

Isolated Sonographic Markers for Detection of Fetal Down Syndrome in the Second Trimester of Pregnancy

David A. Nyberg, MD, Vivienne L. Souter, MD,
Amira El-Bastawissi, MBCB, PhD, Scott Young, MD,
Fred Luthardt, MD, David A. Luthy, MD

Objective. To determine whether sonographic “markers” are associated with fetal Down syndrome during the second trimester and to estimate the degree of risk of individual markers using likelihood ratios. **Methods.** Second-trimester (14–20 weeks) sonographic findings in 186 fetuses with trisomy 21 were compared with a control group of 8728 consecutive control fetuses. Six markers were evaluated: nuchal thickening, hyperechoic bowel, shortened femur, shortened humerus, echogenic intracardiac focus, and renal pyelectasis. **Results.** Major or structural abnormalities were observed in 31 fetuses with trisomy 21 (16.7%) and 53 control fetuses (0.6%) ($P < .001$). Some type of sonographic finding (major abnormality, minor marker, or both) was observed in 68.8% of fetuses with trisomy 21 compared with 13.6% of control fetuses ($P < .001$). An isolated minor or “soft” marker was the only sonographic finding in 42 (22.6%) of 186 fetuses with trisomy 21 compared with 987 (11.3%) of 8728 control fetuses ($P < .001$). Nuchal thickening ($P < .001$; likelihood ratio, 11) and hyperechoic bowel ($P < .001$; likelihood ratio, 6.7) showed the strongest association with trisomy 21 as isolated markers, followed by shortened humerus (likelihood ratio, 5.1), echogenic intracardiac focus (likelihood ratio, 1.8), shortened femur (likelihood ratio, 1.5), and pyelectasis (likelihood ratio, 1.5). Echogenic intracardiac focus was the single most common isolated marker in both affected fetuses (7.1%) and control fetuses (3.9%) but carried a low risk ($P = .046$; likelihood ratio, 1.8). **Conclusions.** A single soft marker is commonly encountered during the second trimester among fetuses with trisomy 21. The risk of fetal Down syndrome, reflected by likelihood ratios, was determined for 6 individual markers. This information can be combined with the a priori risk to estimate the individual patient risk for fetal Down syndrome. **Key words:** Down syndrome; fetus, abnormalities; prenatal diagnosis; chromosome abnormalities; prenatal sonography.

Abbreviations

AAURA, age-adjusted ultrasound risk assessment; CI, confidence interval; EIF, echogenic intracardiac focus; LR, likelihood ratio; ROC, receiver operating characteristic

Received June 6, 2001, from Seattle Ultrasound Associates (D.A.N., V.L.S., S.Y.), Group Health Cooperative of Puget Sound, Center for Health Promotion (A.E.-B.), and Department of Cytogenetics (F.L.) and Center for Perinatal Studies (D.A.L.), Swedish Medical Center, Seattle, Washington. Dr Souter is now with the Genetics Department, University of Washington Medical Center, Seattle, Washington. Dr Young is now with the Radiology Department, Mayo Medical Foundation, Scottsdale, Arizona. Revision requested June 15, 2001. Revised manuscript accepted for publication June 26, 2001.

Address correspondence and reprint requests to David A. Nyberg, MD, Seattle Ultrasound Associates, 1229 Madison St, 1150, Seattle, WA 98115.

A variety of sonographic findings may be seen in fetuses with Down syndrome, including both structural abnormalities and nonstructural abnormalities or “markers.”^{1–7} Sonographic findings that are generally accepted as potential markers of trisomy 21 during the second trimester include nuchal thickening,^{8–10} hyperechoic bowel,^{11–15} echogenic intracardiac focus (EIF),^{16–21} shortened femur or humerus,^{22,23} and renal pyelectasis.^{24,25} These markers are nonspecific, are also present in fetuses without abnormalities, are often transient, and can be readily detected during the second trimester.

