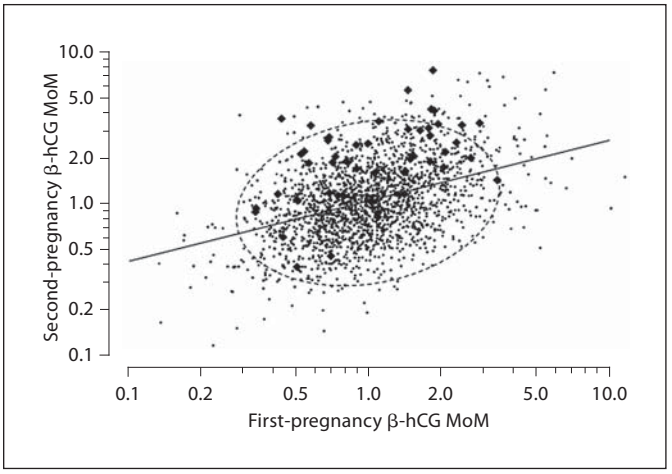

Fig. 2. Scatter diagram showing the relationship between the first unaffected pregnancy and second trisomy 21 (black diamonds) and unaffected (small dots) pregnancies with regression lines and 95% contours (interrupted line) for unaffected pregnancies. β-hCG MoM values are shown. For the purposes of the display, random samples of 2,000 unaffected pregnancies are shown.

relations depended on the time interval between the pregnancies or whether the fetuses had trisomy 21 or were unaffected. The relationships of free β-hCG MoM and PAPP-A MoM between the first and second pregnancies are shown in figures 1 and 2. The correlation of fetal NT between consecutive pregnancies (r = 0.167) was much weaker than with the biochemical tests. Consequently, only 2.8% of the variation in the second-pregnancy NT is explained by the first-pregnancy NT.

Risks were computed using the biochemical data and maternal age of the second pregnancy with and without adjustment for the biochemical results of the first unaffected pregnancy. Estimation of the adjusted risk involved modifying the second-pregnancy mean log MoM

