Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy

K Spencer[1], JA Crossley[2], DA Aitken[2], ABJ Nix[3], FDJ Dunstan[3] and K Williams[3]

Addresses
[1]Endocrine Unit
Clinical Biochemistry Department
Harold Wood Hospital
Gubbins Lane
Romford
Essex RM3 0BE, UK
[2]Duncan Guthrie Institute
of Medical Genetics
Yorkhill
Glasgow G3 8SJ
Scotland, UK
[3]Department of Medical Computing and Statistics
University of Wales College of Medicine
Heath Park
Cardiff CF14 4XN
Wales, UK

Correspondence
Dr Kevin Spencer
E-mail: KevinSpencer1@aol.com

Abstract

Background Many maternal serum markers show concentration changes in Down's syndrome pregnancies but the magnitude of the change in median marker levels varies with gestation. To date these changes have not been accurately specified.

Methods The trends in marker median levels between 6 and 20 weeks of gestation were examined for alphafetoprotein (AFP), free β human chorionic gonadotrophin (Fβ-hCG), total human chorionic gonadotrophin (ThCG) and pregnancy-associated plasma protein A (PAPP-A) by a meta-analysis of data obtained from our collaborative studies and routine screening programmes for Down's syndrome over a 10-year period. Data were available from between 709 and 1082 Down's syndrome pregnancies and from between 14 607 and 153 909 unaffected pregnancies for each marker. The median multiple of the median (MoM) and mean $\log_{10}$MoM for each marker at each completed week of gestation were estimated and the trend with gestation smoothed using a weighted least squares regression model.

Results The gestational ages at which maximum separation of marker levels occurred, comparing affected and unaffected pregnancies, and the respective regressed median MoMs and mean $\log_{10}$MoMs, were: for AFP at 16 weeks, 0·72 MoM, $-0{\cdot}14288$ $\log_{10}$MoM; for Fβ-hCG at 15 weeks, 2·24 MoM, 0·35034 $\log_{10}$MoM; for ThCG at 16 weeks, 1·93 MoM, 0·28548 $\log_{10}$MoM, as well as before 8 weeks ($<0{\cdot}65$ MoM, $-0{\cdot}18853$ $\log_{10}$MoM); and for PAPP-A before 8 weeks, $<0{\cdot}33$ MoM, $-0{\cdot}47727$ $\log_{10}$MoM.

Conclusion There is significant temporal variation in mean $\log_{10}$MoM values for the screening markers investigated. Screening algorithms, modified to take account of this variation, should allow more accurate gestation-specific risks to be calculated in individual pregnancies.

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Introduction

Maternal serum screening for trisomy 21 has, over the past decade, become an established part of obstetric practice in many countries. Double, triple and quadruple combinations of the markers alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), free β subunit of hCG (Fβ-hCG), unconjugated oestriol (UE3) and inhibin A (InhA) have been used in the second trimester, giving detection rates of around 65 to 75% with a 5% false positive rate.[1–4] The next major development in prenatal screening is already under way – a gradual move from second to first trimester screening in some centres, based on a combination of the ultrasound marker fetal nuchal translucency thickness (NT) and the maternal serum markers Fβ-hCG and pregnancy-associated plasma protein A (PAPP-A). It has been shown that a combination of these markers can identify 90% of trisomy 21 cases, with a 5% false positive rate[5,6] – a marked improvement in sensitivity over second trimester screening, which is maintained even when spontaneous fetal loss is taken into account.[7]

The principle of multiple marker risk assessment in screening for trisomy 21 was established by Wald and co-workers in 1988[8] and the statistical methodology has been explained in detail by Reynolds and Penney.[9] One of the key assumptions underlying such methodology is that the median marker value in the affected pregnancy group is a constant proportion of the median for the unaffected cases across the 14th to 20th week (second trimester) or the 9th to 13th week (first trimester) gestational windows. Over the last decade evidence has been accumulating that this is not the case, with many markers having optimum predictive performance at a specific stage of pregnancy.[10–13] This raises two questions: (a) are the population screening protocols presently used in the first and second trimesters optimized for the selected markers to ensure that detection and false positive rates are also optimized, and (b) do women receive accurate gestation-specific risks?

Since cases of trisomy 21 are a relatively rare event, even in major screening centres, it has not been possible for individual centres to quantify these marker changes. As a result of collaboration over many years, our group has accumulated sufficient data to attempt in this paper an analysis of marker trends in affected pregnancies across the first and second trimesters.

