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A reassessment of biochemical marker distributions in trisomy 21-affected and unaffected twin pregnancies in the first trimester

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KEYWORDS: chorionicity; first trimester; free β-hCG; PAPP-A; trisomy 21; twins

ABSTRACT

Objective *To estimate the difference between levels of the two biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and maternal serum free β-human chorionic gonadotropin (free β-hCG) in twin pregnancies relative to singleton pregnancies and establish an improved screening procedure for chromosomal abnormalities such as trisomy 21 in twin pregnancies.*

Methods *4843 unaffected and 47 trisomy 21-affected twin pregnancies were included in the study. Chorionicity-specific medians were generated for PAPP-A and free β-hCG from gestational ages 8 to 14 weeks. Multiple of the median values for each of the biochemical markers were calculated. Detection rates and false-positive rates were estimated for screening tests incorporating nuchal translucency and maternal age, with and without biochemistry.*

Results *Medians for the two biochemical markers for monochorionic and dichorionic twins in unaffected pregnancies show a gestational age-specific increase relative to singleton medians. Allowing for gestation and chorionicity, twin pregnancies affected with trisomy 21 had higher levels of free β-hCG and lower levels of PAPP-A. Adding biochemistry into the risk assessment using a fixed risk cut-off of 1 in 100 increased the detection rate for fetal trisomy 21 in dizygotic twin pregnancies from 78 to 90%, and decreased the false-positive rate from 8.0 to 5.9%.*

Conclusion *Generation of chorionicity-specific medians for the biochemical markers and their use in risk assessment can improve the performance of first-trimester screening for chromosomal abnormalities in twins to a level comparable with that in singleton pregnancies.*

INTRODUCTION

First-trimester screening for chromosomal defects using the combined test incorporating measurements of fetal nuchal translucency (NT) and the biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and maternal serum free β-human chorionic gonadotropin (free β-hCG) is now well established. Numerous studies have demonstrated that this test detects over 85% of fetal trisomy 21 pregnancies for a false-positive rate (FPR) of less than 3%[1]. The combined test can also detect over 90% of pregnancies affected by trisomies 13 or 18[2–4].

Twin pregnancies account for about 2% of all pregnancies in the Western world. With this prevalence of twin pregnancies, there is a need for a non-invasive screening test with low FPR and high detection rate (DR). Serum biochemical marker concentrations in twin pregnancies reflect the presence of two fetuses rather than one. Around gestational weeks 11–13 maternal serum concentrations are approximately double those found in singleton pregnancies. Ultrasound assessment in the first trimester provides accurate diagnosis of chorionicity[5,6]. In monochorionic twins the average of the two NT measurements can be used to calculate the pregnancy risk

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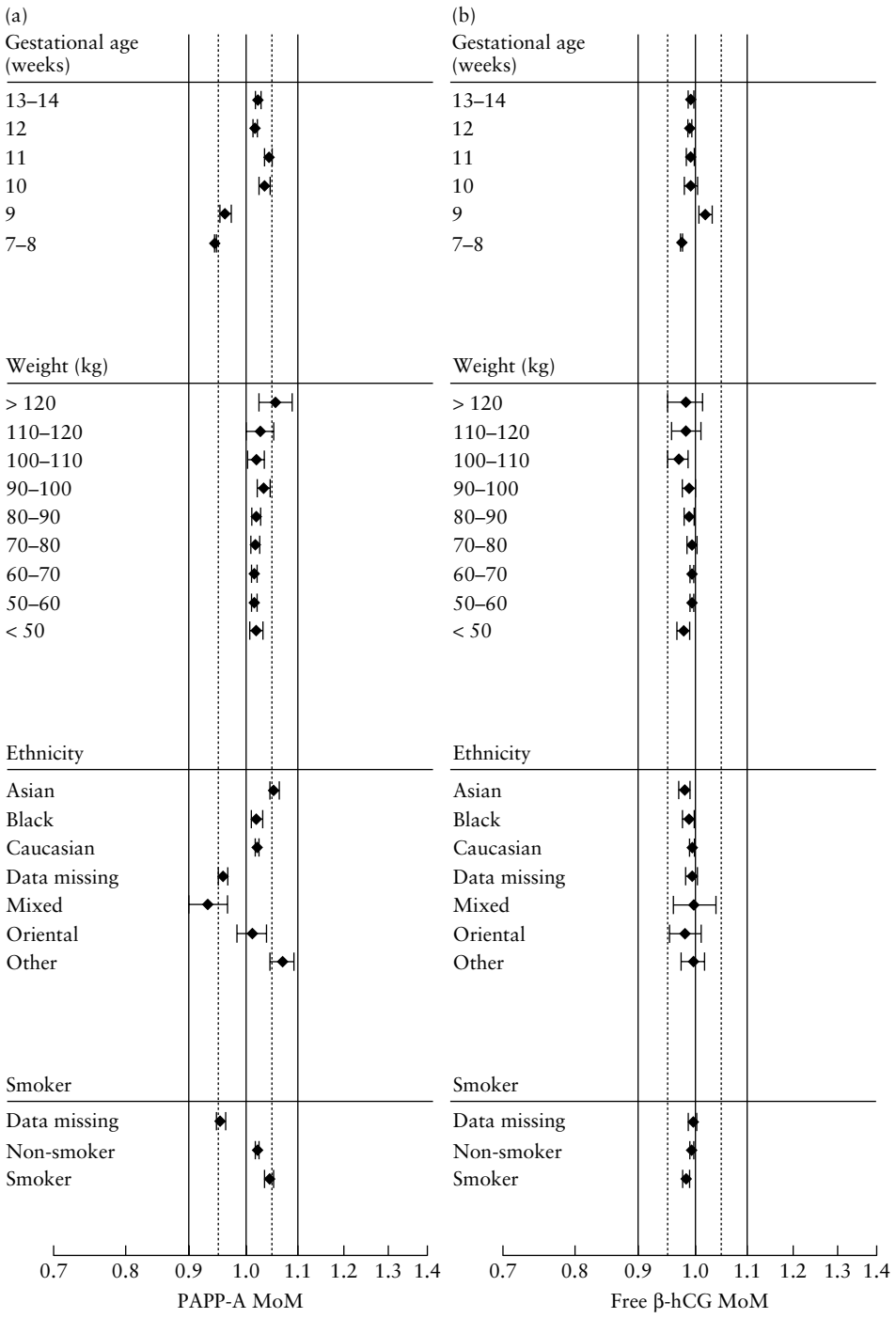