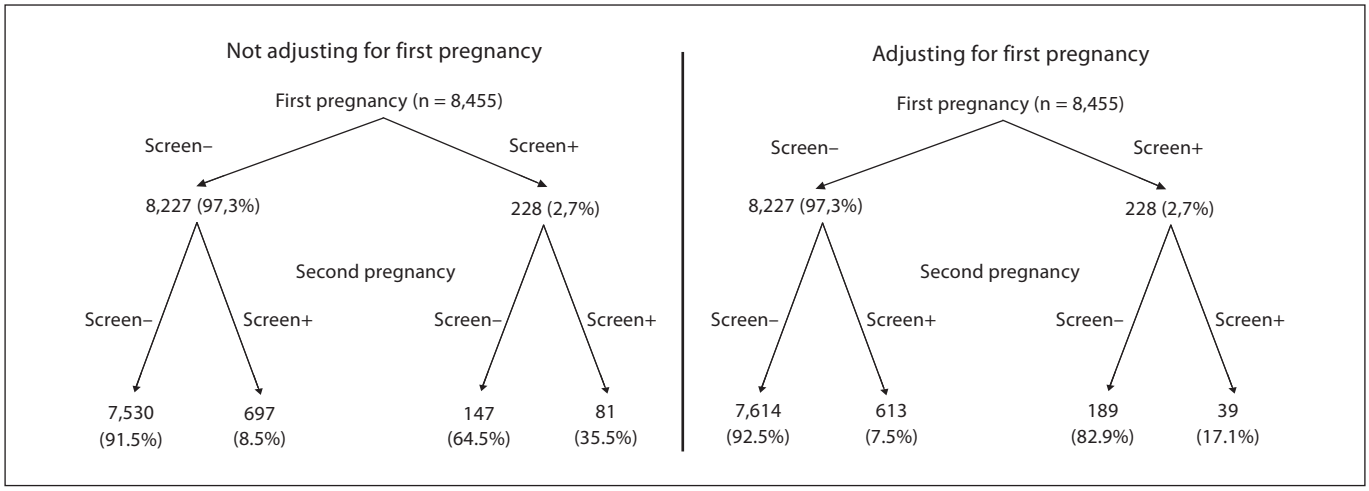

Fig. 3. Effect of deriving the risk for trisomy 21 in the second pregnancy after adjustment for the biochemical results of the first pregnancy in 8,455 cases where both the first and second pregnancies were unaffected by trisomy 21. In the first pregnancy, 2.7% of cases were screen positive and 97.3% were screen negative. In the second pregnancy, the screen-positive rate was 35.5% amongst women who were screen positive in their first pregnancy and 8.5% in those who were screen negative in their first pregnancy (left). Adjustment of the estimated risks in the second pregnancy by taking into account the biochemistry results in the first pregnancy reduced the screen-positive rates to 17.1 and 7.5%, respectively (right).

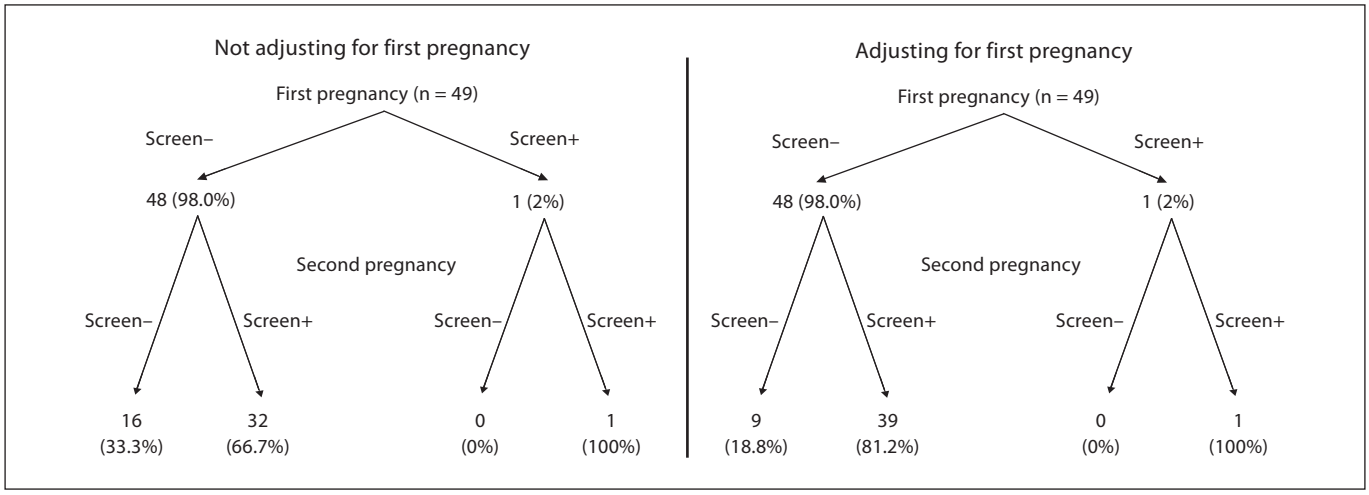

Fig. 4. Effect of deriving the risk for trisomy 21 in the second pregnancy after adjustment for the biochemical results of the first pregnancy in 49 cases where the first pregnancies were normal but the second pregnancies were affected by trisomy 21. The screen-positive rate (detection rate) in those with a screen-negative result in the first pregnancy was lower in those without (left) than with (right) adjustment for the biochemical results of the first pregnancy (66.7 vs. 81.2%).

values through a regression on the first-pregnancy log MoM values and using the correlations and standard deviations from the conditional distribution. The regression coefficients and reduced spread standard deviations and correlations are given in Appendix I.