Methods

Data were available from our various published and unpublished studies and routine screening programmes for the maternal serum biochemical markers Fβ-hCG, total hCG (ThCG), AFP and PAPP-A, measured in unaffected and trisomy 21 pregnancies at various stages of gestation. The numbers of affected and unaffected pregnancies at each week of gestation for each marker are shown in Tables 1–4. The uneven distribution of sample numbers across the gestational range reflects the timing of attendance at the antenatal clinic by women seeking first or second trimester screening for Down's syndrome. All affected pregnancies were confirmed cytogenetically, either by analysis of fetal cells from amniocentesis or of chorionic villus biopsies, or by lymphocyte analysis following the birth of an affected infant. Affected pregnancies were identified from routine screening programmes where there was complete ascertainment of cases (both detected and missed by screening), or from non-interventional, prospective studies. Gestational age was estimated from ultrasound measurement of crown–rump length (CRL) or bi-parietal diameter (BPD) before 14 weeks or by the best available estimate of gestation at the time of screening after 14 weeks (mainly ultrasound dating or in a few cases from the date of the last menstrual period). Since some of the pregnancies at the beginning of the study period were dated only in completed weeks of pregnancy, all data were analysed on the basis of completed weeks of gestation.

A variety of analytical methods was used in our various screening studies and routine testing over a 10-year period. For Fβ-hCG these included, in the second trimester, the CIS (High Wycombe, UK) enzyme-linked immunosorbent assay (ELISA)[14,15] and the single label ELISA Fβ-hCG assay[11,16] or the dual label ELISA Fβ-hCG/AFP assay.[12,17] These three assays have been shown to have comparable performance.[16] In the first trimester Fβ-hCG was measured with the Kryptor[1] (Brahms, Germany) homogeneous time-resolved fluoroimmunoassay method,[5] the single label ELISA assay[11] or the Perkin Elmer Delfia[1] (Perkin Elmer Applied Biosystems, Foster CA, USA) assay. For AFP, the assays used were in-house methods based on immunoradiometric assays (IRMAs)[18] or polyethylene glycol (PEG)-assisted second antibody radio immunoassay (RIA).[19] Commercial AFP assays used included IRMAs (IDS, Boldon, UK), the dual label ELISA Fβ-hCG/AFP assay,[17] the Kryptor homogeneous time-resolved fluoroimmunoassay[20] and the Perkin Elmer Delfia dual label Fβ-hCG/AFP assay. Two methods were used for ThCG analysis, the MAIAClone assay (Serono, Welwyn Garden City, UK)[21] and the Kryptor ThCG assay.[20] For PAPP-A three methods were used, the Kryptor PAPP-A assay,[5] an ELISA PAPP-A assay[22] and the Perkin Elmer Delfia monoclonal-monoclonal PAPP-A assay.

When data are obtained using a variety of different methods over an extended period, consideration needs to be given to the comparability of results from different assays for the same marker before they can be combined. All analyte results were expressed as multiples of the median (MoM), the reference medians being the normal medians calculated by the respective laboratories at the time the analysis was performed. A check of the median MoM values in unaffected pregnancies for each individual assay method showed that all were close to 1·00, suggesting that reference medians were appropriate. Also, the mean of the $\log_{10}$MoM value in unaffected pregnancies at each individual week of gestation was calculated for the combined data for each marker to check for evidence of bias across the gestational range.

Results

Tables 1–4 summarize the biochemical data on the trisomy 21 and unaffected pregnancies included in this meta-analysis. Numbers are displayed, by week of gestation, for the biochemical markers listed above, together with the corresponding observed mean $\log_{10}$MoM values, the regressed $\log_{10}$MoM values and

Table 1. *Number of cases and log_{10} alphafetoprotein (AFP) multiple of the median (MoM) values for Down's syndrome and unaffected pregnancies between 6 and 20 weeks of gestation*