Minor or “soft” markers are more common than major or structural abnormalities in fetuses with trisomy 21 when multiple markers are sought during the second trimester. Major abnormalities are observed in fewer

than 25% of affected fetuses in most studies,^{1,7,26,27} whereas 1 or more soft markers may be observed in 50% or more cases.^{5,7,28,29} This emphasizes the importance of soft markers for detection of fetal trisomy 21 on second-trimester sonography, especially among high-risk women in whom high sensitivity is desirable. On the other hand, the false-positive rate may be unacceptably high (13%–17%) if any one of a panel of markers is detected among low-risk women.^{5,7,28–30}

Sonographic markers are often associated with other markers or structural abnormalities. Therefore, the risk of fetal Down syndrome increases with the number of markers present,^{4–7} whereas the risk from an isolated marker is less certain. Estimating the risk of an isolated marker from a panel of multiple markers requires evaluation of a large number of fetuses with trisomy 21.

The goals of the present study were (1) to determine whether sonographic markers are associated with trisomy 21 when isolated and (2) to estimate the degree of risk with calculated likelihood ratios (LRs).

Materials and Methods

We identified 201 fetuses with trisomy 21 who underwent second-trimester sonography (14–20 weeks) over a 9-year period (March 1990 to March 1999) at a single high-risk center. To minimize bias of sonographic findings, 15 patients who were referred because of abnormal outside sonographic findings were excluded, leaving 186 affected fetuses. This group included 132 fetuses who were included in a previous study.⁷ Cytogenetic, birth, and pathologic records were matched with sonographic records to maximize inclusion of affected fetuses.

Control fetuses consisted of 8728 consecutive fetuses who underwent second-trimester sonography (14–20 weeks) between April 1996 and March 1999 and who had a normal ($n = 3233$ [37%]) or presumably normal ($n = 5495$ [63%]) fetal karyotype.

In all instances, the sonograms were obtained without knowledge of the karyotype and before genetic evaluation. Sonography was performed in a standardized manner that followed the general guidelines of the American Institute of Ultrasound in Medicine with additional views obtained of the posterior fossa, outflow tracts of

the heart, hands, feet, nose, and lips whenever possible. Measurements were made of biparietal diameter, head circumference, abdominal circumference, femur length, humerus length, nuchal thickness (transcerebellar view), and renal pelvis in the anteroposterior dimension. Examinations were performed by highly qualified sonographers registered by the American Registry of Diagnostic Medical Sonographers and were performed under the direct supervision of a physician sonologist who usually also scanned the patient. The scheduled examination time was 45 minutes.

Major abnormalities were considered to be cardiac defects, hydrops, cystic hygroma with internal septation, and duodenal atresia. Nonstructural markers that were evaluated included nuchal thickening (≥ 5 mm),^{7,10} pyelectasis (renal pelvis ≥ 3 mm),⁷ hyperechoic bowel (grades 2 and 3),¹² EIF, and extremity shortening (humerus or femur shortening). Mild cerebral ventricular dilatation (lateral ventricle 10–15 mm) was not considered a structural abnormality and was not separately considered as a sonographic marker in this series.

The femur was considered shortened when the measured-to-expected ratio was 0.91 or less; the humerus was considered shortened when the measured-to-expected ratio was 0.89 or less.²³ An echogenic intracardiac focus was seen as single or multiple discrete foci within the cardiac ventricles, typically the left, with echogenicity equal to or greater than that of bone.⁷ The echogenic intracardiac focus was prospectively evaluated only after April 1996; for cases of trisomy 21 detected before that time, retrospective identification of EIF was accomplished by blindly mixing and blindly reviewing those cases together with cases from fetuses with a known normal karyotype. EIF was defined to be present when 2 independent reviewers (D.A.N. and S.Y.) agreed on its presence. For all other sonographic markers and since April 1996 for EIF, the measurements and presence or absence of the various markers were recorded in a computer database at the time of the sonographic examination.

The frequency of sonographic markers was calculated both overall and as isolated findings. Sonographic markers were considered isolated when they were not associated with major abnormalities or any other of the markers evaluated. Because major or structural anomalies are considered and treated differently from cases

with soft markers alone, the frequency of sonographic markers was also calculated after exclusion of major anomalies.