The effects on the false-positive and detection rates in screening for trisomy 21 by maternal age and serum biochemistry in the second pregnancy after adjustment for the biochemical results of the first pregnancy are shown in figures 3 and 4, respectively. At a risk cutoff for trisomy 21 of 1 in 100, in the unaffected second pregnancies the crude screen-positive rate was 35.5% for those with a screen-positive result in the first pregnancy, which was over four times higher than in those with a screen-neg-

ative result in the first pregnancy (8.5%). These screen-positive rates were reduced to 17.1 and 7.5%, respectively, after adjustment for the biochemical results of the first pregnancy (fig. 3). Although such adjustment resulted in halving the screen-positive rate in women who were screen positive in their first pregnancy (17.1 vs. 35.5%), this was still twice as high as in women with a screen-negative result in their first pregnancy (17.1 vs. 7.5%).

At a risk cutoff for trisomy 21 of 1 in 100, in the trisomy 21 second pregnancies the crude detection rate in those with a screen-negative result in the first pregnancy was lower in those without than in those with adjustment for the biochemical results of the first pregnancy (66.7 vs. 81.2%; fig. 4). This difference in detection rates was more pronounced amongst women with very low risks in their first pregnancy. In 18 women who had a second pregnancy with trisomy 21 and whose first-pregnancy risk was lower than 1 in 5,000, the crude detection rate was 39% (7 of 18) without adjustment compared to 83% (15 of 18) after adjustment.

The expected and observed numbers of trisomy 21 pregnancies divided into three groups according to the likelihood ratio tertile for the first pregnancy are shown in table 3. The expected number of trisomy 21 pregnancies was derived from the sum of the estimated risks. The expected numbers of trisomy 21 pregnancies were not significantly different from the observed numbers when the risks were estimated on the basis of maternal age and gestational age alone ($p = 0.22$) or by a combination of maternal age and serum biochemistry after adjustment for the biochemical results of the previous pregnancy ($p = 0.27$). The expected frequencies from the unadjusted risks were significantly different from the observed frequencies ($p < 0.001$). The unadjusted risks underestimated the risks in women whose first-trimester biochemistry gave a low likelihood of trisomy 21 (bottom tertile) and overestimated the risks in women whose first-trimester biochemistry gave a high likelihood of trisomy 21 (bottom tertile).

Estimated detection and false-positive rates in screening for trisomy 21 by maternal age and serum PAPP-A and free β-hCG stratified according to the tertiles of the first-pregnancy likelihood ratio for trisomy 21 with and without adjustment for biochemical results in the previous pregnancy are given in table 4. Overall, the false-positive rates were slightly lower and the detection rates were higher with the adjustment. The unadjusted results showed a clear trend toward increasing positive rates with the first-pregnancy likelihood ratio tertile. This effect was reduced when NT was incorporated in the risk calculations (table 5). However, even with the inclusion of NT, the false-positive rates in the top tertile were three or more times higher than those in the bottom tertile. The screening performance was improved by adjusting for the biochemical results of the previous pregnancy.

Table 3. Expected and observed number of trisomy 21 pregnancies by likelihood ratio (trisomy 21 to unaffected ratio) tertile from the first pregnancy

Likelihood ratio tertile	Expected cases of trisomy 21			Observed
	maternal age	age and biochemistry		
		adjusted	unadjusted	
Top	15.5663	15.2025	33.1522	10
Middle	15.4750	17.0231	16.6248	20
Bottom	14.9058	14.1852	8.9447	19
Total	45.9470	46.4108	58.7217	49
p	0.21787	0.26854	<0.0001	

Risks based on biochemistry and maternal age alone.
p values are for the lack of fit between the observed and expected number of cases of trisomy 21.

The effects on the patient-specific risk for trisomy 21 following adjustment of the results of the second pregnancy on the basis of biochemical findings in the first pregnancy are illustrated in table 6. In one of the cases, the biochemical profile in the second pregnancy was typical of trisomy 21, and in another case the profile was typical of an unaffected pregnancy. The fetal NT was fixed in these two pregnancies to demonstrate the effect of different first-trimester profiles. Adjusted risks are given where first-pregnancy MoM values for PAPP-A and free β-hCG were 0.5, 1.0 and 2.0. In the first case (trisomy 21 profile), the adjusted risk was higher than the unadjusted risk, whereas in the second case (unaffected profile), the adjusted risk was lower. This is because of the reduced spread in the adjusted distributions as reflected by the reduced standard deviations in Appendix I. In cases where the first pregnancy had a trisomy 21 biochemical profile (PAPP-A MoM = 0.5 and free β-hCG MoM = 2.0), the adjusted MoM values for PAPP-A in the second pregnancy were higher than the unadjusted values and those for free β-hCG were lower with a net effect of substantial reduction in risks. In cases where the second pregnancy had a biochemical profile opposite to that in trisomy 21 (PAPP-A MoM = 2 and free β-hCG MoM = 0.5), the adjusted MoM values were decreased for PAPP-A and increased for free β-hCG with the net effect of a substantial increase in risks.