	Trisomy 21				Unaffected	
Gestation week	*n*	Log_{10}MoM	Regressed log_{10}MoM	Median MoM	*n*	Log_{10}MoM
6	5	−0·0691			12	−0·0073
7	12	−0·1033			73	−0·0401
8	13	−0·0548			85	−0·0453
9	7	−0·0249			85	0·0039
10	15	−0·0797	−0·03403	0·925	86	0·0118
11	45	−0·0567	−0·06600	0·859	82	0·0326
12	106	−0·0884	−0·09244	0·808	77	−0·0450
13	68	−0·1119	−0·11335	0·770	77	−0·0078
14	38	−0·1068	−0·12872	0·743	17 831	−0·0028
15	210	−0·1404	−0·13857	0·727	57 407	0·0014
16	316	−0·1472	−0·14288	0·720	52 666	0·0023
17	159	−0·1367	−0·14166	0·722	16 271	0·0043
18	52	−0·1547	−0·13491	0·733	5486	0·0044
19	15	−0·0509	−0·12263	0·754	2342	0·0028
20	9	−0·1244	−0·10482	0·786	1329	−0·0036
Total	1070	−0·1261			153 909	0·0016

The regressed log_{10} AFP MoM and derived median AFP MoM values are shown for the range of gestations (10- 20 weeks) for which the quadratic model is an adequate fit (*see* Fig. 2).

Table 2. *Number of cases and log_{10} free β human chorionic gonadotrophin (Fβ-hCG) multiple of the median (MoM) values for Down's syndrome and unaffected pregnancies between 6 and 20 weeks of gestation*

	Trisomy 21				Unaffected	
Gestation week	*n*	Log_{10}MoM	Regressed log_{10}MoM	Median MoM	*n*	Log_{10}MoM
6	7	0·0469	−0·01445	0·967	12	−0·0167
7	19	0·1604	0·06298	1·156	64	0·0241
8	28	0·0387	0·13119	1·353	62	−0·0151
9	17	0·2275	0·19017	1·549	250	0·0065
10	50	0·2549	0·23993	1·738	1183	−0·0058
11	117	0·2586	0·28046	1·907	3741	0·0126
12	187	0·3054	0·31177	2·050	5261	0·0154
13	126	0·3203	0·33385	2·157	2500	0·0003
14	46	0·4167	0·34671	2·222	18 776	0·0537
15	150	0·3890	0·35034	2·240	39 325	0·0242
16	193	0·3176	0·34475	2·212	28 357	0·0135
17	97	0·3543	0·32994	2·138	7952	0·0022
18	29	0·2410	0·30589	2·023	2801	0·0197
19	12	0·3009	0·27263	1·873	1311	0·0372
20	4	0·1761	0·23014	1·699	820	0·0519
Total	1082	0·3080			112 415	0·0234

The regressed log_{10} Fβ-hCG MoM and derived median Fβ-hCG MoM values are shown for the range of gestations (6- 20 weeks) for which the quadratic model is an adequate fit (*see* Fig. 3).

the corresponding median MoM. The mean log_{10}MoM values for the unaffected cases are clearly close to zero for each marker, indicating that appropriate reference medians were used and that there was little evidence of assay bias across the gestational range. The overall and within-gestational group log_{10} standard deviations (SD) for each analyte MoM are presented in Table 5.

Table 3. *Number of cases and log_{10} total human chorionic gonadotrophin (ThCG) multiple of the median (MoM) values for Down's syndrome and unaffected pregnancies between 6 and 20 weeks of gestation*

	Trisomy 21				Unaffected	
Gestation week	*n*	Log_{10}MoM	Regressed log_{10}MoM	Median MoM	*n*	Log_{10}MoM
6	4	−0·3450	−0·44035	0·363	10	−0·1025
7	9	−0·3389	−0·30777	0·492	71	−0·0001
8	9	−0·2140	−0·18853	0·648	88	0·0073
9	3	0·1343	−0·08262	0·827	90	−0·0137
10	15	0·0316	0·00996	1·023	127	−0·0079
11	68	0·0610	0·08920	1·228	417	−0·0910
12	148	0·1484	0·15512	1·429	417	−0·0013
13	73	0·2267	0·20771	1·613	161	−0·0239
14	14	0·3583	0·24696	1·766	65	−0·0233
15	98	0·2724	0·27288	1·874	18 165	0·0167
16	149	0·2790	0·28548	1·930	24 338	−0·0014
17	76	0·2989	0·28474	1·926	8328	−0·0116
18	39	0·2461	0·27067	1·865	2705	−0·0030
19	1	−0·2366	0·23713	1·726	1037	−0·0322
20	3	0·3644	0·20253	1·594	518	−0·0159
Total	709	0·2026			56 537	0·0004

The regressed log_{10} ThCG MoM and derived median ThCG MoM values are shown for the range of gestations (6-20 weeks) for which the quadratic model is an adequate fit (*see* Fig. 4).