Figure 1 Diagnostic plots for pregnancy-associated plasma protein-A (PAPP-A) (a) and free β-human chorionic gonadotropin (β-hCG) (b) multiples of the median (MoM) values in unaffected singleton pregnancies.

and in dichorionic twins the individual NT measurements can be used to calculate the fetus-specific risk[7,8]. Previous studies have shown that while NT alone can be used successfully to screen twins with a similar DR and FPR to those in singleton pregnancies, the combination of both first-trimester NT and maternal serum biochemistry markers can improve the overall DR to around 80% at a 5% FPR[9], and this has been borne out in prospective screening practice[10,11]. On the basis of data obtained at 11–13 weeks the effect of gestation on serum PAPP-A and free β-hCG in twin pregnancies has been assumed to be constant[4,12]. In this way serum and ultrasound adjustments can be combined and a 'pseudo risk' – in which the twin pregnancy is considered to be a singleton and interpreted using singleton distribution parameters – calculated.

Recent studies have indicated that first-trimester screening in singleton pregnancies can be performed with the serum biochemistry parameters measured as early as gestational weeks 8–10[13,14]. This tendency towards earlier biochemistry testing has increased the demand for determination of medians for PAPP-A and free β-hCG in twin

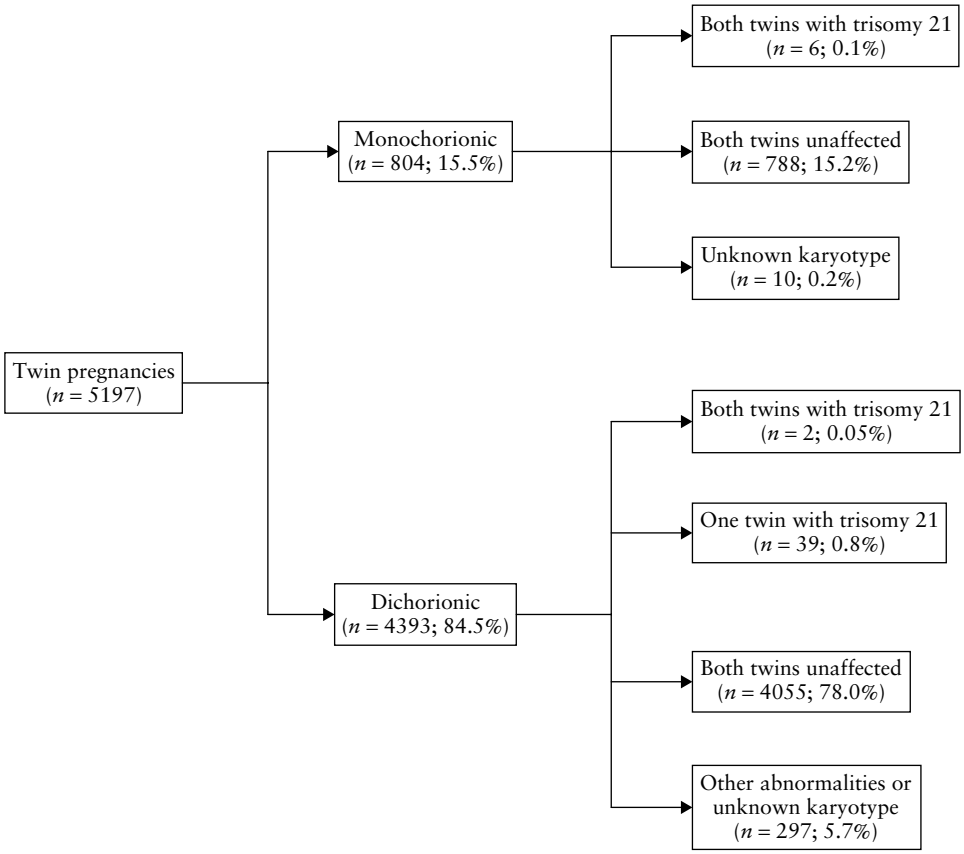

Figure 2 Flowchart for the data on twin pregnancies.

pregnancies. In this study we examine chorionicity-specific and gestational age-related distributions of biochemical markers in euploid and trisomic twin pregnancies and propose an improved algorithm for screening.

METHODS

Data on more than 200 000 singleton and 5197 twin pregnancies were obtained from the centers described below. From the UK: King's College Hospital, London; Fetal Medicine Centre, London (between June 2000 and February 2009); Harold Wood Hospital, Romford; King George Hospital, Goodmayes; Kent and Canterbury Hospital, Canterbury; William Harvey Hospital, Ashford; and Queen Elizabeth The Queen Mother's Hospital, Margate (between October 1999 and July 2009). From Denmark (between January 2004 and June 2009): Aarhus University Hospital, Skejby; Rigshospitalet, Copenhagen University Hospital; Viborg Hospital; Southwestjutland Hospital, Esbjerg; Southjutland Hospital, Sønderborg; Aalborg University Hospital; Hvidovre University Hospital; Regional Hospital Kolding; Herning Hospital; and Regional Hospital Horsens.

