Prenatal screening for Down syndrome in twin pregnancies: estimates of screening performance based on 61 affected and 7,302 unaffected twin pregnancies

Nicholas J Wald*,[1] Jonathan Bestwick,[1] Wayne Huttly,[1] Jonathan Aldis[2] Antoni Borrell,[3] Sandy Goodburn,[4] Ian Mills[5]

Running head: Prenatal Down syndrome screening performance in twin pregnancies

1 Wolfson Institute of Preventive Medicine, Queen Mary University of London
2 Sheffield Teaching Hospitals NHS Foundation Trust
3 BCNatal Hospital Clinic of Barcelona
4 Cambridge University Hospitals NHS Foundation Trust
5 Birmingham Women's and Children's NHS Foundation Trust

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*CORRESPONDING AUTHOR: Professor Nicholas Wald, Wolfson Institute of Preventive Medicine, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK. N.j.wald@qmul.ac.uk

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What's already known about this topic?

- Prenatal Down syndrome screening markers in unaffected twin pregnancies are approximately double those in singleton pregnancies
- It has generally been assumed that the proportional differences in serum marker levels in affected and unaffected singleton pregnancies can be applied to levels found in twin pregnancies

What does this study add?

- The median late first trimester free β-hCG level in monochorionic affected twin pregnancies is lower than levels based on the proportionality assumption, and the median PAPP-A level in dichorionic affected twin pregnancies is higher.
- Combined test screening performance in monochorionic twins is similar to that in singletons, but lower in dichorionic twins
- Different Combined test risk cut-offs in monochorionic and dichorionic twin pregnancies would be needed to achieve similar false-positive rates

ABSTRACT

Objective: To determine whether assumptions used in prenatal screening for Down syndrome in twin pregnancies are valid, and derive estimates of risk and screening performance in twin pregnancies using observed data.

Methods: Data were collected on nuchal translucency, chorionicity, pregnancy associated plasma protein-A (PAPP-A) and free ß human chorionic gonadotrophin (free ß-hCG) from 61 twin pregnancies with Down syndrome and 7,302 unaffected twin pregnancies. Distribution parameters were determined, and used to estimate screening performance.

Results: The assumption that proportional differences in serum marker levels in affected and unaffected singleton pregnancies apply to twin pregnancies was not confirmed. Median free β-hCG value in monochorionic affected twin pregnancies (2.63 multiples of the median (MoM), 95% CI 1.79-3.22MoM)) was lower than that assuming proportionality (3.76 MoM), but the median PAPP-A value in dichorionic affected twin pregnancies (1.88 MoM (95% CI 1.60-2.17 MoM)) was higher than based on proportionality (1.33 MoM). The detection rate was 87% for a 3% false-positive rate in monochorionic twin pregnancies, and 74% in dichorionic twin pregnancies compared with 86% in singleton pregnancies.

Conclusions: Estimates of screening performance in Down syndrome twin pregnancies do not need to rely on assumption and can take account of chorionicity and gestational age.

KEY WORDS: Prenatal screening, Down syndrome, twin, chorionicity, combined test

Introduction

Prenatal screening for Down syndrome is more complex in twin pregnancies than in singleton pregnancies. Because of a lack of data on twin pregnancies with Down syndrome, estimates of the risk of having an affected twin pregnancy have relied on the assumption that the proportional differences in serum marker levels in affected and unaffected singleton pregnancies can be applied to levels found in twin pregnancies, which tend to be about twice as high.[1] On this basis the median marker level in affected monochorionic twin pregnancies is taken as the median level in affected singleton pregnancies multiplied by the median level in unaffected twin pregnancies. Similarly the median marker level in dichorionic twins is taken as the average of the medians in unaffected and affected singleton pregnancies, multiplied by the median in unaffected twin pregnancies.[1] With marker levels expressed as multiple of the median (MoM) in unaffected singleton pregnancies (MoM), and based on these assumptions, the median late first trimester pregnancy associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotrophin (hCG) levels have been estimated to be 0.80 and 3.76 MoM respectively in monochorionic affected twin pregnancies and 1.33 and 2.93 MoM in dichorionic affected twin pregnancies respectively. [1] The assumptions on which these estimates are based have not been empirically tested. We here do so using data from five screening centres.

