Chapter 4 First trimester maternal serum markers for trisomy 18

4.1 Introduction

The first reports for maternal serum markers for Down syndrome were made in the early 1980s. This was quickly followed by a large number of reports confirming the findings in the second trimester. However, there are fewer earlier reports for first trimester markers for two reasons. Second trimester screening was already established for neural tube defects at that time, so sample banks were readily available to document performance of Down syndrome markers. In addition, diagnostic testing in the first trimester (chorionic villus sampling - CVS) was less commonly performed, and this led to fewer cases of aneuploidy being identified that could be used in research. In contrast to methods used to remove smaller studies from analysis for second trimester markers (Chapter 3), attempts were made to be as all inclusive as possible when examining first trimester serum markers. Mainly, this was due to the smaller numbers of useable studies that, in general, had smaller numbers of cases examined. Another clear finding was that the less common aneuploidies (*e.g.*, trisomy 18 and trisomy 13) were far more prevalent in the first trimester than in the second trimester, or at term. In Chapters 1 through 3, trisomy 18 was the sole focus. However, because trisomy 13 (Patau syndrome) is more common in the first trimester and because both trisomy 18 and 13 are sometimes included together in relevant publications on serum and ultrasound markers, readily available information about those marker levels in trisomy 13 pregnancies will also summarized at the end of the chapter. Rather than creating a separate algorithm for trisomy 13, it may be possible to model the proportion of these fetuses that might be identified as part of a risk algorithm for trisomy 18.

Literature search

A PubMed search was performed (through 2009) using search terms “trisomy 18”, “first trimester” and “serum”. Identified abstracts were reviewed and selected articles retrieved. Reference lists of identified articles were also searched for relevant articles. As before, many articles were aimed at Down syndrome screening and only tangentially referred to trisomy 18 (or trisomy 13). The included articles were then stratified into two groups: those in which the serum marker of interest was not used to assign risk for decision-making about diagnostic testing and/or other follow-up (*e.g.*, assessment at birth), and the remaining articles. This first group (32 articles) might be considered ‘unbiased’, as they would likely represent the marker distribution in an unselected group

of affected pregnancies (Aitken *et al.*, 1993; Akolekar *et al.*, 2010; Bersinger *et al.*, 1994; Biagiotti *et al.*, 1998; Bogart *et al.*, 1989; Brambati *et al.*, 1994; Brizot *et al.*, 1994; Brizot *et al.*, 1995; Jauniaux *et al.*, 1996; Johnson *et al.*, 1991; Koster *et al.*, 2010; Koster *et al.*, 2009; Kratzer *et al.*, 1991; MacIntosh *et al.*, 1993; Miell *et al.*, 1997; Ozturk *et al.*, 1990; Palomaki, 2004; Poon *et al.*, 2009; Scott *et al.*, 1996; Sifakis, 2010; Spencer *et al.*, 1992; Spencer *et al.*, 1997; Spencer *et al.*, 2000b; Spencer *et al.*, 2000a; Spencer *et al.*, 2001b; Spencer *et al.*, 2001a; Spencer *et al.*, 2007; Lambert-Messerlian, 2004b). One additional study (Spencer *et al.*, 1994) appears to duplicate data from another, more complete report (Aitken *et al.*, 1993) and was not included.

The second group of 20 or more articles was generally composed of demonstration studies and is not considered in this section. They will be summarized in Chapter 6 where first trimester intervention trials relying on both biochemistry and ultrasound measurements are reviewed. These could be viewed as reporting 'biased' estimates of biochemistry distributions. True positive results (pregnancies with abnormal marker levels) will be identified early, when the prevalence is higher. In contrast, false negative results (pregnancies with more normal marker levels) will not usually be identified until birth, after many of the affected pregnancies have been spontaneously lost. This "bias of ascertainment" is well described for Down syndrome screening and is even more important when screening for trisomy 18 (or trisomy 13), due to the higher rate of fetal loss. Figure 4.1-1 provides a schematic representation of this bias in a hypothetical cohort of 100 first trimester trisomy 18 pregnancies.

Statistical methods are similar to those utilized in Chapter 4, including summary medians and rates determined using random effects modeling, and examining heterogeneity using the Q-statistic and I^2. Summary ROC curves were computed using public domain software (MetaDisc available at www.hrc.es/investigacion/metadisc.html). Smaller studies that provided individual patient results were grouped together and fitted to a probability plot to estimate a representative log mean and log standard deviation for these data.