Table 4. *Number of cases and log_{10} pregnancy-associated plasma protein A (PAPP-A) multiple of the median (MoM) values for Down's syndrome and unaffected pregnancies between 7 and 20 weeks of gestation*

	Trisomy 21				Unaffected	
Gestation week	*n*	Log_{10}MoM	Regressed log_{10}MoM	Median MoM	*n*	Log_{10}MoM
7	12	−0·3582			23	−0·0974
8	22	−0·4904	−0·47727	0·333	24	0·0748
9	21	−0·4384	−0·42710	0·374	216	0·0300
10	47	−0·3360	−0·37515	0·422	1140	−0·0012
11	115	−0·3269	−0·32141	0·477	3696	−0·0210
12	192	−0·2785	−0·26588	0·542	5223	−0·0171
13	114	−0·1883	−0·20856	0·619	2459	−0·0073
14	29	−0·1691	−0·14946	0·709	1100	−0·0102
15	71	−0·0579			164	−0·0020
16	68	−0·0608			145	−0·0089
17	25	−0·0724			124	−0·0153
18	5	−0·1370			137	−0·0088
19	3	−0·3555			155	0·0033
20					1	0·3820
Total	724	−0·2325			14 607	−0·0134

The regressed log_{10} PAPP-A MoM and derived median PAPP-A MoM values are shown for the range of gestations (8-14 weeks) for which the quadratic model is an adequate fit (*see* Fig. 6).

The mean log_{10}MoM values for the affected cases, together with their corresponding 95% confidence intervals, are shown in Figs 1, 2, 3 and 4. For much of the gestational age range considered, the confidence intervals are seen not to include zero, confirming the usefulness of each marker to discriminate between unaffected and affected fetuses. This is, of course, well known and lies at the heart of the screening algorithm

Table 5. *Within-group and overall log_{10} standard deviations (in multiples of the median) for each analyte in Down's syndrome and unaffected pregnancies*

	Trisomy 21		Unaffected	
	Within-group	Overall	Within-group	Overall
AFP	0·1718	0·1732	0·1601	0·1601
Fβ-hCG	0·2787	0·2858	0·2613	0·2618
ThCG	0·2238	0·2526	0·2174	0·2179
PAPP-A	0·2822	0·3027	0·2361	0·2362

AFP = alphafetoprotein; Fβ-hCG = free β human chorionic gonadotrophin; ThCG = total human chorionic gonadotrophin; PAPP-A = pregnancy-associated plasma protein A.

Table 6. *Coefficients in the quadratic regression models for the mean log_{10} multiple of the median values for trisomy 21*

	Coefficients in the quadratic regression model		
Marker	Constant (*a*)	Linear (*b*)	Quadratic (*c*)
AFP	0·590	−0·0901	0·002766
Free β-hCG	−0·673	0·137	−0·00461
PAPP-A	−0·814	0·0350	0·0008935
ThCG	−1·516	0·219	−0·00667

for Down's syndrome. However, current screening models assume these differences are not dependent on gestational age. The temporal patterns shown in Figs 1, 2, 3 and 4 clearly indicate that this assumption is highly questionable. An analysis of variance performed on the log_{10}MoM values for affected cases produced P values that were, to three decimal places, 0·006, <0·001, <0·001 and <0·001 for AFP, free β-hCG, PAPP-A and ThCG, respectively, confirming a significant departure from a null hypothesis of constancy of mean values. This leads to the conclusion that screening algorithms should be modified to account for this temporal variation in mean log_{10}MoM values if more accurate patient-specific risks are to be produced. It is also clear from the figures that the change in mean levels is non-linear. As a result we have fitted quadratic smoothers (weighted least squares regressions) over ranges where this model seemed adequate. These smoothed mean log_{10}MoM values are shown in Figs 2, 3, 5 and 6, and appear to fit the data well over the selected gestational age range. The models can be written in generic form as:

$$\text{Mean } \log_{10}(\text{MoM})\ \text{T21} = a + b \times (\text{gestation in weeks}) + c \times (\text{gestation in weeks})^2$$

The coefficients a, b and c are shown in Table 6 for each of the markers considered, over the gestational age range stated. Although the true relationship to describe the temporal pattern for each marker is unknown, it is reasonable to assume that any changes in marker concentration will be smooth, as they are a result of the biological process of fetal growth and the suggested quadratic model appears to be an adequate descriptor of this process. Non-constancy of the mean log_{10}MoM values also raises questions about the optimal time at which screening should take place for each marker. This issue is discussed below for each marker.

