Chapter 8 Second trimester ultrasound markers for trisomy 18

8.1 Introduction

In many developed countries, a second trimester ultrasound examination at 18 to 22 weeks' gestation is recommended as part of routine prenatal care to identify major structural anomalies. It is scheduled at this relatively late gestational age, because some of the targets (*e.g.,* heart defects, face, hand and foot) are more easily visualized. At a somewhat earlier time in pregnancy (15 to 20 weeks' gestation), ultrasound is also commonly performed prior to amniocentesis. Thus, there is an existing literature regarding the ability of early second trimester ultrasound markers to identify fetuses with aneuploidy. Although the major focus of this literature is on Down syndrome, some information is available concerning trisomy 18. This chapter will focus on ultrasound performed between 15 and 20 weeks' gestation, but will include some later second trimester, and even third trimester, results to aid in determining whether individual markers may be gestational age dependent. Lastly, it is important to acknowledge that the majority of observations will be made in high risk pregnancies, where the ultrasound is performed as part of a diagnostic testing protocol. This has the strength of having the availability of a routine karyotype, but is associated with several important limitations that might bias performance estimates. The most important of these include: the indication for amniocentesis is sometimes because of an ultrasound marker, and the result of the ultrasound and subsequent counseling might influence the uptake of amniocentesis, thus missing cases that do not have that marker.

For this review, ultrasound markers have been stratified into two separate groups: structural anomalies and non-structural anomalies.

- Structural anomalies are real fetal defects that will be maintained during pregnancy and will usually result in birth defects. These include: open defects (abdominal and spinal), cardiac defects, and anomalies of the hand and foot (clenched fingers, rocker bottom feet, talipes). Some groups only reported 'gross' abnormalities that could include combinations of these outcomes.
- Non-structural anomalies will usually not result in birth defects, but may be found in association with aneuploidy during pregnancy or even after delivery. These include: choroid plexus cysts, nuchal skinfold thickening, shortened long bone measurements, echogenic bowel and pyelectasis.

For each of these ultrasound markers, a structured literature search was conducted that included a review of the reference lists from retrieved articles. The Medline search strategy included the names (or names) of the marker, and the terms 'second trimester or mid-trimester', 'aneuploidy' and 'trisomy 18'. Case reports are not included. Studies were included if they provided gestational age, study type (*e.g.*, case/control, cohort, case only), whether the population was at high, population or low risk for trisomy 18, and a suitable description of the ultrasound marker(s) studied. Relevant information is shown in an evidence table, and summary detection and false positive rates are estimated using a random effects model. Overall heterogeneity is evaluated, and sources for that heterogeneity are examined (*e.g.*, year of publication, study type, and gestational age). In general, gestational ages are grouped into earlier (15 to 20 weeks' gestation, inclusive), moderate (15 through 23 weeks) and later (15 through 24 weeks and later) categories. The lower limit of the gestational age is less important than the upper in this classification scheme. For example, a study of 13 through 22 weeks will be classified as moderate, while a study of 17 through 20 will be considered early. If studies provided information on individual pregnancies, the summary used may differ from that published. For example, if a study reported the individual ultrasound marker results for 20 pregnancies from 15 through 30 weeks' gestation, it might be possible to create two summary detection rates. One for the early group (15 to 20 weeks), with the remaining pregnancies (21 to 30 weeks) assigned to the late grouping.

Although all of the included studies provided an estimate of the detection rate, many did not provide sufficient information for the associated false positive rate. Sometimes this was due to study design (*e.g.,* case only), but not always. In general, studies reporting both detection and false positive rates were examined separately from the studies reporting only a detection rate. Table 8.1-1 summarizes the 46 included publications for 10 of the 11 second trimester ultrasound markers summarized in this chapter. The publications for choroid plexus cysts (CPCs) are not included in the Table, as many of them focus exclusively on CPCs (Figure 8.3.1-2). Unlike previous chapters, some of the results have been stratified by study design and/or whether the population studied was high-risk or low-risk. This allowed for evaluation of heterogeneity and examine potential biases.

Table 8.1-1. Included publications for second ultrasound markers and trisomy 18, excluding those for choroid plexus cysts

	Structural anomalies				Non-structural anomalies					
Reference	Gross defect	Open defect	Cardiac defect	Hands & feet	Nuchal Skinfold	Long bone	Hyper. bowel	Pyele-ctasis	2 vessel cord	EICF
(Bahado-Singh *et al.*, 2003)	X	X		X					X	
(Benacerraf *et al.*, 1988)	X	X	X	X						
(Benacerraf *et al.*, 1990)	X	X	X	X						
(Benacerraf *et al.*, 1992)	X	X	X		X	X				
(Benacerraf *et al.*, 1994)	X	X	X	X	X	X	X	X		
(Benacerraf *et al.*, 1994)					X					
(Borrell *et al.*, 1997)					X					
(Bottalico *et al.*, 2009)	X				X	X	X	X		X
(Bronsteen *et al.*, 2004)	X		X	X						
(Brumfield *et al.*, 2000)	X	X	X	X					X	
(Bundy *et al.*, 1986)						X				
(Cheng *et al.*, 2006)										
(Cho *et al.*, 2009)	X		X	X	X	X	X	X	X	X
(Coco and Jeanty, 2004)										
(DeVore, 2000)	X		X		X		X	X		
(Dicke *et al.*, 1989)						X				
(Droste *et al.*, 1990)						X				
(Drugan *et al.*, 1996)					X		X	X		
(Ghidini *et al.*, 2000)										
(Ginsberg *et al.*, 1990)					X	X				
(Grandjean *et al.*, 1998)	X									
(Gray *et al.*, 1996)		X		X						
(Gupta *et al.*, 1997)	X	X	X	X						
(Havutcu et al., 2002)								X		

Table 8.1-1. (Continued)

	Structural anomalies				Non structural anomalies					
Reference	Gross defect	Open defect	Cardiac defect	Hands & feet	Nuchal Skinfold	Long bone	Hyper. bowel	Pyele-ctasis	2 vessel cord	EICF
(Jelliffe-Pawlowski *et al.*, 2008)			X							
(Lubusky *et al.*, 2007)									X	
(Moran *et al.*, 2002)	X									
(Nadel *et al.*, 1992)						X				
(Nicolaides *et al.*, 1992b)			X	X						
(Nyberg *et al.*, 1993)	X	X	X	X						
(Papp *et al.*, 2007)	X	X	X	X			X			X
(Papp *et al.*, 2008)						X		X		
(Picklesimer *et al.*, 2005)	X									
(Roberts *et al.*, 1993)	X									
(Saller *et al.*, 1990)									X	
(Seoud *et al.*, 1994)			X		X	X	X	X		
(Sepulveda *et al.*, 1995)										X
(Shields *et al.*, 1998)	X	X	X	X						
(Taslimi *et al.*, 2005)	X									
(Tongsong *et al.*, 2002)	X	X	X	X						
(Viora *et al.*, 2007)		X	X	X			X			
(Watson *et al.*, 1994)					X					
(Watson *et al.*, 2008)		X	X	X						
(Watson *et al.*, 2008)						X	X		X	
(Wax *et al.*, 2000)										X
(Yeo *et al.*, 2003)		X	X	X			X	X		X
Total number (46)	20	15	19	17	11	12	10	9	6	6

ICEF = intracardiac echogenic focus, hyper = hyperechoic bowel

8.2 Structural anomalies

This section includes three structural anomalies associated with trisomy 18 in early second trimester sonographic examinations and a 'summary' grouping including any structural anomaly. The three individual defects include: open ventral/neural tube defects (most often omphalocele), cardiac structural anomalies (excluding blood flow anomalies), and hand/feet anomalies (including clenched hands, overlapping digits, club feet). Since some studies did not always report individual defects, an additional group designated as a 'any structural defect' was also included. In general, structural anomalies have moderate sensitivity, but high specificity for trisomy 18 (or other aneuploidies).

Overview of the literature search: A total of 26 publications (1988 to 2009) were identified that satisfied inclusion criteria and reported structural anomalies in trisomy 18 pregnancies. These were found via formal literature searching (described in Chapter 8.1) and examining the lists of retrieved publications (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1988; Benacerraf *et al.*, 1990; Benacerraf *et al.*, 1992; Benacerraf *et al.*, 1994; Bottalico *et al.*, 2009; Bronsteen *et al.*, 2004; Brumfield *et al.*, 2000; Cho *et al.*, 2009; DeVore, 2000; Grandjean *et al.*, 1998; Gupta *et al.*, 1997; Jelliffe-Pawlowski *et al.*, 2008; Moran *et al.*, 2002; Nicolaides *et al.*, 1992b; Nyberg *et al.*, 1993; Papp *et al.*, 2007; Picklesimer *et al.*, 2005; Roberts *et al.*, 1993; Seoud *et al.*, 1994; Shields *et al.*, 1998; Taslimi *et al.*, 2005; Tongsong *et al.*, 2002; Viora *et al.*, 2007; Watson *et al.*, 2008; Yeo *et al.*, 2003). Inclusion criteria have been specified earlier. Two studies were excluded because they required that an ultrasound finding (choriod plexus cysts) be present in all included patients (Gray *et al.*, 1996; Nadel *et al.*, 1992). If a study contained no instances of a specific structural anomaly in cases or controls, it was assumed that data were not collected, unless the Methods clearly stated that it was reportable. For example, if a study contained no cases or controls with an omphalocele, this category would be left blank, unless methods explicitly stated that omphalocele was considered a reportable outcome.

Figure 8.2-1 shows the number of trisomy 18 pregnancies reported by each of the 26 included studies, along with the study design (shape of the symbol) and whether the study was derived from a high risk population (filled symbols) or a general pregnancy population (open symbols). No study was performed in a low risk population (*i.e.,* an important proportion of trisomy 18 pregnancies identified and removed from the

population earlier in pregnancy). Not all studies provided information on all four structural anomaly groups included in this analysis, but each contributed at least one. Only three of 26 studies reported structural anomalies on fewer than 10 cases; seven studies reported on 50 or more cases. The majority of these ultrasound studies are case-only. The most common scenario was the review of an existing clinical database, with ultrasound information extracted only on pregnancies diagnosed with trisomy 18.

A pair of articles (Papp *et al.*, 2007; Papp *et al.*, 2008) are also of interest, as they compare the results of routine US in trisomy 18 pregnancies, with the corresponding second trimester autopsy results following termination of pregnancy. Some of the US findings were found to be extremely reliable (*e.g.,* hydrops, CNS defects), while others were quite poor (*e.g.,* only four of the 70 trisomy 18 fetuses had hand/feet anomalies identified by ultrasound, but 46 of 70 had such findings at autopsy).

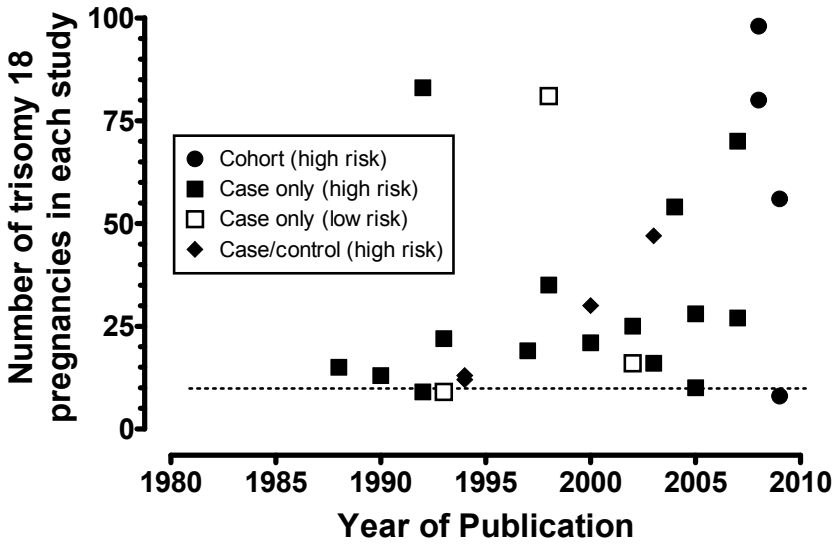

Figure 8.2-1. The included articles of structural anomalies among trisomy 18 fetuses. Each symbol represents a single study, with the shape indicating the study design. The symbol is filled if the study was performed in a high risk population (usually prior to amniocentesis), and open if performed in a general population.

8.2.1 Open fetal defects

Definition: This category mainly includes omphalocele and open spina bifida, which were sometimes reported as an 'abdominal wall defect' or 'neural tube defect'. Occasionally other open defects were also reported and included in this category (*e.g.*, anencephaly).

Literature review: Based on a structured literature search, fifteen studies met inclusion criteria on the proportion of trisomy 18 pregnancies with open fetal defects (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1990; Benacerraf *et al.*, 1988; Benacerraf *et al.*, 1994; Benacerraf *et al.*, 1992; Brumfield *et al.*, 2000; Gray *et al.*, 1996; Gupta *et al.*, 1997; Nyberg *et al.*, 1993; Papp *et al.*, 2008; Shields *et al.*, 1998; Tongsong *et al.*, 2002; Viora *et al.*, 2007; Watson *et al.*, 2008; Yeo *et al.*, 2003).

Results: Table 8.2.1-1 provides a listing of the 15 studies, the observed number of trisomy 18 pregnancies, the number and proportion with open fetal defects. It also includes the approximate time in gestation the ultrasound study was performed (early, middle and late as defined earlier). Figure 8.2.1 -1 shows a forest plot for the proportion of trisomy 18 fetuses associated with open defects for the 15 studies. The summary estimate is 23%, (95% CI 18% to 28%). Heterogeneity is moderate (Q=20, I^2=29%, p<0.001). There is no difference in detection rates in the 19 estimates by study design (p = 0.8), but there is some indication that the rate is slowing decreasing over time (p = 0.055, for slope). There is a much stronger effect seen when the rates are stratified by the time in gestation when the ultrasound was performed. Among the seven estimates from studies with early results (≤20 weeks), the detection rate is 27% (95% CI 19% to 37%), similar to the results found for the six middle estimates of 25% (95% CI 20% to 31%). Overall, the rate is 26% (95% CI 21% to 31%) for these 13 studies. However, the two studies that included results from the late second trimester (no upper gestational age limit) were quite low, 10% (95% CI 6% to 17%). These differences are significant (p = 0.003). Each of the larger groups are homogeneous (I^2=0% and 10%, respectively). It is possible that the later two studies were performed in populations where open fetal defects were identified much earlier in pregnancy and would not represent results found in the 15 to 20 week period. Results from the 15 studies were also examined for publication bias using two methods. Neither was statistically significant (Eggers regression intercept, p = 0.9, trim and fill method

estimates a positive bias of 3%). Only two studies reported the rates of open fetal defects in the control population, and there were too few for reliable estimates. However, a reasonable estimate from the literature for the combination of open neural tube and ventral wall defects is about 1:500 (0.2%).

Summary: A total of 15 articles was identified and included for analysis of open fetal defects, with information on 441 cases of trisomy 18. There was a change in detection rate by gestational age, but among the 13 studies reporting ultrasound exams in the early to mid second trimester, the proportion of 18 fetuses with an open defect was constant at 26%. Two studies including later ultrasound exams found a much lower rate of 10%. When two late gestational age studies with very low detection rates (10%) were removed, the detection rate remained constant over time (p=0.8). No differences by study type were noted. Few studies reported the rate of open defects in the population without any chromosome abnormalities. Best estimate would be 26% detection at a 0.2% false positive rate.

