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Meta-analysis of second-trimester markers for trisomy 21

M. AGATHOKLEOUS*, P. CHAVEEVA*, L. C. Y. POON*, P. KOSINSKI* and K. H. NICOLAIDES*†

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Department of Fetal Medicine, University College Hospital, London, UK*

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ABSTRACT

Objective *To summarize by meta-analysis the accumulated data on the screening performance of second-trimester sonographic markers for fetal trisomy 21.*

Methods *We conducted a literature search to identify studies between 1995 and September 2012 that provided data on the incidence of sonographic markers in trisomy 21 and euploid fetuses at 14–24 weeks' gestation. Weighted independent estimates of detection rate, false-positive rate and positive and negative likelihood ratios (LR) of markers were calculated.*

Results *A total of 48 studies was included in the analysis. The pooled estimates of positive and negative LR were, respectively: 5.83 (95% CI, 5.02–6.77) and 0.80 (95% CI, 0.75–0.86) for intracardiac echogenic focus; 27.52 (95% CI, 13.61–55.68) and 0.94 (95% CI, 0.91–0.98) for ventriculomegaly; 23.30 (95% CI, 14.35–37.83) and 0.80 (95% CI, 0.74–0.85) for increased nuchal fold; 11.44 (95% CI, 9.05–14.47) and 0.90 (95% CI, 0.86–0.94) for hyperechogenic bowel; 7.63 (95% CI, 6.11–9.51) and 0.92 (95% CI, 0.89–0.96) for mild hydronephrosis; 3.72 (95% CI, 2.79–4.97) and 0.80 (95% CI, 0.73–0.88) for short femur; 4.81 (95% CI, 3.49–6.62) and 0.74 (95% CI, 0.63–0.88) for short humerus; 21.48 (95% CI, 11.48–40.19) and 0.71 (95% CI, 0.57–0.88) for aberrant right subclavian artery (ARSA); and 23.27 (95% CI, 14.23–38.06) and 0.46 (95% CI, 0.36–0.58) for absent or hypoplastic nasal bone. The combined negative LR, obtained by multiplying the values of individual markers, was 0.13 (95% CI, 0.05–0.29) when short femur but not short humerus was included and 0.12 (95% CI, 0.06–0.29) when short humerus but not short femur was included.*

Conclusion *The presence of sonographic markers increases, and absence of such markers decreases, the risk for trisomy 21. In the case of most isolated markers there is only a small effect on modifying the pre-test odds for trisomy 21, but with ventriculomegaly, nuchal fold thickness and ARSA there is a 3–4-fold increase in risk and with hypoplastic nasal bone a 6–7-fold increase.*

INTRODUCTION

Several studies have reported that certain features detected during second-trimester ultrasound examination are potential markers for fetal trisomy 21[1–48]. The most widely examined markers are lateral cerebral ventriculomegaly, absent or hypoplastic nasal bone, increased nuchal fold thickness, intracardiac hyperechogenic focus, aberrant right subclavian artery (ARSA), hyperechogenic bowel, mild hydronephrosis and shortening of the femur or humerus. Assessment of the risk for trisomy 21 based on each of these markers necessitates knowledge of their prevalence in trisomic and euploid fetuses.

The aim of this meta-analysis was to examine the screening performance of second-trimester sonographic markers for the detection of trisomy 21.

METHODS

Relevant citations on second-trimester markers for trisomy 21 were extracted from EMBASE and PubMed from 1995 to September 2012 to identify English language articles. Keywords and MeSH terms were combined to generate lists of studies: 'soft markers', 'intracardiac echogenic focus/foci', 'ventriculomegaly', 'nuchal fold', 'nuchal thickness', 'echogenic bowel', 'hydronephrosis', 'pyelectasis', 'short humerus', 'short femur', 'aberrant right subclavian artery', 'ARSA', 'nasal bone hypoplasia', 'absent nasal bone', 'nasal bone length', 'trisomy 21' and 'Down

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: kypros@fetalmedicine.com)

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syndrome'. The inclusion criteria were studies reporting on the incidence of one or multiple markers in trisomy 21 and proven or assumed to be euploid fetuses, publication in or after 1995 and minimum and maximum gestational age at examination of 14 and 24 weeks, respectively. The first reviewer (P.C.) sorted all articles by citations and abstract for more detailed evaluation. The second sort was revised by three reviewers (P.C., M.A., K.N.) and all relevant studies were entirely reviewed by the same reviewers. All studies were carefully compared to ensure that we avoided using duplicate reports on the same subjects.

Studies on second-trimester sonographic markers were eligible if first, they included and described both euploid and trisomy 21 fetuses (so that 2×2 tables for diagnostic performance of the markers could be constructed), second, the fetal karyotype was unknown at the time of sonographic examination (so as to avoid overt diagnosis bias) and third, chromosomal status of the fetuses was confirmed by either karyotype (the gold standard) or postnatal clinical examination. Prospective and retrospective cohort studies were considered eligible for inclusion if the above criteria were met. In the case of ARSA and absent or hypoplastic nasal bone the number of studies fulfilling these criteria was small and we expanded the selection to include case–control studies. In case of data duplication or overlap, only the largest or most recent study with available data was included.

Information was extracted on study population characteristics, time in pregnancy at which sonography was performed, inclusion and exclusion criteria, study design, outcome assessment and potential verification bias and the main results and conclusions of the study.

Quality and integrity of this review were validated with PRISMA: preferred reporting items for systematic reviews and meta-analyses[49].

Statistical analysis

A meta-analysis was performed to provide a quantitative summary of the test performance of each second-trimester sonographic marker. The Newcastle–Ottawa scale was used to assess the methodological quality of the

Table 1 Performance of intracardiac echogenic focus in screening for trisomy 21

		Trisomy 21		*Euploid*			
Study	*Type*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Manning 1998[9]	HR	3/17	17.6 (6.2–41.0)	21/887	2.4 (1.6–3.6)	7.45 (2.46–22.63)	0.84 (0.68–1.05)
Sohl 1999[10]	HR	13/55	23.6 (14.4–36.4)	150/2639	5.7 (4.9–6.6)	4.16 (2.52–6.86)	0.81 (0.70–0.94)
Wax 2000[13]	HR	2/7	28.6 (8.2–64.1)	25/772	3.2 (2.2–4.7)	8.82 (2.57–30.28)	0.74 (0.46–1.18)
Winter 2000[14]	HR	16/53	30.2 (19.5–43.5)	147/3192	4.6 (3.9–5.4)	6.56 (4.23–10.17)	0.73 (0.61–0.87)
Sacco 2007[36]	HR	3/9	33.3 (12.1–64.6)	45/965	4.7 (3.5–6.2)	7.15 (2.72–18.80)	0.70 (0.44–1.11)
Vergani 2008[41]	HR	1/24	4.2 (0.7–20.2)	18/1129	1.6 (1.0–2.5)	2.61 (0.36–18.79)	0.97 (0.90–1.06)
Bottalico 2009[43]	HR	4/12	33.3 (13.8–60.9)	30/628	4.8 (3.4–6.7)	6.98 (2.92–16.71)	0.70 (0.47–1.05)
Prefumo 2001[16]	Sc	2/8	25.0 (7.2–59.1)	239/7688	3.1 (2.7–3.5)	8.04 (2.41–26.88)	0.77 (0.52–1.16)
Coco 2004[25]	Sc	3/11	27.3 (9.8–56.6)	476/12 648	3.8 (3.5–4.1)	7.25 (2.75–19.10)	0.76 (0.53–1.09)
Schluter 2005[30]	Sc	27/73	37.0 (26.8–48.5)	951/16 891	5.6 (5.3–6.0)	6.60 (4.87–8.97)	0.67 (0.56–0.80)
Weisz 2007[38]	Sc	3/12	25.0 (8.9–53.2)	104/2320	4.5 (3.7–5.4)	5.58 (2.06–15.13)	0.79 (0.57–1.09)
Aagaard-Tillery 2009[42]	Sc	15/53	28.3 (18.0–41.6)	345/7725	4.5 (4.0–5.0)	6.34 (4.08–9.85)	0.75 (0.63–0.89)
Shanks 2009[44]	Sc	34/218	15.6 (11.4–21.0)	2223/62 111	3.6 (3.4–3.7)	4.36 (3.19–5.95)	0.88 (0.83–0.93)
Huang 2010[46]	Sc	7/25	28.0 (14.3–47.6)	237/7093	3.3 (3.0–3.8)	8.38 (4.42–15.91)	0.75 (0.58–0.95)
Analysis: total							
Pooled estimate		133/577	24.4 (20.9–28.2)	5011/126 688	3.9 (3.4–4.5)	5.83 (5.02–6.77)	0.80 (0.75–0.86)
Heterogeneity							
Model			Fixed effects		Random effects	Fixed effects	Random effects
I^2			0.451		0.946	−0.276	0.564
Q			21.874		222.667	9.404	27.515
P			0.057		< 0.0001	0.742	0.011
Analysis: high risk							
Pooled estimate		42/177	25.8 (19.6–33.1)	436/10 212	3.7 (2.8–4.8)	5.82 (4.42–7.66)	0.82 (0.73–0.93)
Heterogeneity							
Model			Fixed effects		Random effects	Fixed effects	Random effects
I^2			0.171		0.878	−0.381	0.640
Q			6.031		40.886	3.620	13.893
P			0.420		< 0.0001	0.728	0.031
Analysis: screened							
Pooled estimate		91/400	25.8 (18.1–35.5)	4575/116 476	4.0 (3.3–4.7)	5.83 (4.88–6.97)	0.78 (0.70–0.86)
Heterogeneity							
Model			Random effects		Random effects	Fixed effects	Random effects
I^2			0.679		0.972	0.135	0.575
Q			15.599		172.681	5.783	11.771
P			0.016		< 0.0001	0.448	0.067