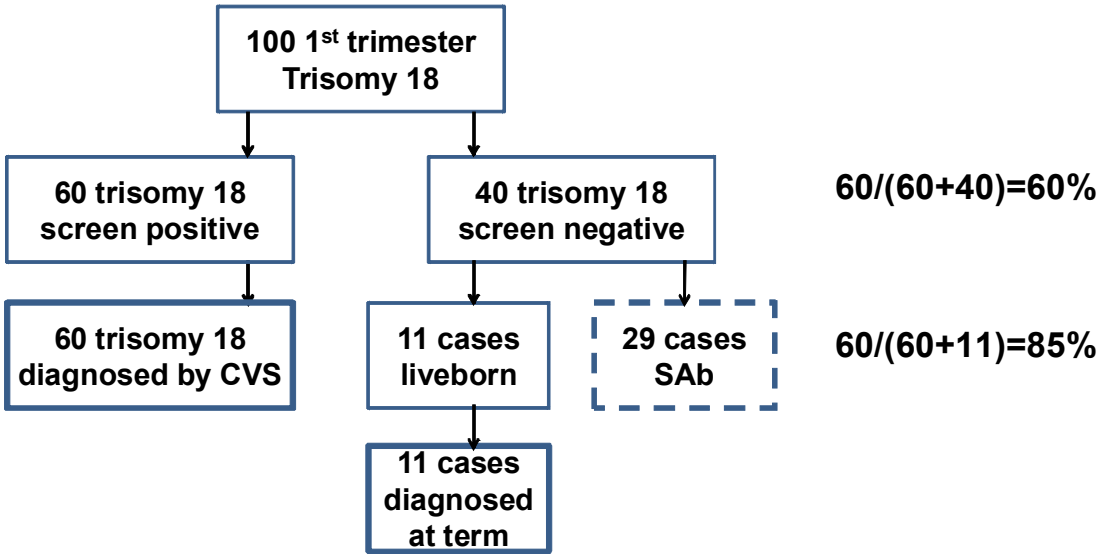

Figure 4.1-1. Schematic description of the 'ascertainment bias' present in demonstration studies. Initially, 100 cases of trisomy 18 are known to be present in a population of women undergoing a first trimester screening test. Assume that the test actually detects 60% of the cases (60 true positives in bold outline) and, therefore, misses 40% of the cases. The fetal loss rate for trisomy 18 from the late first trimester to term is 72% (Morris and Savva, 2008) and is assumed, for now, to be independent of the test results. Thus, of the 40 false negative cases, only 11 can be expected to survive and be diagnosed at term (bold outline). However, the 29 false negative cases will not be identified, as they are spontaneously lost (dashed bold outline). This leads to an overestimated detection rate of 85%, if the bias is not accounted for. If, for example, the screening test were simply "How old are you?" with a cut-off of 35 years of age, then all 60 true positive results would be in women age 35 and older, while the 40 false negative results would all occur in women under age 35. However, as part of an demonstration study, only the 11 cases diagnosed at term would be counted, and it would appear as though 85% of women with trisomy 18 fetuses are age 35 or older, rather than the correct estimate of 60%. This same phenomenon can occur with other markers (*e.g.*, biochemical, ultrasound). To obtain a correct distribution of results for the population (100 cases in this example), it is important to choose study designs that do not suffer from the bias of ascertainment described here, or account for it mathematically.

Characteristics of follow-up for included studies

The group of 32 'unbiased' studies of first trimester serum markers and trisomy 18 were then assigned to one of three categories, depending on study design and ascertainment methods.

- complete ascertainment – in 19 studies all cases were identified, regardless of the analyte level (Aitken *et al.*, 1993; Bersinger *et al.*, 1994; Biagiotti *et al.*, 1998; Bogart *et al.*, 1989; Brambati *et al.*, 1994; Brizot *et al.*, 1994; Brizot *et al.*, 1995; Jauniaux *et al.*, 1996; Johnson *et al.*, 1991; Kratzer *et al.*, 1991; MacIntosh *et al.*, 1993; Ozturk *et al.*, 1990; Palomaki, 2004; Scott *et al.*, 1996; Spencer *et al.*, 1992; Spencer *et al.*, 2000a; Van Lith, 1992; Zaragoza *et al.*, 2009; Zimmermann *et al.*, 1996). For example, serum samples were collected routinely from women of advanced maternal age prior to a CVS. Although this group has a higher rate of trisomy 18 due to age, there is no evidence suggesting that phenotypic presentation is dependent on the mother's age.
- Screened, but not correlated – in 12 studies some form of phenotypic screening has occurred, but the marker used is known to be uncorrelated (or have low correlation) with the marker of interest (Akolekar *et al.*, 2010; Koster *et al.*, 2010; Koster *et al.*, 2009; Miell *et al.*, 1997; Poon *et al.*, 2009; Sifakis, 2010; Spencer *et al.*, 1997; Spencer *et al.*, 2000b; Spencer *et al.*, 2001b; Spencer *et al.*, 2001a; Spencer *et al.*, 2007; Tul *et al.*, 1999). For example, serum samples were collected from women screen positive due to a combination of maternal age and NT measurements. NT measurements have been shown to be independent of biochemistry for Down syndrome and other aneuploidies. Another example would be serum samples from a combined testing program (NT, PAPP-A and free β) that examined the performance of ADAM-12, and found low correlations with the other markers.
- Screened, but follow-up occurs later – in one study (Lambert-Messerlian, 2004b) first trimester screening results were not acted upon until the second trimester. At that time, women were also provided with their second trimester screening test results (quadruple test). This protocol will detect a very high proportion of cases and, therefore, will not be subject to a strong effect of ascertainment bias.

Included studies and associated numbers of affected pregnancies

Overall, 30 of the 32 included publications provided 28 datasets that totaled 508 trisomy 18 pregnancies (open circles in Figure 4.1-2). Two sets of paired studies used the same dataset to examine different markers (Brizot *et al.*, 1994; Brizot *et al.*, 1995; Spencer *et al.*, 2001b; Spencer *et al.*, 2001a) and these will be displayed only once. One dataset

was published only in abstract form (Palomaki, 2004), and another is unpublished data from the FASTER trial (Lambert-Messerlian, 2004b). Raw data were available from both of these studies for analysis. Data from the included studies most often provide information on one or more of the three most common first trimester serum markers for Down syndrome (*i.e.,* PAPP-A, free β hCG, and intact/total hCG), but data are also available for 14 other first trimester biochemical markers.

Of the 32 publications, 17 also reported marker levels in 232 trisomy 13 affected pregnancies (Figure D.1) (Akolekar *et al.*, 2010; Bersinger *et al.*, 1994; Bogart *et al.*, 1989; Brambati *et al.*, 1994; Brizot *et al.*, 1995; Johnson *et al.*, 1991; Koster *et al.*, 2009; Koster *et al.*, 2010; Kratzer *et al.*, 1991; MacIntosh *et al.*, 1993; Poon *et al.*, 2009; Spencer *et al.*, 1997; Spencer *et al.*, 2000b; Spencer *et al.*, 2000a; Spencer *et al.*, 2007; Zaragoza *et al.*, 2009). Again, two sets of paired studies used the same dataset to examine different markers (Brizot *et al.*, 1994; Brizot *et al.*, 1995; Spencer *et al.*, 2000b; Spencer *et al.*, 2000a). Although this number appears quite large, the majority of included publications for trisomy 13 did not focus on the more common first trimester markers. Instead, this literature is dominated by relatively recent, large studies of 'fringe' markers using stored samples collected during first trimester combined demonstration studies (from the 'screening but not correlated' group) (Akolekar *et al.*, 2010; Koster *et al.*, 2010; Poon *et al.*, 2009; Spencer *et al.*, 2007; Zaragoza *et al.*, 2009). Unfortunately, due to the biases mentioned earlier, these reports cannot be utilized for the marker levels used in the interpretive algorithm (*e.g.*, PAPP-A, free β hCG). Only 93 affected pregnancies are available, at most, for the common markers; 65 coming from only two datasets (Koster *et al.*, 2009; Spencer *et al.*, 2000b; Spencer *et al.*, 2000a).