AFP

From Figs 1 and 2 it can be seen that there is only limited ability for the AFP assay to discriminate between affected and unaffected fetuses for gestational ages below 10 weeks. There is maximum

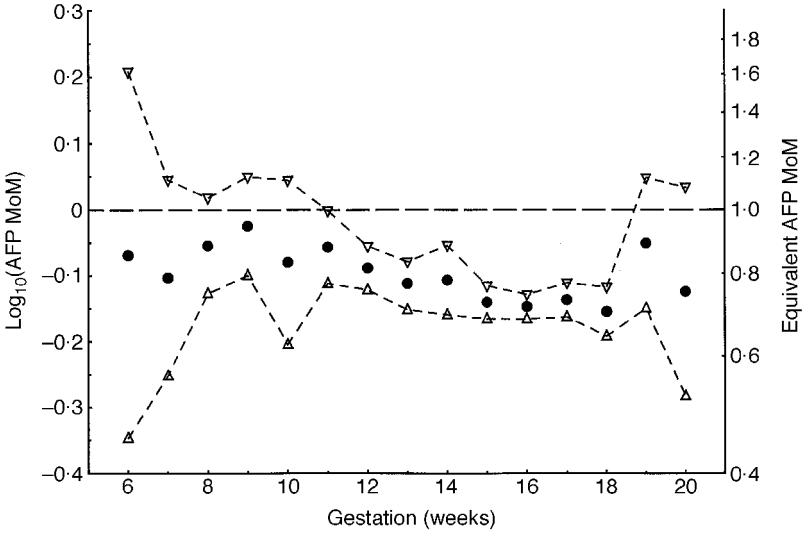

Figure 1. *Mean log_{10}(alphafetoprotein multiple of the median) [log_{10}(AFP MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age.*

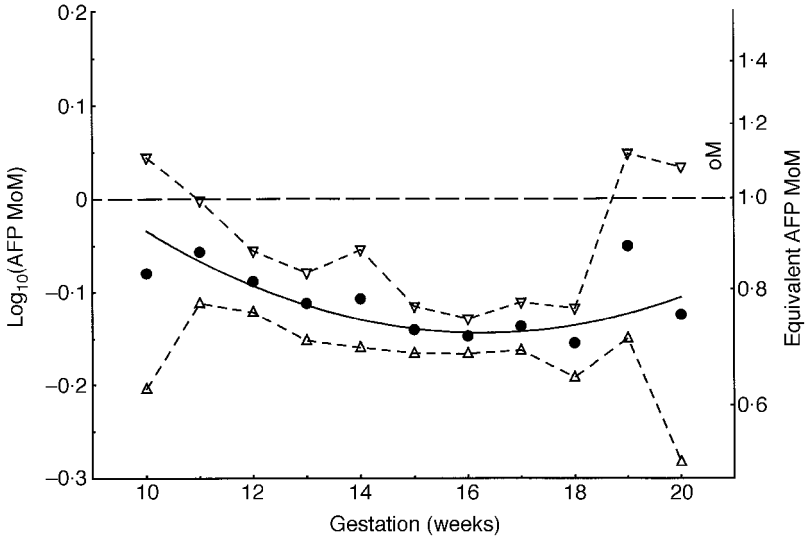

Figure 2. *Quadratic fit (Ð) for mean log_{10}(alphafetoprotein multiple of the median) [log_{10}(AFP MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age between 10 and 20 weeks of gestation.*