Free β-hCG and PAPP-A were measured in maternal blood samples collected at between 7 and 14 weeks' gestation using the Kryptor analyzer (Brahms AG, Berlin, Germany) or the DelfiaXpress system (Perkin Elmer, Waltham, MA, USA). In the UK, data on pre- and postnatal fetal trisomy 21 were identified by chromosomal analysis obtained from cytogenetic laboratories, the National Register, the patients themselves, their GP or the maternity unit in which they delivered. In Denmark, these data were obtained from the Danish central cytogenetic registry.

Fetal crown–rump length (CRL) and NT were measured at between 11 + 2 and 13 + 6 weeks' gestation (CRL, 45–84 mm). Gestational age was based on CRL at the time of the ultrasound scan[15,16], the larger of the two CRL measurements being used in the calculation of gestational age in twin pregnancies. NT and CRL were measured using standard procedures (www.fetalmedicine.com) performed by sonographers certified by The Fetal Medicine Foundation.

Existing models for singleton pregnancies[17] were used to produce multiples of the median (MoM) values specific to gestational age, maternal weight, ethnicity, smoking status, parity and the analytical instrumentation. Summaries of these MoM values were obtained for both singleton and twin pregnancies. Results for singletons were used to confirm the adequacy of the methodology for producing MoM values in singleton pregnancies. The MoM values for twin pregnancies were examined in unaffected monochorionic and dichorionic twins to determine how they differ from those in singleton pregnancies. Multiple regression analysis was then used to model the difference on the log scale between twin and singleton pregnancies. This led to gestational age-specific adjustment factors for monochorionic and dichorionic twins specified in terms

Table 1 Characteristics of the sample of twin pregnancies (trisomy 21-affected and unaffected)

	Center			
Parameter	*King's/FMC* (n = *2437*)	*King George* (n = *1421*)	*Denmark* (n = *1339*)	*Pooled data* (n = *5197*)
Maternal age (years)	34.8 (31.3–37.8)	33.3 (29.6–36.4)	31.5 (28.6–34.3)	33.6 (29.9–36.8)
Maternal weight (kg)	65.4 (59.0–73.1)	65.4 (58.4–75.8)	67.5 (60.0–77.0)	66.0 (59.0–75.0)
Ethnicity				
Asian	112 (4.6)	111 (7.8)	0 (0.0)	223 (4.3)
Black	137 (5.6)	70 (4.9)	3 (0.2)	210 (4.0)
White	2124 (87.2)	956 (67.3)	835 (62.4)	3 915 (75.3)
Mixed	31 (1.3)	9 (0.6)	0 (0.0)	40 (0.8)
Oriental	33 (1.4)	14 (1.0)	7 (0.5)	54 (1.0)
Other	0 (0.0)	9 (0.6)	15 (1.1)	24 (0.5)
Data missing	0 (0.0)	252 (17.7)	479 (35.8)	731 (14.1)
Smoking status				
Smoker	126 (5.2)	115 (8.1)	94 (7.0)	335 (6.4)
Non-smoker	2310 (94.8)	1301 (91.6)	881 (65.8)	4492 (86.4)
Not reported	1 (0.04)	5 (0.4)	364 (27.2)	370 (7.1)
Mode of conception				
Spontaneous	988 (40.5)	77 (5.4)	470 (35.1)	1535 (29.5)
In-vitro fertilization	1180 (48.4)	191 (13.4)	247 (18.4)	1618 (31.1)
Drugs for induction of ovulation	269 (11.0)	8 (0.6)	63 (4.7)	340 (6.5)
Other	0 (0.0)	29 (2.0)	129 (9.6)	158 (3.0)
Not reported	0 (0.0)	1116 (78.5)	430 (32.1)	1546 (29.7)
Gestational age (weeks)				
7	0 (0.0)	0 (0.0)	9 (0.7)	9 (0.2)
8	0 (0.0)	0 (0.0)	229 (17.1)	229 (4.4)
9	0 (0.0)	0 (0.0)	474 (35.4)	474 (9.1)
10	0 (0.0)	8 (0.6)	372 (27.8)	380 (7.3)
11	155 (6.4)	83 (5.8)	178 (13.3)	416 (8.0)
12	1337 (54.9)	752 (52.9)	67 (5.0)	2156 (41.5)
13	848 (34.8)	541 (38.1)	10 (0.7)	1399 (26.9)
14	55 (2.3)	37 (2.6)	0 (0.0)	92 (1.8)
Data missing	42 (1.7)	0 (0.0)	0 (0.0)	42 (0.8)
Karyotype (pregnancy)				
Normal	2113 (86.7)	1405 (98.9)	1325 (99.0)	4843 (93.2)
Trisomy 21	17 (0.7)	16 (1.1)	14 (1.0)	47 (0.9)
Other abnormalities	9 (0.4)	0 (0.0)	0 (0.0)	9 (0.2)
Not known	298 (12.2)	0 (0.0)	0 (0.0)	298 (5.7)
Chorionicity				
Dichorionic	2065 (84.7)	1231 (86.6)	1097 (81.9)	4393 (84.5)
Monochorionic	372 (15.3)	190 (13.4)	242 (18.1)	804 (15.5)

Data are given as median (interquartile range) or *n* (%). FMC, Fetal Medicine Centre, London, UK; King George, King George Hospital, Goodmayes, UK; King's, King's College Hospital, London, UK.

of quadratic functions on the log scale. These adjustment factors were applied to produce twin-specific MoM values. The adequacy of this adjustment was assessed using diagnostic plots to examine twin-specific median MoM values by gestation, weight, ethnicity, smoking status, chorionicity, center and mode of conception.