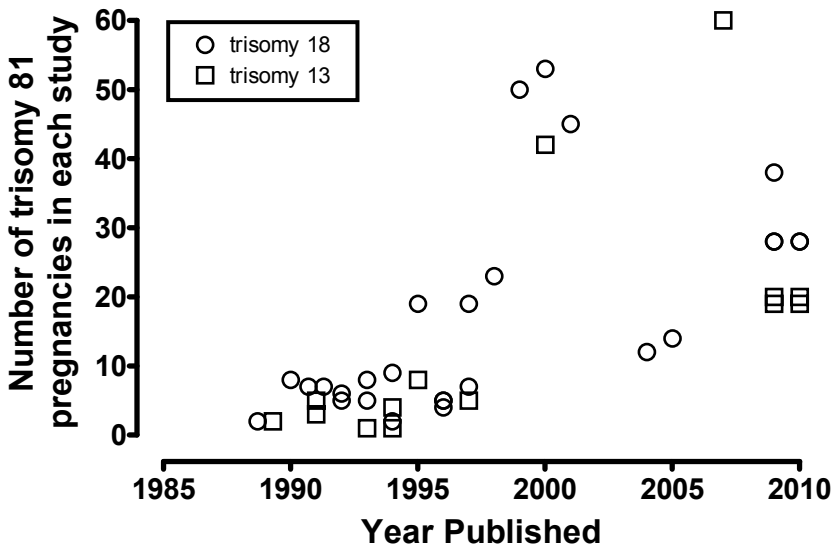

Figure 4.1-2. Relevant publications for biochemical marker levels in 1st trimester pregnancies affected with trisomy 18 or trisomy 13. Open circles indicate the number of trisomy 18 pregnancies, while the open squared indicate trisomy 13 pregnancies. Some of the largest studies are for rarely used markers.

4.2 Common first trimester serum markers

Table 4.2-1 summarizes the numbers of included studies in the first phase of analysis, along with the number of cases of trisomy 18 and 13, and the median MoM levels for the three common first trimester serum markers (free β, hCG and PAPP-A). Each of these had at least five published reports for trisomy 18. In general, these findings provided consistent estimates of effect size, even though some included a relatively small number of cases. Overall, 13 studies reported the free β subunit of hCG measurements in 195 trisomy 18 pregnancies affected with trisomy 18. The overall estimate for free β was about 0.28 MoM. A total of 10 studies reported intact (or total) hCG measurements in 130 pregnancies with trisomy 18, with a summary of 0.37 MoM. For PAPP-A measurements, there were seven studies of 149 women with a summary MoM of 0.21. These estimates will be further refined in subsequent sections.

The data are sparser for trisomy 13 and these serum markers. For the free β subunit of hCG, there are three studies with 29 samples having a summary of 0.43 MoM. There were five studies reporting levels of hCG, with 60 samples having a summary MoM of 0.49. Only two studies reported PAPP-A levels in trisomy 13, with the summary of 0.24 MoM based on 21 samples (20 coming from one study). It is this lack of unbiased (or only modestly biased) data that makes confident modeling of trisomy 13 difficult.

Table 4.2-1. Summary of biochemical markers for trisomy 18 (and trisomy 13) in the late first trimester having five or more included studies of any size

Marker	First Author	Country	GA (range)	Trisomy 18		Trisomy 13	
				Number	MoM	Number	MoM
Free β hCG	(Brambati *et al.*, 1994)	Italy	8 - 12	2	0.13	1	0.15
	(Aitken *et al.*, 1993)	UK	6 - 14	5	0.15	0	-
	(Lambert-Messerlian, 2004a)	US/multiple	10 - 13	14	0.15	0	-
	(Spencer *et al.*, 1992)	UK	7 - 13	5	0.17	0	-
	(Jauniaux *et al.*, 1996)	Belgium	10 - 11	5	0.19	0	-
	(Koster *et al.*, 2010)	Netherlands	11 - 13	43	0.22	20	0.49
	(Palomaki, 2004)	US/multiple	9 - 13	12	0.23	0	-
	(Spencer *et al.*, 1997)	UK	10 – 14	7	0.24	5	0.64
	(Tul *et al.*, 1999)	UK	11 - 13	50	0.28	0	-
	(Scott *et al.*, 1996)	Australia	10 - 13	4	0.30	0	-
	(Zimmermann *et al.*, 1996)	Switz/Austria	10 - 13	5	0.33	0	-
	(Biagiotti *et al.*, 1998)	Italy	8 - 13	23	0.34	0	-
	(Ozturk *et al.*, 1990)	Italy/Neth	8 - 12	8	0.48	0	-
	(Brizot *et al.*, 1995)	UK	10 - 13	19	0.50	8	0.30
	All			**202**	**0.28**	**34**	**0.46**

Table 4.2-1. (continued)

Marker	First Author	Country	GA (range)	Trisomy 18		Trisomy 13	
				Number	MoM	Number	MoM
Intact hCG	(Bogart *et al.*, 1989)	US/CA	9 - 11	2	0.12	2	3.42
	(Aitken *et al.*, 1993)	UK	6 - 14	5	0.27	0	-
	(Jauniaux *et al.*, 1996)	Belgium	10 - 11	5	0.36	0	-
	(Van Lith, 1992)	Netherlands	9 - 11	6	0.81	0	-
	(Johnson *et al.*, 1991)	US/PA	10 - 11	7	0.32	5	0.65
	(Kratzer *et al.*, 1991)	US/CA	9 - 12	7	0.32	3	0.34
	(Palomaki, 2004)	US/multiple	9 - 13	12	0.37	0	-
	(Lambert-Messerlian, 2004a)	US/multiple	10 - 13	14	0.27	0	-
	(Brizot *et al.*, 1995)	UK	10 - 13	19	0.40	8	0.30
	(Spencer *et al.*, 2000b)	UK	10 - 13	53	0.38	42	0.38
	All			**130**	**0.37**	**60**	**0.49**