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened.

Table 2 Performance of ventriculomegaly in screening for trisomy 21

			Trisomy 21		*Euploid*			
Study	*Type*	*Definition*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Nyberg 1995[5]	HR	V ≥ 10 mm	1/18	5.6 (1.0–25.8)	0/232	0.0 (0.0–1.6)	—	0.94 (0.84–1.06)
Deren 1998[8]	HR	V 10–15 mm	2/35	5.7 (1.6–18.6)	4/3674	0.2 (0.0–0.3)	52.49 (9.94–277.26)	0.94 (0.87–1.02)
Sohl 1999[10]	HR	V 10–15 mm	3/55	5.5 (1.9–14.9)	13/2639	0.5 (0.3–0.8)	11.07 (3.25–37.76)	0.95 (0.89–1.01)
Wax 2000[13]	HR	V ≥ 10 mm	2/7	28.6 (8.2–64.1)	1/772	0.1 (0.0–0.1)	220.57 (22.51–2161.20)	0.72 (0.45–1.14)
Aagaard-Tillery 2009[42]	Sc	V ≥ 10 mm	3/54	5.6 (1.9–15.1)	17/7767	0.2 (0.1–0.4)	25.38 (7.66–84.09)	0.95 (0.89–1.01)
Analysis: total								
Pooled estimate			11/169	7.5 (4.2–12.9)	35/15 084	0.2 (0.1–0.4)	27.52 (13.61–55.68)	0.94 (0.91–0.98)
Heterogeneity								
Model				Fixed effects		Random effects	Fixed effects	Fixed effects
I^2				0.322		0.679	0.495	−0.993
Q				4.423		9.336	5.939	1.505
P				0.352		0.053	0.204	0.826
Analysis: high risk								
Pooled estimate			8/115	8.6 (3.9–17.9)	18/7317	0.2 (0.1–0.6)	38.24 (9.97–146.64)	0.94 (0.90–0.99)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Fixed effects
I^2				0.505		0.750	0.662	−0.341
Q				4.037		7.988	5.912	1.492
P				0.257		0.046	0.114	0.684

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened; V, lateral cerebral ventricle diameter.

studies. We assessed the overall diagnostic performance by weighted independent estimation of detection rate (sensitivity), false-positive rate (1 − specificity), positive likelihood ratio (LR; sensitivity / (1 − specificity)) and negative LR ((1 − sensitivity) / specificity). We used both fixed and random effects models to estimate weighted detection rate, false-positive rate and positive and negative LR across studies. The fixed-effects model weighs each study by the inverse of its variance. Random effects incorporate both within-study and between-study variation[50]. Random effects tend to provide wider CIs and are generally preferable, especially in the presence of between-study heterogeneity. Heterogeneity between studies was analyzed using both Higgins' I^2 and Q-test and was considered to be high if I^2 was over 0.50[51]. To explore the potential effect of different study populations on heterogeneity we performed such analysis for the whole dataset and in the subgroups of studies classified as high risk and screening.

The statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and Meta-Analyst (Tufts Medical Center, Boston, MA, USA) were used for data analysis.

RESULTS

The literature search identified 434 potentially eligible studies that were completely reviewed. The inclusion criteria were met by 48 studies (Table S1 online). In the case of ARSA, because there was only one publication, we included an additional study in which the maximum gestational age was 26 rather than 24 weeks[39]. The Newcastle–Ottawa scale assessments for the included studies are presented in Table S1.

Definitions of the markers

In all included studies ventriculomegaly was considered to be present if the diameter of the lateral cerebral ventricle was 10 mm or more, increased nuchal fold thickness was present if the thickness was 6 mm or more and the diagnosis of echogenic bowel required that this was of equal echogenicity to that of bone. The diagnosis of mild hydronephrosis was based on a minimum anteroposterior diameter of the renal pelvis, which varied between studies from 3 mm to 4 or 5 mm. The definitions of short femur, short humerus and hypoplastic nasal bone were based on a cut-off of the respective bone length as a function of gestational age or biparietal diameter, and the cut-offs differed between studies.

Screening performance of sonographic markers for trisomy 21

Screening performances of sonographic markers for trisomy 21 are presented in Tables 1–10 and Figure 1. The pooled estimates of detection rate, false-positive rate and positive and negative LR for trisomy 21 for each marker are summarized in Table 11.

Estimation of combined likelihood ratio of multiple markers for trisomy 21

The LR for trisomy 21 of individual isolated markers is given in the last column of Table 11. This was derived by multiplying the positive LR for the given marker by the negative LR of each of all other markers, except for short humerus.