Table 8.2.1-1. Rates of open fetal defects in trisomy 18 and control pregnancies

Author	Location	Design (prev)[1]	Gestational Age (weeks)[2]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Gupta *et al.*, 1997)	UK	Ca only (high)	16 – 20 E	4/19 21%	
(Benacerraf *et al.*, 1990)	US/MA	Ca only (high)	13 – 20 E	3/13 23%	
(Benacerraf *et al.*, 1988)	US/MA	Ca only (high)	15 – 19 E	2/ 8 25%	
(Nyberg *et al.*, 1993)	US/WA	Ca only (high)	15 – 19 E	5/20 25%	
(Gray *et al.*, 1996)	US/MO	Ca only (high)	14 – 20 E	3/11 27%	
(Benacerraf *et al.*, 1992)	US/MA	Ca only (high)	14 – 20 E	3/ 9 33%	
(Yeo *et al.*, 2003)	US/NJ	Ca only (high)	15 – 19 E	6/16 38%	
(Brumfield *et al.*, 2000)	US/AL	Ca only (high)	14 – 22 M	3/30 10%	
(Viora *et al.*, 2007)	Italy	Ca only (high)	16 – 23 M	6/27 22%	
(Watson *et al.*, 2008)	US/NC	Ca only (high)	15 – 21 M	23/98 23%	
(Shields *et al.*, 1998)	US/WA	Ca only (high)	14 – 22 M	10/35 29%	
(Benacerraf *et al.*, 1994)	US/MA	Case/Cont (high)	14 – 21 M	4/13 31%	0 / 106 0.0%
(Tongsong *et al.*, 2002)	Thailand	Ca only (high)	16 – 22 M	9/25 36%	
(Papp *et al.*, 2007)	Hungary	Ca only (high)	13 – 24 L	5/70 7%	
(Bahado-Singh *et al.*, 2003)	US/CT	Case/Cont (high)	14 – 24 L	6/47 13%	2 / 1,214 0.2%
All Studies				**92/441 23%** (95% CI 18% to 28%)	**2 / 1320 0.2%**

[1] prev = population prevalence. High = referral population, general = unscreened population with background risk

[2] first and last completed week, or mean (SD). The letters indicate a gestational age grouping (E=early, M=moderate, L=late)

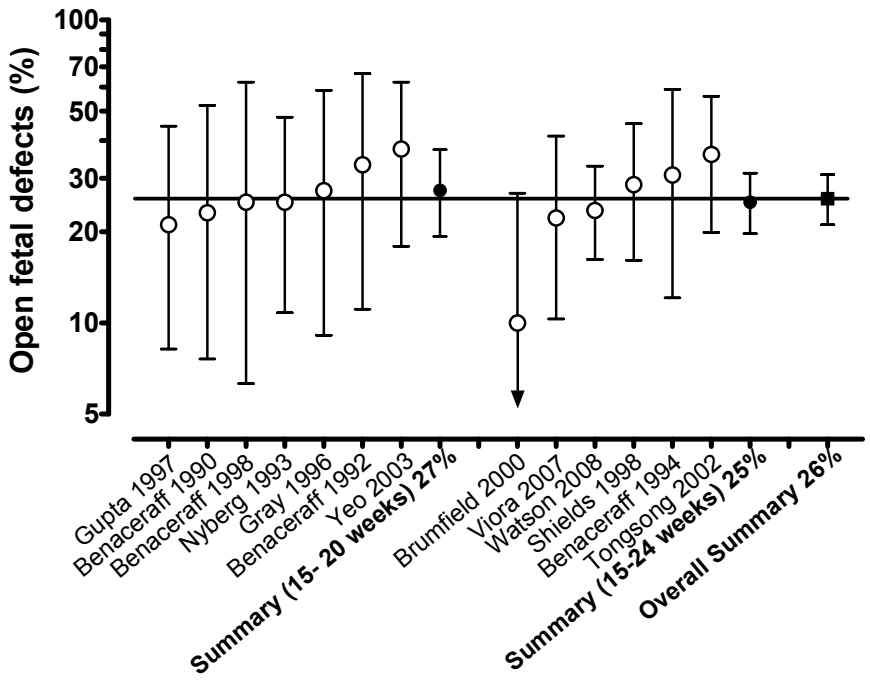

Figure 8.2.1-1. Forest plot showing the proportion of trisomy 18 pregnancies with open fetal defects. Two studies that included third trimester studies or observations are not included. Among the remaining 13 studies, the summary detection rate is 26%.

8.2.2 Cardiac defects

Definition: There was more variability in the reporting of this category, most likely due to improvement in ultrasound imaging equipment over time. The more recent studies routinely reported 4-chambered views of the heart prior to 20 weeks' gestation. The most commonly reported anomaly was a ventricular septal defect (VSD), but other descriptions include 'cardiac abnormalities', 'cardiac structural problems', 'heart defects', and 'major abnormalities of the heart'. When possible, cases having only an abnormal Doppler flow result were excluded, as these would probably not be part of routine testing, due to availability, costs and expertise in interpretation.

Literature search: Nineteen studies reported the proportion of trisomy 18 pregnancies with cardiac defects (Benacerraf *et al.*, 1990; Benacerraf *et al.*, 1994; Benacerraf *et al.*, 1992; Bronsteen *et al.*, 2004; Brumfield *et al.*, 2000; Cho *et al.*, 2009; DeVore, 2000; Gupta *et al.*, 1997; Jelliffe-Pawlowski *et al.*, 2008; Nyberg *et al.*, 1993; Papp *et al.*, 2008; Seoud *et al.*, 1994; Shields *et al.*, 1998; Tongsong *et al.*, 2002; Viora *et al.*, 2007; Watson *et al.*, 2008; Yeo *et al.*, 2003).

Results: Table 8.2.2-1 provides a listing of the 15 studies, the observed number of trisomy 18 pregnancies, the number and proportion with open fetal defects. It also includes additional information, including the approximate time in gestation (15-20 weeks - early, 15 through 24 weeks - middle and 15 through 40 weeks - late), as defined previously, as well as the study design and prevalence of trisomy 18 in the population studied. Figure 8.2.2-1 shows a forest plot for the proportion of trisomy 18 fetuses associated with heart defects for the 19 studies, as shown in Table 8.2.2-1. In one study (Benacerraf *et al.*, 1988), it was possible to divide the data into two groups by gestational age. The overall summary estimate is 49%, (95% CI 31% to 67%). Heterogeneity is high ($Q=61$, $I^2=69\%$, $p<0.001$). When grouped by study design, the summary detection rate for the 15 case-only studies is 40%. This is confirmed by the two cohort studies (42%), but the findings for the three case/control studies are much higher (69%). This difference is statistically significant ($p=0.04$). When the results were regressed by year of publication, there was a positive, but non-significant, slope ($p = 0.13$). There are also important differences by time in gestation when the ultrasound was performed. Among the six early estimates, the detection rate is 38% (95% CI 26% to 51%). The nine middle and five late estimates both showed a 47% detection rate. These

differences are not significant (p = 0.56). There is significant heterogeneity in both the earlier and middle gestational age groups (I^2=72% and 80%, respectively). Results from the 19 studies were also examined for publication bias using two methods; neither was statistically significant (Eggers regression intercept, p = 0.2, trim and fill method found no imputed values).

Five of the studies were either of case/control or cohort design and, therefore, both the detection and false positive rates could be compared. One study (DeVore, 2000) stands out as having a high rate of heart defects in control pregnancies (5.9%), compared to the other four studies (1.2% or lower). It is also associated with the highest detection rate (80%), compared to the other four studies (38%, 46%, 54% and 67%). This may be an indication that those sites reporting high detection rates may be defining 'heart disease' in a different way, and are including heart defects that are either not considered major findings or, perhaps, these authors are using a more refined technique. One case-only study (Yeo *et al.*, 2003) also reported a high detection rate (81%). In that study, nearly all cases were associated with multiple major and minor malformations, perhaps indicating a referral population or, again, a differing technique. However, these two studies are not solely responsible for the high heterogeneity. If they were to be removed, heterogeneity would be reduced from an I^2 value of 69% to 60%. Results from the 17 studies were also examined for publication bias. None was found to be statistically significant (Eggers regression intercept, p = 0.2). The trim and fill method (Duval and Tweedie, 2000) imputed four studies to the right of the mean, changing the detection rate from 61% to 62%, a negligible bias.

Summary: A total of 19 articles (20 datasets) were identified and included for analysis of heart defects, with information on 315 cases of trisomy 18. All studies were performed in a high risk setting. An estimated 44% of trisomy 18 fetuses can be identified as having a heart defect by 20 weeks' gestation. However, there is considerable heterogeneity. The sources of this heterogeneity may be due to: 1) the gestational age at testing, with lower rates associated with earlier scanning; 2) improvement in equipment/technique, with more recent studies showing somewhat higher rates; 3) differences in the definition of a major heart defect; and 4) other less well described factors. This latter group could include sonographer experience, whether the site receives ultrasound referrals and other, yet unknown factors. Publication bias is unlikely to influence the effect size. A conservative detection rate to use is the rate found in the early studies (38% 95% CI 26% to

51%), as these are less likely to be referrals, more likely to reflect general practice and are more relevant to the setting of prenatal screening for trisomy 18. The corresponding rate of heart defects seen in pregnancies without trisomy 18 is about 1.5%. Thus, a reasonable estimate in non-specialized centers is a detection rate of 36% and a false positive rate of 1.5%.

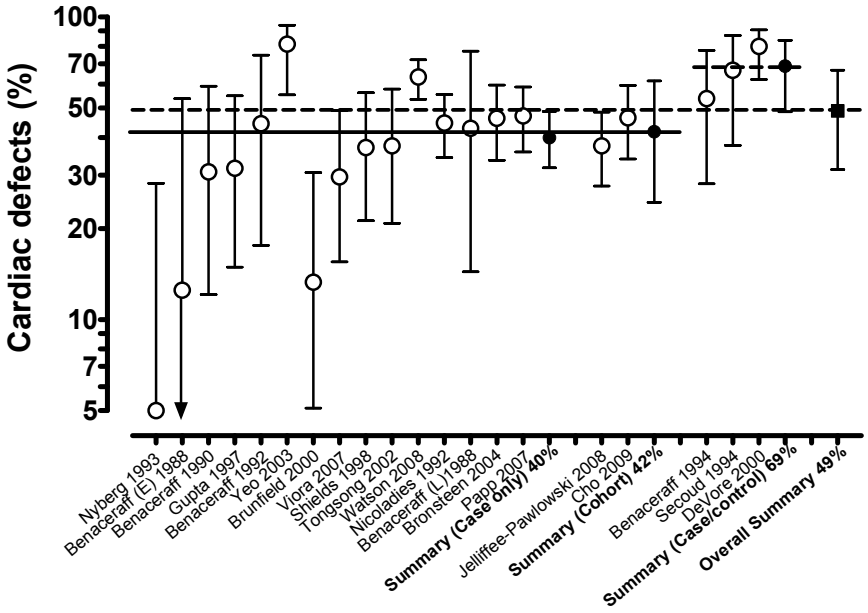

Figure 8.2.2-1. Forest plot showing the proportion of trisomy 18 pregnancies with a major cardiac defect. The summary detection rate among all studies (except the three case/control studies) is 40%, but with considerable heterogeneity. The first group of studies (Case only) are stratified by gestational age (early, middle, later).

Table 8.2.2-1. Rates of cardiac defects in trisomy 18 and control pregnancies

Author	Location	Design (prev)[1]		Gestational Age (weeks)[2]		Trisomy 18 positive / total (%)		Controls positive / total (%)
(Nyberg *et al.*, 1993)	US/WA	Ca only	(high)	15 – 19	E	1/20	5%	
(Benacerraf *et al.*, 1988) (early)	US/MA	Ca only	(high)	15 – 19	E	1/ 8	13%	
(Benacerraf *et al.*, 1990)	US/MA	Ca only	(high)	13 – 20	E	4/13	31%	
(Gupta *et al.*, 1997)	UK	Ca only	(high)	16 – 20	E	6/19	32%	
(Benacerraf *et al.*, 1992)	US/MA	Ca only	(high)	14 – 20	E	4/ 9	44%	
(Yeo *et al.*, 2003)	US/NJ	Ca only	(high)	15 – 19	E	13/16	81%	
(Brumfield *et al.*, 2000)	US/AL	Ca only	(high)	14 – 22	M	4/30	13%	
(Viora *et al.*, 2007)	Italy	Ca only	(high)	16 – 23	M	8/27	30%	
(Shields *et al.*, 1998)	US/WA	Ca only	(high)	14 – 22	M	10/27	37%	
(Tongsong *et al.*, 2002)	Thailand	Ca only	(high)	16 – 22	M	9/24	38%	
(Watson *et al.*, 2008)	US/NC	Ca only	(high)	15 – 21	M	62/98	63%	
(Nicolaides *et al.*, 1992b)	UK	Ca only	(high)	14 – 39	L	37/83	45%	
(Benacerraf *et al.*, 1988) (late)	US/MA	Ca only	(high)	20 – 38	L	3/ 7	43%	
(Bronsteen *et al.*, 2004)	US/MI	Ca only	(high)	15 – 24	L	25/54	46%	
(Papp *et al.*, 2007)	Hungary	Ca only	(high)	13 – 24	L	33/70	47%	
(Jelliffe-Pawlowski *et al.*, 2008)	US/CA	Cohort	(high)	Early 2nd	M	30/80	38%	191 / 20,005 1.0%
(Cho *et al.*, 2009)	US/CA	Cohort	(high)	15 – 21	M	26/56	46%	108 / 8,606 1.2%
(Benacerraf *et al.*, 1994)	US/MA	Case/Cont	(high)	14 – 21	M	7/13	54%	1 / 106 0.9%
(DeVore, 2000)	US/CA	Case/Cont	(high)	15 – 23	M	24/30	80%	118 / 2,000 5.9%
(Seoud *et al.*, 1994)	US/VA	Case/Cont	(high)	22 / 5	L	8/12	67%	0 / 50 0.0%
All Studies						**315/696**	**46%**	
(random effects)						(95% CI 42% to 50%)		
Studies with controls						**87/179**	**56%**	**418/30,868 1.4%**
						(95% CI 39% to 71%)		(95% CI 1.2% to 1.5%)

[1] prev = population prevalence. High = referral population, general = unscreened population with background risk

[2] first and last completed week, or mean/SD. The letters indicate a gestational age grouping (E=15-20 weeks, M=15-24 and L=15-40)

8.2.3 Hand and foot anomalies

Definition: Most study protocols attempted to visualize both hands to look for clenched fists and overlapping fingers. Highly correlated with these findings are problems with the feet, including clubbing. In addition to these specific terms, alternative descriptions include 'defects of the extremities', 'abnormal extremities', and 'abnormal hands/feet'.

Literature search: Seventeen studies reported the proportion of trisomy 18 pregnancies with abnormalities of the hand and foot (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1988; Benacerraf *et al.*, 1990; Benacerraf *et al.*, 1994; Bronsteen *et al.*, 2004; Brumfield *et al.*, 2000; Cho *et al.*, 2009; Gray *et al.*, 1996; Gupta *et al.*, 1997; Nyberg *et al.*, 1993; Papp *et al.*, 2008; Shields *et al.*, 1998; Tongsong *et al.*, 2002; Viora *et al.*, 2007; Watson *et al.*, 2008; Yeo *et al.*, 2003).

Results: Table 8.2.3-1 provides a listing of the 17 studies, the observed number of trisomy 18 pregnancies, the number and proportion with hand and foot anomalies. It also provides additional information, including the approximate time in gestation (15-20 weeks - earlier, 15-24 weeks - middle and 15-40 weeks - later), as defined earlier. In one study (Benacerraf *et al.*, 1988), it was possible to divide the data into two groups by gestational age. Figure 8.2.3-1 shows a forest plot for the proportion of trisomy 18 fetuses associated with hand and foot anomalies. The summary is 51%, (95% CI 38% to 63%). Heterogeneity is high (Q=95, I^2=83%, p<0.001). When grouped by study design, the detection rates for the 15 case-only study estimates (51%) and two case/control study estimates (37%) are not significantly different (p=0.11). When the results were regressed by year of publication, there was a negative and significant, slope (p = 0.007, -0.04117 per year of the logit of the slope, intercept=82.388). This translates into about an eight to 10% decline every five years. There does not appear to be clear trend by time in gestation when the ultrasound was performed. Among the six studies performed earlier in gestation, the detection rate is 41% (95% CI 22% to 64%). The eight middle and three later gestational age group studies showed increasing rates at 50% and 70%, respectively. However, these differences are not significant (p = 0.40), perhaps due to the high heterogeneity in all three groups (I^2=56%, 94% and 81%, respectively). Results from the 17 studies were also examined for publication bias and none was found (Eggers regression, p = 0.6; trim and fill method did not produce any imputed values).

Only three of the studies reported associated rates of hand and feet abnormalities in the control population, with a summary rate of 0.06% (6 per 10,000). This indicates that the presence of such an abnormality provides a high suspicion of an affected pregnancy. Of interest is the trend towards higher detection rates with advancing gestational age. Although not statistically significant, this may be an indication that as the fetus becomes larger, it is easier to visualize the fetal extremities and identify these abnormalities.

Summary: A total of 17 articles were identified and included for analysis of hand and feet abnormalities, with information on 615 cases of trisomy 18. For ultrasound exams performed between 15 and 20 weeks' gestation, the detection rate was 41%. However, there was a change in detection rate over time, with lower rates reported in more recent studies. This is likely to indicate both an improvement in equipment, and a general awareness among sonographers of the importance of examining the fetal extremities. Hand and feet anomalies such as described here, are rare in the few control populations examined, with a rate of about 6 per 10,000. In most setting, it should be possible to achieve a detection rate of about 41% at a false positive of 0.06%.