In chromosomally abnormal dichorionic twins a model in which the mean logMoM was assumed to be half of that in singleton pregnancies with the same chromosomal abnormality was assessed for adequacy[2,17]. In chromosomally abnormal concordant monochorionic twins, a model in which the mean logMoM was the same as that in singleton pregnancies with the same chromosomal abnormality was similarly assessed. The standard deviations of logMoM values in unaffected twins and in trisomy 21-affected twins (after removing the gestation-specific mean) together with the correlations between logMoM values were produced and compared with those in singleton pregnancies.

Risks for twin pregnancies were produced for maternal age with NT and for maternal age with biochemistry and NT combined using the models for mean logMoM values described above. Standard deviations and correlations in twin pregnancies were assumed to be the same as for singleton pregnancies. The mixture model was used for NT in the calculation of risks[18]. Using a risk cut-off of 1 in 100 at the time of screening, crude and maternal age-standardized DRs and FPRs were produced[19,20]. All logs were to the base 10.

RESULTS

A total of 212 308 unaffected singleton pregnancies were considered in the assessment of validity of the model

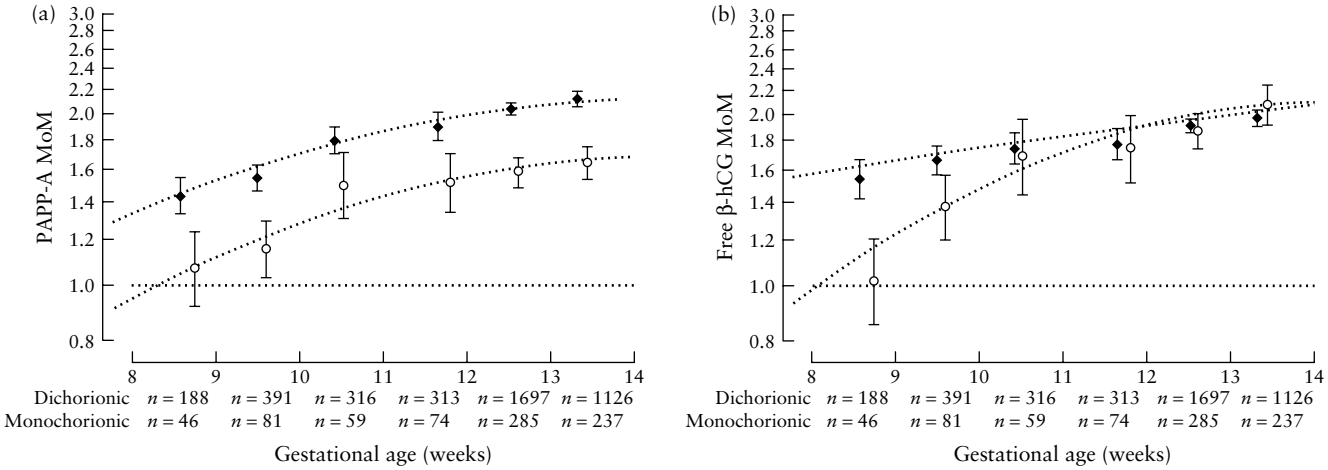

Figure 3 Median pregnancy-associated plasma protein-A (PAPP-A) (a) and free β-human chorionic gonadotropin (β-hCG) (b) multiples of the median (MoM) values and 95% CIs by chorionicity (◆, dichorionic; ○, monochorionic) and gestation, with fitted regression lines, for unaffected twin pregnancies. The medians plotted at 8 weeks are those for 7 and 8 weeks combined and are plotted at the median gestation over this period. Similarly, the medians plotted at 13 weeks are those for 13 and 14 weeks combined and are plotted at the median gestation over this period.

Table 2 Multiple regression coefficients for the gestational age-specific effect of twins relative to singletons, for pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG)

	Estimate			
Chorionicity	*Log scale*	*Original scale**	*SE†*	P
PAPP-A				
Dichorionic	0.2702	1.8629	0.0063	0.0000
Monochorionic	0.1552	1.4296	0.0141	0.0000
Dichorionic × (GA-77)	0.0048	1.0112	0.0004	0.0000
Monochorionic × (GA-77)	0.0059	1.0137	0.0008	0.0000
Dichorionic × $(GA\text{-}77)^2$	−0.0001	0.9998	0.0000	0.0052
Monochorionic × $(GA\text{-}77)^2$	−0.0001	0.9997	0.0001	0.0917
Free β-hCG				
Dichorionic	0.2636	1.8347	0.0069	0.0000
Monochorionic	0.2340	1.7141	0.0156	0.0000
Dichorionic × (GA-77)	0.0029	1.0066	0.0004	0.0000
Monochorionic × (GA-77)	0.0079	1.0183	0.0009	0.0000
Dichorionic × $(GA\text{-}77)^2$	−0.0000	1.0000	0.0000	0.6802
Monochorionic × $(GA\text{-}77)^2$	−0.0002	0.9996	0.0001	0.0325

*Calculated as 10^(log scale estimate). †Standard error for log scale estimate. GA, gestational age (days).

used for producing MoM values. These pregnancies were selected so that they were tested over the same time period as that for the twin pregnancies. Figure 1 shows diagnostics for MoM values, obtained from models in the paper by Wright *et al.*[17], for these singleton pregnancies. The estimated median MoM values all fall well within 10% of 1 MoM, confirming the adequacy of the models used for producing MoM values.

A total of 5197 twin pregnancies were collected; 1339 from Denmark and 3858 from the UK. Forty-seven (0.9%) were affected with trisomy 21. There were 39 trisomy 21 affected discordant dichorionic twin pregnancies and two trisomy 21 concordant dichorionic twin pregnancies; altogether 43 affected dichorionic twins from 41 separate pregnancies. Of the 5197 pairs of twins, 4393 (84.5%) were dichorionic and 804 (15.5%) were monochorionic. Data for this sample of twin pregnancies are shown in Figure 2, while Table 1 shows the characteristics of the sample.