Table 3 Performance of increased nuchal fold in screening for trisomy 21

		Trisomy 21		*Euploid*			
Study	*Type*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Bahado-Singh 1995[1]	HR	4/8	50.0 (21.5–78.5)	9/640	1.4 (0.7–2.7)	35.56 (13.76–91.86)	0.51 (0.25–1.01)
DeVore 1995[2]	HR	4/32	12.5 (5.0–28.1)	13/2000	0.6 (0.4–1.1)	19.23 (6.63–55.78)	0.88 (0.77–1.00)
Grandjean 1995[3]	HR	17/44	38.6 (25.7–53.4)	273/3205	8.5 (7.6–9.5)	4.54 (3.07–6.70)	0.67 (0.53–0.85)
Nyberg 1995[5]	HR	3/18	16.7 (5.8–39.2)	1/232	0.4 (0.1–2.4)	38.67 (4.23–353.10)	0.84 (0.68–1.03)
Vintzileos 1996[6]	HR	9/14	64.3 (38.8–83.7)	6/406	1.5 (0.7–3.2)	43.50 (17.95–105.40)	0.36 (0.18–0.73)
Deren 1998[8]	HR	5/29	17.2 (7.6–34.6)	22/3674	0.6 (0.4–0.9)	28.79 (11.71–70.80)	0.83 (0.71–0.98)
Tannirandorn 1999[11]	HR	2/19	10.5 (2.9–31.4)	51/2114	2.4 (1.8–3.2)	4.36 (1.14–16.64)	0.92 (0.79–1.07)
Vergani 1999[12]	HR	6/22	27.3 (13.2–48.2)	16/898	1.8 (1.1–2.9)	15.31 (6.63–35.37)	0.74 (0.57–0.96)
Wax 2000[13]	HR	0/7	0.0 (0.0–35.4)	1/772	0.1 (0.0–0.7)	0.0	1.00 (1.00–1.00)
Viora 2001[17]	HR	3/33	9.1 (3.1–23.6)	8/2069	0.4 (0.2–0.8)	23.51 (6.53–84.69)	0.91 (0.82–1.02)
Bahado-Singh 2002[18]	HR	28/108	25.9 (18.6–34.9)	42/5619	0.7 (0.6–1.0)	34.69 (22.37–53.78)	0.75 (0.67–0.83)
Sacco 2007[36]	HR	4/9	44.4 (18.9–73.3)	7/965	0.7 (0.4–1.5)	61.27 (21.69–173.07)	0.56 (0.31–1.00)
Vergani 2008[41]	HR	8/23	34.8 (18.8–55.1)	16/1118	1.4 (0.9–2.3)	24.30 (11.58–51.02)	0.66 (0.49–0.89)
Bottalico 2009[43]	HR	2/12	16.7 (4.7–44.8)	4/628	0.6 (0.3–1.6)	26.17 (5.29–129.40)	0.84 (0.65–1.08)
Schluter 2005[30]	Sc	24/73	32.9 (23.2–44.3)	142/16 891	0.8 (0.7–1.0)	39.11 (27.11–56.41)	0.68 (0.58–0.80)
Weisz 2007[38]	Sc	3/12	25.0 (8.9–53.2)	46/2320	2.0 (1.5–2.6)	12.61 (4.54–35.00)	0.77 (0.55–1.06)
Aagaard-Tillery 2009[42]	Sc	6/33	18.2 (8.6–34.4)	24/6473	0.4 (0.3–0.6)	49.04 (21.46–112.08)	0.82 (0.70–0.97)
Analysis: total							
Pooled estimate		128/496	26.0 (20.3–32.9)	681/50 024	1.0 (0.5–1.9)	23.30 (14.35–37.83)	0.80 (0.75–0.86)
Heterogeneity							
Model			Random effects		Random effects	Random effects	Random effects
I^2			0.532		0.983	0.843	0.526
Q			32.072		879.956	95.451	31.625
P			0.010		< 0.0001	< 0.0001	0.011
Analysis: high risk							
Pooled estimate		95/378	25.8 (18.7–34.4)	469/24 340	1.0 (0.5–2.1)	21.87 (12.32–38.81)	0.81 (0.75–0.88)
Heterogeneity							
Model			Random effects		Random effects	Random effects	Random effects
I^2			0.594		0.979	0.839	0.536
Q			29.557		560.512	74.328	25.854
P			0.005		< 0.0001	< 0.0001	0.018
Analysis: screened							
Pooled estimate		33/118	27.6 (18.9–38.3)	212/25 684	0.9 (0.4–1.9)	32.17 (17.04–60.71)	0.75 (0.66–0.86)
Heterogeneity							
Model			Random effects		Random effects	Random effects	Random effects
I^2			0.589		0.979	0.791	0.643
Q			2.432		48.490	4.789	2.799
P			0.296		< 0.0001	0.091	0.247

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened.

The same approach can be used when any combination of two or more markers is detected. For example the positive LRs for mild hydronephrosis and ventriculomegaly are 7.63 and 27.52, respectively. When the ultrasound examination detects these two markers the combined positive LR is 209.98 (7.63×27.52) and this must be multiplied by the combined negative LR of all other markers that were not present ($0.80 \times 0.80 \times 0.90 \times 0.80 \times 0.71 \times 0.46 = 0.15$) to derive a combined LR of 31.50 (209.98×0.15). A spreadsheet to automatically perform these calculations is available online (Appendix S1).

Incidence of trisomy 21 and euploid fetuses in the absence of sonographic markers

The literature search identified 12 studies that examined multiple sonographic markers and reported on the incidence of no markers in trisomy 21 and euploid fetuses (Table 12). In the absence of sonographic markers, the pooled incidences of trisomy 21 and euploid fetuses were 30.9% (95% CI, 23.1–39.9%) and 88.1% (95% CI, 85.3–90.4%), respectively. The LR for trisomy 21 in the absence of sonographic markers was 0.37 (95% CI, 0.29–0.47). Consequently, in the absence of all markers the risk for trisomy 21 was reduced by 2.7-fold.

In nine studies high-risk pregnancies were examined in specialist units[5,12,13,15,17,19,21,23,36] and in three the patients had routine second-trimester ultrasound examination[37,38,42]. The LR for trisomy 21 in the absence of sonographic markers in specialist units was 0.32 (95% CI, 0.24–0.42) and in the routine examination studies it was 0.52 (95% CI, 0.44–0.62). Consequently, in the absence of all markers the risk for trisomy 21 was reduced by 3.1-fold and 1.9-fold, respectively.

Table 4 Performance of echogenic bowel in screening for trisomy 21

		Trisomy 21		*Euploid*			
Study	*Type*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
DeVore 1995[2]	HR	6/32	18.8 (8.8–35.3)	31/2000	1.6 (1.1–2.2)	12.10 (5.43–26.96)	0.83 (0.70–0.98)
Nyberg 1995[5]	HR	1/18	5.6 (1.0–25.8)	5/232	2.2 (0.9–4.9)	2.58 (0.32–20.90)	0.97 (0.86–1.08)
Vintzileos 1996[6]	HR	0/14	0.0 (0.0–21.5)	4/406	1.0 (0.4–2.5)	—	1.01 (1.00–1.02)
Vergani 1999[12]	HR	0/22	0.0 (0.0–14.9)	7/898	0.8 (0.4–1.6)	—	1.01 (1.00–1.01)
Sohl 1999[10]	HR	9/55	16.4 (8.9–28.3)	63/2639	2.4 (1.9–3.0)	6.86 (3.60–13.07)	0.86 (0.76–0.96)
Wax 2000[13]	HR	1/7	14.3 (2.6–51.3)	4/772	0.5 (0.2–1.3)	27.57 (3.51–216.56)	0.86 (0.64–1.17)
Bahado-Singh 2002[18]	HR	23/108	21.3 (14.6–29.9)	116/5619	2.1 (1.7–2.5)	10.32 (6.88–15.46)	0.80 (0.73–0.89)
Sacco 2007[36]	HR	0/9	0.0 (0.0–29.9)	12/965	1.2 (0.7–2.2)	—	1.01 (1.01–1.02)
Vergani 2008[41]	HR	3/24	12.5 (4.3–31.0)	9/1129	0.8 (0.4–1.5)	15.68 (4.53–54.32)	0.88 (0.76–1.03)
Bottalico 2009[43]	HR	2/12	16.7 (4.7–44.8)	10/628	1.6 (0.9–2.9)	10.47 (2.56–42.73)	0.85 (0.66–1.09)
Schluter 2005[30]	Sc	13/73	17.8 (10.7–28.1)	252/16 891	1.5 (1.3–1.7)	11.94 (7.18–19.84)	0.83 (0.75–0.93)
Weisz 2007[38]	Sc	1/12	8.3 (1.5–35.4)	5/2320	0.2 (0.1–0.5)	38.67 (4.88–306.65)	0.92 (0.78–1.09)
Aagaard-Tillery 2009[42]	Sc	8/55	14.5 (7.6–26.2)	40/7778	0.5 (0.4–0.7)	28.28 (13.89–57.60)	0.86 (0.77–0.96)
Analysis: total							
Pooled estimate		67/441	16.7 (13.4–20.7)	558/42 277	1.1 (0.8–1.5)	11.44 (9.05–14.47)	0.90 (0.86–0.94)
Heterogeneity							
Model			Fixed effects		Random effects	Fixed effects	Random effects
I^2			−0.220		0.894	0.297	0.526
Q			9.014		103.581	15.646	23.223
P			0.702		< 0.0001	0.208	0.026
Analysis: high risk							
Pooled estimate		45/301	17.1 (13.1–22.1)	261/15 288	1.5 (1.1–1.9)	9.50 (7.13–12.68)	0.91 (0.86–0.96)
Heterogeneity							
Model			Fixed effects		Random effects	Fixed effects	Random effects
I^2			0.019		0.711	−0.236	0.574
Q			8.158		27.646	6.475	18.782
P			0.518		0.001	0.692	0.027
Analysis: screened							
Pooled estimate		22/140	15.9 (10.7–23.0)	297/26 989	0.6 (0.2–1.6)	19.07 (9.21–39.50)	0.86 (0.80–0.92)
Heterogeneity							
Model			Fixed effects		Random effects	Random effects	Fixed effects
I^2			−0.307		0.982	0.774	−0.138
Q			0.765		55.504	4.417	0.879
P			0.682		< 0.0001	0.110	0.644

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened.