Table 8.2.3-1. Rates of hand and foot anomalies in trisomy 18 and control pregnancies

Author	Location	Design (prev)[1]	Gestational Age (weeks)[2]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Nyberg *et al.*, 1993)	US/WA	Ca only (high)	15 – 19 E	5/20 25%	
(Gupta *et al.*, 1997)	UK	Ca only (high)	16 – 20 E	5/19 26%	
(Gray *et al.*, 1996)	US	Ca only (high)	14 – 20 E	3/11 27%	
(Benacerraf *et al.*, 1990)	US/MA	Ca only (high)	13 – 20 E	6/13 46%	
(Benacerraf *et al.*, 1988) (early)	US/MA	Ca only (high)	15 – 19 E	4/ 8 50%	
(Yeo *et al.*, 2003)	US/NJ	Ca only (high)	15 – 19 E	16/16 100%	
(Brumfield *et al.*, 2000)	US/AL	Ca only (high)	14 – 22 M	3/30 10%	
(Cho *et al.*, 2009)	US/CA	Cohort (high)	15 – 21 M	20/56 36%	6 / 8,707 0.1%
(Tongsong *et al.*, 2002)	Thailand	Ca only (high)	16 – 22 M	10/25 40%	
(Watson *et al.*, 2008)	US	Ca only (high)	15 – 21 M	43/98 44%	
(Benacerraf *et al.*, 1994)	US/MA	Case/Cont (high)	14 – 21 M	6/13 46%	0 / 106 0.0%
(Viora *et al.*, 2007)	Italy	Ca only (high)	16 – 23 M	22/27 81%	
(Shields *et al.*, 1998)	US/WA	Ca only (high)	14 – 22 M	16/18 89%	
(Papp *et al.*, 2007)	Hungary	Ca only (high)	13 – 24 L	4/70 6%	
(Bahado-Singh *et al.*, 2003)	US/CT	Case/Cont (high)	14 – 24 L	16/47 34%	0 / 1,214 0.0%
(Bronsteen *et al.*, 2004)	US/MI	Ca only (high)	15 – 24 L	31/54 57%	
(Nicolaides *et al.*, 1992b)	UK	Ca only (high)	14 – 39 L	71/83 86%	
(Benacerraf *et al.*, 1988) (late)	US/MA	Ca only (high)	20 – 38 L	7/ 7 100%	
All Studies				**288/615 51%**	**6 / 10,027 0.06%**
(random effects)				(95% CI 38% to 63%)	(95% CI 0.02% to 1.3%)

[1] prev = population prevalence. High = referral population, general = unscreened population with background risk

[2] first and last completed week. The letters indicate a gestational age grouping (E=15-20 weeks, M=15-24, L=15-40)

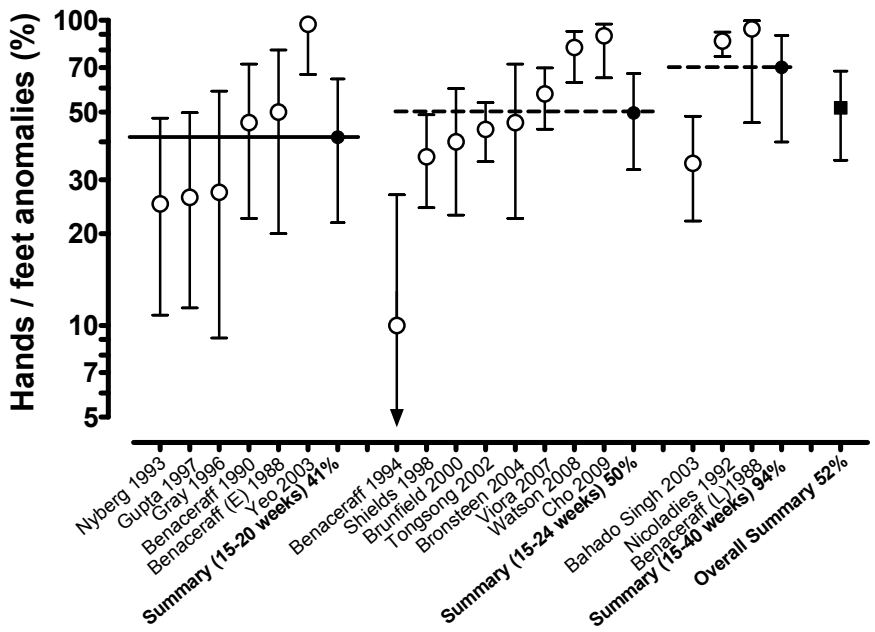

Figure 8.2.3-1. Forest plot showing the proportion of trisomy 18 pregnancies with hand and foot anomalies. The summary detection rate for ultrasound exams done between 15 and 20 weeks' gestation is 41%, with high unexplained heterogeneity. The proportion of trisomy 18 pregnancies with hand and foot anomalies is significantly higher, when exams are performed later in gestation.

8.2.4 Any structural anomaly

Definition: Descriptions for this category include non-specific terms for structural anomalies: 'gross defect', 'major defect', any 'structural defect', and 'structural abnormality'. This often included multiple outcomes (*e.g.,* both a heart defect and club feet). Specifically excluded were any non-structural anomalies covered in a later section (*e.g.,* choroid plexus cysts, nuchal skin fold thickness).

Literature search: Twenty articles reported the proportion of trisomy 18 pregnancies with any structural anomaly (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1994; Benacerraf *et al.*, 1992; Benacerraf *et al.*, 1988; Bottalico *et al.*, 2009; Bronsteen *et al.*, 2004; Brumfield *et al.*, 2000; Cho *et al.*, 2009; DeVore, 2000; Grandjean *et al.*, 1998; Gupta *et al.*, 1997; Moran *et al.*, 2002; Nyberg *et al.*, 1993; Papp *et al.*, 2008; Picklesimer *et al.*, 2005; Roberts *et al.*, 1993; Shields *et al.*, 1998; Taslimi *et al.*, 2005; Tongsong *et al.*, 2002). In two of these studies, it was possible to divide the reported cases into two separate gestational age groups (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1988), resulting in a total of 22 estimates of the detection rate.

Results: Table 8.2.4-1 provides a listing of the 20 studies and 22 datasets, the observed number of 18 trisomy pregnancies, the number and proportion with gross abnormalities as well as additional information, including the approximate time in gestation (early, middle and late), as defined earlier. The entries are ordered by increasing detection rate, within the three gestational age groupings. Among the 588 trisomy 18 pregnancies, 428 were reported to also have any structural anomaly. The summary estimate for the detection rate is 71%, (95% CI 61% to 79%). Heterogeneity is high ($Q=85$, $I^2=75\%$, $p<0.001$). Two of the three general population studies have very low detection rates, even though all three were in the late gestational age grouping. One from Australia (Roberts *et al.*, 1993) found 11% and another from Ireland (Moran *et al.*, 2002) found 33%. This is in contrast to the third from France (Grandjean *et al.*, 1998) that reported 79%. The difference between these findings may be that, in France, there is a systematic program of routine ultrasound aimed at identifying structural abnormalities in aneuploidies. This was not true for the two other general population studies. Because of this, it may reasonable to remove the other two studies because they do not represent routine practice at a referral center (Roberts *et al.*, 1993; Moran *et al.*, 2002). There is also one very high detection rate of 97% from a large US study (DeVore, 2000). This group is known for its intensive surveillance that is demonstrated by their associated false positive rate of over 15% (last column, Table

8.2.1-1). Other centers report false positive rates of about 1% to 3%. This level of surveillance is unlikely to represent routine practice at an average referral site, and it was also removed for the subsequent analyses. Figure 8.2.4-1 shows a forest plot for the trisomy 18 detection rates shown in Table 8.2.4-1, after these three outlying datasets were removed. The summary detection rate is now 72.4%. Among these remaining 19 estimates (17 studies), there is no difference in the detection rates by study design (p = 0.5), or when the results were regressed by year of publication (p = 0.8, for significant slope). However, there are important differences by time in gestation when the ultrasound was performed. Detection rates are 60% (95% CI 44% to 74%), 68% (95% CI 51% to 81%), and 83% (95% CI 72% to 90%) among the 7, 5 and 7 studies in the early, moderate and late gestational age groupings, respectively These differences are significant (p=0.01). However, there remains important unexplained heterogeneity in all of the groups (p-values 0.07, 0.01 and 0.001, with I^2 values of 49%, 44% and 80%, respectively). The 19 datasets were also examined for publication bias using two methods, but neither was statistically significant (Eggers regression intercept, p = 0.4, and the trim and fill method estimated a positive bias of 2% (observed detection of 72%, adjusted rate of 70%). Using all five reports for the rate in control pregnancies, the summary estimate is 2.6% (95% CI 0.8% to 8.3%) with very high heterogeneity (Q=510, I^2=99%, p<0.001). Were the one high estimate (DeVore, 2000) to be removed, the estimate is reduced to 1.6% (95% CI 0.8% to 3.2%), but the heterogeneity is still relatively high (Q=19, I^2=85%, p<0.001).

Summary: A total of 20 articles were identified and 22 datasets were included for analysis, with information on 588 cases of trisomy 18. When restricted to reports of routine and standardized ultrasound at an average referral center (average defined as finding 1% to 3% of chromosomally normal fetuses having any structural anomaly), 60% of trisomy 18 fetuses would be detected between 15 to 20 weeks' gestation. This rate has remained constant from the late 1980s to present. The detection rate for trisomy 18 appears to increase as the ultrasound exams are performed later in gestation, reaching a maximum of about 83% by the early third trimester. The remaining unexplained heterogeneity is likely due to the definition of gross anomalies, and/or sonographer(s) training, expertise or experience. The false positive rate is highly heterogeneous, but with one outlying high value removed, a reasonable estimate is 1.6%. In most settings, the detection rate is expected to be 60% at a 1.6% false positive rate.

Table 8.2.4-1. Rate of 'any structural anomaly' in trisomy 18 and control pregnancies

Author	Location	Design (prev)[1]	Gestational Age (weeks)[2]	Trisomy 18 positive / total (%)	Control positive / total (%)
(Gupta *et al.*, 1997)	UK	Case only (high)	16 – 20 E	6/19 32%	
(Bahado-Singh *et al.*, 2003)	US/CT	Case/Cont (high)	14 – 17 E	14/29 57%	9 / 1,214 0.7%
(Picklesimer *et al.*, 2005)	US/NC	Case only (high)	14 – 17 E	6/10 60%	
(Benacerraf *et al.*, 1988)	US/MA	Case only (high)	15 – 19 E	5/ 8 63%	
(Nyberg *et al.*, 1993)	US/WA	Case only (high)	15 – 19 E	15/20 75%	
(Benacerraf *et al.*, 1994)	US/MA	Case/Cont (high)	15 – 19 E	10/13 77%	2 / 106 1.9%
(Brumfield *et al.*, 2000)	US/AL	Case only (high)	14 – 22 M	10/30 33%	
(Cho *et al.*, 2009)	US/CA	Cohort (high)	15 – 21 M	36/56 64%	244 / 8,707 2.9%
(Taslimi *et al.*, 2005)	US/IL	Case only (high)	15 – 23 M	19/28 68%	
(Benacerraf *et al.*, 1990)	US/MA	Case/Cont (high)	14 – 21 M	9/13 69%	
(Shields *et al.*, 1998)	US/WA	Case only (high)	14 – 22 M	30/35 86%	
(Bottalico *et al.*, 2009)	US/NJ	Cohort (high)	15 – 22 M	7/ 8 88%	9 / 628 1.4%
(Tongsong *et al.*, 2002)	Thailand	Case only (high)	16 – 22 M	24/25 96%	
(DeVore, 2000)	US/CA	Case/Cont (high)	14 – 23 M	29/30 97%	314 / 2,000 15.7%
(Roberts *et al.*, 1993)	Australia	Case only (gen)	16 – 24 L	1/ 9 11%	
(Moran *et al.*, 2002)	Ireland	Case only (gen)	2nd trim. L	7/16 44%	
(Bahado-Singh *et al.*, 2003)	US/CT	Case/Cont (high)	18 – 24 L	13/18 72%	9 / 1,214 0.7%
(Grandjean *et al.*, 1998)	France	Case only (gen)	20 (7.1) L	64/81 79%	
(Papp *et al.*, 2007)	Hungary	Case only (high)	13 – 24 L	61/70 87%	
(Bronsteen *et al.*, 2004)	US/MI	Case only (high)	15 – 24 L	47/54 87%	
(Benacerraf *et al.*, 1992)	US/MA	Case only (high)	20 – 38 L	8/ 9 89%	
(Benacerraf *et al.*, 1988)	US/MA	Case only (high)	20 – 34 L	7/ 7 100%	
All				**428/588 71%** 95% CI (61 - 79%)	**587/13,869 2.6%** 95% CI (0.8% to 8.3%)

[1] prev = population prevalence. High = referral population, general = unscreened population with background risk
[2] first and last completed week, or mean (SD). The letters indicate a gestational age grouping (E=earlier, M=moderate, L=later)

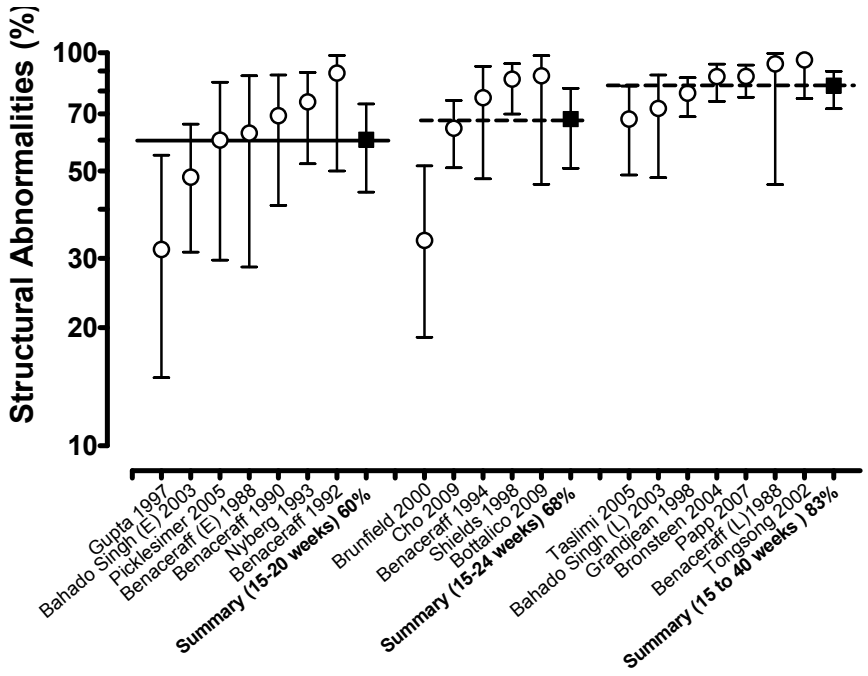

Figure 8.2.4-1. Forest plot showing the proportion of trisomy 18 pregnancies with any structural anomaly. Results are after excluding three datasets (two low, one high). Although the summary detection rate is 72% (not shown), some of the heterogeneity is explained by the gestational ages at the time of ultrasound that are included. The solid line is the estimate relevant to early second trimester ultrasound testing for trisomy 18 (solid summary line at 60%). Higher proportions are found when later ultrasound studies are also included in the publications (dashed summary lines at 68% and 83%).

8.3 Non-structural Anomalies

The non-structural anomalies in this section are sometimes referred to as 'soft' ultrasound markers. They differ from structural anomalies (Chapter 8.2), in that they do not, by themselves, cause problems for the fetus or newborn. Most of these markers were originally studied because of a relationship with Down syndrome. Often, these are physical characteristics known to manifest in children with Down syndrome and/or trisomy 18. For example, children with Down syndrome and trisomy 18 are growth retarded at birth, and this has led to the observation of shortened long bone measurements in the second trimester (*e.g.*, humerus, femur). Another example is the excess skin at the back of the neck in newborns and children with Down syndrome. This led to measuring the nuchal skin fold (NSF) thickness in Down syndrome and in other fetuses with chromosomal abnormalities. The exception to this rule is the finding of cysts in the choroid plexus (CPC). CPCs are generally considered benign and usually resolve by late in the second trimester. In general, these non-structural anomalies can be characterized as being of moderate sensitivity, but low specificity.

8.3.1 Choriod plexus cyst (CPC)

Definition: The first identification of a CPC in a fetus was reported in 1984 from a group in London, England (Chudleigh *et al.*, 1984) using early second trimester ultrasound. Their description reads:

> Ultrasound scanning during the second trimester pregnancy revealed cysts of the choroid plexus in the posterior horn of the lateral ventricle. In two (of the five) cases, they were obviously bilateral. Careful follow up high resolution ultrasound examination showed that all the cysts disappeared spontaneously between 20 -23 weeks' gestation and in no cases was there any suggestion of hydrocephaly.

The choroid plexuses are located in the lateral ventricles and are responsible for the production of cerebrospinal fluid. In about 1 to 3% of fetuses, there is an echoic region within the choroid plexus – a CPC. Figure 8.3.1-1 shows an ultrasound image of a choroid plexus cyst (CPC).

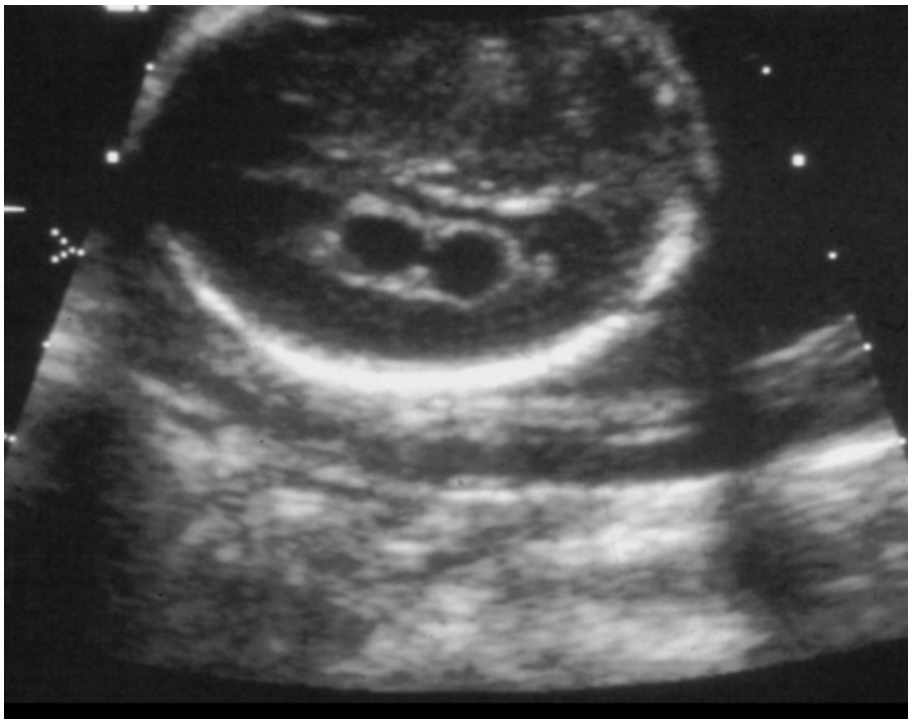

Figure 8.3.1-1. Choriod plexus cyst (CPC). A cross-sectional view of a fetal head at 17.9 weeks' gestation showing the outline of the skull, the lateral ventricles, and, within the choroid plexus, two large cysts (from Alan Donnenfeld, M.D, personal communication).