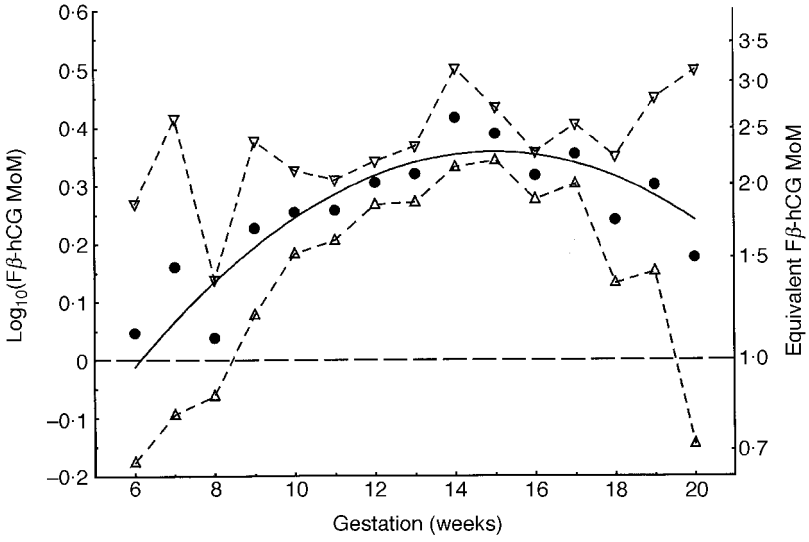

Figure 3. *Quadratic fit (Ð) for mean log_{10}(free β human chorionic gonadotrophin multiple of the median) [log_{10}(Fβ-hCG MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age between 6 and 20 weeks of gestation.*

separation at approximately 16 weeks, suggesting that optimum efficiency is achieved at that gestation.

Fβ-hCG

From Fig. 3 it can be seen that, as with AFP, there is limited ability for Fβ-hCG assay to discriminate between unaffected and affected fetuses for gestational ages below 10 weeks. Although Fβ-hCG can be regarded as a viable marker within the 10–20 week gestational window, maximum separation and hence optimum efficiency is achieved when screening is performed at 15 weeks gestation.

Total hCG

Figure 4 shows a very different pattern for the mean log_{10}MoM values for the ThCG than do Figs 1 and 2 for AFP and Fβ-hCG, respectively. For AFP and Fβ-hCG a single gestational age range can be defined for each marker that seems appropriate for screening. However, Fig. 4 clearly indicates the possibility of screening at very early gestational ages with ThCG, for example, less than 8 weeks. For second trimester screening, optimum efficiency is achieved at about 16 weeks. The data seem to suggest that for gestational ages of between 10 and 12 weeks ThCG values for both the unaffected and affected pregnancies are similar, making the marker ineffective for Down's screening at that time.

PAPP-A

The trend for PAPP-A differs markedly from those of the three markers already discussed. As can be seen

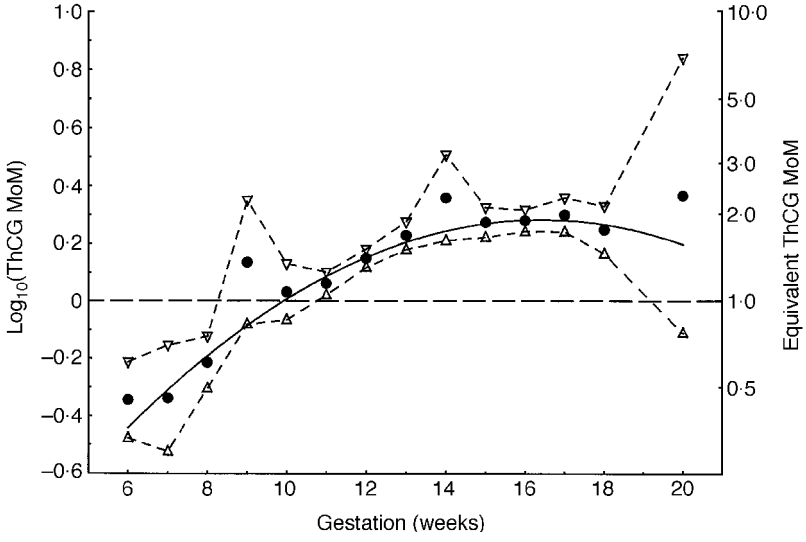

Figure 4. *Quadratic fit (Ð) for mean log_{10}(total human chorionic gonadotrophin multiple of the median) [log_{10}(ThCG MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age between 6 and 20 weeks of gestation.*