Table 4. Estimated detection and false-positive rates in screening for trisomy 21

Risk cutoff	Detection rate				False-positive rate			
	top (n = 10)	middle (n = 20)	bottom (n = 19)	all (n = 49)	top (n = 2,823)	middle (n = 2,813)	bottom (n = 2,814)	all (n = 8,450)
Unadjusted								
1 in 50	83 (54–96)	51 (35–67)	35 (21–51)	51 (41–62)	4.4 (3.9–5.0)	1.5 (1.2–1.8)	0.6 (0.4–0.8)	2.2 (1.9–2.4)
1 in 100	90 (35–100)	61 (46–75)	45 (30–61)	61 (50–71)	8.0 (7.3–8.8)	3.1 (2.7–3.6)	1.3 (1.0–1.6)	4.2 (3.8–4.5)
1 in 150	92 (45–100)	68 (53–81)	53 (37–69)	67 (57–76)	11.3 (10.4–12.2)	4.7 (4.2–5.2)	2.0 (1.7–2.4)	6.0 (5.6–6.4)
1 in 200	94 (56–100)	73 (58–85)	57 (41–74)	71 (61–80)	14.2 (13.3–15.3)	6.3 (5.7–6.9)	2.7 (2.3–3.1)	7.7 (7.3–8.2)
1 in 250	95 (65–100)	77 (62–88)	62 (45–77)	75 (65–83)	16.9 (15.8–18.0)	7.8 (7.1–8.5)	3.4 (3.0–3.9)	9.4 (8.9–9.8)
1 in 300	96 (73–100)	80 (66–90)	64 (47–80)	77 (68–85)	19.3 (18.1–20.5)	9.3 (8.5–10.1)	4.2 (3.7–4.7)	10.9 (10.4–11.4)
Adjusted								
1 in 50	72 (48–90)	54 (39–68)	51 (34–68)	56 (46–67)	2.3 (1.9 –2.8)	2.3 (1.9 –2.7)	1.8 (1.5–2.3)	2.1 (1.9–2.4)
1 in 100	84 (52–98)	64 (50–76)	64 (46–79)	68 (58–78)	4.1 (3.5–4.7)	4.1 (3.6–4.6)	3.3 (2.9–3.9)	3.8 (3.5–4.2)
1 in 150	89 (59–99)	70 (56–81)	69 (52–84)	74 (64–82)	5.6 (4.9–6.3)	5.8 (5.2–6.4)	4.7 (4.2–5.4)	5.4 (5.0–5.7)
1 in 200	92 (65–99)	74 (61–85)	73 (56–86)	77 (68–85)	7.0 (6.3–7.8)	7.4 (6.7–8.1)	6.0 (5.5–6.7)	6.8 (6.4–7.2)
1 in 250	94 (58–100)	78 (64–87)	75 (60–87)	80 (71–87)	8.3 (7.5–9.2)	8.8 (8.0–9.5)	7.2 (6.7–8.0)	8.1 (7.7–8.6)
1 in 300	95 (65–100)	81 (68–90)	77 (62–88)	82 (73–89)	9.6 (8.7–10.5)	10.1 (9.3–10.9)	8.5 (7.9–9.3)	9.4 (8.9–9.9)

Figures are percentages with 95% confidence intervals in parentheses. Screening was performed by maternal age and serum PAPP-A and free β-hCG stratified according to the tertiles of the first-pregnancy likelihood ratio for trisomy 21 with and without adjustment for biochemistry results in the previous pregnancy. The rates are standardised to the maternal age distribution of pregnancies in England and Wales in 2000–2002.