Publications, study designs and confounders: Sonographers in the mid 1980s thought CPCs were rare, and suggested that the benign nature of the cysts might warrant a repeat ultrasound examination at 24 weeks' gestation to confirm their disappearance (Chudleigh *et al.*, 1984). Over the next five years, at least 12 studies were published that looked at the presence of CPC in the second trimester and associated abnormalities; mainly trisomy 18 (Figure 8.3.1-1). The number of publications now exceeds 60, and to make sense of them, it is necessary to stratify results by study design. The following sections define three main study designs: cohort, case/control, and those studies in which all enrollees have an identified CPC (*e.g.,* test positives only). Since all of these women are, in a sense, screen positive, the term for this type of study will be 'screen positive'. Each of these designs has been used in both general population and high risk settings (*e.g.*,

referred pregnancies, women over age 34). Several additional covariates will be also be included in the analyses. All of these factors combine into making the interpretation of the literature on CPC and trisomy 18 difficult, and give rise to the multitude of conflicting summary analyses that characterize this literature.

Impact of various study designs

- Cohort: a consecutive series of pregnant women having ultrasound testing in a given region or at a given center. In these studies, it is possible to determine the false positive rate (proportion of women without trisomy 18 or other aneuploidy) with a CPC, as well as the detection rate (proportion of trisomy 18 cases with a CPC) and OAPR (odds for the women with a CPC having a fetus with trisomy 18). The prevalence of trisomy 18 in the population can also be determined.
- Case/control: a series of ultrasound results from women with a trisomy 18 pregnancy are compared to a matched (or unmatched) series of results from women without trisomy 18 pregnancies. Both detection and false positive rates can be determined, but OAPR and prevalence cannot be computed directly.
- Case-only: a series of ultrasound results from women with an affected pregnancy are collected. Only the detection rate can be computed.
- Screen positive: a series of women whose ultrasound shows a CPC are all followed to determine pregnancy outcome (*e.g.,* trisomy 18, normal). Only the OAPR (and perhaps the false positive rate) can be computed. Neither the detection rate nor prevalence can be computed. Comparison of these rates to those derived from other study types is problematic.

Impact of the prevalence of trisomy 18 in the population studied

- General population: an unselected pregnancy population in which the incidence of trisomy 18 should be representative (*i.e.,* about 1:1,500 in the second trimester).
- High risk: a selected population that is likely to have either a higher rate of CPC than expected (due to referrals) and/or a higher rate of trisomy 18 (due to indications for referral such as maternal age, positive serum biochemistry, or ultrasound finding of gross abnormalities).
- Low risk: a selected population that is likely to have a lower rate of trisomy 18 than expected, usually due to active screening (either biochemical or ultrasound based) that occurs earlier in the pregnancy (*e.g.,* screen negative results for the first trimester combined testing).

Consideration of other important covariates

- Improvement in technique/equipment: There is little question that the equipment has vastly improved since the 1984 report of second trimester CPC identification (Chudleigh *et al.*, 1984). This may allow the detection of smaller or less well-defined CPC, resulting in a higher proportion of the population being screen positive. Earlier studies rarely included CPC that did not measure at least 4 to 5 mm in diameter. As equipment improved, CPC of 1 or 2 mm could be visualized and were reported. Currently, there are formal recommendations to ignore these small CPCs. In 2005, for example, the Society of Obstetricians and Gynecologists of Canada suggested that anything smaller than 3 mm not be considered a CPC (www.SOGC.org). Thus, the definition of a CPC may have changed over time from rather large and easy to distinguish to all sizes visualized, and now to only those of a certain size being considered.
- Isolated CPC versus CPC with other US findings (non-isolated): After the first flurry of reports, some researchers began to look for patterns that might subdivide pregnancies with a CPC into higher and lower risk. Such findings included laterality, size and whether or not the CPC was associated with another US finding. Only isolated/non-isolated seems to be of importance. However, given the improvement of equipment, it is also possible that the definition of 'isolated' has changed over time, with earlier studies more often unable to locate other abnormalities. In addition, the definition and number of 'abnormalities' is changing. Originally, only gross structural defects were considered (*e.g.,* omphalocele, major heart defect, clenched fist), while some more recent studies include several of the 'soft' ultrasound markers (*e.g.,* femur length, nuchal fold thickness) as abnormalities.
- Prospective/retrospective: Many authors pay careful attention to the nature of the observations, but do not correctly classify them. Often, a large series of non-structural anomalies and pregnancy outcomes are available. These could be collected with the intent of performing an analysis involving CPC where the US information is recorded prior to the collection of outcome (prospective collection/analysis). However, it is more common that both findings have been recorded in a retrospective analysis of prospectively collected data (US information recorded prior to knowledge of the outcome). True retrospective studies (finding those with/without trisomy 18 and then interpreting the results of

the stored US image) are relatively rare. Such studies might be subject to a strong reviewer bias. Blinding would not be sufficient, as the US would contain strong indications of trisomy 18 (*e.g.*, gross structural anomalies) that might influence the interpretation of the CPC.

- Trimester of ultrasound: Many of the non-structural anomalies are more, or less, likely to occur (or be identified) as gestation advances. Heart defects are hard to identify early in the second trimester because the heart is relatively small. This is the reason behind the anomaly scan being generally targeted at 20 weeks' gestation. However, CPCs usually disappear by 24 to 28 weeks' gestation, and any study looking at this later time period might fail to find the association. For this reason, the current analysis focuses only on the 15 to 20 week time period. If the mean gestational age or range is outside of this window for more than a small proportion, the study might be eliminated. Alternatively, if sufficient individual data are presented, a subset of cases can be analyzed that do fall within the targeted range.
- Timing of diagnosis: in many of the case/control or case-only studies, the women were at high risk and had already chosen amniocentesis. This has the advantage of knowing about all cases of trisomy 18 regardless of associated defect, but has the disadvantage of selection bias. For example, if women know about gross abnormalities found prior to the procedure, their choice will almost certainly be influenced towards acceptance of amniocentesis based on the non-structural anomalies. This would result in a higher proportion of trisomy 18 pregnancies having amniocentesis also having any structural anomaly. In a cohort study the result could be unbiased, if the women who do not choose amniocentesis eventually have the diagnosis made at or near birth. However, given the high rate of spontaneous loss for trisomy 18 pregnancies, this would be unlikely.

Figure 8.3.1-2 displays the 61 studies that will be included in the analysis of CPC and trisomy 18 by year of publication and also shows the type of study (symbol) and whether it was performed in a general (larger and open) or high risk (smaller and filled) population. As expected, some designs will have relatively large numbers of trisomy 18 pregnancies studied (*e.g.*, case/control, case-only), while others will have smaller numbers of affected fetuses (*e.g.*, cohort or screen positive). In general, the studies using the screen positive study design were focused only on CPC and not other abnormalities, while most of the case-only and

case/control studies provided information on other possible trisomy 18 markers (both structural anomalies and non-structural anomalies).

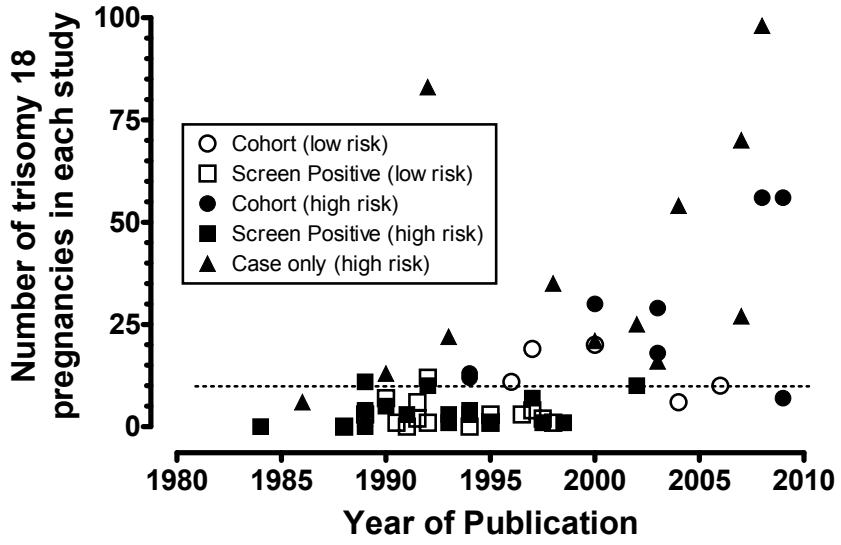

Figure 8.3.1-2. Sixty-one published studies regarding choroid plexus cysts (CPC) and trisomy 18. Each symbol represents a single publication. Open symbols indicate studies performed in the general population (background risk of both CPC and trisomy 18), while filled symbols indicate those studies that have a high risk (of either CPC, trisomy 18, or both). Circles indicate cohort or case/control studies, squares indicate studies that reported only on pregnancies in which a CPC had been identified (screen positive design), and diamonds indicate studies that only included pregnancies with trisomy 18 (case-only). A horizontal dotted line is drawn at 10 cases to indicate studies with relatively low power.

Results: Population-based cohort studies

Table 8.3.1-1 shows the four population-based cohort studies of CPCs and trisomy 18 (Cheng *et al.*, 2006; Coco and Jeanty, 2004; Ghidini *et al.*, 2000; Gray *et al.*, 1996). The one 'case-only' study from the general population has also been included in the analysis of the detection rate (Gupta *et al.*, 1997). Among the four cohort studies, a total of 53,151 women in the early second trimester of pregnancy (usually <20 weeks' gestation) were scanned for CPCs. Overall, 47 cases of

trisomy 18 were identified. Among these five studies the rate of CPCs was 1.4% (range 0.98% to 2.9%). The analysis shows high heterogeneity between these rates (Q=247, I^2=99%). One way to validate the general population nature and completeness of follow-up for these studies is to examine the prevalence of trisomy 18. Overall the second trimester prevalence is expected to be about 1:1500, and the observed rate of 1:1300 is consistent with that expectation. All five studies in Table 8.3.1-1 can be used to determine the overall, detection rate of 29% for any CPC and trisomy 18. The estimates have low heterogeneity (Q=1, I^2=0%, p=NS), perhaps due to the small number of cases analyzed. When CPCs are the only finding (*i.e.,* isolated), the likelihood of identifying trisomy18 appears low (none observed in 765 pregnancies with isolated CPCs). However, the rate is not likely to be zero, as the one included case-only study reported two trisomy 18 fetuses where CPC was isolated. On the other hand, when CPCs were associated with another ultrasound abnormality (usually a structural anomaly), the likelihood of trisomy 18 fetus was high (15 cases in 89 pregnancies).

Table 8.3.1-1. Choroid plexus cysts (CPCs) and trisomy 18: Population based cohort studies

Study	Location	GA weeks	Number		Rate	Trisomy 18			OAPR for CPC	
			Total	CPC		Total	Prev (1:n)	w/CPC (%)	Isolated	w/abnorm
(Cheng *et al.*, 2006)	Taiwan	16-20	7,795	98	1.3%	10	1: 780	3 (30)	0/ 82	3/13 (1: 4)
(Coco and Jeanty, 2004)	Italy	16-18	12,670	364	2.9%	6	1:2,112	2 (33)	0/311	2/51 (1:25)
(Ghidini *et al.*, 2000)	Italy	16-23	23,842	199	0.8%	20	1:1,192	7 (35)	0/181	7/11 (1: 2)
(Gray *et al.*, 1996)	US	14-20[1]	18,844	208	1.1%	11	1:1,713	3 (27)	0/191	3/14 (1: 5)
All (random effects)			**53,151**	**869**	**1.4%**	**47**	**1:1300**	**19 (32)**	**0/765**	**15/89 (1: 3)**
					(0.7%-2.5%)	(66)	(1:880-1:1900)	(20% -47%)	**<1:765**	(1:1 - 1:15)
(Gupta *et al.*, 1997)[2]	UK	14-20				19		4[3] (21)	2/ NR[3]	2 / NR[3]

GA = gestational age, Prev = prevalence, OAPR = odds of being affected given a positive result, w/abnorm = other important ultrasound abnormality identified during the same examination.

[1] Originally 14-26 weeks, but trisomy 18 cases after 20 weeks have been removed

[2] Case-only design from the general population. Data not included in the analyses

[3] Two of these four were isolated CPCs

Results: Population-based 'screen positive' studies:

Table 8.3.1-2 summarizes the 16 studies reporting on the results of a routine ultrasound finding of CPCs in a general pregnancy population. They contain the outcomes for only those with a CPC identified (e.g., screen positive for CPCs) (Achiron *et al.*, 1991; Camurri and Ventura, 1989; Chinn *et al.*, 1991; Chitty *et al.*, 1998; Clark *et al.*, 1988; Digiovanni *et al.*, 1997; Geary *et al.*, 1997; Howard *et al.*, 1992; McHugo *et al.*, 1991; Ostlere *et al.*, 1990; Perpignano *et al.*, 1992; Reinsch, 1997; Twining *et al.*, 1991; Beke *et al.*, 2008; Gupta *et al.*, 1995; Walkinshaw *et al.*, 1994). One of these studies (Gupta *et al.*, 1995) reports on separate populations from Dundee and Yorkshire that are listed separately, yielding 17 datasets for analysis. Three identified studies were not included in the analysis: two did not report the number of women with a CPC identified (Ricketts *et al.*, 1987; Hertzberg *et al.*, 1989) and one reported on gestational ages outside of the early second trimester (Chitkara *et al.*, 1988).

These studies reported results in over 375,000 early second trimester pregnancies with widely varying definitions of a CPC. Overall, CPCs were identified in 0.8%, with high heterogeneity (Q=1830, I^2=98%, p<0.001). This is somewhat lower than the 1.4% found in the cohort studies (Table 8.3.1-1). This difference may be related to the dates of publication. The median year of publication for the screen positive studies is 1992, while the earliest publication date for the general population cohort studies is 1996. Figure 8.3.1-3 shows a meta-regression of publication year versus proportion of pregnancies with a CPC for the 21 datasets (17 screen positive and 4 cohort). There is a statistically significant relationship (slope=0.11454, intercept=-233.637) that predicts CPC rates of 0.6%, 1.0% and 1.8% in 1995, 2000 and 2005, respectively. This relationship is potentially explained by the changing definition for a CPC. In the late 1980s and early 1990s, it was common to define CPCs as being 3, 4 or even 5 mm or larger. Now, the definition usually includes CPCs as small as 2 or 3 mm. For example, an earlier report (Gupta *et al.*, 1995) used a definition of >5 mm (expanding it later in the study to smaller cysts) and reported a rate of 0.3%. In a more recent study (Beke *et al.*, 2008), the definition of ≥2mm was used, with a reported rate over 10 times higher (4.0%). Among those pregnancies identified with a CPC, an average of 2.2% were identified with trisomy 18 with low heterogeneity between studies (Q=17, I^2=6%, p=NS).

Not all of the studies reported whether the CPC occurred as an isolated finding, or in combination with one or more additional non-structural anomalies (last two columns in Table 8.3.1-2). Among the 12 studies reporting this information, 13 of the 44 cases of trisomy 18 (30%, 95% CI 17% to 45%) were found to have CPCs as the only finding. Half of the studies found at least one instance of a fetus with trisomy 18 having CPCs as the only ultrasound finding. The prevalence of trisomy 18 among pregnancies with isolated CPCs was 1:217 (95% CI 1:125 – 1:400). Among those with ultrasound anomalies in addition to CPCs the prevalence was much higher 1:10 (95% CI 1:7 – 1:15). Although this is similar to the pattern found in the population cohort studies (Table 8.3.1-1), there is a less striking difference between isolated and non-isolated CPCs in these studies from the general population.

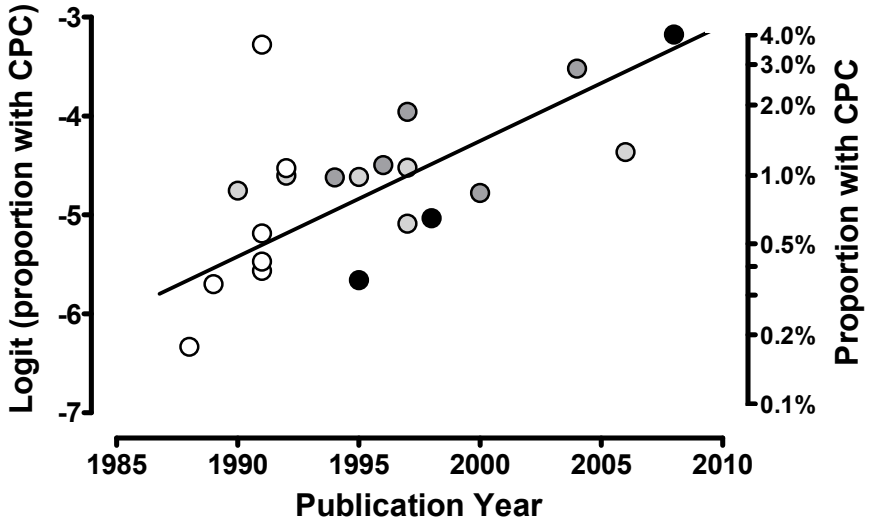

Figure 8.3.1-3. Relationship between the proportion of women with a CPC identified and year of publication, for studies in the general population. The darkness of the data points indicates the relative weight (darker circles indicating studies with more observations).