Most studies examining multiple markers did not include absent or hypoplastic nasal bone and ARSA. The combined negative LR obtained by multiplying the values of the individual markers in Table 11, but excluding short humerus, absent or hypoplastic nasal bone and ARSA was 0.40 (95% CI, 0.29–0.58).

Estimation of combined likelihood ratio in the absence of all markers for trisomy 21

The combined negative LR, obtained by multiplying the values of individual markers in Table 11, was 0.13 (95% CI, 0.05–0.29) when short femur but not short humerus was included and 0.12 (95% CI, 0.06–0.29) when short humerus but not short femur was included. Consequently, in the absence of all markers the risk for trisomy 21 would be reduced by 7.7-fold and 8.3-fold, respectively.

DISCUSSION

The findings of this meta-analysis confirm that the incidence of each of the selected second-trimester sonographic markers is higher in trisomy 21 than in euploid fetuses. The pooled estimate of the positive LR was about 5 for intracardiac echogenic focus and short femur or humerus, about 10 for echogenic bowel and mild hydronephrosis, 20 for increased nuchal fold thickness and ARSA and about 25 for ventriculomegaly and absent or hypoplastic nasal bone. Absence of all markers, apart from short humerus, was associated with a combined negative LR of 0.13 and therefore a 7.7-fold reduction in risk. If assessment for ARSA and absent or hypoplastic nasal bone was not included in the ultrasound examination the negative LR was 0.40, with a consequent 2.5-fold reduction in risk.

Our results on intracardiac echogenic focus were similar to those of a previous meta-analysis that included 11 studies, published between 1995 and 2001, on a total of 51 831 pregnancies, of which 333 had trisomy 21, and that reported that the positive LR was 6.2[52]. However, our findings differ from those of Smith-Bindman *et al.*[53], who examined multiple markers on a combined total of 1930 fetuses with trisomy 21 and 130 365 unaffected fetuses in 56 articles published between 1980 and 1999.

Table 5 Performance of mild hydronephrosis in screening for trisomy 21

			Trisomy 21		*Euploid*			
Study	*Type*	*Renal pelvis*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
DeVore 1995[2]	HR	AP ≥ 4 mm	6/32	18.8 (8.9–35.3)	26/2000	1.3 (0.9–1.9)	14.42 (6.38–32.62)	0.82 (0.70–0.97)
Nyberg 1995[5]	HR	AP ≥ 4 mm	3/18	16.7 (5.8–39.2)	5/232	2.2 (0.9–4.9)	7.73 (2.01–29.79)	0.85 (0.69–1.05)
Vintzileos 1996[6]	HR	AP ≥ 4 mm	4/14	28.6 (11.7–54.7)	16/406	3.9 (2.4–6.3)	7.25 (2.78–18.89)	0.74 (0.53–1.04)
Deren 1998[8]	HR	AP ≥ 4 mm	1/34	2.9 (0.5–14.9)	22/3674	0.6 (0.4–0.9)	4.91 (0.68–35.41)	0.98 (0.92–1.04)
Sohl 1999[10]	HR	AP ≥ 4 mm	1/55	1.8 (0.3–9.6)	42/2639	1.6 (1.2–2.1)	1.14 (0.16–8.15)	1.00 (0.96–1.04)
Vergani 1999[12]	HR	AP ≥ 4 mm	4/22	18.2 (7.3–38.5)	18/898	2.0 (1.3–3.2)	9.07 (3.35–24.59)	0.84 (0.69–1.02)
Wax 2000[13]	HR	AP ≥ 4 mm	1/7	14.3 (2.6–51.3)	14/772	1.8 (1.1–3.0)	7.88 (1.19–52.01)	0.87 (0.65–1.18)
Viora 2001[17]	HR	AP ≥ 4 mm	1/33	3.0 (0.5–15.3)	26/2069	1.3 (0.9–1.8)	2.41 (0.34–17.25)	0.98 (0.92–1.04)
Sacco 2007[36]	HR	AP ≥ 4 mm	2/9	22.2 (6.3–54.7)	19/965	2.0 (1.3–3.1)	11.29 (3.07–41.45)	0.79 (0.56–1.13)
Vergani 2008[41]	HR	AP ≥ 4 mm	4/24	16.7 (6.7–35.9)	11/1129	1.0 (0.5–1.7)	17.11 (5.86–49.90)	0.84 (0.70–1.01)
Bottalico 2009[43]	HR	AP ≥ 4 mm	3/12	25.0 (8.9–53.2)	9/628	1.4 (0.8–2.7)	17.44 (5.39–56.50)	0.76 (0.55–1.06)
Coco 2005[29]	Sc	AP ≥ 4 mm	2/11	9.1 (1.6–37.7)	364/12 648	2.9 (2.6–3.2)	6.32 (1.80–22.22)	0.84 (0.64–1.11)
Schluter 2005[30]	Sc	AP ≥ 4 mm	15/73	20.5 (12.9–31.2)	355/16 891	2.1 (1.9–2.3)	9.78 (6.16–15.23)	0.81 (0.72–0.91)
Weisz 2007[38]	Sc	AP ≥ 5 mm	0/12	0.0 (0.0–24.3)	27/2320	1.2 (0.8–1.7)	—	1.01 (1.01–1.02)
Aagaard-Tillery 2009[42]	Sc	AP ≥ 3 mm	4/55	7.3 (2.9–17.3)	103/7777	1.3 (1.1–1.6)	5.49 (2.10–14.38)	0.94 (0.87–1.01)
Carbone 2011[48]	Sc	AP ≥ 4 mm	23/218	10.6 (7.1–15.3)	1213/61 730	2.0 (1.9–2.1)	5.37 (3.63–7.93)	0.91 (0.87–0.96)
Analysis: total								
Pooled estimate			74/629	13.9 (11.2–17.2)	2270/116 778	1.7 (1.4–2.0)	7.63 (6.11–9.51)	0.92 (0.89–0.96)
Heterogeneity								
Model				Fixed effects		Random effects	Fixed effects	Random effects
I^2				0.389		0.897	0.182	0.569
Q				22.910		135.854	17.117	32.492
P				0.086		< 0.0001	0.312	0.006
Analysis: high risk								
Pooled estimate			30/260	15.9 (11.3–21.9)	208/15 412	1.5 (1.2–2.0)	9.63 (6.67–13.92)	0.93 (0.88–0.98)
Heterogeneity								
Model				Fixed effects		Random effects	Fixed effects	Random effects
I^2				0.384		0.784	0.139	0.519
Q				14.599		41.651	10.453	18.714
P				0.147		< 0.0001	0.402	0.044
Analysis: screened								
Pooled estimate			44/369	12.6 (7.8–19.7)	2062/101 366	1.9 (1.5–2.3)	6.68 (5.07–8.81)	0.91 (0.87–0.96)
Heterogeneity								
Model				Random effects		Random effects	Fixed effects	Random effects
I^2				0.592		0.959	0.292	0.511
Q				7.358		73.479	4.238	6.132
P				0.118		< 0.000	0.375	0.190

Only the first author of each study is given. AP, anteroposterior diameter; DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened.