Figure 3 shows median MoM values with 95% confidence intervals (CIs), on the log scale, against gestation for unaffected twin pregnancies. These MoM values were obtained using the multiple regression models for singleton pregnancies tested in Figure1[17]. The median MoM values in twins increase with gestational age. For PAPP-A, the median MoM values are higher in dichorionic twins than in monochorionic twins, approaching 2.1 MoM and 1.6 MoM, respectively, at week 13. Free β-hCG-MoM values in dichorionic and monochorionic twins increase to a similar level with gestation, approaching 2.0 MoM at 13 weeks' gestation. Multiple regression models for the gestational age-specific effect of twins relative to singletons are summarized in Table 2. The estimated median MoMs relative to

singletons obtained from these regression models are given in Table 3. Median MoM values with gestational age- and chorionicity-specific adjustment for unaffected twin pregnancies are shown with 95% CIs in Figure 4. Although there is some evidence of a decreasing trend with maternal weight for PAPP-A, the median MoM values in twins shown in Figure 4 are generally well within 10% of 1 MoM. The standard deviations and correlations of logMoM-PAPP-A and free β-hCG adjusted for gestational age- and chorionicity-specific twin effects are given in Table 4. LogMoM values, adjusted for gestational age- and chorionicity-specific effects, for twins affected by trisomy 21 are shown in Figure 5. Summary statistics are presented in Table 5. In dichorionic twins affected with trisomy 21, a purported model in which the mean logMoM was assumed to be half of that in singleton pregnancies with trisomy 21 was tested[17]. There was no evidence of any departure from this model ($P = 0.72$ for PAPP-A and 0.64 for free β-hCG). In monochorionic twins with trisomy 21, a model in which the mean logMoM was the same as that in singleton pregnancies with trisomy 21 was tested. While MoM values for PAPP-A were consistent with this model ($P = 0.60$), there was some evidence of lack of fit for free β-hCG ($P = 0.04$).

Table 3 Estimated median multiples of the median of biochemical markers for unaffected twin pregnancies relative to singleton pregnancies at each week of gestational age

	Estimate (95% CI)	
Week	*Monochorionic*	*Dichorionic*
PAPP-A		
7 + 3	0.8501 (0.6740–1.0722)	1.2262 (1.0967–1.3710)
8 + 3	1.0194 (0.8946–1.1616)	1.4182 (1.3318–1.5101)
9 + 3	1.1886 (1.1034–1.2803)	1.6044 (1.5494–1.6614)
10 + 3	1.3475 (1.2643–1.4362)	1.7756 (1.7255–1.8271)
11 + 3	1.4855 (1.3969–1.5797)	1.9221 (1.8706–1.9750)
12 + 3	1.5923 (1.5196–1.6685)	2.0353 (1.9954–2.0761)
13 + 3	1.6597 (1.5691–1.7554)	2.1082 (2.0555–2.1624)
14 + 3	1.6820 (1.4769–1.9156)	2.1362 (2.0088–2.2716)
Free β-hCG		
7 + 3	0.8487 (0.6566–1.0969)	1.5205 (1.3440–1.7203)
8 + 3	1.0868 (0.9407–1.2557)	1.6101 (1.5020–1.7259)
9 + 3	1.3382 (1.2326–1.4530)	1.6988 (1.6344–1.7656)
10 + 3	1.5842 (1.4763–1.7000)	1.7859 (1.7303–1.8433)
11 + 3	1.8031 (1.6846–1.9301)	1.8708 (1.8155–1.9278)
12 + 3	1.9732 (1.8738–2.0779)	1.9527 (1.9103–1.9959)
13 + 3	2.0760 (1.9512–2.2088)	2.0308 (1.9746–2.0886)
14 + 3	2.1000 (1.8188–2.4248)	2.1045 (1.9662–2.2525)

β-hCG, β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A.

Risk calculations for twins

The risk of trisomy 21 was calculated using maternal age together with NT and biochemistry and with maternal age and NT alone using the mixture model[18] for NT and the model described above for biochemistry. The results are presented for dichorionic and monochorionic pregnancies

Table 4 SD log multiples of the median (MoM) values and correlations by week and by chorionicity for unaffected twin pregnancies

	Monochorionic		*Dichorionic*	
Week	n	*SD logMoM (95% CI) or correlation coefficient (95% CI)*	n	*SD logMoM (95% CI) or correlation coefficient (95% CI)*
PAPP-A				
8	44	0.2026 (0.1674 to 0.2567)	184	0.2186 (0.1983 to 0.2435)
9	81	0.2234 (0.1935 to 0.2643)	391	0.2150 (0.2009 to 0.2312)
10	59	0.1954 (0.1654 to 0.2387)	316	0.2178 (0.2020 to 0.2362)
11	74	0.2144 (0.1846 to 0.2559)	313	0.2131 (0.1976 to 0.2312)
12	285	0.2202 (0.2035 to 0.2399)	1 693	0.2048 (0.1981 to 0.2119)
13	216	0.2093 (0.1912 to 0.2311)	1 064	0.1993 (0.1912 to 0.2082)
All		0.2117 (0.2036 to 0.2197)		0.2108 (0.2046 to 0.2170)
Free β-hCG				
8	44	0.2407 (0.1989 to 0.3050)	184	0.2074 (0.1882 to 0.2311)
9	81	0.2263 (0.1960 to 0.2677)	391	0.2276 (0.2127 to 0.2448)
10	59	0.2252 (0.1907 to 0.2752)	316	0.2248 (0.2086 to 0.2439)
11	74	0.2545 (0.2191 to 0.3037)	313	0.2275 (0.2109 to 0.2468)
12	285	0.2531 (0.2339 to 0.2758)	1 693	0.2298 (0.2223 to 0.2378)
13	216	0.2585 (0.2362 to 0.2855)	1 064	0.2338 (0.2243 to 0.2442)
All		0.2442 (0.2320 to 0.2563)		0.2260 (0.2200 to 0.2320)
Correlation*				
8	44	0.1734 (−0.1302 to 0.4472)	184	0.1067 (−0.0386 to 0.2475)
9	81	0.0585 (−0.1619 to 0.2734)	391	0.1285 (0.0297 to 0.2248)
10	59	0.3544 (0.1082 to 0.5597)	316	0.1698 (0.0606 to 0.2750)
11	74	0.2781 (0.0530 to 0.4763)	313	0.2703 (0.1643 to 0.3700)
12	285	0.1321 (0.0162 to 0.2446)	1 693	0.2103 (0.1643 to 0.2554)
13	216	0.1176 (−0.0161 to 0.2472)	1 064	0.1044 (0.0446 to 0.1635)
All		0.1558 (0.0836 to 0.2239)		0.1686 (0.1123 to 0.2207)