Table 8.3.1-2. Choroid plexus cysts (CPCs) and trisomy 18: General population 'screen positive' studies

Study	Location	GA Weeks[1]	CPC Size[2]	Number studied	All CPCs N (%)	All T18 N (%)	OAPR for CPC Isolated	OAPR for CPC w/abnorm
(Achiron *et al.*, 1991)	Israel	20 / NR	≥8 mm	5,400	30 (0.6)	2 (6.7)	1: NR	1: NR
(Beke *et al.*, 2008)	Hungary	15 -21	≥2 mm	10,875	436 (4.0)	6 (1.4)	3:278	3:112
(Camurri and Ventura, 1989)	Italy	16-20	NR	3,000	10 (0.3)	1 (10)	0: NR	1: NR
(Chinn *et al.*, 1991)	US/CA	15-24	>2 mm	1,045	38 (3.6)	0 (0.0)	0: 36	0: 2
(Chitty *et al.*, 1998)	UK	14-24	≥3 mm	101,600	658 (0.6)	12 (1.8)	2:603	10: 55
(Clark *et al.*, 1988)	US/UT	16-22	≥3 mm	2,820	5 (0.2)	0 (0.0)	NR	NR
(Digiovanni *et al.*, 1997)	US/IL	15-22	NR	8,270	89 (1.1)	3 (3.4)	NR	NR
(Geary *et al.*, 1997)	UK	18-20	pres/abs	13,690	84 (0.6)	3 (3.6)	0: 78	3: 6
(Gupta *et al.*, 1995)	Dundee	16-21	NR	7,250	71 (1.0)	1 (1.4)	1: 62	0: 9
(Gupta *et al.*, 1995)	Yorkshire	16-20	>5, any	151,000	524 (0.3)	7 (1.3)	0:486	7: 38
(Howard *et al.*, 1992)	UK	18-20	NR	4,765	51 (1.1)	1 (1.2)	NR	NR
(McHugo *et al.*, 1991)	UK	mid-trim.	pres/abs	4,984	19 (0.4)	0 (0.0)	NR	NR
(Ostlere *et al.*, 1990)	UK	16-18	pres/abs	11,700	100 (0.9)	3 (3.0)	0: NR	3: NR
(Perpignano *et al.*, 1992)	US/NY	18 / 2.7	≥2 mm	8,769	87 (1.0)	4 (4.6)	3: NR	1: NR
(Reinsch, 1997)	US/CA	18-21	pres/abs	16,059	301 (1.9)	2 (0.7)	0:263	2: 38
(Twining *et al.*, 1991)	UK	18-20	≥3 mm	4,541	19 (0.4)	1 (5.3)	NR	NR
(Walkinshaw *et al.*, 1994)	UK	17-19	>5 mm	15,565	152 (1.0)	3 (2.0)	3:151	0: 1
				377,621	**2,715 (0.8)**	**49 (2.2)**	**9:1,957**	**25:261**
					(0.5 - 1.3%)	(1.7 – 3.0%)	**1:217** (114 – 475)	**1:10** (7 – 16)

[1] Gestational age range, or mean / standard deviation
[2] lower cutoff (*e.g.,* >3 mm), lowest reported size (*e.g.,* 8 mm) or not specified (*i.e.,* presence/absence)
NR = not reported

Results - High risk case/control and cohort studies:

The next series of studies is from high risk populations (*e.g.*, screen positive via biochemistry, advanced maternal age). Table 8.3.1-3 summarizes the data from eight studies (Bahado-Singh *et al.*, 2003; Benacerraf *et al.*, 1994; DeVore, 2000; Seoud *et al.*, 1994; Bottalico *et al.*, 2009; Cho *et al.*, 2009; Watson *et al.*, 2008; Goetzinger *et al.*, 2008). One study (Bahado-Singh *et al.*, 2003) provided sufficient data so that the data could be separated into those less than 17.5 weeks, and those at or beyond 17.5 weeks. Thus, nine datasets were available for analysis. One study (Drugan *et al.*, 1996) was excluded because sufficient information could not be obtained from the report. Case control and cohort studies were reported and analyzed separately. Only one study specified the size of >3 mm (Bottalico *et al.*, 2009), but since most of the studies were performed after 1999, it is likely they used a relatively consistent definition of greater than 2 or 3 mm.

Among the four case/control studies (five datasets), a total of 3,370 unaffected pregnancies were included, with a rate of CPCs of 4.4%. One study (Seoud *et al.*, 1994) had a relatively high rate of 18%, but this estimate was based on only 50 observations. It is mainly responsible for the identified heterogeneity (Q=30, I^2=87%, p<0.001). These four studies also included 102 trisomy 18 pregnancies (prevalence 1:33). Among these cases, 43% (44 cases) had a CPC identified during the ultrasound examination. Heterogeneity was low (Q=2.5, I^2=0%, p=0.6).

The three cohort studies included 148,484 unaffected pregnancies, with a 3.5% rate of CPCs (Q=100, I^2=97%, p<0.001). The smallest study (Bottalico *et al.*, 2009) reported a high rate of 8.3%, but included only 628 unaffected pregnancies. Two of these studies reported CPC measurements on a total of 112 trisomy 18 pregnancies (prevalence 1:707). CPCs were identified in 47% (52 cases). Heterogeneity was again low (Q=1.3, I^2=22%, p=0.3).

Although there were some differences in the rate of identifying CPCs in the unaffected population, the overall rate for all eight studies is 4.0% (I^2>90%). There is, however, good consistency among the detection rates, with 43% of cases having a CPC identified via ultrasound (Q=4.7, I^2=0%, p=0.7).

It is possible to stratify the results further, based on whether the CPC was an isolated finding. Three studies reported whether the CPC was an isolated finding, for both the cases and controls (Benacerraf *et al.*, 1994; Cho *et al.*, 2009;

Goetzinger *et al.*, 2008). Among the 114 trisomy 18 fetuses, 7% (seven cases) had an isolated CPC, and the associated heterogeneity was low (Q=3.8, I^2=58%, p=0.15). According to these studies, about 1 in 14 second trimester trisomy 18 fetuses might have CPCs as the only ultrasound finding. Among the corresponding 70,924 unaffected pregnancies, 0.19% had isolated CPCs. Heterogeneity of this finding was also low (Q=0.3, I^2=0%, p=0.8).

Table 8.3.1-3. Choroid plexus cysts (CPCs) and trisomy 18: High risk case/control and cohort studies

Study	Location	GA[1] weeks	Unaffected pregnancies			Trisomy 18			Isolated CPC	
			Total	CPC	Rate (%)	Total	Prev	w/CPC (%)	T18	UA
Case/control										
(Bahado-Singh *et al.*, 2003)	US/CT	14-17.5	767	24	3.1%	29	1: 26	9 (31)		
(Bahado-Singh *et al.*, 2003)	US/CT	17.6-24	447	21	4.7%	18	1: 25	8 (44)		
(Benacerraf *et al.*, 1994)	US/MA	14-21	106	2	1.9%	13	1: 8	6 (46)	1/ 13	2/ 106
(DeVore, 2000)	US/CA	14-23	2,000	56	2.8%	30	1: 67	15 (50)		
(Seoud *et al.*, 1994)	US/VA	22 / 5	50	9	18.0%	12	1: 4	6 (50)		
Subtotal			**3,370**	**112**	**4.4%**	**102**	**1: 33**	**44 (43)**		
(random effects)					(2.7-7.2)			(34-53)		
Cohort										
(Bottalico *et al.*, 2009)	US/NJ	15-22	628	52	8.3%	7	1: 90	NR		
(Cho *et al.*, 2009)	US/CA	15-21	8,707	221	2.5%	56	1: 155	29 (52)	6/ 45	167/ 8707
(Goetzinger *et al.*, 2008)	US/MO	15-21	63,222	1,322	2.1%	56	1:1,129	23 (41)	1/ 56	111/62111
Subtotal			**72,557**	**1,595**	**3.5%**	**112**	**1: 605**	**52 (47)**		
(random effects)					(2.0-5.9)			(37-56)		
All[2]			**75,927**	**1,707**	**4.0%**	**214**		**96 (45)**	**7/114**	**280/70924**
(random effects)					(2.8-5.7)			(38-52)	**7.2%**	**0.19%**
									(2.2-21)	(0.17-0.21)

[1] Gestational age range, or mean / standard deviation

Results - High risk 'screen positive' studies:

The next series of 17 studies (Table 8.3.1-4) reported only on women who had a CPC identified as part of the second trimester ultrasound study (*i.e.,* screen positive only). The first seven studies (Chan *et al.*, 1989; DeRoo *et al.*, 1988; Gabrielli *et al.*, 1989; Kupferminc *et al.*, 1994; Morcos *et al.*, 1998; Platt *et al.*, 1991; Porto *et al.*, 1993) report the total number of pregnancies scanned (denominator), while the latter 10 do not (Bakos *et al.*, 1998; Benacerraf *et al.*, 1989; Bird *et al.*, 2002; Chudleigh *et al.*, 1984; Gross *et al.*, 1995; Nadel *et al.*, 1992; Nava *et al.*, 1994; Oettinger *et al.*, 1993; Thorpe-Beeston *et al.*, 1990; Jou, 1997).

The first seven studies reported the number of women scanned in the second trimester (30,325) and found 492 (1.6%) with CPCs (95% CI 1.1% - 2.4%). Heterogeneity was high (Q=102, I^2=94%, p<0.001). Among these 429 fetuses, trisomy 18 was diagnosed in 25 (5%). In the remaining 10 studies, a total of 1,687 women with a CPC were identified, and 39 (2%) were diagnosed with trisomy 18. Among all 17 studies, eight provided information on other non-structural anomalies in addition to CPCs for both cases and controls. One study (Gabrielli *et al.*, 1989) reported this information for cases only and, therefore, is not included in the analysis. The odds for trisomy 18 among fetuses with an isolated CPC are 1:156, compared to 1:7 when additional ultrasound anomalies are present.

Table 8.3.1-4. Choroid plexus cysts (CPCs) and trisomy 18: High risk 'screen positive' studies

Study	Location	GA[1] (weeks)	CPC Size	Number Studied	N (%) All CPCs	N (%) All T18	Isolated OAPR for CPC	w/abnormality OAPR for CPC
(Chan *et al.*, 1989)	US/PA	15-24	≥3 mm	513	13 (2.5)	11 (85)	-	
(DeRoo *et al.*, 1988)	US/NH	14-21	>2 mm	1,565	12 (0.8)	0 (0)	-	
(Gabrielli *et al.*, 1989)	US/Italy	16-28	NR	933	21 (2.3)	4 (19)	0 / NR	4 / NR
(Kupferminc *et al.*, 1994)	US/IL	19.5/2.8	NR	9,100	102 (1.1)	3 (3)	1 / 98	2 / 4
(Morcos *et al.*, 1998)	US/CA	2nd trim	≥2 mm	7,617	210 (2.8)	1 (<1)	0 / 181	1 / 29
(Platt *et al.*, 1991)	US/CA	15-22	NR	7,350	71 (1.0)	3 (4)	-	
(Porto *et al.*, 1993)	US/CA	15-22	>2 mm	3,247	63 (1.9)	3 (5)	-	
Subtotal				**30,325**	**492 (1.6)** (1.1 – 2.4)	**25 (5)**		
(Bakos *et al.*, 1998)	Sweden	11-20	≥3 mm	-	50	1 (2)	-	
(Benacerraf *et al.*, 1989)	US/MA	16-18.4	NR	-	14	0 (0)	-	
(Bird *et al.*, 2002)	US/CA	2nd trim	NR	-	395	10 (3)	1 / 341	1 / 54
(Chudleigh *et al.*, 1984)	UK	17-19	NR	-	5	0 (0)	-	
(Gross *et al.*, 1995)	US/TN	14-22	NR	-	80	1 (1)	0 / 74	1 / 6
(Jou, 1997)	Taiwan	13-24	NR	-	108	7 (7)	5 / 95	2 / 6
(Nadel *et al.*, 1992)	US/MA	14-22	NR	-	234	10 (4)	0 / 220	10 / 14
(Nava *et al.*, 1994)	US/PA	19.6/3	≥4 mm	-	211	4 (2)	1 / 193	3 / 18
(Oettinger *et al.*, 1993)	Israel	2nd trim	NR	-	14	1 (7)	-	
(Thorpe-Beeston *et al.*, 1990)	UK	15-18	≥3 mm	-	83	5 (6)	0 / 49	5 / 34
Subtotal					**1,687**	**39 (2)**		
All					**2,179**	**64 (3)**	**8 / 1,251** **1:156**	**25 / 165** **1:7**

[1] Gestational age range, or mean/standard deviation

Results - High risk case-only studies

The next series of CPC studies is case-only, performed in a high risk setting. A total of 13 second trimester studies were identified (Benacerraf *et al.*, 1990; Bronsteen *et al.*, 2004; Brumfield *et al.*, 2000; Bundy *et al.*, 1986; Fitzsimmons *et al.*, 1989; Nicolaides *et al.*, 1992b; Papp *et al.*, 2008; Shields *et al.*, 1998; Tongsong *et al.*, 2002; Viora *et al.*, 2007; Yeo *et al.*, 2003; Nyberg *et al.*, 1993; Watson *et al.*, 2008) (Table 8.3.1-5). Among the 568 cases, 41% were found to have CPCs. Heterogeneity was low (Q=14, I^2=14, p=0.3). Eight of these studies reported whether the CPC was an isolated finding. Overall, 8% of the 260 cases from these studies had a CPC as the only ultrasound finding. Two of these studies (Papp *et al.*, 2008; Shields *et al.*, 1998) reported 10 of the 14 cases with isolated CPCs. One possibility for this might be that both studies included a small number of pregnancies prior to 15 weeks' gestation, when some of the other non-structural anomalies in trisomy 18 fetuses are difficult to visualize and/or measure. Regardless, the heterogeneity was still low (Q=11, I^2=34%, p=0.2).

Table 8.3.1-5. Choroid plexus cysts (CPCs) and trisomy 18: High risk positive case only studies

Study	Location	GA (weeks)	CPC size	Trisomy 18				
				Total	CPC	CPC (%)	Isolated CPC	N (%)
(Benacerraf *et al.*, 1990)	US/MA	13-20	NR	13	4	31%	1	8%
(Bronsteen *et al.*, 2004)	US/MI	15-24	NR	54	22	41%	NR	NR
(Brumfield *et al.*, 2000)	US/AL	14-22	NR	21	9	43%	NR	NR
(Bundy *et al.*, 1986)	US/MA	15-18	NR	6	1	17%	1	17%
(Fitzsimmons *et al.*, 1989)	US/WA	18-19	NR	2	2	100%	1	50%
(Nicolaides *et al.*, 1992b)	UK	14-39	NR	83	39	47%	NR	NR
(Nyberg *et al.*, 1993)	US/WA	15-19	NR	20	7	35%	1	5%
(Papp *et al.*, 2008)	Hungary	13-24	NR	70	27	39%	5	7%
(Shields *et al.*, 1998)	US/WA	14-22	NR	35	15	43%	5	14%
(Tongsong *et al.*, 2002)	Thailand	16-22	NR	25	10	40%	NR	NR
(Viora *et al.*, 2007)	Italy	16-23	NR	27	8	30%	NR	NR
(Watson *et al.*, 2008)	US/MN	15-21	NR	98	39	40%	0	0%
(Yeo *et al.*, 2003)	US	15-19	AIUM	16	16	100%	0	0%
All				**568**	**238**	**41%**	**14**	**8%**
(random effects)						(36 – 46)		(4 to 16)

AIUM = American Institute of Ultrasound Medicine

Conclusions: trisomy 18 and CPCs:

Given the large number of studies with widely varying designs, settings, equipment and sonographer training, it can be difficult to come to a simple conclusion. However, two findings are clear:

- Prospective population-based studies find both a lower detection rate (29%) and false positive rate (0.8% to 1.4%) than do studies in high risk settings (detection rates of 44%, 52% and 41% at false positive rates of 3.5%, 2.1% and 1.6%). This is most likely due to the setting, as population-based studies are more likely to rely on less comprehensive ultrasound scans being done by less skilled personnel with equipment that may not be as up-to-date. The data from the prospective studies will be used as the summary estimates, appropriate for use in the general population.
- The finding that isolated CPCs are far less likely to be associated with trisomy 18 is obvious, once the evidence on associated anomalies is taken into account. Over time, with the addition of more 'soft' markers, improved equipment and sonographer training, the likelihood of not finding at least one other marker in a fetus with trisomy 18 is becoming smaller.