Methods

Twenty-four twin pregnancies affected with Down syndrome were identified from the Wolfson Institute Screening Service, together with 7,302 unaffected twin pregnancies between January 2007 and November 2016. An additional 37 affected twin pregnancies were identified from screening centres in Barcelona (12), Cambridge (5), Birmingham (15), and Sheffield.(5) Among the 61 affected twin pregnancies, 12 were monochorionic and 49 were dichorionic, and among the unaffected twin pregnancies 1,490 were monochorionic and 5,812 dichorionic. Marker levels for PAPP-A and free β-hCG, together with measurements of the ultrasound marker nuchal translucency (NT) (ie the

Combined test markers) were reported to the study centre by each centre as multiples of the median (MoM) values so that the data could be combined without introducing bias.

Median MoM values were calculated in monochorionic and dichorionic affected and unaffected twin pregnancies. For monochorionic twin pregnancies the geometric mean NT MoM value of the two separate fetus MoM values was used. Differences in median MoM values between monochorionic and dichorionic twin pregnancies were compared using the Wilcoxon rank sum test. Median regression was used to determine whether MoM values were influenced by gestational age. MoM values in twin pregnancies were calculated based on median values in unaffected twin pregnancies by dividing the reported MoM values by the median MoM in unaffected twin pregnancies given gestation and chorionicity and designated MOM_T to indicate that the reference population is unaffected twin pregnancies, not singleton pregnancies.

Medians, standard deviation and correlation coefficients were estimated for the markers in affected and unaffected twin pregnancies, together with truncations limits, to define the multivariate Gaussian distributions in affected and unaffected twin pregnancies. In unaffected twin pregnancies, for the two serum markers, standard deviations were estimated from a regression of values on probability plots between the 10^{th} and 90^{th} centile to avoid the influence of outliers. In affected twin pregnancies a regression of all values was used. For NT, standard deviations were estimated using all data to reflect the positive skew. [2] In unaffected pregnancies, correlation coefficients were estimated after excluding outlying values greater than 3.5 standard deviations from the mean for affected pregnancies, because of the small numbers, this was done by inspection of scatterplots (one excluded). Truncation limits were set on where the MoM values deviated from a Gaussian distribution i.e. deviated from the straight line on the probability plots in affected and unaffected pregnancies, or at the point of risk reversal.[3]

Screening performance was estimated using Monte Carlo simulation and multivariate Gaussian analyses. 100,000 affected and 100,000 unaffected pregnancies were simulated each separately for monochorionic and dichorionic pregnancies. This was done for the Combined test marker values (including a single NT MoM value for monochorionic twin pregnancies, and two NT MoM values for dichorionic twin pregnancies, taking into account any correlations between the two). Each pregnancy was assigned a maternal age based on the 2014-2016 maternal age distribution of England and Wales.[4] For each simulated monochorionic twin pregnancy, the risk of being affected with Down syndrome at term was calculated by multiplying the maternal age specific odds of having an affected singleton live birth [5], adjusted by the 66% reduction in risk of a pregnancy being affected if it is a monochorionic twin pregnancy compared with a singleton pregnancy [6], by the likelihood ratio for being affected (for the simulated set of marker values) calculated from the multivariate Gaussian distributions of marker levels in affected and unaffected pregnancies. For each simulated dichorionic twin pregnancy, the risk of being affected with Down syndrome at term was calculated by multiplying half the maternal age specific odds of having an affected singleton live birth [5], adjusted by the 34% increase in risk of at least one of the fetuses being affected in a dichorionic twin pregnancy compared with a singleton pregnancy [6], by the likelihood ratio for each NT MoM value, summing the two and then multiplying that by the likelihood ratio for the serum markers. Risks were also calculated based on using NT alone, with maternal age. Screening performance was estimated as detection rates (DRs) for specified false-positive rates (FPRs), FPRs for specified DRs and DRs and FPRs for specified risk cut-offs. Screening performance in twin pregnancies was compared with that in singleton pregnancies using updated parameters from the Serum, Urine and Ultrasound Screening Study (SURUSS) [7][8] with a revised standard deviation for NT in unaffected pregnancies to take account of improvements in measuring NT over time, leading to decreases in the standard deviation of the log NT MoM values. The revised NT standard deviation was based on data from an audit of reflex DNA screening for Down syndrome, trisomy 18 and trisomy 13 [9]; among 22,706 unaffected pregnancies the $\log_{10}$ standard deviation was 0.0963 in

pregnancies screened in the 11th week of pregnancy and 0.0843 between 12 and 13 weeks, lower than the values we previously reported (0.1275 and 0.1105 respectively).[2] The lower standard deviation of 0.0843 was used in estimating screening performance in singleton pregnancies.