Table 4.2-1. (continued)

Marker	First Author	Country	GA (range)	Trisomy 18		Trisomy 13	
				Number	MoM	Number	MoM
PAPP-A	(Brambati *et al.*, 1994)	Italy	8 - 12	2	0.51	1	0.59
	(Zimmermann *et al.*, 1996)	Switz/Austria	10 - 13	5	0.08	0	-
	(Benacerraf *et al.*, 1994)	UK	10 - 14	9	0.07	4	0.28
	(Palomaki, 2004)	US/multiple	9 - 13	12	0.28	0	-
	(Lambert-Messerlian, 2004a)	US/multiple	10 - 13	14	0.23	0	-
	(Brizot *et al.*, 1994)	UK	10 - 13	19	0.17	8	0.25
	(Biagiotti *et al.*, 1998)	Italy	8 - 13	23	0.25	0	-
	(Koster *et al.*, 2010)	Netherlands	11 - 13	43	0.19	20	0.22
	(Tul *et al.*, 1999)	UK	11 - 13	50	0.18	0	-
	All			**177**	**0.20**	**33**	**0.25**

4.3 Other first trimester serum markers

Table 4.3-1 shows data for the 14 additional markers identified in the literature search, in alphabetical order. Both AFP and the free alpha-subunit of hCG have results from four studies. For AFP, the results from the 80 observations are variable, ranging from 0.62 to 1.25 MoM. For free alpha, there are 22 observations from four studies, with results ranging from 0.98 to 2.09 MoM. Neither appears to be useful for trisomy 18 in the late first trimester. All of the remaining 12 markers have one (9 markers) or two (3 markers) included studies. Due to these limited data, the reported estimates for these should be considered preliminary. Of interest, however, is the single finding of very low uE3 measurements (0.34 MoM) (Aitken *et al.*, 1993). However, this was based on only five observations and needs confirmation.

Also included in Table 4.3-1 are the corresponding results for trisomy 13 pregnancies. Several relatively large studies for selected markers in this category are promising. In two studies (80 cases), ADAM-12 median MoM levels were 0.58 and 0.66 (Poon *et al.*, 2009; Spencer *et al.*, 2007). Several other markers had low levels reported (*e.g.,* PlGF, PP13), but each was based on a single study.

Table 4.3-1. Summary of other biochemical markers for trisomy 18 (and trisomy 13) in the late first trimester having fewer than five included studies

Marker	First Author	Country	GA (range)	Trisomy 18		Trisomy 13	
				Number	MoM	Number	MoM
Activin-A	(Spencer, 2001)	UK	10 - 13	45	1.23	0	-
A disintegrin and metalloprotease	(Poon *et al.*, 2009)	UK	11 - 13	28	0.70	20	0.58
(ADAM-12)	(Spencer *et al.*, 2007)	UK	10 - 13	0	-	60	0.66
Alpha-fetoprotein (AFP)	(Zimmermann *et al.*, 1996)	Switz/Austria	10 - 13	5	0.62	0	-
	(Aitken *et al.*, 1993)	UK	6 - 14	5	0.71	0	-
	(Spencer *et al.*, 2000a)	UK	10 - 13	53	0.91	42	0.92
	(Johnson *et al.*, 1991)	US/PA	10 - 11	7	1.25	5	0.50
Free-alpha subunit of hCG	(Bogart *et al.*, 1989)	US/CA	9 - 11	2	0.98	2	1.25
	(Kratzer *et al.*, 1991)	US/CA	9 - 12	7	1.01	3	0.32
	(Jauniaux *et al.*, 1996)	Belgium	10 - 11	5	1.58	0	-
	(Ozturk *et al.*, 1990)	Italy/Nether.	8 - 12	8	2.09	0	-
human Placental Lactogen (hPL)	(Sifakis, 2010)	UK	11 - 13	28	0.62	0	-
IGFBP-1	(Miell *et al.*, 1997)	UK	11 - 13	19	2.78	0	-
IGFBP-2	(Miell *et al.*, 1997)	UK	11 - 13	19	0.39	0	-

Table 4.3-1. (continued)

Marker	First Author	Country	GA (range)	Trisomy 18		Trisomy 13	
				Number	MoM	Number	MoM
Dimeric Inhibin-A	(Spencer *et al.*, 2001a)	UK	10 - 13	45	0.74	0	-
	(Lambert-Messerlian, 2004a)	US/multiple	10 - 13	14	0.78	0	-
hyperglycosoylated hCG	(Palomaki, 2004)	US/multiple	9 - 13	12	0.15	0	-
Placental growth factor (PlGF)	(Zaragoza *et al.*, 2009)	UK	11 - 13	28	0.48	19	0.4
	(Spencer *et al.*, 2001b)	UK	10 - 13	45	0.89	0	-
Placental protein 13 (PP13)	(Koster *et al.*, 2009)	Netherlands	8 - 14	38	0.64	23	0.46
Progesterone	(Kratzer *et al.*, 1991)	US/CA	9 - 12	7	0.63	3	0.60
Schwangerschafts protein 1	(Bersinger *et al.*, 1994)	UK	10 - 14	9	0.62	4	0.70
(SP1)	(MacIntosh *et al.*, 1993)	Italy	6 - 11	8	1.15	1	0.46
unconjugated estriol (uE3)	(Aitken *et al.*, 1993)	UK	6 - 14	5	0.34	0	-

4.4 Population parameters for first trimester serum markers

Introduction

Table 4.2-1 provided central estimates for the three common first trimester markers (PAPP-A, free β and total/intact hCG). In this section, those estimates will be refined, and additional population parameters (logarithmic standard deviations, correlation coefficients and truncation limits) will be estimated. These parameters, along with age-associated birth prevalences and fetal loss rates set the stage for modeling patient-specific risks for trisomy 18. It is current practice to not offer stand-alone serum screening for trisomy 18 (*i.e.,* without nuchal translucency – NT measurements), but these parameters will be used in Chapter 7 for modeling combined (and eventually integrated) testing.