*Correlation between pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) log MoMs at each week of gestational age.

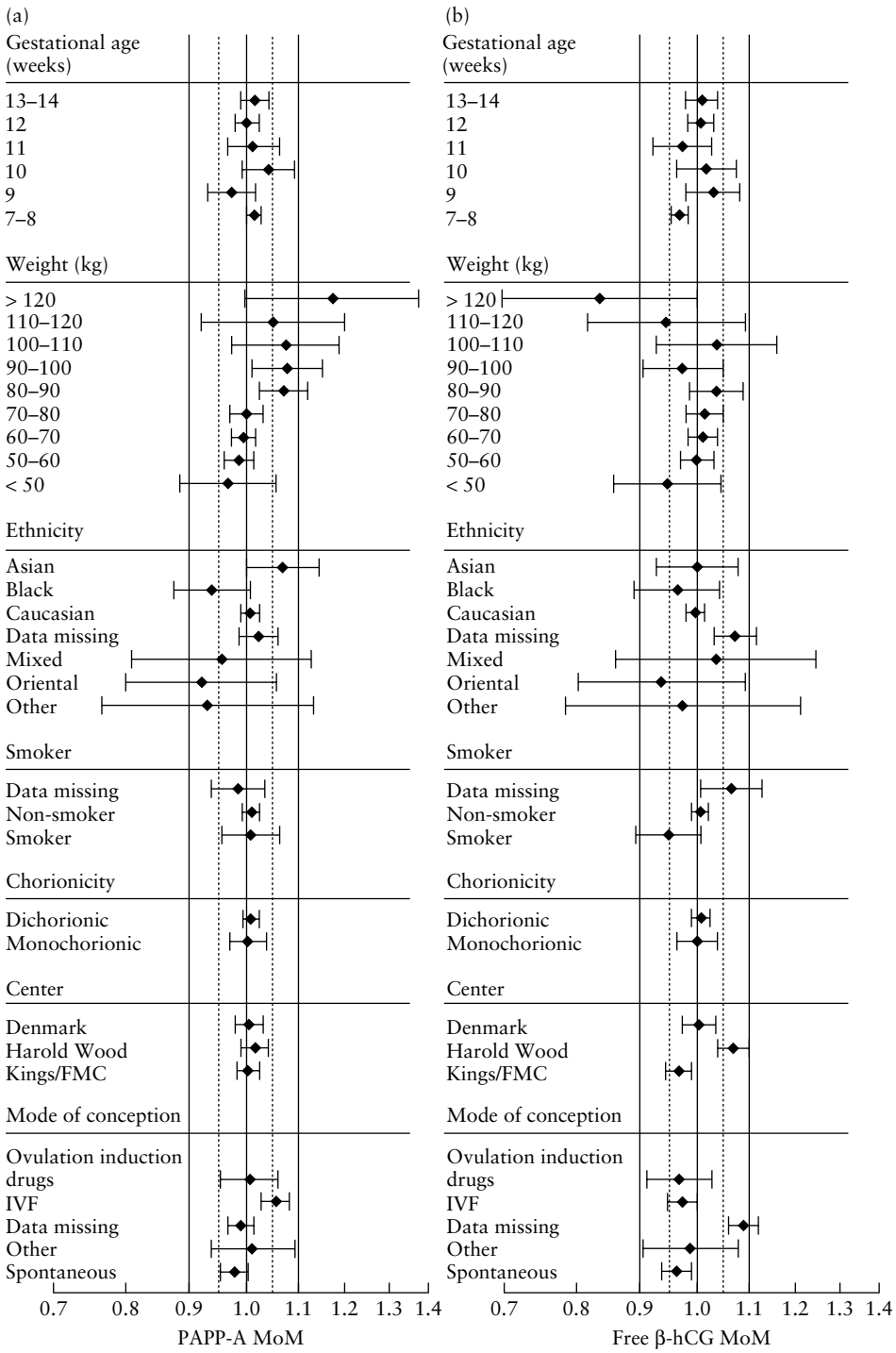

Figure 4 Diagnostic plots for pregnancy-associated plasma protein-A (PAPP-A) (a) and free β-human chorionic gonadotropin (β-hCG) (b) multiples of the median (MoM) values in unaffected twin pregnancies, having corrected for the effect of twin pregnancy and how this varies with gestational age.

in Tables 6 and 7, respectively. Receiver–operating characteristics curves for screening dichorionic twins using the combined test and NT alone are shown in Figure 6. The addition of biochemistry markers to NT in the risk calculation for dichorionic pregnancies produces a worthwhile reduction in FPR and an increase in DR. With the relatively small number of monochorionic pregnancies there is little to suggest any benefit or harm from the addition of biochemistry markers to NT and maternal age. It should be noted that the alternative, likelihood based approach of combining the two NT measurements produces a lower FPR than does the standard process of taking averages of the two risks.