Results

Table 1 shows median NT, free β-hCG and PAPP-A MoM values for all twin pregnancies and according to chorionicity in Down syndrome and unaffected pregnancies. The table shows anticipated results, for example that the median NT MoM values for affected twin pregnancies are approximately double those in unaffected pregnancies (1.95 MoM) and two unexpected results, both relating to the two serum markers. First, the median free β -hCG value in monochorionic affected twin pregnancies was lower than that based on the proportionality assumption (3.76 MoM [1]), with an estimate of 2.63 MoM (95% confidence interval [CI] 1.79-3.22 MoM), an observation not observed in dichorionic affected twin pregnancies; the median (2.60 MoM, 95% CI 2.23-3.30 MoM) was consistent with that based on the proportionality assumption (2.93 MoM [1). Second, the median PAPP-A value in dichorionic affected twin pregnancies was higher (1.88 MoM, 95% CI 1.60-1.27 MoM) than that based on the proportionality assumption (1.33 MoM [1), an observation not made in monochorionic affected twin pregnancies; the median (0.83 MoM) was similar to that based on the proportionality assumption (0.80 MoM).[1]

Figure 1 shows free β-hCG and PAPP-A MoM values in unaffected pregnancies according to gestational age, separately for monochorionic and dichorionic twin pregnancies. In monochorionic unaffected twin pregnancies there was no statistically significant change in free β -hCG MoM values, but in dichorionic unaffected twin pregnancies free β -hCG MoM values increased by 4.2% per week of gestation (95% CI 0.9% to 7.6%, p=0.003). In both monochorionic and dichorionic unaffected twin pregnancies there was a statistically significant increase in PAPP-A MoM values of 14.6% (95% CI 8.7% to 20.9%, p<0.001) and 2.8% (95% CI 0.1% to 5.5%, p=0.039) respectively. Table 2 shows the parameters used to estimate risks and screening performance.

Figure 2 shows free β-hCG and PAPP-A expressed in MoM values for unaffected twin pregnancies in affected pregnancies according to gestational age, separately for monochorionic and dichorionic twin pregnancies. There was no indication of an effect of gestation on either marker in either monochorionic or dichorionic affected twin pregnancies but the statistical power to show an effect is limited.

Figure 3 shows the detection rates for a 3% false-positive rate using NT alone with maternal age and the Combined test (which includes maternal age) in singleton pregnancies, monochorionic twin pregnancies and in dichorionic twin pregnancies. In singleton pregnancies the addition of the serum markers increased the detection rate by 10 percentage points (from 76% to 86%), by 13 percentage points in monochorionic twin pregnancies (from 74% to 87%) and by 4 percentage points in dichorionic twin pregnancies (from 70% to 74%). Table 3 shows estimates of screening performance for five specified false-positive rates and five specified detection rates. For example, the detection rate at a 3% false-positive rate was, respectively, 87% and 74% for monochorionic and dichorionic twin pregnancies. Table 4 shows detection rates and false-positive rates for a range of term risk cut-offs. For example at a 1 in 150 risk cut-off the screening performance is 77% for a 0.6% false-positive rate in monochorionic twin pregnancies and 81% for a 6.4% false-positive rate in dichorionic twin pregnancies. The detection rates and the false-positive rates at a given risk cut-off are higher in dichorionic twin pregnancies than in monochorionic twin pregnancies.

Table 5 shows the (rounded) risk cut-offs in singleton pregnancies, monochorionic, and dichorionic twin pregnancies that could be used to achieve the same false-positive rates. For example, at a 2% false-positive rate a risk cut-off of 1 in 150 could be used for singleton pregnancies, 1 in 500 for monochorionic twin pregnancies, and 1 in 60 for dichorionic twin pregnancies.