After exclusion of cases with major defects the estimated positive LR was 2.8 for intracardiac echogenic focus, 2.7 for short femur, 7.5 for short humerus, 6.1 for echogenic bowel, 1.9 for mild hydronephrosis and 17 for increased nuchal fold. These LRs are considerably lower than in our analysis. The most likely explanation for this is that the majority of studies were in high-risk pregnancies and the sensitivity of the markers in the meta-analysis of Smith-Bindman *et al*. was lower than in our analysis, presumably because awareness of the potential importance of these markers and therefore the search for their presence was not as widespread in the 1980s and early 1990s as in later years.

There was high heterogeneity in results between the studies, which presumably reflects the large differences in design and focus, including prospective and retrospective cohort studies or case–control studies, with the scans performed in specialist units or routine ultrasound departments, reporting on either one or multiple markers and using different definitions for the presence of a marker. The problem of high heterogeneity in results was not overcome by subanalysis of data derived from screening studies and those involving examination of high-risk pregnancies. Particularly big differences in LRs were observed for nuchal fold thickness and echogenic bowel, presumably reflecting the subjective nature of these markers and greater susceptibility to allocation bias.

We excluded studies published before 1995 because awareness of the possible importance of markers and consequently the specific search for their presence or absence was limited before this time. We focused

Table 6 Performance of short humerus in screening for trisomy 21

			Trisomy 21		*Euploid*			
Study	*Type*	*Definition*	*n/N*	*DR (%) (95% CI)*	*n/N*	*FPR (%) (95% CI)*	*LR+ (95% CI)*	*LR− (95% CI)*
Vintzileos 1996[7]	HR	H ≤ 0.89 MoM for BPD	10/22	45.5 (26.9–65.3)	49/493	9.9 (7.6–12.9)	4.57 (2.69–7.76)	0.61 (0.41–0.89)
Wax 2000[13]	HR	H ≤ 0.89 MoM for BPD	1/7	14.3 (2.6–51.3)	3/772	0.4 (0.1–1.1)	36.76 (4.34–311.62)	0.86 (0.64–1.16)
Viora 2001[17]	HR	H ≤ 0.90 MoM for BPD	9/33	27.3 (15.1–44.2)	258/2069	12.5 (11.1–14.0)	2.19 (1.24–3.86)	0.83 (0.67–1.03)
Bahado-Singh 2002[18]	HR	H < 0.90 MoM for BPD	30/108	27.8 (20.2–36.9)	320/5619	5.7 (5.1–6.3)	4.88 (3.53–6.73)	0.77 (0.68–0.86)
Bottalico 2009[43]	HR	H ≤ 0.89 MoM for GA	2/12	16.7 (4.7–44.8)	8/628	1.3 (0.7–2.5)	13.08 (3.10–55.24)	0.84 (0.66–1.09)
Schluter 2005[30]	Sc	H ≤ 0.92 MoM for BPD	47/73	64.4 (52.9–74.4)	1956/16 891	11.6 (11.1–12.1)	5.56 (4.66–6.63)	0.40 (0.30–0.55)
Aagaard-Tillery 2009[42]	Sc	H ≤ 0.90 MoM for BPD	3/26	11.5 (4.0–29.0)	89/3840	2.3 (1.9–2.8)	4.98 (1.68–14.72)	0.91 (0.79–1.04)
Analysis: total								
Pooled estimate			102/281	30.3 (17.1–47.9)	2683/30 312	4.6 (2.8–7.4)	4.81 (3.49–6.62)	0.74 (0.63–0.88)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Random effects
I^2				0.862		0.989	0.658	0.799
Q				36.273		450.252	14.600	24.926
P				< 0.0001		< 0.0001	0.024	< 0.0001
Analysis: high risk								
Pooled estimate			52/182	29.0 (22.8–36.2)	638/9581	4.2 (2.3–7.7)	4.80 (2.80–8.23)	0.78 (0.72–0.86)
Heterogeneity								
Model				Fixed effects		Random effects	Random effects	Fixed effects
I^2				0.324		0.981	0.754	−0.039
Q				4.438		154.944	12.205	2.888
P				0.350		< 0.0001	0.016	0.577
Analysis: screened								
Pooled estimate			50/99	34.0 (3.8–87.1)	2045/20 731	5.3 (1.0–23.0)	5.54 (4.66–6.59)	0.61 (0.28–1.35)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Random effects
I^2				—		—	—	—
Q				15.832		241.575	0.039	22.029
P				< 0.0001		< 0.0001	0.844	< 0.0001

Only the first author of each study is given. BPD, biparietal diameter; DR, detection rate; FPR, false-positive rate; GA, gestational age; H, humerus; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MoM, multiples of the median; Sc, screened.

Table 7 Performance of short femur in screening for trisomy 21

			Trisomy 21		*Euploid*			
Study	*Type*	*Definition*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Grandjean 1995[4]	HR	F ≤ 0.91 MoM for BPD	15/34	44.1 (28.9–60.6)	495/2763	17.9 (16.5–19.4)	2.46 (1.67–3.63)	0.68 (0.51–0.92)
Nyberg 1995[5]	HR	F ≤ 0.91 MoM for BPD	5/18	27.8 (12.5–50.9)	14/232	6.0 (3.6–9.9)	4.60 (1.87–11.34)	0.77 (0.58–1.03)
Vintzileos 1996[7]	HR	F ≤ 0.88 MoM for BPD	5/22	22.7 (10.1–43.4)	50/493	10.1 (7.8–13.1)	2.24 (0.99–5.06)	0.86 (0.68–1.08)
Sohl 1999[10]	HR	F ≤ 0.91 MoM for BPD	9/55	16.4 (8.9–28.3)	42/2639	1.6 (1.2–2.1)	10.28 (5.27–20.06)	0.85 (0.76–0.96)
Wax 2000[13]	HR	F ≤ 0.91 MoM for BPD	1/7	14.3 (2.6–51.3)	2/772	0.3 (0.1–0.9)	55.14 (5.63–540.30)	0.86 (0.64–1.16)
Viora 2001[17]	HR	F ≤ 0.91 MoM for BPD	10/33	30.3 (17.4–47.3)	213/2069	10.3 (9.1–11.7)	2.94 (1.73–5.02)	0.78 (0.62–0.97)
Bahado-Singh 2002[18]	HR	F < 0.90 MoM for BPD	30/108	27.8 (20.2–36.9)	503/5619	9.0 (8.2–9.7)	3.10 (2.26–4.25)	0.79 (0.71–0.89)
Vergani 2008[41]	HR	F ≤ 0.91 MoM for GA	4/24	16.7 (6.7–35.9)	145/1110	13.1 (11.2–15.2)	1.28 (0.52–3.16)	0.96 (0.80–1.15)
Bottalico 2009[43]	HR	F ≤ 0.91 MoM for GA	2/12	16.7 (4.7–44.8)	7/628	1.1 (0.5–2.3)	14.95 (3.46–64.64)	0.84 (0.65–1.09)
Schluter 2005[30]	Sc	F ≤ 0.93 MoM for BPD	46/73	63.0 (51.6–73.2)	2534/16 891	15.0 (14.5–15.6)	4.20 (3.51–5.03)	0.44 (0.32–0.59)
Weisz 2007[38]	Sc	F < 5th percentile for GA	1/12	8.3 (1.5–35.4)	111/2320	4.8 (4.0–5.7)	1.74 (0.26–11.48)	0.96 (0.81–1.14)
Aagaard-Tillery 2009[42]	Sc	F ≤ 0.91 MoM for BPD	16/56	28.6 (18.4–41.5)	514/7761	6.6 (6.1–7.2)	4.31 (2.83–6.58)	0.77 (0.65–0.90)
Analysis: total								
Pooled estimate			144/454	27.7 (19.3–38.1)	4630/43 297	6.4 (4.7–8.8)	3.72 (2.79–4.97)	0.80 (0.73–0.88)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Random effects
I^2				0.779		0.989	0.706	0.630
Q				45.344		870.638	33.977	26.993
P				< 0.0001		< 0.0001	< 0.0001	0.005
Analysis: high risk								
Pooled estimate			81/313	26.7 (21.9–32.0)	1471/16 325	5.7 (3.7–8.7)	3.79 (2.47–5.83)	0.83 (0.78–0.88)
Heterogeneity								
Model				Fixed effects		Random effects	Random effects	Fixed effects
I^2				0.347		0.983	0.76	−0.245
Q				10.714		407.439	29.132	5.623
P				0.218		< 0.0001	< 0.0001	0.689
Analysis: screened								
Pooled estimate			63/141	34.1 (11.8–66.8)	3159/26 972	7.9 (3.8–15.7)	4.19 (3.55–4.94)	0.70 (0.48–1.02)
Heterogeneity								
Model				Random effects		Random effects	Fixed effects	Random effects
I^2				0.948		0.998	−0.174	0.951
Q				19.253		446.995	0.852	20.472
P				< 0.0001		< 0.0001	0.653	< 0.0001

Only the first author of each study is given. BPD, biparietal diameter; DR, detection rate; F, femur; FPR, false-positive rate; GA, gestational age; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MoM, multiples of the median; Sc, screened.