DISCUSSION

This study, using a large dataset for twin pregnancies, has established chorionicity- and gestational age-specific

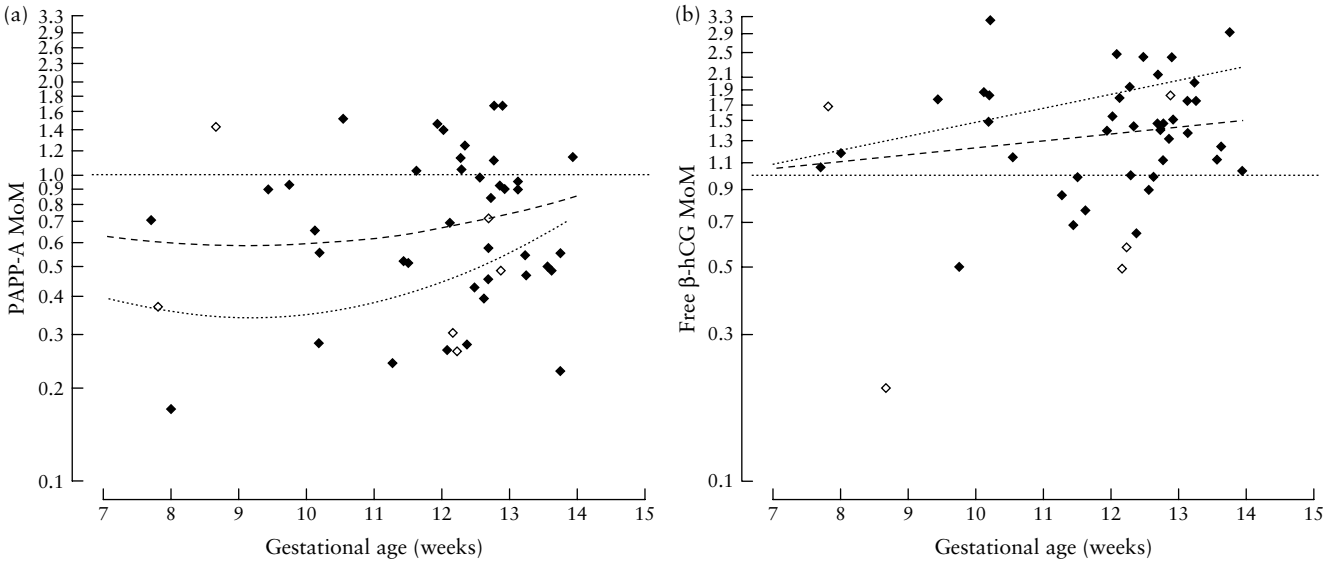

Figure 5 Adjusted pregnancy-associated plasma protein-A (PAPP-A) (a) and free β-human chorionic gonadotropin (β-hCG) (b) multiples of the median (MoM) in trisomy 21 twin pregnancies by chorionicity (dichorionic (◆) and monochorionic (◇)) and gestation. The dotted lines show existing regression models for PAPP-A- and free β-hCG-MoMs in trisomy 21 singleton pregnancies; the dashed lines show regression models in which these effects are halved on the log scale.

Table 5 Estimated effects, SD log multiples of the median (MoM) and correlations by chorionicity for trisomy 21 twin pregnancies

Chorionicity	n	*Estimated effect (95% CI) or correlation coefficient (95% CI)*	*SD logMoM (95% CI)*
PAPP-A			
All twins	47		0.2206 (0.1612 to 0.2801)
Monochorionic	6	0.4910 (0.3193 to 0.7549)	0.1848 (0.1107 to 0.5310)
Dichorionic	41	0.6648 (0.5641 to 0.7835)	0.2457 (0.2017 to 0.3144)
Free β-hCG			
All twins	47		0.2428 (0.1095 to 0.3761)
Monochorionic	6	0.8485 (0.5273 to 1.3654)	0.3227 (0.1933 to 0.9273)
Dichorionic	41	1.4130 (1.1783 to 1.6944)	0.1816 (0.1491 to 0.2323)
Correlation*			—
All twins	47	0.64411 (−0.7874 to 0.9821)	—
Monochorionic	6	0.9303 (0.2678 to 0.9955)	—
Dichorionic	41	−0.1034 (−0.3984 to 0.2110)	—

*Correlation between pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) log MoMs.

medians for PAPP-A and free β-hCG from 8 to 14 gestational weeks. The results show – contrary to previous assumptions – that the medians for PAPP-A and free β-hCG are highly dependent upon the time of sampling in the first trimester and that it is necessary to generate chorionicity-specific medians for twin pregnancies in order to use the biochemical markers in risk assessment for fetal aneuploidy in first-trimester screening of twin pregnancies. We show that using this new algorithm including chorionicity-specific medians for biochemistry in twin pregnancies, the performance of first-trimester screening in twins is improved to a level comparable with that in singletons.

In twins the levels of serum PAPP-A and free β-hCG change with gestation and these levels are lower in monochorionic twins than in dichorionic twins. In dichorionic twins both markers increase from approximately 1.5 MoM of the singleton median in gestational weeks 8–9 to approximately 2.0 MoM at 13–14 weeks. In monochorionic twins the levels of both biochemical markers are approximately equal to the singleton median at 8–9 weeks and increase at 13–14 weeks to 2.0 MoM for free β-hCG and 1.5 MoM for PAPP-A. These results are in agreement with those of Spencer *et al.*[4], who reported significantly lower corrected PAPP-A-MoM in monochorionic twins than in dichorionic twins, and Linskens *et al.*[12], who reported decreased levels of PAPP-A and free β-hCG-MoMs in monochorionic twins. The reason for this relatively lower increase in PAPP-A in maternal serum from women with monochorionic twins is not known, but may reflect the relatively lower amount of fetoplacental units in monochorionic twins[9].