Discussion

Our results show that the assumption that the pattern of marker levels seen in affected and unaffected singleton pregnancies will be proportionately the same in twin pregnancies is not the case. The median PAPP-A value in dichorionic affected twin pregnancies was lower than in unaffected twin pregnancies (0.79 times lower, Table 2) but not as low as in affected singleton pregnancies compared with unaffected singleton pregnancies (0.50 at 12 weeks' gestation).[8] Also, the median free β-hCG level in affected twin pregnancies (monochorionic and dichorionic twin pregnancies combined) was higher than in unaffected twin pregnancies (1.43 times higher, Table 2) but not as high as in affected singleton pregnancies compared with unaffected singleton pregnancies (2.19 at 12 weeks' gestation).[8]

In monochorionic twin pregnancies screening performance is similar to that in singleton pregnancies. In dichorionic pregnancies the discrimination of PAPP-A and free β-hCG between affected and unaffected pregnancies is lower than that in singleton pregnancies. This means that screening performance in dichorionic twin pregnancies using the Combined test is less than has been previously estimated [7], but still improved over using nuchal translucency and maternal age alone. All monochorionic twins are monozygotic (identical) so averaging NT values in monochorionic twins is valid, however, about 10% of dichorionic pregnancies are monozygous [10] and in these cases not averaging the NT measurements will introduce some error in relation to how NT is used in the screening algorithm. This, however has a negligible effect on screening performance; among such pregnancies the detection rate of NT alone with age is only one percentage point lower than that for dichorionic dizygous twin pregnancies at a 3% false-positive rate, with no change in the detection rate of the Combined test .

Our results are in general consistent with those of Madsen et al.[11] The median PAPP-A levels in in monochorionic and dichorionic affected twin pregnancies reported by Madsen et al were, respectively, 0.49 (95% CI 0.32-0.75) and 0.66 (0.56-0.78) compared with monochorionic and dichorionic unaffected twin pregnancies; in our study they were 0.42 (0.33-0.62) and 0.79 (0.67-0.93). With respect to free β-hCG the corresponding values reported by Madsen et al are 0.85 (0.53-1.37) and 1.41 (1.18-1.69) (not statistically significantly different) and 1.32 combined compared with 1.43 (1.23-1.61) for all affected twins in our paper.

Table 4 shows that for a given risk cut-off screening performance differs significantly between monochorionic and dichorionic twin pregnancies. This is mainly due to Down syndrome pregnancies being 66% less common in monochorionic twin pregnancies than in singleton pregnancies, but 34% more common in dichorionic than in singleton pregnancies [6], which leads to a 4-fold difference in the background maternal age related risk between monochorionic and dichorionic twin pregnancies.

As an ultrasound scan is performed at 11-13 weeks' to measure crown-rump length and NT, the chorionicity status can also be determined, and if chorionicity status in twin pregnancies is known, consideration could be given to the use of different risk cut-off levels in monochorionic and dichorionic twin pregnancies from those in singleton pregnancies to achieve a similar screening performance (see Table 5).

In summary, our results indicate that it is still appropriate to use PAPP-A and free β-hCG as well as nuchal translucency in screening twin pregnancies, but appropriate revisions are needed to update the distribution parameters for the screening markers.

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Table 1: Median marker values (95% confidence intervals) in twin pregnancies expressed in singleton based median MoM values, together with median MoM values in affected twin pregnancies based on the proportionality assumption (square brackets)

	Down syndrome pregnancies				Unaffected pregnancies			
Marker	Monochorionic (n=12)	Dichorionic (n=49)	p	All twins (n=61)	Monochorionic (n=1490)	Dichorionic (n=5130)	p	All twins (n=7302)
NT								
Affected twin(s)	1.94 (1.22-2.16)	1.95 (1.62-2.36)*	0.654	1.95 (1.64-2.15)	-	-	-	-
Unaffected twin(s)	-	1.03 (0.96-1.08)**	-	-	0.98 (0.97-0.99)	1.00 (1.00-1.01)	<0.001	1.00 (0.99-1.00)
Free β-hCG	2.63 (1.79-3.22) [3.76]	2.60 (2.23-3.30) [2.93]	0.870	2.62 (2.45-3.13)	1.84 (1.78-1.90)	1.98 (1.94-2.01)	<0.001	1.94 (1.91-1.97)
PAPP-A	0.83 (0.65-1.27) [0.80]	1.88 (1.60-2.17) [1.33]	<0.001	1.62 (1.47-2.03)	2.03 (1.95-2.08)	2.37 (2.33-2.40)	<0.001	2.28 (2.26-2.32)

[1]The affected twin was not identified in 6 dichorionic pregnancies

[2]In one dichorionic pregnancy one twin was affected with Down syndrome and the other twin was affected with trisomy 13. The NT from the fetus with trisomy 13 was not included in calculation of the median NT

Table 2: Distribution parameters for the Combined test markers in Down syndrome and unaffected twin pregnancies expressed in MoM values for unaffected twin pregnancies (MoM_T)