Statistical analysis was performed using Stata software (Stata Corporation, College Station, TX). Comparisons were performed using the Pearson χ^2 test. Significance was taken at the 5% level. Confidence intervals were calculated at the 95th percentile confidence limits. The LR is defined as sensitivity/false-positive rate. An LR of greater than 1 suggests a positive association with a particular finding. We also performed logistic regression analysis and receiver operating characteristic (ROC) curve analysis to determine which markers were significant.

Results

The mean (SD) maternal age for the 186 patients who proved to have fetal Down syndrome was 36.3 (4.9) years, and the mean maternal age for the control group was 32.4 (5.5) years (Table 1). The mean gestational ages at the time of sonography were 16.9 (1.7) weeks for affected fetuses and 17.6 (1.9) weeks for the control group ($P < .01$).

Among affected fetuses with trisomy 21, the maternal indication for scanning was advanced maternal age (35 years and older) with or without abnormal biochemical screening results in 68.3% of cases, abnormal biochemical screening results in women younger than 35 years in 17.2%, and routine second-trimester sonography in women younger than 35 years in 14.5% (Table 1). Of the control group, 39.9% were referred for advanced maternal age, and 1.9% were referred for abnormal biochemical screening results. The remaining patients were referred for routine indications, most commonly a routine fetal survey.

The risk of trisomy 21 was related to both major abnormalities and the number of soft markers (Table 2). Among 186 fetuses with trisomy 21, 31 (16.7%) had structural or major abnormalities, including cystic hygroma, hydrops, or both ($n = 16$), cardiac defects ($n = 14$), and duodenal atresia ($n = 1$). In comparison, of 8728 control fetuses, 53 (0.6%) had major anomalies shown on sonography ($P < .01$). Some type of sonographic finding (major abnormality, minor marker, or both) was observed in 68.8% of fetuses with trisomy 21 compared with 13.6% of control fetuses ($P < .01$). Conversely, no abnormalities were shown on sonography in 31.2% of

affected fetuses compared with 86.4% of control fetuses (LR, 0.36). Overall, markers had a sensitivity of 52.2% (97 of 186) for trisomy 21 and a false-positive rate of 13% (1134 of 8728) in the comparison group.

Each sonographic marker was observed with significantly greater frequency in fetuses with trisomy 21 (Table 3). In affected fetuses, nuchal thickening (39.4%) was the most common sonographic marker, followed by short femur (31.6%), EIF (27.7%), short humerus (21.9%), hyperechoic bowel (20.6%), and pyelectasis (13.5%). Among control fetuses, short femur (5.2%) was the most common false-positive

Table 1. Comparison of 186 Fetuses With Down Syndrome and 8728 Consecutive Control Fetuses Examined During the Second Trimester (14–20 Weeks)

Characteristic	Down Syndrome (n = 186)	Control (n = 8728)	P
Maternal age, y	36.3 (SD, 4.9)	32.4 (SD, 5.5)	<.01
Gestational age, wk	16.9 (SD, 1.7)	17.6 (SD, 1.9)	<.01
Indication advanced maternal age, %	68.3	39.9	<.01
Abnormal biochemical screening results, %	17.2	1.9	<.01

Table 2. Comparison of Number of Markers in Fetuses With Down Syndrome and Control Fetuses

No. of Markers or Major Abnormality	Down Syndrome (n = 186)	Control (n = 8728)	LR*
0, n (%)	58 (31.2)	7541 (86.7)	0.36
1, n (%)	42 (22.6)	987 (11.3)	2.0
2, n (%)	28 (15.1)	136 (1.6)	9.7
≥3, n (%)	27 (14.5)	11 (0.1)	115.2
Major abnormality, n (%)	31 (16.7)	63 (0.6)	27.5

*Percent fetuses with Down syndrome/control fetuses.

Table 3. Comparison of Sonographic Findings After Exclusion of Major Abnormalities in 155 Fetuses With Fetal Down Syndrome and 8675 Control Fetuses

Sonographic Marker	Down Syndrome (n = 155)	Control (n = 8675)	P
Nuchal thickening, n (%)	36 (23.2)	56 (0.6)	<.001
Hyperechoic bowel, n (%)	21 (13.5)	53 (0.6)	<.001
Short humerus, n (%)	29 (18.7)	124 (1.4)	<.001
Short femur, n (%)	44 (28.4)	451 (5.2)	<.001
EIF, n (%)	37 (23.8)	382 (4.4)	<.001
Pyelectasis, n (%)	21 (13.5)	226 (2.6)	<.001

marker, followed by EIF (4.4%), pyelectasis (2.6%), short humerus (1.4%), hyperechoic bowel (0.6%), and nuchal thickening (0.6%).