PAPP-A measurement in late first trimester trisomy 18 pregnancies

Seven studies reported PAPP-A measurements (Biagiotti *et al.*, 1998; Brambati *et al.*, 1994; Koster *et al.*, 2010; Palomaki, 2004; Tul *et al.*, 1999; Zimmermann *et al.*, 1996; Lambert-Messerlian, 2004b). Of these, three reported over 20 cases (total of 116 cases) as well as summary population parameters (Biagiotti *et al.*, 1998; Koster *et al.*, 2010; Tul *et al.*, 1999). The remaining four studies (33 cases) provided sufficient information to estimate the individual MoM levels and thus estimate a combined 'small study' dataset. Figure 4.4-1 shows a probability plot for the 33 cases comprising the small study dataset. The resulting four sets of parameters (from the three larger studies and the small study combined dataset) are summarized in Table 4.4-1. One of the estimates of the standard deviation (Koster *et al.*, 2010) is much larger than the other three (*i.e.*, 0.42 versus about 0.31). No probability plot or other descriptions of the data were provided, except for listing the parameters.

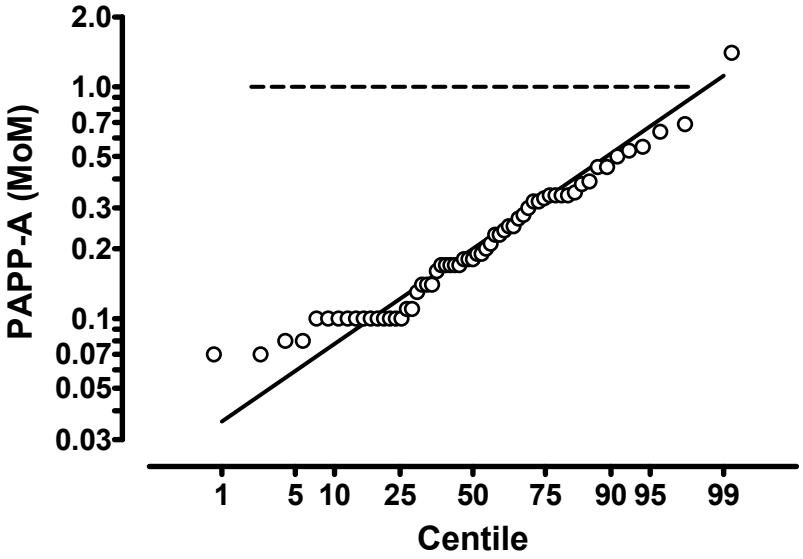

Figure 4.4-1. Probability plot for PAPP-A measurements in first trimester trisomy 18 pregnancies from four smaller studies (33 observations). The straight line indicates the regression line for the observed data (Table 4.4-1). The median MoM level in the general population (1.00) is shown as a horizontal dashed line.

Free β hCG measurement in late first trimester trisomy 18 pregnancies

Thirteen studies reported free β measurements (Aitken *et al.*, 1993; Biagiotti *et al.*, 1998; Brambati *et al.*, 1994; Brizot *et al.*, 1995; Jauniaux *et al.*, 1996; Koster *et al.*, 2010; Ozturk *et al.*, 1990; Palomaki, 2004; Scott *et al.*, 1996; Spencer *et al.*, 1992; Tul *et al.*, 1999; Zimmermann *et al.*, 1996; Lambert-Messerlian, 2004b). Of these, three reported over 20 cases (total of 116 cases) as well as summary population parameters (Biagiotti *et al.*, 1998; Koster *et al.*, 2010; Tul *et al.*, 1999). The remaining nine studies (79 cases) provided sufficient information to estimate the individual MoM levels and thus estimate a combined 'small study' dataset. Figure 4.4-2 shows a probability plot for the 79 cases comprising the small study dataset for free β. The resulting four sets of parameters (from the three larger studies and the small study combined dataset) are summarized in the second section of Table 4.4-1.

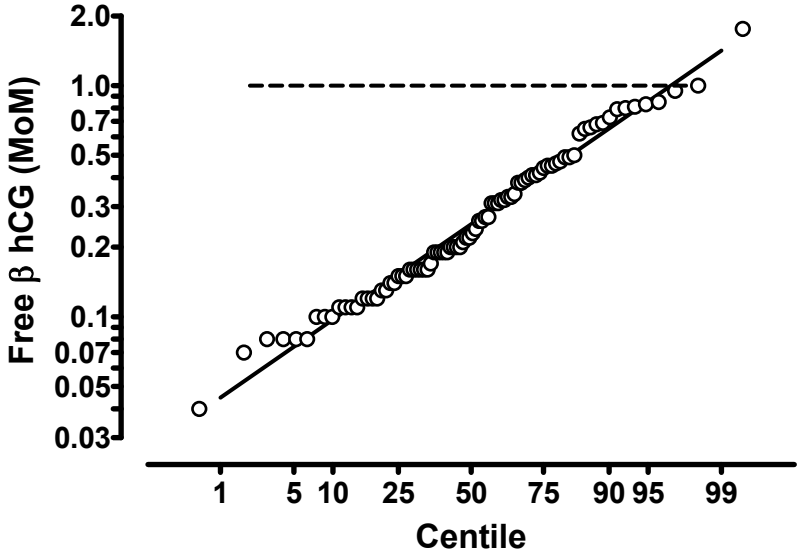

Figure 4.4-2. Probability plot for free β hCG measurements in first trimester trisomy 18 pregnancies from 10 smaller studies (79 observations). The straight line indicates the regression line for the observed data (Table 4.4-1). The median MoM level in the general population (1.00) is shown as a horizontal dashed line.

hCG in measurement in late first trimester trisomy 18 pregnancies

Ten studies reported total/intact hCG measurements (Aitken *et al.*, 1993; Bogart *et al.*, 1989; Brizot *et al.*, 1995; Jauniaux *et al.*, 1996; Johnson *et al.*, 1991; Kratzer *et al.*, 1991; Palomaki, 2004; Spencer *et al.*, 2000a; Van Lith, 1992; Lambert-Messerlian, 2004b). Of these, only one reported over 20 cases (53 cases) as well as summary population parameters (Spencer *et al.*, 2000a). Eight of the remaining nine studies (70 cases) provided sufficient information to estimate the individual MoM levels and thus estimate a combined 'small study' dataset. One study (Kratzer *et al.*, 1991) did not provide individual data. Figure 4.4-3 shows a probability plot for the 70 cases comprising the small study dataset. The resulting two sets of parameters (from the one large study and the small study combined dataset) are summarized in the final section of Table 4.4-1.