Table 5. Estimated detection and false-positive rates in screening for trisomy 21

Risk cutoff	Detection rate				False-positive rate			
	top (n = 10)	middle (n = 20)	bottom (n = 19)	all (n = 49)	top (n = 2,823)	middle (n = 2,813)	bottom (n = 2,814)	all (n = 8,450)
Unadjusted								
1 in 50	92 (70–99)	76 (58–88)	82 (62–94)	82 (72–89)	2.1 (1.7–2.4)	1.0 (0.7–1.3)	0.4 (0.3–0.6)	1.1 (1.0–1.3)
1 in 100	98 (79–100)	84 (69–92)	84 (64–95)	87 (78–93)	3.8 (3.3–4.4)	1.6 (1.4–2.1)	0.9 (0.6–1.2)	2.1 (1.9–2.4)
1 in 150	100	89 (75–96)	86 (65–96)	90 (82–95)	5.2 (4.6–5.8)	2.3 (1.9–2.8)	1.3 (1.0–1.6)	2.9 (2.7–3.2)
1 in 200	100	93 (78–98)	87 (66–97)	92 (84–97)	6.5 (5.8–7.2)	2.9 (2.5–3.5)	1.7 (1.3–2.1)	3.7 (3.4–4.0)
1 in 250	100	95 (72–100)	88 (66–97)	93 (86–98)	7.7 (7.0–8.5)	3.5 (3.1–4.2)	2.0 (1.6–2.5)	4.4 (4.1–4.8)
1 in 300	100	96 (76–100)	89 (61–98)	94 (86–98)	8.9 (8.1–9.8)	4.2 (3.6–4.8)	2.4 (1.9–2.9)	5.2 (4.8–5.5)
Adjusted								
1 in 50	82 (45–97)	78 (61–89)	87 (53–98)	82 (73–89)	1.1 (0.8–1.4)	1.3 (0.9–1.7)	1.1 (0.8–1.4)	1.1 (1.0–1.3)
1 in 100	88 (37–100)	87 (73–95)	90 (46–99)	88 (80–94)	2.0 (1.6–2.4)	2.2 (1.7–2.6)	2.0 (1.6–2.4)	2.0 (1.8–2.3)
1 in 150	91 (47–100)	92 (75–98)	90 (40–100)	91 (83–96)	2.8 (2.3–3.3)	2.9 (2.4–3.5)	2.7 (2.3–3.2)	2.8 (2.5–3.1)
1 in 200	93 (55–100)	94 (78–99)	90 (41–100)	92 (85–97)	3.5 (2.9–4.1)	3.6 (3.0–4.2)	3.4 (2.9–3.9)	3.5 (3.2–3.8)
1 in 250	94 (64–100)	96 (81–99)	91 (43–100)	93 (86–98)	4.2 (3.6–4.8)	4.2 (3.6–4.9)	4.0 (3.4–4.6)	4.1 (3.8–4.5)
1 in 300	95 (69–100)	97 (85–100)	91 (45–100)	94 (87–98)	4.7 (4.1–5.4)	4.8 (4.1–5.5)	4.4 (3.9–5.1)	4.7 (4.3–5.0)

Figures are percentages, with 95% confidence intervals in parentheses when all pregnancies were screen positive for all maternal ages. Screening was performed by maternal age, fetal NT thickness and maternal serum PAPP-A and free β-hCG stratified according to the tertiles of the first-pregnancy likelihood ratio for trisomy 21 with and without adjustment for biochemistry results in the previous pregnancy. The rates are standardised to the maternal age distribution of pregnancies in England and Wales in 2000–2002.