The implication of our results is that in first-trimester screening in twin pregnancies the levels of PAPP-A and free β-hCG cannot be described adequately by a constant multiple of singleton medians throughout the

Table 6 Screening performance for dichorionic twins

Karyotype			*Risk > 1 in 100* (n *(%))*		
Twin 1	*Twin 2*	n	*Twin 1*	*Twin 2*	*Either*
Screening performance based on maternal age, NT and biochemistry					
Normal karyotype		4023	164	182	237 (5.9)
Abnormal karyotype					
Normal	Trisomy 21	37	10	33	33 (89.2)
47XXX	Trisomy 21	1	1	0	1 (100.0)
Trisomy 18	Trisomy 21	1	1	1	1 (100.0)
Trisomy 21	Trisomy 21	2	2	2	2 (100.0)
Total		41			37 (90.2)
Screening performance based on maternal age and NT					
Normal karyotype		4023	199	210	321 (8.0)
Abnormal karyotype					
Normal	Trisomy 21	37	2	28	28 (75.7)
47XXX	Trisomy 21	1	1	0	1 (100.0)
Trisomy 18	Trisomy 21	1	1	1	1 (100.0)
Trisomy 21	Trisomy 21	2	2	2	2 (100.0)
Total		41			32 (78.0)

Of the original 4055 unaffected dichorionic twin pregnancies, 32 (0.8%) have been excluded due to missing data on maternal age, gestational age, nuchal translucency (NT) or biochemistry.

Table 7 Screening performance for monochorionic twins

Karyotype	n	*Risk > 1 in 100* (n *(%))*
Screening performance based on maternal age, NT and biochemistry		
Normal	782	36 (4.6)
Trisomy 21	6	6 (100.0)
Screening performance based on maternal age and NT		
Normal	782	37 (4.7)
Trisomy 21	6	6 (100.0)

Of the original 788 unaffected monochorionic twin pregnancies, six have been excluded due to missing data. NT, nuchal translucency.

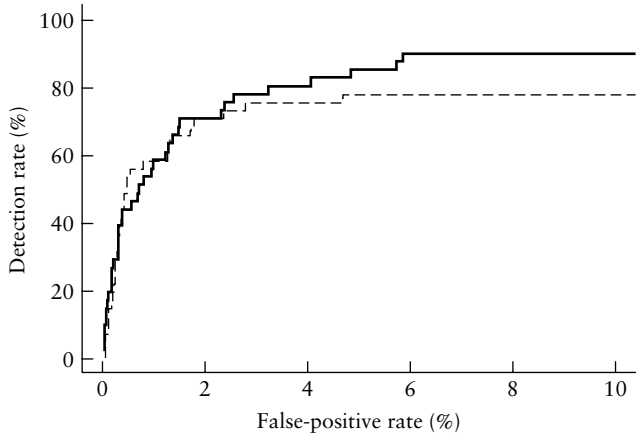

Figure 6 Receiver–operating characteristics curves for screening dichorionic twins with nuchal translucency (NT) alone (– – –) and NT + biochemistry (——).

first trimester from 8 to 14 weeks, but must be based on individual chorionicity-specific medians.

Twin pregnancies affected by fetal trisomy 21 show a similar tendency to trisomy 21-affected singletons, with decreased PAPP-A-MoM and increased free β-hCG-MoM. In dichorionic twins affected by trisomy 21, the mean logMoM fitted well with the model assuming half the level of that in singleton pregnancies with trisomy 21. In monochorionic twins with trisomy 21, the model assuming similar logMoM values to those in singleton pregnancies fitted well with PAPP-A and less well with free β-hCG. However, the number of trisomy 21 cases of monochorionic twin pregnancies was limited, which may influence the conclusion.

The addition of likelihood ratios for PAPP-A and free β-hCG to risk assessment by maternal age and fetal NT in dichorionic twins increased the DR for fetal trisomy 21 from 78 to 90% and decreased the FPR from approximately 8 to 6%, using a fixed risk cut-off of 1 in 100. This brings the performance of first-trimester screening in twins close to the standard for screening in singletons. While the benefit for screening performance from the addition of biochemistry to maternal age and NT in dichorionic twins is clear, there appears to be no improvement in the detection of fetal aneuploidy in monochorionic twins. Our results show that neither the DR nor the screen positive rate of prenatal screening for fetal trisomy 21 in monochorionic twin pregnancies is affected by the addition of biochemical markers. Based on the number of monochorionic twin pregnancies in the dataset it is questionable whether biochemistry will affect the screen positive rate, but more cases of fetal trisomy 21 in twins are needed in order to draw reliable conclusions regarding the DR. Whether risk calculation based on chorionicity-specific twin medians is superior to the 'pseudo risk' approach in gestational weeks 12–14 is

unknown, but it is a prerequisite for risk calculation in the extended window from gestational weeks 8–14.

The risk of aneuploidy is in general higher in twin pregnancies because of increased maternal age. It has been debated whether the age-specific risk could be applied per dichorionic twin fetus instead of per dichorionic twin pregnancy, resulting in half the risk for a twin fetus compared to a singleton fetus[21,22]. In our calculations we have assumed that the age-specific risk is specific for each fetus. The uncertainty in this factor makes this issue of a combined risk calculation even more relevant. Adding biochemistry parameters to NT and maternal age in the risk assessment will alter risks at the level of the individual. In cases where the biochemistry is typical of chromosomal abnormalities, risks will increase. Conversely, in cases where the biochemistry is typical of a chromosomally normal pregnancy, the risks will decrease relative to those deduced from maternal age and NT alone. Overall the evidence would suggest an improvement in both DR and FPR in screening programs using a fixed risk cut-off. Gestational age-specific adjustments rather than the current fixed adjustments would be expected to reduce FPR at early gestations (before 11 weeks).

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