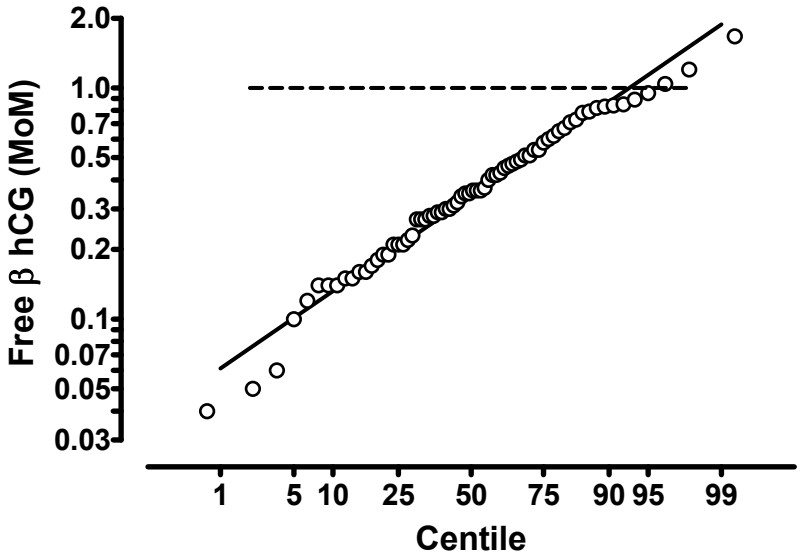

Figure 4.4-3. Probability plot for intact/total hCG measurements in first trimester trisomy 18 pregnancies from 9 smaller studies (70 observations). The straight line indicates the regression line for the observed data (Table 4.4-1). The median MoM level in the general population (1.00) is shown as a horizontal dashed line.

Table 4.4-1 contains the population summary information for PAPP-A, free β and hCG measurements in trisomy 18 pregnancies based on the previous analyses, along with the corresponding information from the both the large and smaller 'combined' studies. Based on these data, and additional information from the original studies in Table 4.2-1, it is possible to create Forest plots for these three markers showing the variability in the median levels for each of the studies used (Figure 4.4-4). Some of the included studies are small and/or did not provide an estimated of the SD. For that reason, a pooled SD was used to estimate the confidence interval. For all three markers, the estimates from all studies are below the median MoM value of 1.00, the expected level in unaffected pregnancies.

Table 4.4-1. Population parameters for three commonly reported first trimester serum markers of trisomy 18

Data source	Number	Median	Log mean (antilog)	Log SD
Pregnancy associated plasma protein-A (PAPP-A)				
(Tul *et al.*, 1999)	50	0.18	-0.7040 (0.20)	0.3060
(Koster *et al.*, 2010)	43	0.19	-0.6500 (0.22)	0.4200
(Biagiotti *et al.*, 1998)	23	0.25	-0.6020 (0.25)	0.3090
'Combined'[1] (various)	33	0.20	-0.6989 (0.20)	0.3207
All[2]	**149**	**0.20**	**-0.6759 (0.21)**	**0.3432**
Free β hCG				
(Tul *et al.*, 1999)	50	0.28	-0.5540 (0.28)	0.3219
(Koster *et al.*, 2010)	43	0.22	-0.6600 (0.22)	0.4100
(Biagiotti *et al.*, 1998)	23	0.34	-0.4690 (0.34)	0.3839
'Combined'[1] various	79	0.22	-0.5992 (0.25)	0.3246
All[2]	**195**	**0.25**	**-0.5861 (0.26)**	**0.3508**
human chorionic gonadotropin (hCG)				
(Spencer *et al.*, 2000a)	53	0.35	-0.4693 (0.38)	0.2686
'Combined'[1] (various)	70	0.38	-0.3790 (0.35)	0.3195
All[2]	**124**	**0.37**	**-0.4179 (0.38)**	**0.2986**

[1] Combination of individual data from 4, 10 and 9 smaller studies, for PAPP-A, free β hCG and hCG, respectively.

[2] Weighted summary estimates (square root of weighted variances for log SD)