	Down syndrome twin pregnancies			Unaffected twin pregnancies			
	Median MoM_T (95% CI)	Median MoM_T (log_{10})	SD (log_{10})	Median MoM_T (95% CI)	Median MoM_T (log_{10})	SD (log_{10})	Truncation limits
NT[1]							
Monochorionic	1.95 (1.67-2.20)	0.2900	0.2400	1.00 (0.99-1.00)	0	0.0868	0.90[3]-2.50
Dichorionic	1.95 (1.67-2.20) 1.00 (0.99-1.00)	0.2900 0	0.2400 0.0963	1.00 (0.99-1.00) 1.00 (0.99-1.00)	0 0	0.0963 0.0963	0.88[3]-2.50
Free β-hCG[2]	1.43 (1.23-1.61)	0.1553	0.2209	1.00 (0.98-1.02)	0	0.2375	0.30-4.00
PAPP-A							
Monochorionic	0.42 (0.33-0.62)	-0.3768	0.2995	1.00 (0.99-1.01)	0.00	0.2017	0.31[2]-1.00
Dichorionic	0.79 (0.67-0.93)	-0.1024	0.2036				0.50-3.00

[1]No statistically significant difference between affected fetuses from monochorionic and dichorionic twin pregnancies so overall median and standard deviation taken (see Table 1)

[2]No statistically significant difference between monochorionic and dichorionic twin pregnancies (see Table 1)

[3]Truncation limit set at point of risk reversal

The correlation between free β-hCG and PAPP-A log_{10} MoM_T values in unaffected twin pregnancies was 0.1790; the correlation coefficient between paired log_{10} NT MoM_T values in unaffected dichorinic twin pregnancies was 0.4585; all other correlation coefficients were not statistically significant and taken as zero

There were no statistically significant change in free β -hCG or PAPP-A MoM_T values with gestational age in affected twin pregnancies (p=0.545 and p=0.345 respectively) Similarly there was no change in NT MoM_T values in monochorionic or dichorionic affected twin pregnancies with gestational age (p=0.703 and p=0.701 respectively).

Table 3: Down syndrome screening performance of the Combined test, and nuchal translucency (NT) with maternal age in twin pregnancies compared with performance in singleton pregnancies (12 completed weeks); detection rate (DR) for specified false-positive rate (FPR) and FPR for specified DR.

	DR (%) for FPR of:-					FPR (%) for DR of:-				
Screening test	1%	2%	3%	4%	5%	70%	75%	80%	85%	90%
Combined test										
Singleton pregnancies	79	83	86	87	88	0.2	0.5	1.2	2.7	6.6
Twin pregnancies										
Monochorionic	81	85	87	89	90	0.2	0.4	0.9	2.1	5.2
Dichorionic	66	71	74	77	79	1.7	3.2	5.9	10.5	18.9
All*	69	74	77	79	81	1.4	2.6	4.9	8.8	16.2
NT + age										
Singleton pregnancies	69	73	76	77	79	1.1	2.8	5.8	11.1	21.2
Twin pregnancies										
Monochorionic	68	72	74	76	78	1.4	3.4	6.7	12.9	22.4
Dichorionic	63	67	70	72	74	2.9	5.6	9.3	15.3	26.1
All*	64	68	71	73	75	2.6	5.2	8.8	14.8	25.4