An isolated soft marker was the only sonographic finding in 42 (22.6%) of 186 affected fetuses. After exclusion of major abnormalities, an isolated soft marker was the only sonographic finding in 42 (27.1%) of 155 affected fetuses, compared with 987 (11.4%) of 8675 control fetuses ($P < .001$; Table 4). Among affected fetuses, EIF was the single most common isolated marker, observed in 11 (7.1%) of 155 fetuses with Down syndrome. This represented 24% (11 of 42) of affected fetuses with an isolated soft marker. Other isolated soft markers, in descending order of frequency, were nuchal thickening (5.8%), short femur (5.8%), hyperechoic bowel (3.2%), pyelectasis (3.2%), and short humerus (1.9%).

An echogenic intracardiac focus and a shortened femur were the 2 most common isolated markers in control fetuses, observed in 3.9% of cases each, followed by pyelectasis (2.2%). Each of the other 3 markers was observed as an isolated finding in 0.5% or less of control fetuses.

The sensitivity of EIF was probably underestimated in this study, because this marker was retrospectively evaluated for fetuses with Down syndrome before April 1996. The sensitivity of EIF during the retrospective time of the study was 17% (22 of 127 cases), and it was 35.6% (21 of 59) when evaluated prospectively.

A shortened femur and pyelectasis as isolated findings were not found to be statistically associated with trisomy 21 (Table 4). If these were excluded, the overall sensitivity of markers would decrease from 52.1% to 44.6% (83 of 186), and the false-positive rate among control fetuses would decrease from 13% to 7% (609 of 8728).

Table 4. Comparison of Isolated Sonographic Findings After Exclusion of Major Abnormalities in Fetuses With Trisomy 21 and Control Fetuses

Sonographic Marker	Down Syndrome (n = 155)	Control (n = 8675)	P
Nuchal thickening, n (%)	9 (5.8)	46 (0.5)	<.001
Hyperechoic bowel, n (%)	5 (3.2)	42 (0.5)	<.001
Short humerus, n (%)	3 (1.9)	33 (0.4)	<.02
Short femur, n (%)	9 (5.8)	338 (3.9)	.2 (NS)
EIF, n (%)	11 (7.1)	341 (3.9)	.046
Pyelectasis, n (%)	5 (3.2)	187 (2.2)	.4 (NS)
Any single marker, n (%)	42 (27.1)	987 (11.4)	<.001

NS indicates not significant.

The risk of each sonographic marker is reflected by the observed LRs (Table 5). Isolated sonographic markers yielded LRs of 11 (95% confidence interval [CI], 5.5–22) for nuchal thickening, 6.7 (CI, 2.7–16.8) for hyperechoic bowel, 5.1 (CI, 1.6–16.5) for short humerus, 1.8 (CI, 1–3.2) for EIF, and 1.5 each for shortened femur (CI, 0.8–2.8) and pyelectasis (CI, 0.6–3.6).

Logistic regression analysis was performed for the 6 markers after excluding major structural abnormalities. This showed that each of the 6 individual sonographic markers was associated with trisomy 21. With the use of modeling, the area under the ROC curve (0.77) was greatest for a saturated model using all 6 markers.

During the time the control group was studied (1996–1999), we identified 59 fetuses with trisomy 21 (prevalence, 1 per 148), including 8 fetuses from low-risk patients (younger than 35 years and with normal biochemical screening results). Given the size of the control group and age distribution alone,³¹ we would have expected to detect 30 fetuses with Down syndrome, including 7.2 from patients younger than 35 years.

Mild cerebral ventricular dilatation (10–15 mm) was observed in 8 (4.3%) of 186 fetuses with trisomy 21 in this series but was not isolated in any case. Of these 8 cases, 3 had other structural defects, 3 had 3 or more soft markers, and 2 also had nuchal thickening.