In reviewing the data on CPCs as a marker for trisomy 18, several reports provide insight and are summarized below:

- Equipment: In one study (Ostlere *et al.*, 1990), an older Hitachi EUB 25 ultrasound machine found a rate of 1:300 for CPC. However, the same sonographers using a higher resolution Hitachi EUB 340 in the same population found a rate of 1:90, three times higher.
- Bilateral CPC/Persistence: Several studies provide evidence that neither size, bilaterality, nor persistence of isolated CPCs are indicators of a trisomy 18 fetus (Kupferminc *et al.*, 1994; Nava *et al.*, 1994; Perpignano *et al.*, 1992).
- CPCs and Down syndrome: A 1998 review of CPC and Down syndrome (Chitty *et al.*, 1998) identified 32 studies reporting on 4,342 fetuses. The overall incidence of Down syndrome was 1:150. However, since some of those studies were in high risk populations due to maternal age, non-structural anomalies and serum screening results, this rate is difficult to interpret. If the data are restricted to the 13 studies performed in the general pregnancy population (1,494 CPC in 247,406 women), the incidence is considerably lower (1:239). In that same group of studies, the risk for Down syndrome among women with isolated CPC is only 1:1,962. This provides

strong evidence that the association of CPC, especially isolated CPC, with Down syndrome is relatively weak.

- Long-term follow-up: One group followed the children of women with isolated CPCs and normal karyotypes after birth (Digiovanni *et al.*, 1997). Among 76 children followed between one and five years, all had normal early childhood development as measured by the modified Denver II Developmental Screening Test.

8.3.2 Nuchal skin fold thickness (NSF)

Definition: Thickening of the skin at the back of the neck is a common finding in newborns and infants with trisomy 18 (earlier analysis). By 1990, sonographers were looking for this finding in the early second trimester. NSF thickening was described in 1992 as (Benacerraf 1992):

> "Measurement from the outer limit of the occipital bone to the skin edge using a modified transverse view of the fetal head, which included the brain stem, cerebellum and occipital bone

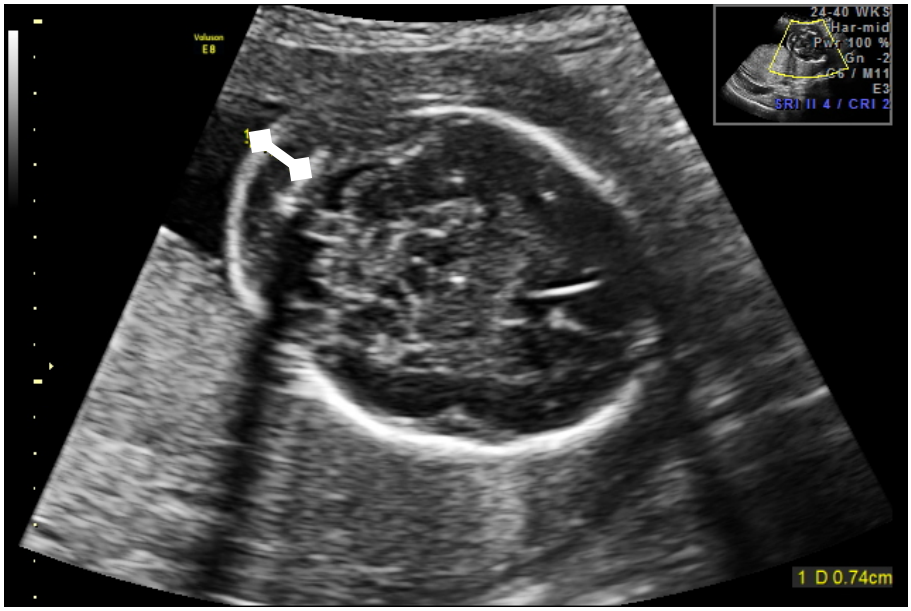

Figure 8.3.2-1. Ultrasound measurement of nuchal skin fold (NSF) thickness. Ultrasound image at 19 weeks, 6 days (personal communication, Alan Donnenfeld, MD). The white oval visible in the center of the image is a cross-sectional view of the fetal skull. The white bar in the upper left quadrant indicates the NSF measurement of 7.4 mm. In most centers, this would be considered a positive NSF measurement

Literature search: A total of 16 studies was identified that reported NSF measurements in trisomy 18 pregnancies in the early second trimester (Borrell *et al.*, 1997; Benacerraf *et al.*, 1992; Ginsberg *et al.*, 1990; DeVore, 2000; Seoud *et al.*, 1994; Gupta *et al.*, 1997; Tongsong *et al.*, 2002; Papp *et al.*, 2008; Viora *et al.*, 2007; Yeo *et al.*, 2003; Nicolaides *et al.*, 1992b; Drugan *et al.*, 1996; Cho *et al.*, 2009; Bottalico *et al.*, 2009; Watson *et al.*, 1994; Benacerraf *et al.*, 1994) (Table 4.3.2-1). All of the studies were performed in a high risk setting (usually prior to amniocentesis due to maternal age or positive biochemistry testing). Three distinct study types were represented (cohort, case/control and case only). Each of these is presented separately in Table 8.3.2-1, so that differences can be seen.

Results: Among the four cohort studies, three reported the rate of elevated NSF thickness measurements in unaffected pregnancies to be 1.2% (95% CI 0.9% - 1.8%). Heterogeneity is high (Q=7.9, I^2=75%, p=0.02). A lower rate for NSF elevations of 0.3% (95% CI 0.2% - 0.6%) was found in the six case/control studies. Heterogeneity was low (Q=2, I^2=0%, p=0.8). For all nine studies combined, the rate was 0.6% (95% CI 0.2% – 2.4%), with high heterogeneity (Q=30, I^2=74%, p<0.001). The point estimate for all three cohort studies is higher than the highest estimate among the case/control studies, indicating that study design is related to variability. Fifteen studies reported the rate of elevated NSF thickness and trisomy 18. There were clear differences by study design. Among the three cohort studies, one increased NSF thickness was identified among 63 trisomy 18 (1.6%, 95% CI <0.1% to 8.5%). The six case/control studies found 20 of 70 identified (30%, 95% CI 20% - 44%), while the six case only studies found 24 of 240 cases (12%, 95% CI 6.7% - 16%). The overall estimate of 15% (95% CI 9.5% - 24%) is heterogeneous (Q=30, I^2=56%, p=0.005).

Summary: A total of 16 studies reported NSF thickening measurements on 373 cases of second trimester trisomy 18 fetuses. The best test performance was reported by case/control studies, with the lowest false positive rate (0.3%) along with the highest detection rate (30.3%). This leads to an overall likelihood ratio (LR) of 10. However, the corresponding LR in the cohort studies is about 1 (1.2% and 1.3%, respectively). With no explanation for this high heterogeneity, the routine use of second trimester NSF thickening may not be a suitable test for trisomy 18 in the early second trimester. To be conservative, the rates from the cohort studies will be used as the summary estimate.

Table 8.3.2-1. Nuchal skin fold thickness (NSF) and trisomy 18

Study	Location	Gestational Age (wks)	Defined NSF+	Unaffected pregnancies			Trisomy 18		
				NSF +	Total	Rate (%)	NSF+	Total	Rate (%)
Cohort									
(Bottalico *et al.*, 2009)	US/NJ	15-22	≥6 mm	4	628	0.6%	NR		
(Cho *et al.*, 2009)	US/CA	15-21	≥6 mm	94	8,707	1.1%	1	56	1.8%
(Drugan *et al.*, 1996)	US	15-19	>5 mm				0	1	0.0%
(Watson *et al.*, 1994)	US/SD	14-21	≥6 mm	27	1,453	1.9%	0	6	0.0%
Subtotal				**125**	**10,788**	**1.2%**	**1**	**63**	**1.6%**
					(95% CI 0.9-1.8%)			(95% CI 0.1-8.5%)	
Case/control									
(Benacerraf *et al.*, 1994)	US/MA	14-21	≥6 mm	0	102	0.0%	4	13	30.8%
(Benacerraf *et al.*, 1992)	US/MA	14-20	≥6 mm	2	588	0.3%	5	9	55.6%
(Borrell *et al.*, 1997)	Spain	15-18	≥6 mm	2	1,365	0.1%	2	7	28.6%
(DeVore, 2000)	US/CA	14-23	>6 mm	7	2,000	0.4%	5	30	16.7%
(Ginsberg *et al.*, 1990)	US	14-20	≥6 mm	0	212	0.0%	2	4	50.0%
(Seoud *et al.*, 1994)	US/VA	22/5	>5 mm	0	50	0.0%	2	7	28.6%
Subtotal				**11**	**4,317**	**0.3%**	**20**	**70**	**30.3%**
					(95% CI 0.2-0.6%)			(95% CI 20-44%)	

Table 8.3.2-1. (continued)

Study	Location	Gestational Age (wks)	Defined NSF+	Unaffected pregnancies NSF+	Unaffected pregnancies Total	Unaffected pregnancies Rate (%)	Trisomy 18 NSF+	Trisomy 18 Total	Trisomy 18 Rate (%)
Case only									
(Gupta *et al.*, 1997)	UK	16-20	>5 mm				1	19	5.3%
(Nicolaides *et al.*, 1992b)	UK	14-39	NR				5	83	6.0%
(Papp *et al.*, 2008)	Hungary	13-24	>6 mm				12	70	17.1%
(Tongsong *et al.*, 2002)	Thailand	16-22	NR				2	25	8.0%
(Viora *et al.*, 2007)	Italy	16-23	≥6 mm				3	27	11.1%
(Yeo *et al.*, 2003)	US/NJ	15-19	NR				1	16	6.3%
Subtotal							**24**	**240**	**10.5%**
									(95% CI 6.7-16%)
All				**136**	**15,105**	**0.6%**	**45**	**373**	**12%**
						(95% CI 0.4-1.1%)			(95% CI 3.8 – 33%)

[1] expressed as range (13-19) or average/standard deviation (18.3/1.7)

NR = not reported

8.3.3 Fetal long bone measurements

Definition: Trisomy 18 newborns are small for gestational age, and intrauterine growth retardation is often a finding by the third trimester. These early findings support the effort to search for shortened long bone measurements in the early second trimester. By the early 1990s, sonographers were reporting results on shortened femur and humeral bone measurements. However, there was not a generally accepted definition of a 'shortened' long bone measurement. Some sonographers used selected centiles or multiples of the standard deviation in known unaffected pregnancies. Others used a cut-off for the ratio of observed to expected length, given the gestational age of the fetus. The two major long bones studied most often are the humerus and the femur, and the relevant studies will be summarized separately.

Humeral length

Literature search: A total of seven studies were identified that reported humeral bone measurements in second trisomy 18 pregnancies (Benacerraf *et al.*, 1992; Benacerraf *et al.*, 1994; Bottalico *et al.*, 2009; Bundy *et al.*, 1986; Cho *et al.*, 2009; Dicke *et al.*, 1989; Droste *et al.*, 1990; Ginsberg *et al.*, 1990; Nadel *et al.*, 1992; Papp *et al.*, 2008; Seoud *et al.*, 1994; Watson *et al.*, 2008) (Table 8.3.3-1). Although there are varied study designs and definitions for a shortened humeral bone, there are too few datasets and no clear pattern. Thus, no stratified analyses will be performed.

Results: Six of the studies reported humeral bone length measurements in 153 second trimester trisomy 18 fetuses. Twenty-three (19%) had shortened humeral bone lengths. Heterogeneity was moderate ($Q=10$, $I^2=50\%$, $p=0.08$). Five studies reported the corresponding rate of shortened humeral bones in 11,491 unaffected pregnancies to be 2.5%. Heterogeneity was high ($Q=47$, $I^2=91\%$, $p<0.001$). At least some of the heterogeneity is due to the definition of 'shortened'. For example, one group (Cho *et al.*, 2009) used a cut-off of less than 0.85 for the observed/expected ratio. This led to a lower detection and false positive rate. Removal of this study reduces heterogeneity and produces a summary likelihood ratio of 7.6 (22% / 2.9%). A reasonable summary estimate for detection and false positive rates are 19% and 2.5%, respectively.

Table 8.3.3-1. Fetal long bone (humeral) measurements and trisomy 18

Author	Location	Design	Definition of 'shortened'	Gestational Age (weeks)[1]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Bottalico *et al.*, 2009)	US/NJ	Cohort	O/E < .90	15-22	NR / 7	8/ 628 (1.3%)
(Cho *et al.*, 2009)	US/CA	Cohort	O/E < .85	15-21	3 /56 (5%)	144/ 8,707 (1.7%)
(Benacerraf *et al.*, 1994)	US/MA	Case / Cont	O/E < .90	14-21	2 / 9 (22%)	3 / 84 (3.6%)
(Benacerraf *et al.*, 1992)	US/MA	Case / Cont	O/E < .90	14-20	3 / 9 (33%)	34/ 588 (5.8%)
(Droste *et al.*, 1990)	US/WI	Case / Cont	O/E < -2SD	18-19	2 / 3 (67%)	4/ 174 (2.5%)
(Bundy *et al.*, 1986)	US/MA	Case only	NR	15-18	1 / 6 (17%)	NR
(Papp *et al.*, 2008)	Hungary	Case only	<10th	13-24	12 /70 (17%)	NR
All (random effects)					**23 / 153 (19%)[2]** (95% CI 10 - 34%)	**150 / 11,491 (2.5%)** (95% CI 1.2 to 5.1)

O/E = observed / expected, SD = standard deviation

[1] expressed as the range of completed weeks

[2] excluded Bottalico 2009

Femur length

Literature search: A total of 11 studies were identified that reported measurements of femur length in trisomy 18 pregnancies (Benacerraf *et al.*, 1994; Benacerraf *et al.*, 1992; Bottalico *et al.*, 2009; Cho *et al.*, 2009; Dicke *et al.*, 1989; Droste *et al.*, 1990; Ginsberg *et al.*, 1990; Nadel *et al.*, 1992; Papp *et al.*, 2008; Seoud *et al.*, 1994; Watson *et al.*, 2008) (Table 4.3.3-2). Six of these studies were of case/control design; the other five were of various other designs. One report (Nadel *et al.*, 1992) reported only on women who were identified with a CPC (screen positive study design) and was not included.

Results: Eleven studies reported that the proportion of the 284 trisomy 18 pregnancies with a shortened femur length was 33%. Heterogeneity was high ($Q=32$, $I^2=72\%$, $p<0.001$). When stratified into the five case/control studies versus other study designs, there was a significant difference. Case/control studies alone had a rate of 54% (95% CI 40% -68%), while other study types had a lower rate of 16% (12% - 21%). Remaining heterogeneity was low in both groups.

Seven studies reported the proportion of 11,491 unaffected pregnancies with shortened femurs to be 5.2%. Heterogeneity was high ($Q=124$, $I^2=95\%$, <0.001). The five case/control studies had a summary rate of 8.2% (5.4% – 12%) versus 2.0% (1.1% – 3.8%) in the two remaining studies. Heterogeneity was low in both groups.

Two separate likelihood ratios can be generated. Data from the case/control studies indicate an LR of 6.6 (54%/8.2%) while the corresponding LR for other study types is 8 (16%/2%). The differences do not appear to be due to varying definitions/cut-off levels. However, there is a clear temporal difference. The case/control studies were all published between 1989 and 1994, while four of the five remaining studies were published in 2008 or later. Given this information, the best LR estimate to use may be the 8.0, based on the non case/control studies.

Table 8.3.3-2. Fetal long bone (femur) measurements and trisomy 18

Author	Location	Design[1]	Definition[2]	Gestational Age (weeks)[3]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Bottalico *et al.*, 2009)	US/NJ	Cohort	O/E < .91	15-22	NR	7 / 628 (1.1%)
(Cho *et al.*, 2009)	US/CA	Cohort	O/E < .85	15-21	10 / 56 (18%)	245 / 8707 (2.8%)
(Watson *et al.*, 2008)	US/MN	Cohort	< 5th	15-21	13 / 98 (13%)	NR
(Benacerraf *et al.*, 1994)	US/MA	Case / Cont	O/E < .91	14-21	8 / 13 (62%)	4 / 106 (3.8%)
(Benacerraf *et al.*, 1992)	US/MA	Case / Cont	O/E < .91	14-20	5 / 9 (55%)	63 / 588 (11%)
(Dicke *et al.*, 1989)	US/MO	Case / Cont	O/E < .91	14-21	5 / 10 (50%)	10 / 142 (7.0%)
(Droste *et al.*, 1990)	US/WI	Case / Cont	O/E > -2 SD	18-19	3 / 4 (75%)	NR
(Ginsberg *et al.*, 1990)	US/IL	Case / Cont	BPD/FL < 1.5 SD	14-20	3 / 4 (75%)	14 / 212 (6.6%)
(Seoud *et al.*, 1994)	US/VA	Case / Cont	BPD/FL < 1.5 SD	22 / 5	3 / 10 (30%)	7 / 50 (14%)
(Papp *et al.*, 2008)	Hungary	Case only	< 10th	13-24	12 / 70 (17%)	NR
(Nadel *et al.*, 1992)	US/MA	Screen pos	NR	14-22	2 / 10 (20%)	NR
All					**64 / 284 (33%)**	**150 / 11,491 (5.2%)**
(random effects)					(95% CI 21-49%)	(95% CI 2.7 to 9.8)

O/E = observed / expected, SD = standard deviation, BPD = biparietal diameter, FL = femur length, NR = not reported

1 All studies were in populations with a high prevalence of trisomy 18

2 Expressed as range (13-19) or average/standard deviation (18.3 / 1.7)

8.3.4 Hyperechoic bowel

Definition: Hyperechoic (or echogenic) bowel occurs when the brightness of the scanned bowel meets or exceeds the echogenicity (brightness) of nearby bone (Figure 8.3.4-1). This definition is generally accepted and widely applied.