*Weighted average based on 20% of twins being monochorionic

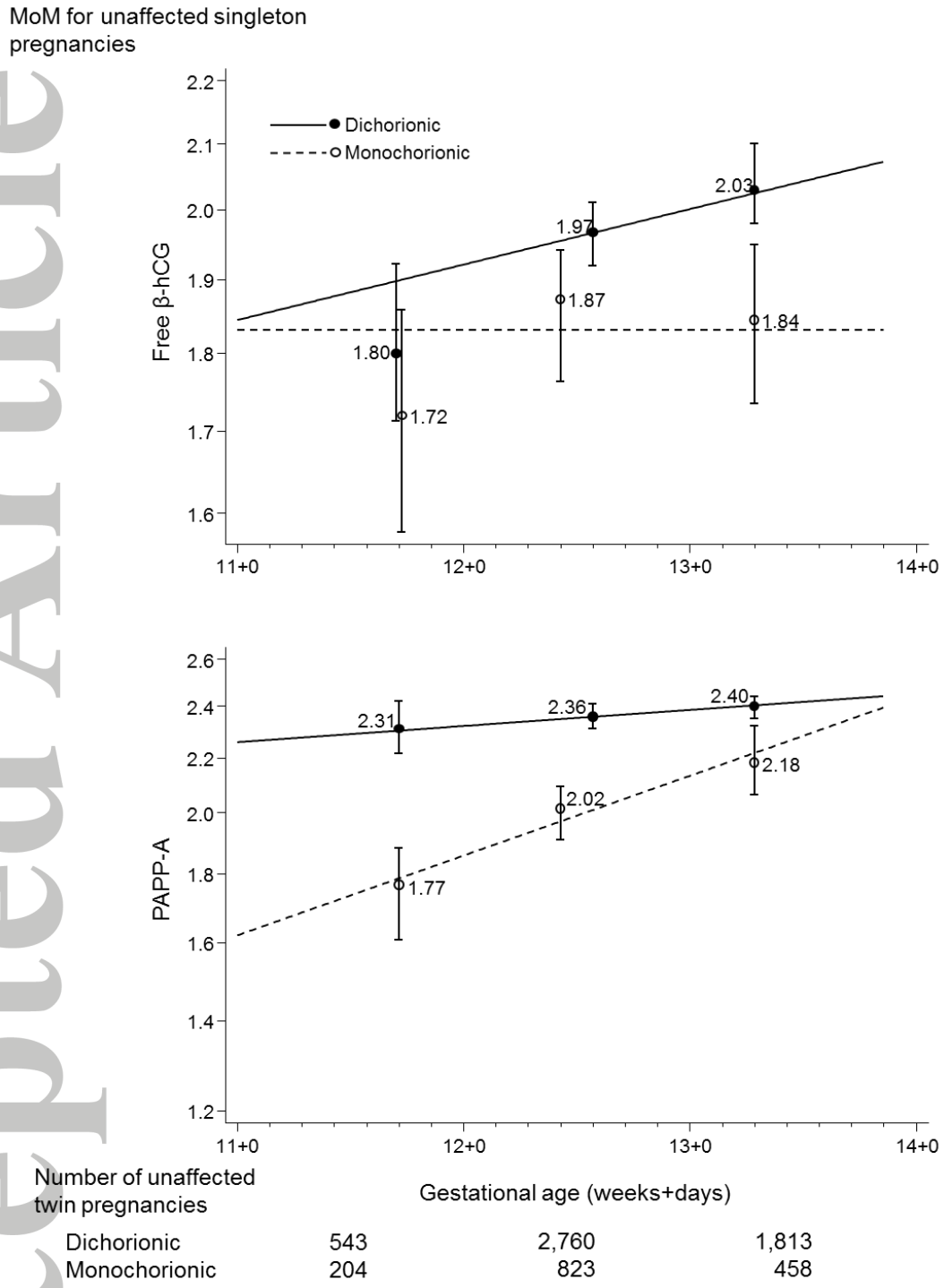
Table 4: Down syndrome screening performance of the Combined test, and nuchal translucency (NT) with maternal age in twin pregnancies compared with performance in singleton pregnancies (12 completed weeks); detection rate (DR) and false-positive rate (FPR) for specified term risk cut-offs.

	Risk cut-off													
	1 in 50		1 in 75		1 in 100		1 in 150		1 in 200		1 in 400		1 in 800	
	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)
Combined test														
Singleton pregnancies	76	0.6	79	1.0	81	1.3	83	1.9	85	2.6	88	4.9	92	9.0
Twin pregnancies														
Monochorionic	70	0.2	73	0.3	75	0.4	77	0.6	79	0.8	83	1.6	87	3.1
Dichorionic	69	1.4	73	2.6	77	3.9	81	6.4	84	9.0	90	18.6	95	34.4
All*	69	1.2	73	2.1	77	3.2	80	5.2	83	7.4	89	15.2	93	28.1
NT + age														
Singleton pregnancies	67	0.6	69	1.0	71	1.3	73	2.1	75	2.9	81	6.6	87	13.8
Twin pregnancies														
Monochorionic	60	0.2	62	0.3	63	0.4	65	0.6	67	0.9	71	1.9	77	4.3
Dichorionic	64	1.0	70	2.6	72	4.1	78	7.7	81	10.6	88	20.0	93	38.8
All*	63	0.8	68	2.1	70	3.4	75	6.3	78	8.7	85	16.4	90	31.9