Table 8 Performance of aberrant right subclavian artery in screening for trisomy 21

	Trisomy 21		*Euploid*			
Study	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Zalel 2008[39]	3/8	37.5 (13.7–69.4)	13/924	1.4 (0.8–2.4)	26.65 (9.38–75.77)	0.63 (0.37–1.08)
Borenstein 2010[45]	8/28	28.6 (15.3–47.1)	14/932	1.5 (0.9–2.5)	19.02 (8.69–41.62)	0.73 (0.57–0.92)
Pooled estimate	11/36	30.7 (17.8–47.4)	27/1856	1.5 (1.0–2.1)	21.48 (11.48–40.19)	0.71 (0.57–0.88)
Heterogeneity						
Model		Fixed effects		Fixed effects	Fixed effects	Fixed effects
I^2		0		0	0	0
Q		0.232		0.029	0.257	0.203
P		0.630		0.864	0.613	0.653

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; LR+, positive likelihood ratio; LR−, negative likelihood ratio.

Table 9 Performance of absent nasal bone in screening for trisomy 21

		Trisomy 21		*Euploid*			
Study	*Type*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Bromley 2002[19]	HR	6/16	37.5 (18.5–61.4)	1/223	0.4 (0.1–2.5)	83.63 (10.71–653.06)	0.63 (0.43–0.92)
Cicero 2003[23]	HR	11/34	32.4 (19.1–49.2)	6/982	0.6 (0.3–1.3)	52.95 (20.81–134.76)	0.68 (0.54–0.86)
Vintzileos 2003[24]	HR	12/29	41.4 (25.5–59.3)	0/102	0.0 (0.0–3.6)	—	0.59 (0.43–0.80)
Cusick 2004[26]	HR	1/4	25.0 (4.6–69.9)	0/422	0.0 (0.0–0.9)	—	0.75 (0.43–1.32)
Tran 2005[31]	HR	11/31	35.5 (21.1–53.1)	1/136	0.7 (0.1–4.1)	48.26 (6.47–360.02)	0.65 (0.50–0.84)
Viora 2005[32]	HR	10/18	55.6 (33.7–75.4)	2/417	0.5 (0.1–1.7)	115.83 (27.36–490.37)	0.45 (0.27–0.75)
Cusick 2007[34]	HR	1/11	9.1 (1.6–37.7)	3/371	0.8 (0.3–2.4)	11.24 (1.27–99.68)	0.92 (0.76–1.11)
Gianferrari 2007[35]	HR	10/21	47.6 (28.3–67.6)	1/2515	0.0 (0.0–0.2)	1197.62 (160.43–8940.55)	0.52 (0.35–0.79)
Persico 2008[40]	HR	7/26	26.9 (13.7–46.1)	0/135	0.0 (0.0–2.8)	—	0.73 (0.58–0.92)
Odibo 2006[33]	Sc	5/22	22.7 (10.1–43.4)	13/2446	0.5 (0.3–0.9)	42.76 (16.67–109.70)	0.78 (0.62–0.97)
Analysis: total							
Pooled estimate		74/212	36.1 (29.8–43.0)	27/7749	0.5 (0.3–0.7)	66.75 (40.62–109.69)	0.71 (0.65–0.78)
Heterogeneity							
Model			Fixed effects		Fixed effects	Fixed effects	Fixed effects
I^2			0.189		0.078	0.387	0.484
Q			9.868		8.674	13.046	15.506
P			0.361		0.468	0.161	0.078
Analysis: high risk							
Pooled estimate		69/190	37.5 (30.8–44.8)	14/5303	0.5 (0.3–0.8)	79.23 (44.16–142.15)	0.67 (0.59–0.77)
Heterogeneity							
Model			Fixed effects		Fixed effects	Fixed effects	Random effects
I^2			0.132		0.186	0.410	0.530
Q			8.065		8.604	11.858	14.887
P			0.427		0.377	0.158	0.061

Only the first author of each study is given. DR, detection rate; FPR, false-positive rate; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; Sc, screened.

on studies reporting within the gestational-age range of 14–24 weeks to minimize the potential effect of gestational age on the incidence of the markers. In the majority of studies the median gestational age of the ultrasound examinations was 17–19 weeks. It remains uncertain whether the relative proportion of trisomic and euploid fetuses with a given marker, and therefore the LR, is constant across the gestational-age range of 14–24 weeks. This is true for categorical variables, such as intracardiac echogenic foci and echogenic bowel and even more so for markers based on fixed measurements, such as increased nuchal fold thickness, mild hydronephrosis and ventriculomegaly.

Some of the studies were in women undergoing routine screening during the second trimester but most studies were performed in patients undergoing amniocentesis because their risk was considered to be high owing to either advanced maternal age or abnormal second-trimester serum biochemistry testing. This is reflected in the high incidence of trisomy 21 in most study populations included in the analysis.

A few of the included studies report results on multiple markers, but the majority examined specifically the value of individual markers. There are no studies that systematically examined the possible interrelationship between markers, and it is therefore assumed that they are independent of each other, apart from short femur and short humerus, which have been shown to be highly correlated in both euploid and trisomic fetuses[42]. Similarly, there is no consistent evidence that

Table 10 Performance of absent or hypoplastic nasal bone in screening for trisomy 21

			Trisomy 21		*Euploid*			
Study	*Type*	*Definition (absent or hypoplastic)*	*n/N*	*DR (95% CI) (%)*	*n/N*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*
Bromley 2002[19]	HR	BPD/NBL ≥ 11	11/16	68.8 (44.4–85.8)	11/223	4.9 (2.8–8.6)	13.94 (7.17–27.08)	0.33 (0.16–0.68)
Cicero 2003[23]	HR	NBL < 2.5 mm	21/34	61.8 (45.0–76.1)	12/982	1.2 (0.7–2.1)	50.50 (27.15–94.09)	0.39 (0.25–0.59)
Bunduki 2003[22]	HR	NBL < 5th percentile for GA	13/22	59.1 (38.7–76.7)	82/1600	5.1 (4.2–6.3)	11.53 (7.68–17.32)	0.43 (0.26–0.71)
Cusick 2004[26]	HR	NBL < 3 mm	4/4	100.0 (51.0–100.0)	0/422	0.0 (0.0–0.9)	—	0.00
Gámez 2004[27]	HR	NBL < 2.5 mm	5/5	100.0 (56.6–100.0)	34/1899	1.8 (1.3–2.5)	55.85 (40.03–77.93)	0.00
Odibo 2004[28]	HR	BPD/NBL ≥ 12	6/16	37.5 (18.5–61.4)	19/508	3.7 (2.4–5.8)	10.03 (4.64–21.68)	0.65 (0.44–0.95)
Tran 2005[31]	HR	BPD/NBL ≥ 18	15/31	48.4 (32.0–65.2)	5/136	3.7 (1.6–8.3)	13.16 (5.17–33.49)	0.54 (0.38–0.76)
Viora 2005[32]	HR	NBL < 2.5 mm	14/18	77.8 (54.8–91.0)	3/417	0.7 (0.2–2.1)	108.11 (34.09–342.88)	0.22 (0.09–0.53)
Cusick 2007[34]	HR	BPD/NBL ≥ 11	7/11	63.6 (35.4–84.8)	16/371	4.3 (2.7–6.9)	14.76 (7.66–28.41)	0.38 (0.17–0.83)
Gianferrari 2007[35]	HR	NBL < 0.75 MoM	18/21	85.7 (65.4–95.0)	74/2515	2.9 (2.4–3.7)	29.13 (21.92–38.71)	0.15 (0.05–0.42)
Sooklim 2010[47]	HR	BPD/NBL ≥ 12	3/10	30.0 (10.8–60.3)	2/386	0.5 (0.1–1.9)	57.90 (10.84–309.25)	0.70 (0.47–1.06)
Odibo 2006[33]	Sc	BPD/NBL ≥ 12	9/22	40.9 (23.3–61.3)	161/2423	6.6 (5.7–7.7)	6.16 (3.65–10.40)	0.63 (0.45–0.90)
Analysis: total								
Pooled estimate			126/210	59.8 (48.9–69.9)	419/11 882	2.8 (1.9–4.0)	23.27 (14.23–38.06)	0.46 (0.36–0.58)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Random effects
I^2				0.547		0.916	0.878	0.537
Q				22.069		118.436	82.043	21.609
P				0.024		< 0.0001	< 0.0001	0.028
Analysis: high risk								
Pooled estimate			117/188	62.0 (50.7–72.2)	258/9459	2.5 (1.7–3.6)	26.31 (16.57–41.77)	0.43 (0.33–0.56)
Heterogeneity								
Model				Random effects		Random effects	Random effects	Random effects
I^2				0.531		0.863	0.842	0.542
Q				19.208		65.483	56.867	19.655
P				0.038		< 0.0001	< 0.0001	0.033