Literature search: A total of 10 studies was identified that reported echogenicity of the bowel in second trimester trisomy 18 fetuses (Benacerraf *et al.*, 1994; Bottalico *et al.*, 2009; Bromley *et al.*, 1994; Cho *et al.*, 2009; DeVore, 2000; Gupta *et al.*, 1997; Papp *et al.*, 2008; Seoud *et al.*, 1994; Viora *et al.*, 2007; Watson *et al.*, 2008; Yeo *et al.*, 2003; Drugan *et al.*, 1996) (Table 8.3.4-1). Although the definition of echogenic bowel was not reported for four of these studies, it is likely that they used the definition provided above. All studies were performed in high risk settings.

Results: Among the ten studies, 322 trisomy 18 fetuses were scanned, and 7.1% were found with echogenic bowel (Table 8.3.4-1). There was high heterogeneity among the study estimates ($Q=17$, $I^2=59\%$, $p=0.02$). Five of those studies also reported the rate of echogenic bowel in unaffected pregnancies. The summary was 1.5%. Again, the heterogeneity was high ($Q=78$, $I^2=95\%$ $p<0.001$). The summary likelihood ratio was 4.7, but the high heterogeneity hinders routine application of this ultrasound finding to identify trisomy 18.

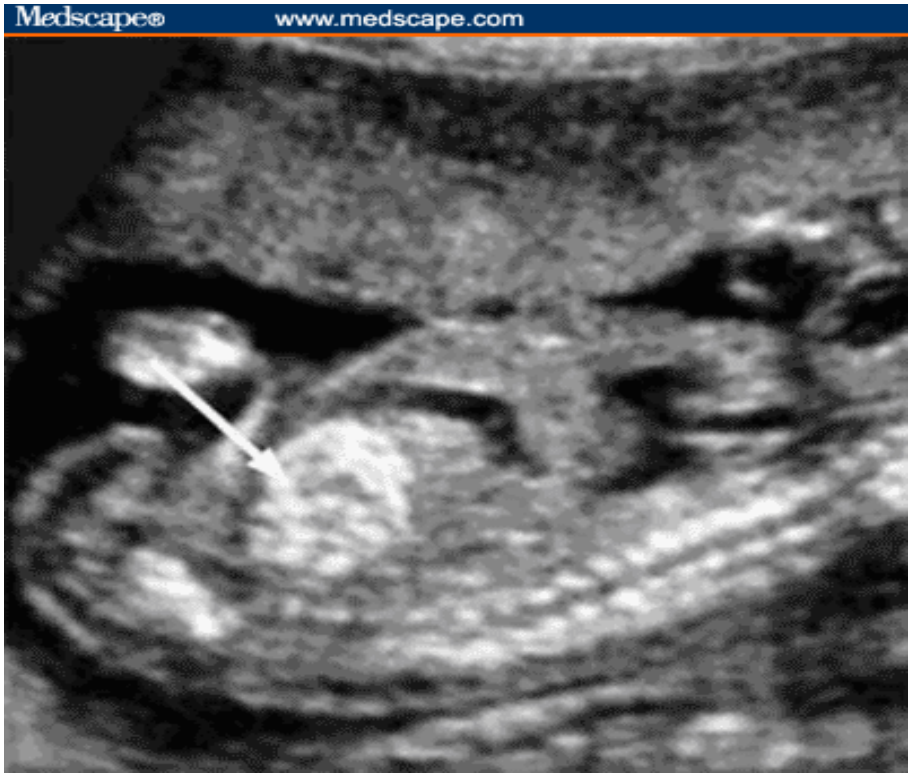

Figure 8.3.4-1. Echogenic bowel. Early second trimester ultrasound scan, in which the bowel (arrow) appears to be as bright as adjacent bone.(echogenic). The fetal spine can be seen across the bottom of the image.

Table 8.3.4-1. Hyperechoic bowel and trisomy 18

Author	Location	Design	Definition	Gestational Age (weeks)[1]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Bottalico *et al.*, 2009)	US/NJ	Cohort	≥ bone	15-22	NR	10 / 628 (1.6%)
(Cho *et al.*, 2009)	US/CA	Cohort	≥ bone	15-21	2 / 56 (4%)	69 / 8707 (0.8%)
(Drugan *et al.*, 1996)	US/MI	Cohort	NR	15-19	0 / 1 (0%)	NR
(Watson *et al.*, 2008)	US/MN	Cohort	NR	15-21	1 / 98 (1%)	NR
(Benacerraf *et al.*, 1994)	US/MA	Case / Cont	≥ bone	14-21	0 / 13 (0%)	1 / 106 (0.9%)
(DeVore, 2000)	US/CA	Case / Cont	≥ bone	14-23	4 / 30 (13%)	70 / 2000 (3.5%)
(Seoud *et al.*, 1994)	US/VA	Case / Cont	bone	22 / 5	4 / 11 (36%)	0 / 50 (0.0%)
(Papp *et al.*, 2007)	Hungary	Case only	NR	13-24	5 / 70 (7%)	NR
(Viora *et al.*, 2007)	Italy	Case only	bone	16-23	1 / 27 (4%)	NR
(Yeo *et al.*, 2003)	US/NJ	Case only	NR	15-19	1 / 16 (6%)	NR
All					**18 / 322 (7.1%)**	**150 / 11,491 (1.5%)**
(random effects)					(95% CI 3.2-15%)	(95% CI 0.6 to 3.8)

NR = not reported

[1] expressed as range (13-19) or average/standard deviation (18.3 / 1.7)

8.3.5 Pyelectasis

Definition: Pyelectasis is defined as the diameter of the renal pelvis (anterior to posterior measurement) being greater than a certain size, usually 4 mm. Some sonographers use a higher cut-off level of 5 mm or even greater.

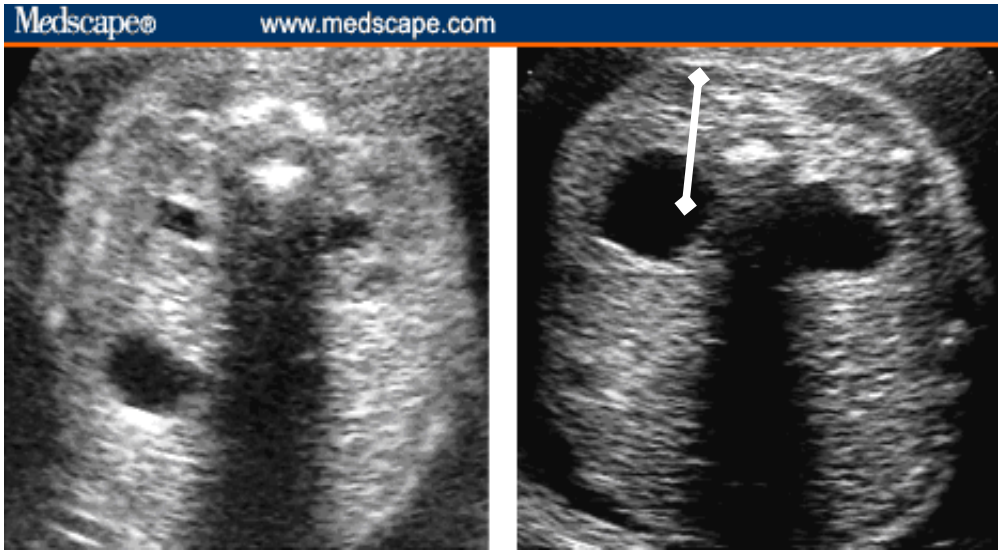

Figure 8.3.5-1. Pyelectasis in the early second trimester. The left sonogram shows the normal sized renal pelvis, while the left photo shows a large dilation of the renal pelvis (pyelectasis).

Literature search: A total of nine studies were identified that reported pyelectasis in trisomy 18 pregnancies (Benacerraf *et al.*, 1994; Bottalico *et al.*, 2009; Cho *et al.*, 2009; DeVore, 2000; Havutcu *et al.*, 2002; Papp *et al.*, 2008; Seoud *et al.*, 1994; Yeo *et al.*, 2003; Drugan *et al.*, 1996). Three were cohort studies, three were case/control studies, two were case only, and one only followed women whose scan indicated pyelectasis (screen positive). All studies were performed in a high risk setting, except for the screen positive study (Havutcu *et al.*, 2002).

Results: All nine studies provided the proportion of the 198 trisomy 18 pregnancies identified with pyelectasis, but in two studies (Drugan *et al.*, 1996; Havutcu *et al.*, 2002), only one, or no trisomy 18 pregnancies were reported (Table 4.3.5-1). Overall, the detection rate was 11% (21 with pyelectasis). Heterogeneity was high ($Q=14$, $I^2=65\%$, $p=0.01$). Six of the nine studies reported the rate of pyelectasis among the 37,077 unaffected pregnancies. The summary was 1.5%, with high

heterogeneity identified (Q=10 I^2=52% p=0.06). The summary likelihood ratio for pyelectasis based on these rates is 7.3 (11% / 1.5%). However, only one of the nine studies actually provided detection and false positive rates with at least one instance of pyelectasis in both groups (Cho *et al.*, 2009) resulting in a likelihood ratio of 2.9. The summary estimates of 11% and 1.5% are reasonable estimates for detection and false positive rates for pyelectasis.

Table 8.3.5-1. Pyelectasis and trisomy 18

Author	Location	Design	Definition	Gestational Age (weeks)[1]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Bottalico *et al.*, 2009)	US/NJ	Cohort	≥4 mm	15-22	NR	9 / 628 (1.4)
(Cho *et al.*, 2009)	US/CA	Cohort	>4 mm	15-21	3 / 56 (5%)	146 /8.707 (1.7)
(Drugan *et al.*, 1996)	US	Cohort	>5 mm	15-19	0 / 1 (0%)	
(Benacerraf *et al.*, 1994)	US/MA	Case / Cont	≥4 mm	14-21	0 / 13 (0%)	0 / 106 (0)
(DeVore, 2000)	US/CA	Case / Cont	≥4 mm	14-23	0 / 30 (0%)	33 /2,000 (1.7)
(Seoud *et al.*, 1994)	US/VA	Case / Cont	≥4 mm	22 / 5	5 / 12 (42%)	0 / 50 (0)
(Papp *et al.*, 2008)	Hungary	Case only	NR	13-24	12 / 70 (17%)	
(Yeo *et al.*, 2003)	US/NJ	Case only	NR	15-19	1 / 16 (6%)	
(Havutcu *et al.*, 2002)	UK	Screen pos (low)	≥5 mm	18-24	0 / 0 (0%)	320 / 25,586 (1.3)
All (random effects)					**21 / 198 (11%)** (95% CI 4 – 25%)	**308 / 37,077 (1.5%)** (95% CI 1.2 – 1.8%)

NR = not reported, mm = millimeters

[1] expressed as range (13-19) or average/standard deviation (18.3 / 1.7)

8.3.6 Two-vessel cord

Definition: In normal pregnancies, the umbilical cord is composed of three vessels; one vein and two arteries. In some instances, only two vessels are present (one vein and one artery). This is labeled a two-vessel cord. Some studies distinguish between whether the right or left artery is absent, but this does not seem to be strongly associated with karyotype.

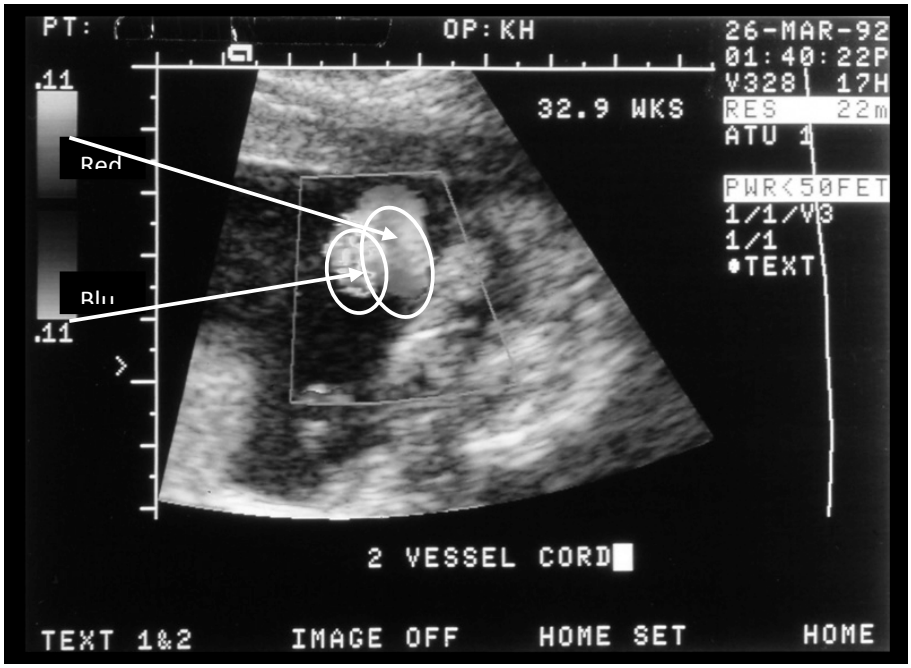

Figure 8.3.6-1. Two vessel cord. A third trimester black & white version of a color Doppler image of the cross-sectional view of an umbilical cord (personal communication, Alan Donnenfeld, MD). A normal umbilical cord has 3 vessels (2 arteries and a vein). This image shows only two vessels (indicated by the white ovals), one would be colored red and the other blue.

Literature search: A total of seven studies were identified that reported two-vessel cords in trisomy 18 pregnancies. All occurred in high risk settings, usually prior to amniocentesis (Bahado-Singh *et al.*, 2003; Brumfield *et al.*, 2000; Cho *et al.*, 2009; Watson *et al.*, 2008; Lubusky *et al.*, 2007; Saller *et al.*, 1990). One of these studies (Bahado-Singh *et al.*, 2003) could be stratified into an early and late gestational age groups resulting in the analysis of four datasets.

Results: The six datasets included 231 fetuses with trisomy 18, and a two-vessel cord was identified in an estimated 14% (Table 8.3.6-1). Heterogeneity was low (Q=2.9, I^2=0%, p=0.7). Four studies also reported the false positive rates. The summary estimate is 1.2% (95% CI 0.3% to 4.6%), with high heterogeneity (Q=135, I^2=98, p<0.001). One study had a very high rate of 4% (Lubusky *et al.*, 2007). If this were removed, the estimate would be reduced to 0.7% (95% CI 0.4% to 1.4%). The heterogeneity is reduced, but still high (Q=7.6, I^2=74%, p=0.02). Using these summary estimates for two-vessel cord, the corresponding likelihood ratio would be 20 (14%/0.7%).

Table 8.3.6-1. Two-vessel cord and trisomy 18

Author	Location	Design	Gestational Age (weeks)[1]	Trisomy 18 positive / total (%)	Unaffected positive / total (%)
(Cho *et al.*, 2009)	US/CA	Cohort	15-21	6 / 56 (11%)	38 / 8,707 (0.4%)
(Lubusky *et al.*, 2007)	Czech Rep	Cohort	Early 2nd trimester	8 / 16 (50%)	81 / 2,020 (4.0%)
(Bahado-Singh *et al.*, 2003)	US/CT	Case / Cont	14-17.5	3 / 25 (12%)	6 / 767 (0.8%)
(Bahado-Singh *et al.*, 2003)	US/CT	Case /Cont	17.6-24	3 / 22 (14%)	6 / 447 (1.3%)
(Brumfield *et al.*, 2000)	US/AL	Case only	14-22	1 / 21 (5%)	NR
(Saller *et al.*, 1990)	US/MD	Case only		2 / 9 (22%)	NR
(Watson *et al.*, 2008)	US/MN	Case only	14-21	16 / 98 (17%)	NR
All				**31 /231 (14%)**	**131 / 11,941 (1.2%)**
(random effects)				(95% CI 10-19%)	(95% CI 0.5-1.5%)

NR = not reported

[1] expressed as range (13-19) or average/standard deviation (18.3/1.7)

8.3.7 Echogenic intra-cardiac focus (EICF)

Definition: Echogenic intra-cardiac focus was reported in the literature as early as 1988 (Levy and Mintz, 1988) and was initially thought to be benign. The EICF is a bright spot (as bright as bone), usually observed in the left ventricle during a four-chambered view of the heart. It may be due to calcification, thickening, or incomplete penetration of the papillary muscles or chordate tendineae (Wax *et al.*, 2000).