*Weighted average based on 20% of twins being monochorionic

Table 5: Screening policy options; risk cut-off (term) according to false-positive rate

False-positive rate	Risk cut-off		
		Twin pregnancies	
	Singleton pregnancies	Monochorionic	Dichorionic
1%	1 in 80	1 in 250	1 in 40
2%	1 in 150	1 in 500	1 in 60
3%	1 in 250	1 in 750	1 in 80
4%	1 in 300	1 in 1000	1 in 100
5%	1 in 400	1 in 1300	1 in 120



Number of unaffected twin pregnancies			
Dichorionic	543	2,760	1,813
Monochorionic	204	823	458

Figure 1: Median free β-hCG and PAPP-A multiple of the median values for unaffected singleton pregnancies in unaffected twin pregnancies according to chorionicity and gestational age together with regression lines*

*Monochorionic unaffected twin pregnancies; slope of the regression line for free β-hCG not statistically significantly different from zero; horizontal line shows the overall median MoM of 1.83. Dichorionic unaffected twin pregnancies; median free β-hCG MoM $=10^{0.07044+0.00254\times\text{gestational age (days)}}$. Monochorionic unaffected twin pregnancies; median PAPP-A MoM $= 10^{-0.44274+0.00847\times\text{gestational age (days)}}$. Dichorionic unaffected twin pregnancies; median PAPP-A MoM $= 10^{0.22143+0.00171\times\text{gestational age (days)}}$.