Table 6. Second-pregnancy risks for various first-pregnancy biochemical profiles

First pregnancy		Second pregnancy adjusted			
PAPP-A MoM	free β-hCG MoM	PAPP-A MoM	free β-hCG MoM	biochemistry risk	combined risk
Case A					
0.5	0.5	0.698	2.780	1 in 57	1 in 167
0.5	1.0	0.696	2.053	1 in 152	1 in 446
0.5	2.0	0.683	1.516	1 in 364	1 in 1,072
1.0	0.5	0.502	2.708	1 in 18	1 in 50
1.0	1.0	0.500	2.000	1 in 47	1 in 138
1.0	2.0	0.498	1.477	1 in 117	1 in 344
2.0	0.5	0.361	2.638	1 in 5	1 in 14
2.0	1.0	0.359	1.948	1 in 14	1 in 38
2.0	2.0	0.358	1.439	1 in 34	1 in 99
Case B					
0.5	0.5	1.397	1.390	1 in 3,795	1 in 359
0.5	1.0	1.391	1.027	1 in 7,842	1 in 749
0.5	2.0	1.385	0.758	1 in 14,732	1 in 1,406
1.0	0.5	1.004	1.354	1 in 1,676	1 in 161
1.0	1.0	1.000	1.000	1 in 3,639	1 in 348
1.0	2.0	0.996	0.739	1 in 7,113	1 in 679
2.0	0.5	0.722	1.319	1 in 666	1 in 64
2.0	1.0	0.719	0.974	1 in 1,503	1 in 144
2.0	2.0	0.716	0.719	1 in 3,056	1 in 292

The table gives the values of PAPP-A and free β-hCG in the first pregnancy and the consequences of adjusting for these values in the second pregnancy. In a woman aged 32 years with fetal CRL of 63 mm, NT of 2.0 mm and with unadjusted serum biochemistry which is typical of a trisomy 21 pregnancy (PAPP-A MoM 0.5 and free β-hCG MoM 2.0), the estimated risk for trisomy 21 by maternal age and biochemistry would be 1 in 78, and by combination of maternal age, fetal NT and biochemistry, the risk would be 1 in 229 (case A). In a woman aged 32 years with fetal CRL of 63 mm, NT of 3.0 mm and with unadjusted serum biochemistry which is typical of an unaffected pregnancy (PAPP-A MoM and free β-hCG MoM 1.0), the estimated risk for trisomy 21 by maternal age and biochemistry would be 1 in 2,606, and by the combined test would be 1 in 250 (case B).

Discussion

The findings of this study demonstrate that there is a significant association in serum free β-hCG and PAPP-A between consecutive pregnancies in the same women, and that about 20% of the variation for each of the two markers in the second pregnancy is explained by the first-pregnancy MoM values. In contrast, less than 3% of the variation in fetal NT is explained by the results of the previous pregnancy. In first-trimester screening for trisomy 21, adjustment for the biochemical findings in the previous pregnancy results in both an increase in the detection rate and a decrease in the false-positive rate. Although such adjustment has a small impact on the overall performance of screening by a combination of maternal age, fetal NT and serum free β-hCG and PAPP-A, there are major effects on individual patient-specific risks.

In the analysis of data on the association in serum free β-hCG and PAPP-A between consecutive pregnancies in the same women, we made the appropriate adjustments in the measured concentrations by expressing the values as MoM to take into account maternal factors, including weight, smoking status and method of conception and gestational age at testing, that may vary between pregnancies [2]. The standard deviations of the log MoM values for free β-hCG and PAPP-A are consistent with those reported in the literature [2]. The correlation coefficients for free β-hCG MoM and PAPP-A MoM between pregnancies of 0.444 and 0.480, respectively, were similar but slightly higher than the 0.398 and 0.437 reported previously [3]. Additionally, we found that the correlations were not affected by the time interval between pregnancies, and the correlation coefficients were similar for the trisomy 21 and unaffected pregnancies.

Our results indicate that a high proportion of women with a biochemical profile resulting in a false-positive result in the first pregnancy will also have an abnormal result in their subsequent pregnancy. In our example of using a risk cutoff for trisomy 21 of 1 in 100 to define screen positivity, the screen-positive rate in the second unaffected pregnancy was over four times higher in those with a screen-positive than -negative result in their first pregnancy. Such high prevalence of repeat false positivity has also been observed with second-trimester biochemical screening for trisomy 21 because of inter-pregnancy correlations for serum α-fetoprotein, unconjugated estriol, total hCG and free β-hCG, with coefficients between 0.3 and 0.4 [10–12].