Only the first author of each study is given. BPD, biparietal diameter; DR, detection rate; FPR, false-positive rate; GA, gestational age; HR, high risk; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MoM, multiples of the median; NBL, nasal bone length; Sc, screened.

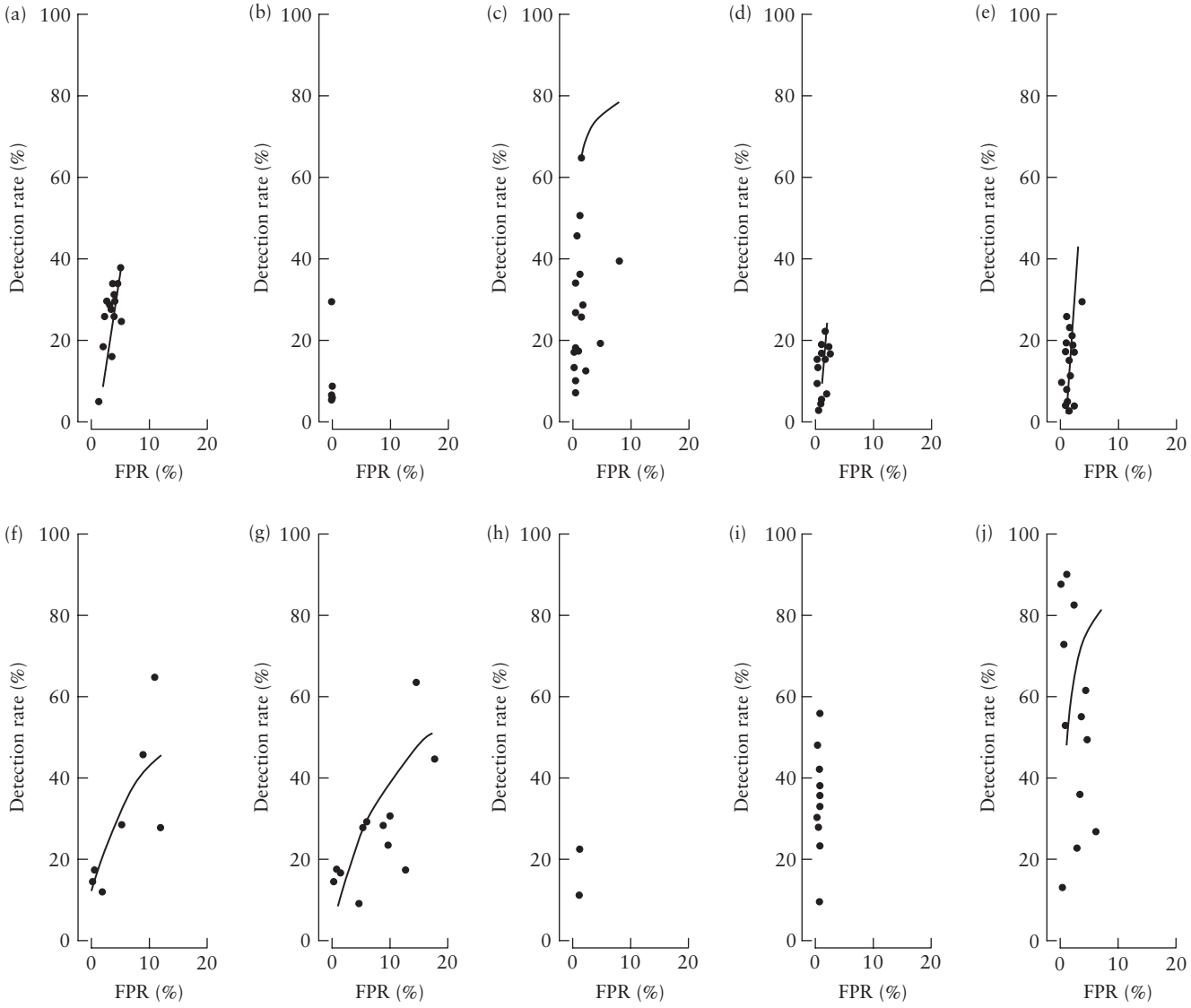

Figure 1 Summary receiver–operating characteristics curves with detection rate and false-positive rate (FPR) of sonographic markers of trisomy 21: (a) intracardiac echogenic foci, (b) ventriculomegaly, (c) nuchal fold thickness, (d) echogenic bowel, (e) hydronephrosis, (f) short humerus, (g) short femur, (h) aberrant right subclavian artery, (i) absent nasal bone and (j) absent or hypoplastic nasal bone.

Table 11 Pooled estimates of detection rate (DR), false positive rate (FPR) and positive and negative likelihood ratios (LR+ and LR−) of sonographic markers for trisomy 21 and estimated likelihood ratio (LR) of individual isolated markers

Marker	*DR (95% CI) (%)*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*	*LR isolated marker**
Intracardiac echogenic focus	24.4 (20.9–28.2)	3.9 (3.4–4.5)	5.83 (5.02–6.77)	0.80 (0.75–0.86)	0.95
Ventriculomegaly	7.5 (4.2–12.9)	0.2 (0.1–0.4)	27.52 (13.61–55.68)	0.94 (0.91–0.98)	3.81
Increased nuchal fold	26.0 (20.3–32.9)	1.0 (0.5–1.9)	23.30 (14.35–37.83)	0.80 (0.74–0.85)	3.79
Echogenic bowel	16.7 (13.4–20.7)	1.1 (0.8–1.5)	11.44 (9.05–14.47)	0.90 (0.86–0.94)	1.65
Mild hydronephrosis	13.9 (11.2–17.2)	1.7 (1.4–2.0)	7.63 (6.11–9.51)	0.92 (0.89–0.96)	1.08
Short humerus	30.3 (17.1–47.9)	4.6 (2.8–7.4)	4.81 (3.49–6.62)	0.74 (0.63–0.88)	0.78
Short femur	27.7 (19.3–38.1)	6.4 (4.7–8.8)	3.72 (2.79–4.97)	0.80 (0.73–0.88)	0.61
ARSA	30.7 (17.8–47.4)	1.5 (1.0–2.1)	21.48 (11.48–40.19)	0.71 (0.57–0.88)	3.94
Absent or hypoplastic NB	59.8 (48.9–69.9)	2.8 (1.9–4.0)	23.27 (14.23–38.06)	0.46 (0.36–0.58)	6.58

*Derived by multiplying the positive LR for the given marker by the negative LR of each of all other markers, except for short humerus. ARSA, aberrant right subclavian artery; NB, nasal bone.