Discussion

A single soft sonographic marker is commonly detected during the second trimester among fetuses without abnormalities. Although 2 or more markers were detected in just 1.6% of control cases in the current series, an isolated marker was identified in 11.3% with the panel of 6 sonographic markers used (nuchal thickening, hyperechoic bowel, shortened femur or humerus, EIF, and renal pyelectasis). The actual frequency of an isolated marker will vary with the type and number of markers sought. Using slightly different panels of sonographic markers, Sohl et al³⁰ observed a single marker in 14.6% of their cases.

Although frequent in fetuses without abnormalities, isolated markers are even more common in fetuses with trisomy 21 and were observed in 22.6% of affected fetuses in our series ($P < .01$). These data suggest that isolated mark-

ers should not be ignored, especially among high-risk patients. The presence of any marker, however, will result in a high false-positive rate if the presence of any single marker is considered a positive finding. This approach can understandably lead to considerable anxiety³² and inconsistent management³³ when a single marker is identified among low-risk patients.

The false-positive rate can be appropriately lowered among younger women by considering the a priori risk.^{34–36} With the use of the model of age-adjusted ultrasound risk assessment (AAURA),⁷ an individual patient-specific risk can be estimated by the presence or absence of sonographic markers combined with the a priori risk based on maternal age. Because sonographic findings appear to be largely independent of both maternal age and biochemical analytes,^{37–39} we think the risk from biochemical screening (serum markers plus maternal age risk) can be substituted for maternal age risk alone when known.

Age-adjusted sonographic risk assessment considers the risk of specific sonographic findings by LRs. By analyzing a large number of fetuses with Down syndrome, we were able to calculate LRs for each of the sonographic markers evaluated (Table 5). It is important to note that these LRs calculated from a single referral center are similar to the LRs previously assumed in the initial model of AAURA⁷ and are very similar to those of the meta-analysis of isolated markers by Smith-Bindman et al⁴⁰ (Table 6). This supports the validity of these LRs when estimating the risk of fetal Down syndrome when using a similar panel of sonographic markers. Application of the LRs is most conveniently performed with a computer package or spreadsheet. We have made such a spreadsheet available on-line (www.fetalcenter.org).

Four markers (nuchal thickening, hyperechoic bowel, shortened humerus, and EIF) were found to be statistically associated with an increased risk of trisomy 21 as isolated findings. The 2 remaining markers (shortened femur and pyelectasis) were also associated with trisomy 21 overall, although this association did not reach statistical significance when these markers were isolated findings. If these 2 markers are omitted, then the false-positive rate of any marker would decrease from 13% to 6.9%, and the sensitivity would decrease from 52.1% to 44.6%. However, the risk is so low for femur shortening and

pyelectasis (LR, 1.5 each) that their inclusion in AAURA increases the age-related risk above our threshold (1:200) only for women 36 years or older. As a result, excluding these markers from AAURA would only lower the sensitivity among high-risk women, for whom high sensitivity is most desirable and for whom the clinical alternative is amniocentesis for all patients (100% false-positive rate). Also, logistic regression showed that all markers were contributory. Therefore, we think that these markers should be excluded as criteria only from protocols that consider a single marker to be a positive finding among low-risk patients younger than 35 years.

Nuchal thickening was the first of the non-structural markers identified⁸ and remains the single most predictive sonographic marker. Not surprisingly, nuchal thickening showed the strongest association with trisomy 21 as an isolated marker in the current study ($P < .001$; LR, 11). Initial studies suggested a cutoff of 6 mm,^{8,9} although subsequent studies with ROC curve analysis suggested that 5 mm is a better single cutoff before 20 weeks.^{10,41} Even more recent studies suggest that gestational age-specific

Table 5. Calculated LRs (Sensitivity/False-Positive Rate) of Sonographic Findings for Fetal Down Syndrome

Sonographic Marker	LR Overall*	LR as Isolated Markert	95% CI
Nuchal thickening	61	11	5.5–22
Hyperechoic bowel	33.8	6.7	2.7–16.8
Short humerus	15.3	5.1	1.6–16.5
Short femur	6.1	1.5	0.8–2.8
EIF	6.3	1.8	1.0–3.2
Pyelectasis	5.2	1.5	0.6–3.6

*LR when a marker was present either as an isolated finding or in combination with other markers.

tLR when a marker was present as an isolated finding.