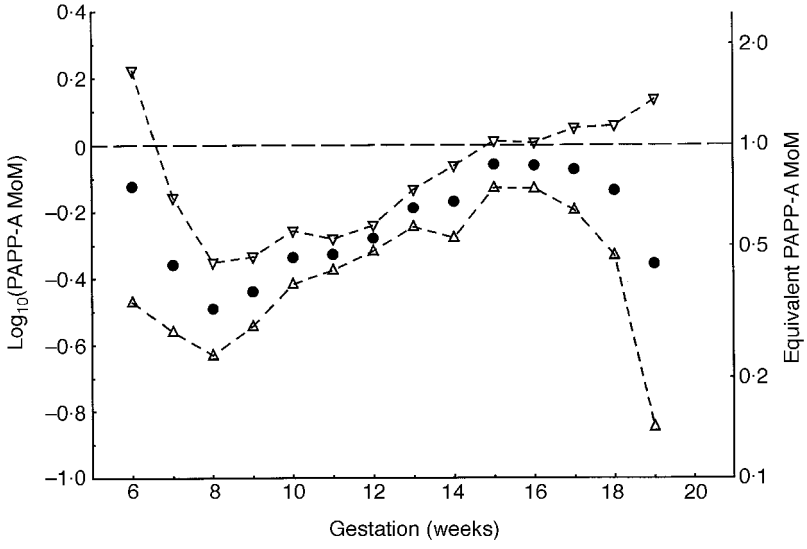

Figure 5. *Mean log_{10}(pregnancy-associated plasma protein A multiple of the median) [log_{10}(PAPP-A MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age.*

from Figs 5 and 6, the trisomy 21 means at each gestation seem to follow a cubic profile, although it should be remembered that the data are limited at both early and late gestational ages, as indicated by the substantial widths of the 95% confidence intervals for these gestations. From a practical point of view it would seem that PAPP-A measurements are not useful indicators of Down's syndrome pregnancies in the second trimester and a quadratic profile (Fig. 6) adequately describes the data across the gestational range for which PAPP-A is most effective. Maximum separation, and hence optimum detection efficiency, is achieved very early in pregnancy, at about 8 weeks. Useful but declining discrimination is available up to the end of the first trimester.

Discussion

The phenomenon of non-constancy of median MoM for Down's syndrome screening markers across a wide gestational range has been recognized for some time. In a preliminary report[10] and in a subsequent extended report[23] it was suggested that serum concentrations of AFP based on a series of 51 cases of trisomy 21-affected pregnancies were most predictive between 16·5 and 17·5 weeks of gestation, since this was the point of lowest median AFP MoM. Either side of this window it was suggested that the levels became less predictive with decreasing or increasing gestation. However, the number of cases was small and the statistical analysis did not show any changes to be

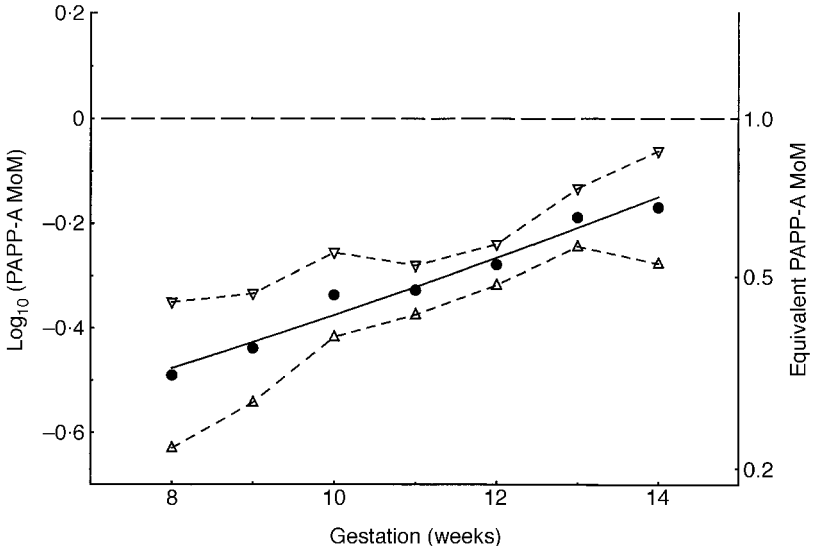

Figure 6. *Quadratic fit (Ð) for mean log_{10}(pregnancy-associated plasma protein A multiple of the median) [log_{10}(PAPP-A MoM)] values (●) together with corresponding lower (△) and upper (▽) 95% confidence limits for each gestational age between 8 and 14 weeks of gestation.*

significant. Waller and co-workers[24] analysed data from the California Maternal Serum AFP Screening Program and found that the lowest maternal serum AFP MoM values occurred between the 16th and 17th week of gestation, although data from Wald and Cuckle[25] suggested that a trend towards higher median AFP MoM levels did not occur until after the 19th week of gestation. Mancini and co-workers[26] examined the AFP and ThCG MoMs in samples collected between the 14th and 16th week and compared them with the results obtained in samples collected from the same patients in the 16th to 20th week. The study group consisted of 12 cases of trisomy 21, 61 unaffected pregnancies with an increased risk for trisomy 21 and 79 unaffected pregnancies not at increased risk for trisomy 21. No significant difference was found for AFP but a highly significant increase in ThCG MoM was observed later in gestation.