We demonstrated that in women with high free β-hCG and/or low PAPP-A, with consequent high risk for trisomy 21 in their first unaffected pregnancy, adjustment of the biochemical profile in the second pregnancy by taking into account the results of their previous pregnancy will improve the performance of screening by reducing the false-positive rate. Additionally, we found that in women with low free β-hCG and/or high PAPP-A, with consequent low risk for trisomy 21 in their first unaffected pregnancy, adjustment of their results in a subsequent pregnancy improves the performance of screening by increasing the detection rate. In the practical implementation of

these results, a reliable approach must be used to ensure accurate recording of the biochemical findings from a previous pregnancy with a normal outcome, excluding aneuploidies as well as adverse outcomes, such as miscarriage, stillbirth or preeclampsia, which are known to be associated with low serum PAPP-A [13].

Appendix I

Risk calculation for the second pregnancy is based on the conditional distribution of $\log_{10}$ MoM in the second pregnancy given the $\log_{10}$ MoM values in the first. Denoting these by vectors x_1 and x_2 for the first and second, respectively, the conditional distribution of x_2 given x_1 is obtained from the joint multivariate gaussian distribution of $\binom{x_1}{x_2}$. In general, this is specified in terms of a mean vector which can be represented in partitioned form as $\binom{\mu_1}{\mu_2}$ and the covariance matrix which can be represented as $\begin{pmatrix}\Sigma_{11} & \Sigma_{12}\\ \Sigma_{21} & \Sigma_{22}\end{pmatrix}$. For details, see Anderson [14].

The distribution of x_2 given x_1 takes a particular value a is then a multivariate gaussian with mean

$$\mu'_2 = \mu_2 + \Sigma_{21}\Sigma_{11}^{-1}(a - \mu_1) \quad (1)$$

and covariance matrix

$$\Sigma' = \Sigma_{22} - \Sigma_{21}\Sigma_{11}^{-1}\Sigma_{12} \quad (2)$$

Equation (1) produces regression models for the second-pregnancy log MoM values on the first-pregnancy log MoM values. This is simplified because, since the first pregnancy is unaffected, $\mu_1 = 0$. Equation (2) produces a covariance matrix that does not depend on a. We applied equations (1) and (2) to the estimates from our study to obtain the regression equations for the log MoM values in the second pregnancy.

For Unaffected Second Pregnancies

The regression equations for updating the second-pregnancy $\log_{10}$ MoM values are as follows: mean $\log_{10}$ MoM PAPP-A_2 = 0.4762592 × $\log_{10}$ MoM $PAPPA_1$ + 0.0059330 × $\log_{10}$ MoM free β-hCG_1, and mean $\log_{10}$ MoM free β-hCG_2 = 0.0378159 × $\log_{10}$ MoM $PAPPA_1$ + 0.4371823 × $\log_{10}$ MoM free β-hCG_1.

The standard deviations for the log MoM values for PAPP-A and free β-hCG obtained from equation (2) are 0.1976 and 0.2248, respectively. The correlation coefficient between the log MoM values is 0.2076.

For Trisomy 21 Second Pregnancies

The regression equations for updating the second-pregnancy $\log_{10}$ MoM values are as follows: mean $\log_{10}$ MoM PAPP-A = μ_p + 0.4808643 × $\log_{10}$ MoM $PAPPA_1$ + 0.0373621 × $\log_{10}$ MoM free β-hCG_1, and mean $\log_{10}$ MoM free β-hCG_2 = μ_f + 0.0304559 × $\log_{10}$ MoM $PAPPA_1$ + 0.4938187 × $\log_{10}$ MoM free β-hCG_1, where μ_p and μ_f are the mean log MoM values for Down's syndrome pregnancies. These were obtained from our previous paper [2].

The standard deviations for log MoM values for PAPP-A and free β-hCG obtained from equation (2) are 0.2191 and 0.2308, respectively. The correlation coefficient between the log MoM values is 0.0737.

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