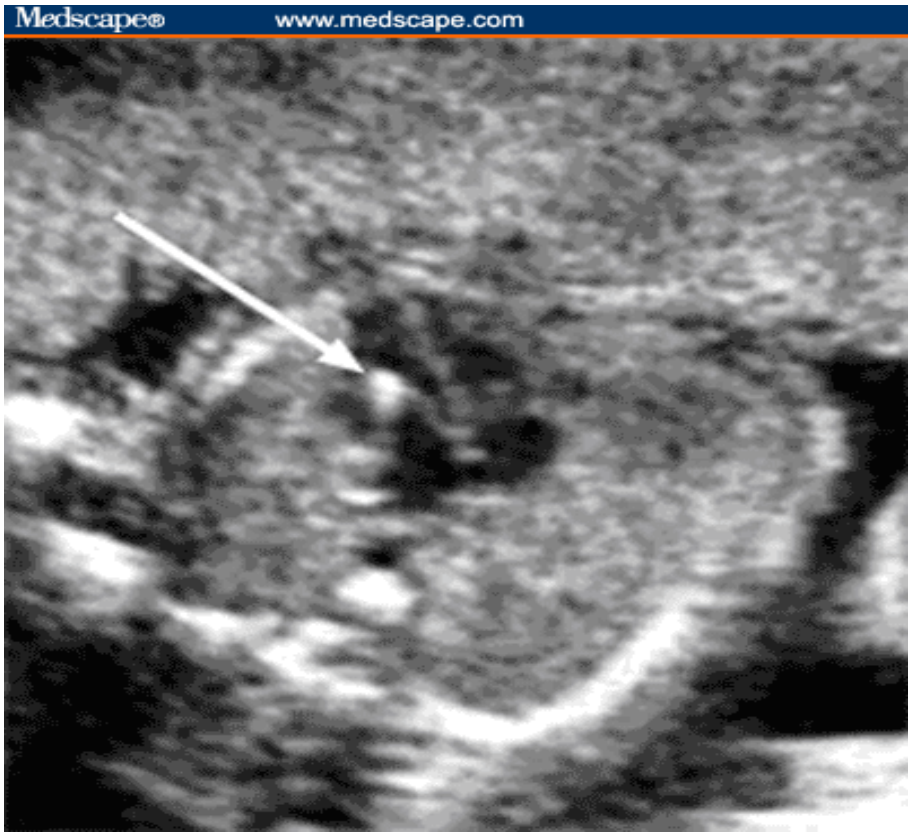

Figure 8.3.7-1 Echogenic intra-cardiac focus (EICF). This ultrasound image shows the fetal heart with a bright spot (arrow) in the left ventricle – the echogenic intra-cardiac focus.

Literature search: A total of six relevant articles was identified (Bottalico *et al.*, 2009; Cho *et al.*, 2009; Papp *et al.*, 2008; Sepulveda *et al.*, 1995; Wax *et al.*, 2000; Yeo *et al.*, 2003). All were performed in a high risk setting, three were of case only design and the three others were cohort studies.

Results: The data are summarized in Table 8.3.7-1. The gestational ages are, in general, early second trimester. For the five studies reporting the detection rates, the summary rate was 1.9% (95% CI 0.4% to 5.5%, based on the binomial distribution); rates ranged from 0 to 8%. The observed rate was used, because two of the five studies detected no cases (Wax *et al.*, 2000; Papp *et al.*, 2008). For the three studies reporting false positive rates, the summary using a random effects model was 3.8% (95% CI 2.9% to 4.8%), with high heterogeneity (Q=4, I^2=61%, p=0.08). The summary likelihood ratio is 0.50 (1.9%/3.8%). Only one study had sufficient data (Cho *et al.*, 2009) to compute an individual OR (0.55, 95% CI 0.08 to 3.7, p=NS). In summary, the data are highly variable with an overall estimate of 1.9% detection rate with a 3.8% false positive. In reality, it is likely that there is little or no association between EICF and trisomy 18.

Table 8.3.7-1. Echogenic intra-cardiac focus (EICF) and trisomy 18

Author	Location	Design	Gestational Age (weeks)[1]	Trisomy 18 positive / total (%)	Controls positive / total (%)
(Bottalico *et al.*, 2009)	US/	Cohort	15-22	NR	30 / 628 (4.8)
(Cho *et al.*, 2009)	US/CA	Cohort	15-21	1 / 56 (2)	280 / 8,707 (3.2)
(Wax *et al.*, 2000)	US/CT	Cohort	18.3/1.7	0 / 2 (0)	27 / 682 (4.0)
(Papp *et al.*, 2007)	Hungary	Case only	13-24	0 / 70 (0)	NR
(Sepulveda *et al.*, 1995)	UK	Case only	mid-trimester	1 / 13 (8)	NR
(Yeo *et al.*, 2003)	US/NJ	Case only	15-19	1 / 16 (6)	NR
All (fixed and random effects)				**3 / 157 (1.9)** (95% CI 0.4 – 5.5)	**337 / 10,017 (3.8)** (95% CI 2.9 - 4.8)

NR = not reported

[1] expressed as average/standard deviation (e.g., 18.3/1.7) or range (e.g., 13-24)

8.3.8 Less commonly reported non-structural anomalies

Several other non-structural anomalies for Down syndrome have been reported, including iliac wing, heart rate, polyhydramnios, ear length, and renal anomalies. These may, or may not, be useful in screening for trisomy 18. They were not included, either because there are relatively few data available for these markers in Down syndrome, or because the reported effect is small.

8.4 Summary of second trimester ultrasound markers

Table 8.4-1 summarizes the findings regarding the structural anomalies and non-structural anomalies that could be potentially used to help identify fetuses with trisomy 18 during the early second trimester. For each ultrasound marker, the number of included studies, as well as total number of unaffected and trisomy 18 pregnancies reported are presented. The reported trisomy 18 detection and false positive rates are the most relevant, and do not necessarily use data from all included studies. For example, the detection and false positive rates for 'any structural anomaly' relies only on those studies reporting their cases between 15 and 20 completed weeks' gestation. The summary likelihood ratio is then provided along with the setting in which it might be acheived.

- Structural anomalies. All four groupings had high likelihood ratios (25-690), but the performance estimates for two of them (cardiac defects and hand/foot anomalies) would be unlikely to be met outside of a high risk referral center. Since these anomalies are all considered serious, their identification is important, regardless of karyotype. The classification of 'false positives' is misleading, but in an epidemiological context is appropriate for evaluation purposes.
- Non-structural anomalies. Only two of the eight ultrasound markers in this category were considered to be suitable for use. The long bone measurement (femur) could be reliably measured in routine settings, but the two-vessel cord requires Doppler ultrasound that may only be available from referral centers.

Summary: Overall, the large literature base is mostly composed of opportunistic study populations that were not properly designed or powered to provide reliable and clinically relevant conclusions. Along with evolving ultrasound techniques and equipment, this results in heterogeneous findings making subsequent evaluation and recommendations difficult. Given the high detection and low false positive rates achievable in the second trimester (maternal age in combination with AFP, uE3, hCG and PAPP-A measurements), the first trimester (maternal age in combination with NT, PAPP-A and hCG or free β measurements), or with an integrated test (combinations of the first and second trimester markers), the addition of second trimester ultrasound measurements does not appear needed, especially in light of the pressure to have the 'anomaly scan' at 19 weeks or later. However, such ultrasound scans clearly have a role in identifying important structural defects such as cardiac anomalies. The next section shows examines the whether an earlier version of such testing might be useful among those with positive serum test results.

Table 8.4-1. Summary of association of second trimester ultrasound markers with trisomy 18

Ultrasound Marker (page)	**Number of**			**Relevant estimates for**			
	Studies	**T18**	**UA**	**DR (%)**	**FPR (%)**	**(LR)**	**Estimates appropriate for**
Structural anomaly							
Hand/foot anomalies (174)	17	615	10,027	41	0.06	680	Referral centers, 15 - 20 wks
Open fetal defects (166)	15	441	1,320	26	0.2	130	All settings, 15 - 20 wks
Any structural anomaly (178)	20	588	13,869	60	1.6	38	All settings, 15 - 20 wks
Cardiac defects (171)	19	696	30,868	38	1.5	25	Non-specialized centers, 15 - 20 wks
Non-structural anomaly							
Two-vessel cord (216)	7	231	9,921	14	0.7	20	Referral centers, 15-20 wks
Long bone – humeral (205)	7	153	11,491	19	2.5	8	All settings, 15 - 20 wks
CPC - all (199)	61	942	536,960	29	1.4	21	Not usable: highly variable
Pyelectasis (213)	9	198	37,077	11	1.5	7.3	Not usable: variable, poor performance
Hyperechoic bowel (209)	10	322	11,491	7.1	1.5	4.7	Not usable: variable, poor performance
Nuchal skin fold (202)	16	373	15,105	1.6	1.2	1.3	Not usable: variable, poor performance
CPC - isolated[1] (200)	-	-	-	low	low	~1	Not usable: poor performance
EICF (219	6	157	10,017	1.9	3.8	0.5	Not usable: poor performance

UA = unaffected, DR = detection rate, FPR = false positive rate, LR = likelihood ratio

CPC = choroid plexus cysts, EICF = echogenic intra-cardiac focus

[1] With current practice (especially at referral centers), few, if any, truly isolated CPCs would be identified among trisomy 18 pregnancies

8.5 Combining second trimester ultrasound markers

The usual method of combining serum markers is to identify those markers that provide reasonable separation between unaffected and affected pregnancies, determine whether those markers are independent of each other, and evaluate various combinations to determine which should be included in a test offering. Ultrasound markers are different in that the sonographer will, during the course of the examination, identify markers that may, or may not, be useful. Thus, a 'set' ultrasound test is more difficult to create. One must also keep in mind that the ultrasound scan is not designed solely to identify trisomy 18, but a whole host of findings relating to overall fetal well-being.

Structural anomalies: The most obvious first combination of tests is the identification of any structural anomaly. Earlier data showed that the combination of open fetal defects, cardiac defects and other gross anomalies can identify over half of trisomy 18 fetuses. In addition, the 'false positives' are not really unaffected pregnancies. They really do have, for the most part, an important fetal defect that is worthwhile identifying, even if the karyotype is normal. It is clear from the data, that an examination of the hands and feet can be quite useful in identifying trisomy 18 (but not Down syndrome). This requires time and high resolution equipment and may be an example of an anomaly that cannot be easily translated into routine practice.

Non-structural anomalies. The literature regarding these markers is characterized by high heterogeneity, even when attempting to account for potentially important covariates. One unexpected finding is the high likelihood ratio found for 2 vessel cords, along with moderate to low heterogeneity. This suggests that it may have potential as a widely applicable test if Doppler analysis is available. The various long bone measurements are surely correlated to some extent, and the literature suggests that both the femur and humeral bone measurements are equally predictive. However, there is more heterogeneity present when the femur is used, suggesting that humeral bone measurements might be preferred. Pyelectasis and hyperechoic bowel could also be potentially useful, with moderate predictive power, but are associated with unexplained high heterogeneity. Identifying CPCs are not very useful as they are not associated with Down syndrome and are, as equipment improves, less likely to be found in isolation. Nuchal skin fold measurements appear far too variable for routine use. It is possible that standardization, such as that currently employed for nuchal translucency measurements, may solve this problem. Until then, NSF measurements do not appear ready for routine use. ECIF appears to be of limited use in testing for trisomy 18.

US screening performance: The screening performance of individual markers (Table 8.4-1) can be used to estimate a combined marker panel by assuming independence of markers. This is reasonable, if careful choices are made. First, the estimate for the combination of structural anomalies (identified in the literature and this review as 'gross anomalies') can be used. This accounts for interdependences among those markers and is likely to be more reproducible than difficult measurements made in referral centers. Second, the number of non-structural anomalies can be limited, and those that are likely to show correlation (*e.g.,* both humerus and femur length) would not be included together. A *monte carlo* simulation can then generate thousands of combinations of markers in both affected and unaffected pregnancies and use the associated detection and false positive rates to estimate combined performance. Using a combination of gross anomalies (at 15 – 20 weeks' gestation), two vessel cord, humerus length and pyelectasis, the model predicts an 87.6% detection rate with a corresponding 8.8% false positive rate.

Combining US and biochemistry: Could second trimester ultrasound markers be combined with serum markers to screen for trisomy 18? The answer from a statistical point of view is yes. A routine scan looking for gross abnormalities and two or three non-structural anomalies (*i.e.,* 2 vessel cord, humerus length and pyelectasis) could potentially be helpful in identifying trisomy 18 in the second trimester. Although some additional information would be needed to develop a reliable model, screening performance could be improved by combining the two strategies rather than choosing one or the other. The real problem is timing, reliability and the necessary resources for the ultrasound. Unlike biochemistry, there are no savings in scale for ultrasound. Most professional organizations for sonographers recommend waiting until 19 weeks or later for an anomaly scan, so that features are more easily visualized. This is too late for a trisomy 18 scan to be part of routine practice. There are also not sufficient numbers of trained sonographers to handle the 2 to 4 million pregnant women that would be potential candidates for such testing. Thus, it is likely that second trimester ultrasound and biochemistry will not be combined into a 'one step' trisomy 18 test.

US markers as a follow-up test: Performing an ultrasound scan among women already screen positive for trisomy 18 via second trimester biochemistry is routine in many referral centers. The stated aim of the scan is to 'refining the assigned risk'. However, follow-up screening tests must be highly sensitive (*i.e.,* high detection rate) in order that women with affected fetuses would not be missed on the follow-up testing. Structural

anomalies are highly specific, but not highly sensitive. The addition of several non-structural anomalies would increase the detection rate, but probably not to the level required (95% detection rate or higher). Figure 8.5-1 shows that follow-up ultrasound testing does not have sufficiently high detection to stratify screen positive women into a low risk group that would not benefit from diagnostic testing.

Second trimester biochemistry can identify a group of women at an odds of 1:16 (or higher) of having trisomy 18 (Chapter 3, Sections 3.10 and 3.11). Among the hypothetical group of 17,000 screen positive women, 1,000 women would be carrying a trisomy 18 fetus. Of these, 876 will have one or more US markers positive (87.6% detection rate as described above), along with 1,408 women with normal pregnancies (8.8% of the 16,000). The risk in screen positive women is now about 1:2 and the offer of diagnostic testing/karyotyping is clearly warranted. However, the risk in the women with a negative ultrasound is about 1:120. Significantly lower, but not so low as to not offer amniocentesis, especially since these women have already been told of their increased risk of trisomy 18. Thus, reclassification using second trimester ultrasound is not sufficiently sensitive to use in determine which of these 'high risk' women should be offered ultrasound. However, many of these fetuses will have a structural anomaly and/or an ultrasound finding. One could respond that relatively few of the trisomy 18 fetuses that would be missing would be likely survive to term, and even fewer would survive more than a few days. However, evidence presented in Chapter 2 indicates that the more severely affected fetuses (such as those identified with gross abnormalities) are more likely to be lost and more likely to survive for a shorter time period. Thus, those that are 'missed' via a second trimester ultrasound scanning protocol as shown in Figure 8.5-1, may actually be more likely to survive, making their identification via diagnostic testing especially important.

Thus, the literature supports the assertion that several of the second trimester ultrasound markers are associated with trisomy 18. However, that data generally is available later (19 weeks or later) than the biochemistry window (15-17 weeks) making it difficult to combine the two into a single interpretation sufficiently early in the second trimester to be clinically useful. Even a combination of second trimester ultrasound markers are not sufficiently sensitive (high enough detection) to allow their use among biochemistry screen positive women. Although not suitable for routine use in screening for trisomy 18 (or Down syndrome), second trimester ultrasound testing may still have use in the detection of other structural anomalies.

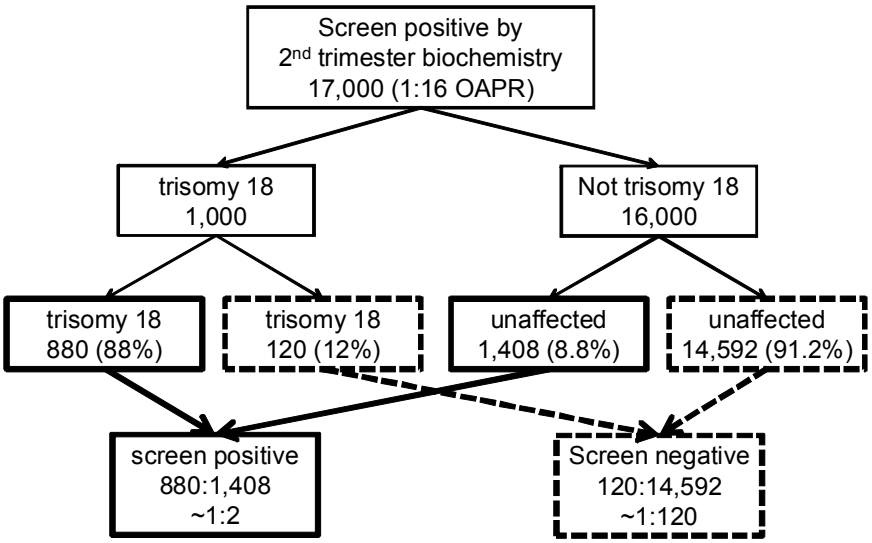

Figure 8.5-1. A hypothetical cohort of 17,000 women at high risk for trisomy 18 in the second trimester showing the effect of follow-up ultrasound testing. The top three entries show the 1000 case, and 16,000 control pregnancies. At that point, all undergo a second trimester ultrasound test that includes gross anomalies (at 15 – 20 weeks' gestation), two vessel cord, humerus length and pyelectasis. The third row shows the results grouped into positive (solid outlined boxes) and negative (dashed boxes) ultrasound exams. The final row shows the computation of the odds of being affected given a positive result for those with positive and negative test results. Those women screen negative still have a relatively high risk (1:120) of having an affected fetus, and an offer for diagnostic testing still appears warranted.