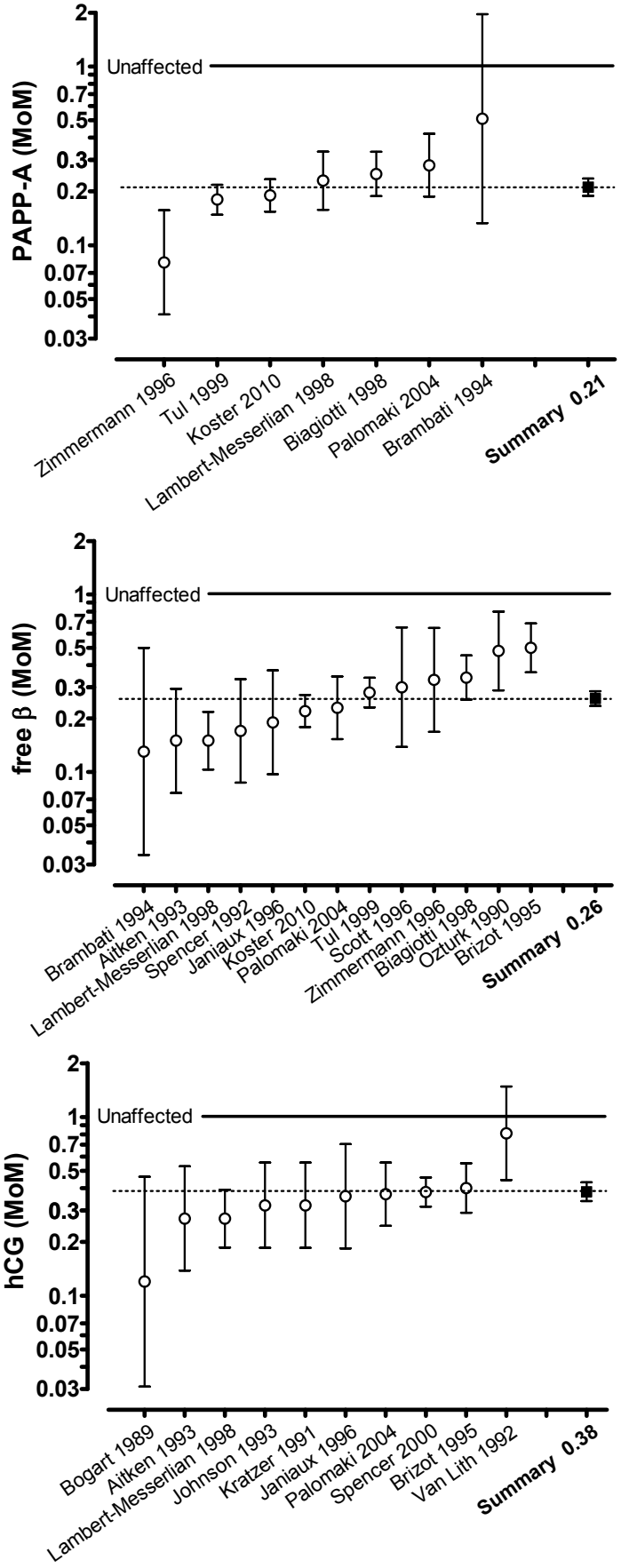

Figure 4.4-4. Forest plots for first trimester maternal serum markers in trisomy 18 pregnancies. The central estimate (usually logarithmic mean) and associated 95% confidence intervals are shown for PAPP-A (upper figure), the free β subunit of hCG (middle figure) and intact hCG (lower figure). The solid line (MoM = 1.00) is the expected value for unaffected pregnancies. The summary median levels are shown as a horizontal dotted line.

Correlations between the three common markers in first trimester trisomy 18 pregnancies

Five studies provided correlation coefficients (or sufficient data to compute it) in at least 10 trisomy 18 pregnancies (Biagiotti *et al.*, 1998; Koster *et al.*, 2010; Palomaki, 2004; Tul *et al.*, 1999; Lambert-Messerlian, 2004b). Other reports based on fewer samples, or those that did not provide multivariate results, were excluded. All five studies provided correlations for PAPP-A and free β hCG measurements. Three showed little correlation (-0.07 to 0.07), one a modest correlation (0.19), and a third showed a moderate correlation (0.44). As a way to help resolve this variability, the largest collection of trisomy 18 results was examined (Kagan *et al.*, 2008b). This study was excluded from this analysis, as the data were collected as part of a demonstration study that utilized both PAPP-A and free β measurements to assign risk. However, among the 122 trisomy 18 pregnancies identified, the PAPP-A/free β correlation was 0.386. This is similar to the highest of the five unbiased estimates (0.44). The potential bias of ascertainment in this large study is unlikely to have a important impact on the correlation. In conclusion, there is wide variability in the correlation reported between PAPP-A and free β measurements in first trimester trisomy 18 pregnancies. The weighted summary of the included studies is 0.1286, and this will be used in modeling. However, this could be an underestimate and should be explored further.

Less data are available for the other two correlations. In all previous reports, the free β and intact/total hCG measurements are highly correlated in both cases and controls, and the two available datasets confirm this (summary r = 0.9033). Conflicting results occur between PAPP-A and intact/total hCG, with one study showing low and the other moderate correlation. Using well defined parameters in the past (*e.g.,* for Down syndrome, unaffected pregnancies), one would expect this correlation to be similar to that found for PAPP-A and free β. The summary of 0.2255 meets this expectation and will be used in future modeling.

Table 4.4-2. Summary of the correlation coefficients in trisomy 18 pregnancies

Study	Number	Correlation coefficient		
		PAPP-A / free β	PAPP-A / hCG	free β / hCG
(Biagiotti *et al.*, 1998)	23	0.0770	NR	NR
(Koster *et al.*, 2009)	43	0.1980	NR	NR
(Lambert-Messerlian, 2004a)	14	-0.0772	0.0587	0.8616
(Palomaki, 2004)	12	0.4490	0.4200	0.9520
(Tul *et al.*, 1999)	50	0.0735	NR	NR
All	**142**	**0.1286**	**0.2255**	**0.9033**
(Kagan *et al.*, 2008b)[1]	122	0.3860	NR	NR

NR = not reported

1 Not included in the analysis, but used to help resolve the wide variability in the PAPP-A/free β correlation coefficients from the five included studies.

Parameters in first trimester unaffected pregnancies

Although it might seem best if the parameters for unaffected pregnancies were derived from the same dataset as the cases, there are advantages to obtaining these parameters elsewhere. Some of the included trisomy 18 studies are relatively old, and improvements in assays and interpretive refinements (*e.g.*, maternal weight adjustment) may have resulted in more refined parameter estimates. I have chosen the SURUSS parameters (Wald *et al.*, 2003), because they are:

- Complete – they include all three common parameters, as well as first trimester NT measurements and second trimester measurements (useful later for integrated testing)
- Up-to-date – the assays were performed after 2000, and revised parameters for NT measurements have just been published (Bestwick *et al.*, 2010)
- Verified – originally collected in the UK, a similar study in the US (FASTER) (Malone *et al.*, 2005) has confirmed the parameters as being reasonable using even more recent data from the United States

Table 4.4-3 contains a summary of all the first trimester serum parameters needed for future modeling for both trisomy 18 and unaffected pregnancies. It also contains a summary estimate of the screening potential for the three markers. The separation between the markers (on a logarithmic scale) is divided by the log standard deviation in unaffected pregnancies. This z-score indicates that the 'best' marker is PAPP-A, followed by the two hCG-related markers that have similar, but lower, z-scores.