Macri and co-workers[11] found a Fβ-hCG median of 2·13 MoM in 15 trisomy 21 pregnancies at 14 to 16 weeks of gestation compared with 1·83 MoM in 14 pregnancies at 17 to 18 weeks of gestation. This difference resulted in a detection rate of 80% in early gestation compared with 64% at the later stage. This greater Fβ-hCG median MoM earlier in the second trimester was confirmed in a study comparing marker levels in pregnancies with trisomy 21 at 16 weeks with those at 17 weeks. The median AFP level was 0·82 MoM at 16 weeks and 0·76 MoM at 17 weeks and the corresponding values for Fβ-hCG were 2·32 MoM and 2·05 MoM.[15] In a much larger series of 90 cases it was shown[27] that detection rates at 14 to 16 weeks were greater (at 77%) than later in gestation (54%) when the combination AFP and Fβ-hCG was used, and that a similar gestation-related difference was apparent when Fβ-hCG was substituted by ThCG (61% versus 46%). This difference in detection rate was shown[28] to be due to a higher Fβ-hCG median MoM at the earlier gestation (2·52 versus 2·32) whilst the AFP median MoMs were not significantly different (0·66 versus 0·73) and the groups had similar maternal age distributions. In another series of 105 cases detection rates of 71% were found in pregnancies at 14 to 16 weeks compared with 54% in pregnancies after 16 weeks.[12] This was again as a result of the median Fβ-hCG being higher in earlier gestation (2·71 versus 2·30), whilst AFP was not different (0·79 versus 0·81). In seven years prospective practice rather than in retrospective studies of stored serum it has been shown[3] that detection rates at the 14 to 16-week period are higher than those found later in gestation when the marker pair AFP and Fβ-hCG is used (79% versus 59%).

Other evidence for a gestation-related difference in the magnitude of serum marker changes in trisomy 21 pregnancies was provided by Berry and co-workers.[13] In this study, two serum samples were obtained from each of 47 trisomy 21 pregnancies, one in the first trimester (between 7 and 14 weeks gestation) and a second sample between 15 and 18 weeks gestation. Comparison of AFP, Fβ-hCG and PAPP-A median levels between each sample series at each stage of pregnancy showed that there was a smaller median shift in the first trimester trisomy 21 pregnancies for AFP (0·80 MoM versus 0·72 MoM) and Fβ-hCG (1·99 MoM versus 2·79 MoM), but a larger median shift for PAPP-A (0·50 MoM versus 0·94 MoM). The lowest PAPP-A values were found early in the first trimester and it was noted that an overall median MoM value for PAPP-A in the first trimester would be influenced by the gestation time at which the samples were collected.

Evidence of a temporal shift for both Fβ-hCG and PAPP-A median MoMs has also been reported[5] across the narrow gestational window of 10 to 14 weeks, and a further analysis has demonstrated temporal changes in PAPP-A median MoMs across the first and second trimester.[29] Similarly, for ThCG in the first trimester, median MoMs in cases of trisomy 21 increased progressively across this period.[20]

The above studies have increasingly raised doubts over the assumption that there is a constant shift in the mean $\log_{10}$MoM marker values in pregnancies with affected fetuses across the first half of pregnancy, and it has been suggested that risk algorithms used in the first and second trimesters need to be specifically optimized to take this change into account.[30] The analyses performed in this paper confirm these doubts and provide regression curves that model the temporal dependence in the mean $\log_{10}$MoM marker values. Our results also indicate that there is likely to be an optimal gestation window for screening purposes for each of the markers considered.

In addition to the use of the appropriate gestation-specific medians, algorithms for the estimation of the risk of a Down's syndrome pregnancy also use the SD of the marker distributions and correlation coefficients between markers. The above data show that it is not appropriate to combine MoM values for affected fetuses over a range of gestations to derive an SD since mixing MoM values from distributions with different mean values can significantly inflate the estimate of the SD (*see* Table 6).

Further analyses are in progress to assess the impact of the temporal shift in marker levels described above on the estimation of patient-specific risks and detection rates for Down's syndrome.

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