Table 12 Studies reporting data on the absence of multiple markers in trisomy 21 and euploid fetuses

			Trisomy 21 – no marker		*Euploid – no marker*		
Study	*Type*	*Sonographic markers*	*n/N*	*% (95% CI)*	*n/N*	*% (95% CI)*	*LR+ (95% CI)*
Nyberg 1995[5]	HR	NF, EB, hydro, SF, ventric	9/18	50.0 (29.0–71.0)	350/374	93.6 (90.6–95.7)	0.53 (0.34–0.85)
Vergani 1999[12]	HR	NF, EF, EB, hydro, SF, SH	10/22	45.5 (26.9–65.3)	870/898	96.9 (95.5–97.8)	0.47 (0.30–0.74)
Wax 2000[13]	HR	NF, EF, EB, hydro, SF, SH, CPC, SUA, clinodactyly	2/7	28.6 (8.2–64.1)	688/772	89.1 (86.7–91.1)	0.32 (0.10–1.04)
Nyberg 2001[15]	HR	NF, EF, EB, hydro, SF, SH	58/186	31.2 (25.0–38.2)	7541/8728	86.4 (85.7–87.1)	0.36 (0.29–0.45)
Viora 2001[17]	HR	NF, EB, hydro, SF, SH, CPC	10/33	30.3 (17.4–47.3)	1497/2069	72.4 (70.4–74.2)	0.42 (0.25–0.70)
Bromley 2002[19]	HR	NF, EF, EB, hydro, SF, SH	32/164	19.5 (14.2–26.3)	575/656	87.7 (84.9–90.9)	0.22 (0.16–0.30)
Vintzileos 2002[21]	HR	NF, EF, EB, hydro, SF, SH, CPC, SUA, clinodactyly, sandal gap, short ear	7/53	13.2 (6.6–24.8)	3291/3700	88.9 (87.9–89.9)	0.15 (0.07–0.30)
Cicero 2003[23]	HR	NF, EF, EB, hydro, SF, SH, CPC, NBH, clinodactyly, sandal gap	4/34	11.8 (4.7–26.6)	694/982	70.7 (67.8–73.4)	0.17 (0.07–0.42)
Sacco 2007[36]	HR	NF, EF, EB, hydro, SF, cardiac markers	2/9	22.2 (6.3–54.7)	915/965	94.8 (93.2–96.1)	0.23 (0.07–0.80)
Smith-Bindman 2007[37]	Sc	NF, EF, EB, hydro, SF, SH, CPC	115/245	46.9 (40.8–53.2)	7467/8707	85.8 (85.0–86.5)	0.55 (0.48–0.63)
Weisz 2007[38]	Sc	NF, EF, EB, hydro, SF	6/12	50.0 (25.4–74.6)	2013/2320	86.8 (85.3–88.1)	0.58 (0.33–1.02)
Aagaard-Tillery 2009[42]	Sc	NF, EF, EB, hydro, SF, SH	21/59	35.6 (24.6–48.3)	6775/7783	87.0 (86.3–87.8)	0.41 (0.29–0.58)
Analysis: total							
Pooled estimate			276/842	30.9 (23.1–39.9)	32 676/37 954	88.1 (85.3–90.4)	0.37 (0.29–0.47)
Heterogeneity							
Model				Random effects		Random effects	Random effects
I^2				0.815		0.985	0.795
Q				54.040		660.089	48.696
P				< 0.0001		< 0.0001	< 0.0001
Analysis: high risk							
Pooled estimate			134/526	26.4 (19.2–35.2)	16 421/19 144	89.0 (84.2–92.4)	0.32 (0.24–0.42)
Heterogeneity							
Model				Random effects		Random effects	Random effects
I^2				0.696		0.989	0.676
Q				23.010		636.644	21.597
P				0.003		< 0.0001	0.006
Analysis: screened							
Pooled estimate			142/316	44.1 (36.6–51.8)	16 255/18 810	86.5 (85.5–87.4)	0.52 (0.44–0.62)
Heterogeneity							
Model				Random effects		Random effects	Random effects
I^2				0.612		0.836	0.600
Q				2.575		6.100	2.500
P				0.276		0.047	0.287

Only the first author of each study is given. Cardiac markers are pericardial effusion, tricuspid regurgitation, ventricular disproportion, ventricular septal defect. CPC, choroid plexus cyst; EB, echogenic bowel; EF, intracardiac echogenic focus; HR, high risk; hydro, mild hydronephrosis; LR+, positive likelihood ratio; NBH, nasal bone hypoplasia; NF, increased nuchal fold; Sc, screened; SF, short femur; SH, short humerus; SUA, single umbilical artery; ventric, ventriculomegaly.

the incidence of markers is related to maternal age or the results of second-trimester serum biochemical testing or first-trimester combined testing[42]. Surprisingly, three studies reported that there is no significant association between nuchal translucency (NT) thickness at 11–13 weeks' gestation and second-trimester nuchal fold thickness[54–56]. Another study in euploid fetuses reported a weak but significant association between NT and nuchal fold thickness, with a correlation coefficient of 0.1[57]. The same study found that in cases with increased NT, compared to those with normal NT, there was a higher frequency of echogenic bowel (2.4 *vs* 0.1%), but not intracardiac echogenic focus, pyelectasia or short femur and short humerus[57]. In contrast, another study reported that the incidence of intracardiac echogenic focus during the second trimester was 2.8-fold higher in fetuses with increased NT compared to those with normal NT[16].

Several studies have reported on the use of ultrasonography to modify the risk of aneuploidy in pregnancies with advanced maternal age or abnormal serum biochemistry[22,58–60]. It was subsequently suggested that, in the estimation of the post-test odds for trisomy 21 based on ultrasound findings during the second trimester of pregnancy, the pre-test odds, derived from maternal age, second-trimester serum biochemical testing or first-trimester combined testing, could be multiplied by the positive LR of each marker found to be present and the negative LR of each marker looked for but not found[61].

In this meta-analysis the combined negative LR of all markers, including short femur but not short humerus, was 0.13, implying that if a systematic ultrasound examination is carried out and all markers are excluded there is a 7.7-fold reduction in risk. This estimated negative LR is similar to the 0.15 reported in a study in which a very detailed scan, including examination for features such as short ears, sandal gap and clinodactyly, was carried out in high-risk pregnancies[21]. However, such reduction in risk requires considerable expertise in scanning and in three studies in women undergoing routine second-trimester ultrasound examination the combined negative LR was 0.52, with a consequent 1.9-fold reduction in risk[37,38,42].

The clinical implications of our findings are that firstly, if a systematic second-trimester ultrasound examination demonstrates the absence of all major defects and markers there is a 7.7-fold reduction in risk for trisomy 21; secondly, the detection of any one of the markers during the scan should stimulate the sonographer to look for all other markers or defects; thirdly, the post-test odds for trisomy 21 is derived by multiplying the pre-test odds by the positive LR for each detected marker and the negative LR for each marker demonstrated to be absent; and fourthly, in the case of most isolated markers, including intracardiac echogenic focus, echogenic bowel, mild hydronephrosis and short femur, there is only a small effect on modifying the pre-test odds.

Further studies are needed to establish reference ranges for each biometric marker and to estimate the effect of gestational age on screening performance. In the era of widespread first-trimester screening and selective termination of most affected fetuses the undertaking of high-quality screening studies may ultimately be impossible. In the interim the data arising from this meta-analysis and their interpretation could form the basis for clinical practice. However, as in the case of fetal NT, it is essential that those performing the second-trimester scan receive appropriate training and certification of competence and subject their results to regular audit.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Characteristics of studies included in the analysis

Appendix S1 Excel spreadsheet allowing automated calculations of the likelihood ratio for any given combination of presence and absence of markers using pooled estimates from the meta-analysis. Please note that this does not provide confidence intervals for estimates of combined likelihood ratios