Table 4.4-3. Summary of population distribution parameters for first trimester maternal serum markers in trisomy 18 and unaffected pregnancies

			Correlation coefficients		
Marker	**Log mean**	**Log SD**	**PAPP-A**	**Free β**	**hCG**
Unaffected pregnancies					
PAPP-A	0.0000	0.2495	-	0.1395	0.2198
Free β	0.0000	0.2651	-	-	0.7178
hCG	0.0000	0.1950	-	-	-
Trisomy 18 pregnancies					
PAPP-A	-0.6989	0.3207	-	0.1286	0.2255
Free β	-0.5992	0.3255	-	-	0.9033
hCG	-0.4179	0.2986	-	-	-
Truncation limits					
PAPP-A	0.3 – 0.7				
Free β	0.3 – 1.2				
hCG	0.4 – 1.2				
Separation in marker levels in case and control pregnancies, expressed as Z-score					
PAPP-A	-2.80				
Free β	-2.26				
hCG	-2.14				

SD = standard deviation

Distribution of first trimester serum markers in trisomy 18 and unaffected pregnancies

Figures 4.4-5 (a through c) display the overlapping Gaussian curves in trisomy 18 and unaffected pregnancies for measurements of maternal serum PAPP-A, free β and hCG, respectively. The figures are plotted on a logarithmic scale. The separation is best for PAPP-A measurements. At a 5% false positive rate, the univariate detection rates for the three markers are 82%, 69% and 63%, respectively. These can be seen in the figure, using the thin vertical line as the guide. The false positive rate of 5% is to the left of the vertical line under the unaffected distribution (dashed curved line). The corresponding detection rate is to the right of the line under the trisomy 18 distribution (solid curved line). At a fixed 60% detection rate, the corresponding false positive rates are 0.7%, 2.6% and 4.0%, respectively. Multivariate modeling for biochemistry and biochemistry plus first trimester ultrasound markers will be presented in Chapter 6.

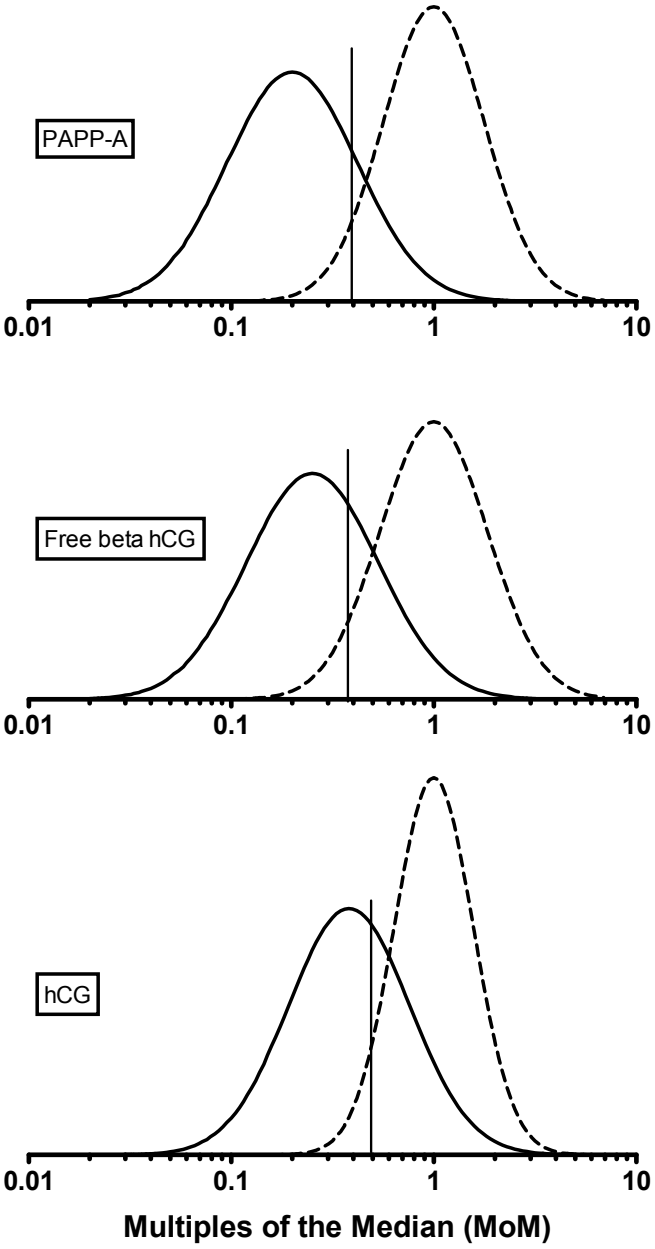

Figure 4.4-5. Overlapping Gaussian distributions in unaffected and trisomy 18 pregnancies. Marker levels for pregnancy associated plasma protein-A (PAPP-A), the free β subunit of hCG (free β hCG) and for total/intact human chorionic gonadotropin (hCG) are expressed as multiples of the median (MoM) on the horizontal logarithmic axis. The relative distributions of results (population density) are shown as varying heights of the curves. For all three markers, trisomy 18 is associated with reduced levels (left bell-shaped curve). The vertical line is drawn at a 5% false positive rate.

4.5. First trimester markers in trisomy 13 pregnancies

Tables 4.2-1 and 4.3-1 also contain estimates for the median PAPP-A, free β and total/intact hCG measurements for trisomy 13 pregnancies (0.25, 0.46 and 0.49 MoM, respectively). For PAPP-A and free β, the majority of samples (20/33 and 20/34, respectively) come from one study (Koster *et al.*, 2010). The corresponding logarithmic standard deviations were 0.43 and 0.30, respectively, and these will be used in any modeling. They are consistent with the logarithmic standard deviation estimated on the 5^{th} to 95^{th} centiles reported in that same study (0.43 and 0.25, respectively). This study also reported the correlation coefficient between PAPP-A and free β to be 0.198. For intact/total hCG measurements, a different study (Spencer *et al.*, 2000a) dominates the results (42/60 observations). The reported logarithmic standard deviation was 0.2656. No correlations between intact/total hCG and PAPP-A were reported in any included, or excluded, study. For any modeling, the correlation for trisomy 18 will be used (0.2255). The PAPP-A measurements in trisomy 18 and 13 are similar, but there is less separation for both free β and hCG. How this might impact the coincidental detection of trisomy 13 during a trisomy 18 risk assessment will be discussed further in Chapter 6, after first trimester ultrasound markers have been examined.