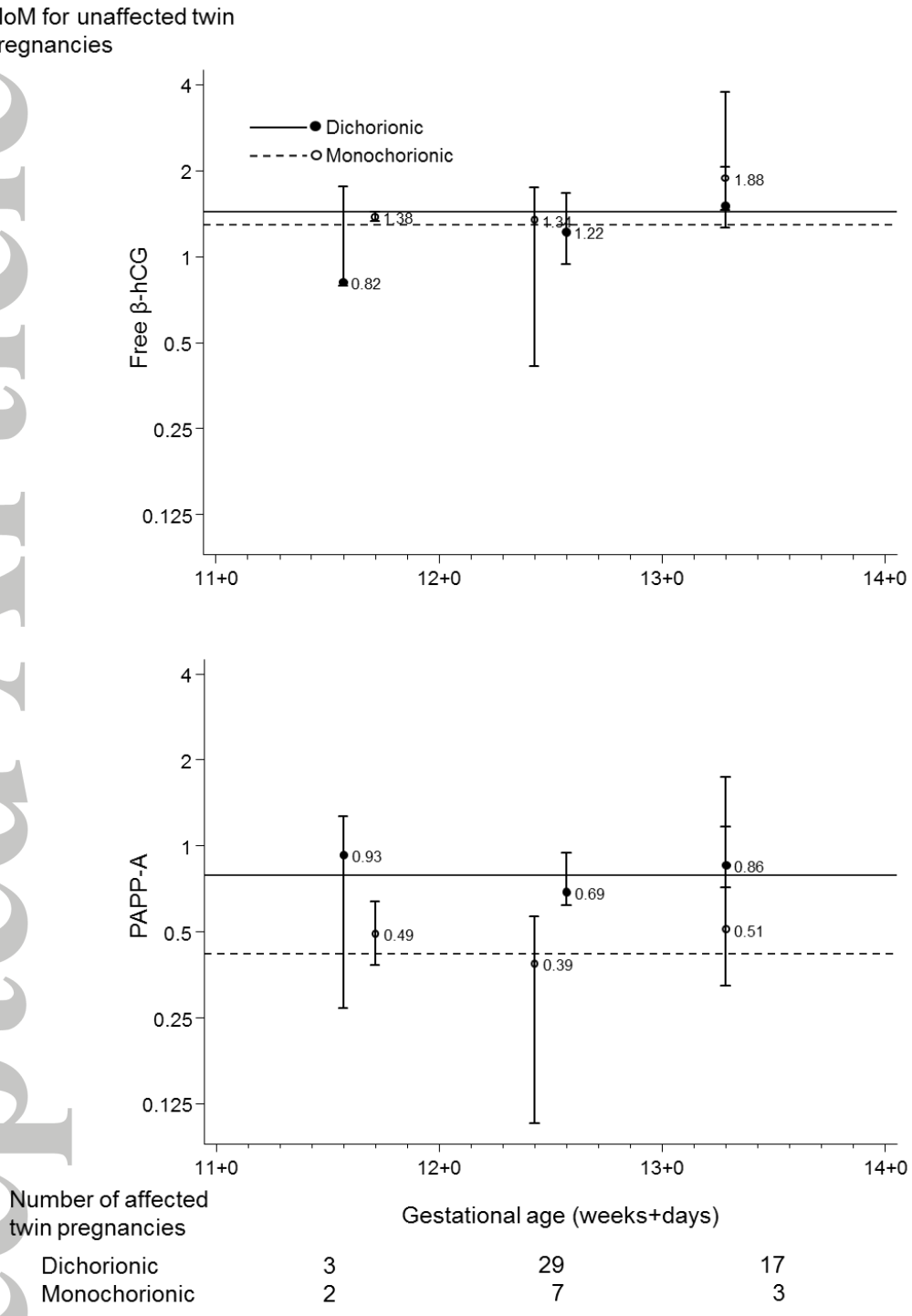


Figure 2: Median free β-hCG and PAPP-A multiple of the median values for unaffected twin pregnancies in affected twin pregnancies according to chorionicity and gestational age together with overall medians (horizontal lines)

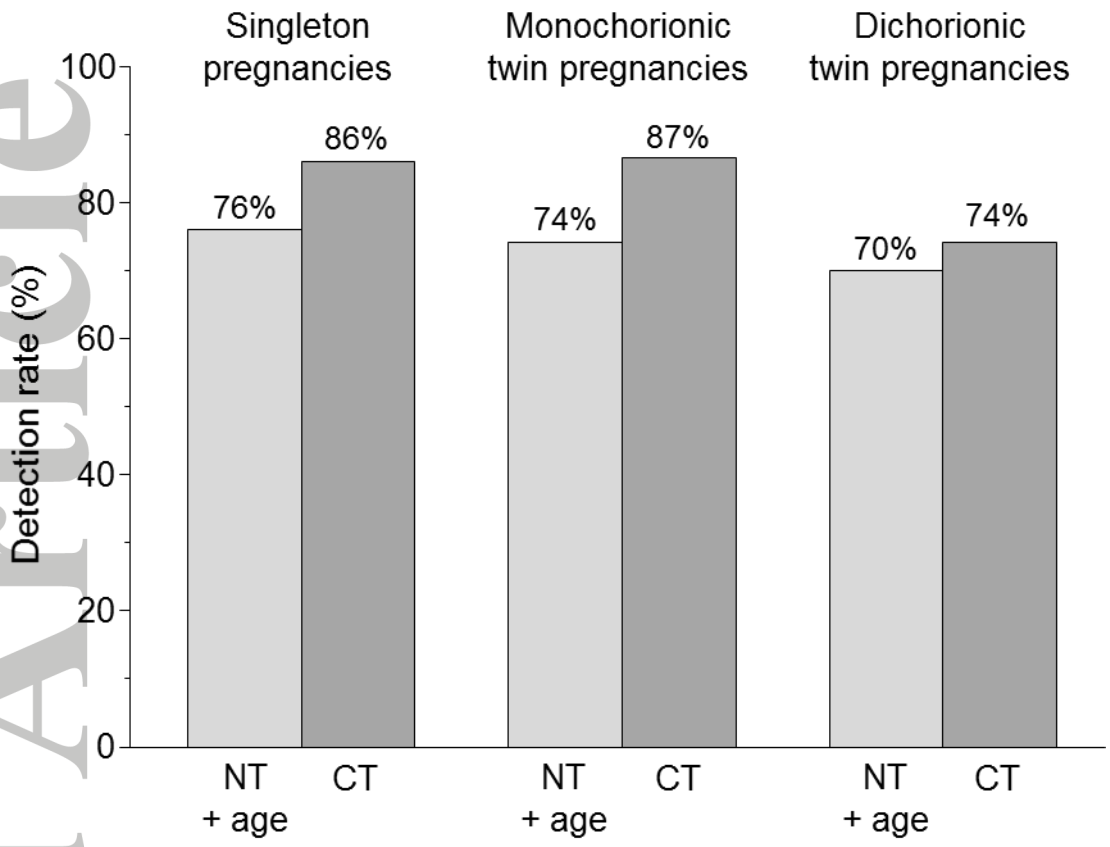


Figure 3: Down syndrome detection rates for a 3% false-positive rate using nuchal translucency (NT) with maternal age or the Combined test (CT), which includes maternal age, in singleton (12 completed weeks), monochorionic twin and dichorionic twin pregnancies