Table 6. Likelihood Ratios and 95% CIs for Isolated Markers Reported in This Study and the Meta-analysis by Smith-Bindman et al⁴⁰ Compared With LRs Assumed by the Original AAURA Model⁷

Sonographic Marker	AAURA ⁷ LR	This Study LR (95% CI)	Smith-Bindman et al ⁴⁰ LR (95% CI)
Nuchal thickening	18.6	11 (5.5–22)	17 (8–38)
Hyperechoic bowel	5.5	6.7 (2.7–16.8)	6.1 (3–12.6)
Short humerus	2.5	5.1 (1.6–16.5)	7.5 (4.7–12)
Short femur	2.2	1.5 (0.8–2.8)	2.7 (1.2–6)
EIF	2	1.8 (1.0–3)	2.8 (1.5–5.5)
Pyelectasis	1.5	1.5 (0.6–3.6)	1.9 (0.7–5.1)

criteria should be used, because nuchal thickness normally increases with gestational age.⁴²⁻⁴⁴ Multiples of the median and associated LRs can then be calculated for the entire range of nuchal thickness measurements.^{42,43}

Hyperechoic bowel has consistently been identified as a sonographic marker for fetal aneuploidy,¹¹⁻¹⁵ although it has remained controversial because its detection is subjective. The echogenicity of normal bowel also increases with transducer frequency,⁴⁵ although this effect is uniform, whereas true hyperechoic bowel tends to be focal.^{11,12} To minimize subjectivity, some authors^{5,15} consider only bowel that is markedly hyperechoic, whereas we and others^{46,47} recognize both moderate and markedly hyperechoic bowel (grades 2 and 3) as indicating increased risk of fetal aneuploidy.

Despite its subjectivity, the prevalence of hyperechoic bowel among fetuses without abnormalities (0.5%) has been remarkably consistent at our center in the last decade^{1,7} and is also similar to that of other reports,^{6,15,30} suggesting that different centers can agree on the presence of hyperechoic bowel. Using criteria described here, isolated hyperechoic bowel was observed in 3.2% of fetuses with trisomy 21 and was the second most predictive marker ($P < .001$; LR, 6.7). Other centers using more strict criteria might be expected to have lower sensitivity but an even higher LR for hyperechoic bowel.

Mildly shortened extremities are known phenotypic traits of both infants and fetuses with trisomy 21. Although this trait might technically be considered "structural," it is appropriately categorized as a soft marker, because it is nonspecific and does not affect patient outcome. A shortened humerus appears to be more specific but also less common than a shortened femur for detection of trisomy 21, at least with the criteria used here. Other studies have also found that a shortened humerus is more predictive than a shortened femur.⁴⁸ Results may vary with gestational age, ethnic groups, possibly fetal gender, and the criteria used, as well as systematic differences in long bone measurements.⁴⁹ Despite these variables, as well as wide overlap between affected and unaffected fetuses, a shortened humerus or femur is commonly used as a sonographic marker.

The most common method for determination of a shortened humerus or femur is comparing

the actual measurement with the expected measurement, typically based on biparietal diameter or another dating parameter rather than gestational age. Another approach proposed by Bahado-Singh and colleagues⁵⁰ is to calculate multiples of the median and associated LRs for the whole range of humerus length measurements, similar to the approach proposed for nuchal thickness. An advantage of this approach is that it permits integration with serum biochemical screening to give a single combined risk estimate.

Renal pyelectasis was confirmed to be associated with trisomy 21 overall, but it did not reach statistical significance as an isolated finding. The lack of association as an isolated finding has also been confirmed by most previous studies,^{51,52} although one center has shown an association as an isolated finding.⁵³

Echogenic intracardiac focus is the most recent, and undoubtedly the most controversial, of the soft markers that have been described. Because EIF is a subjective finding, its detection depends on a variety of factors, including resolution of the sonographic equipment, technique, thoroughness of the examination, and the sonographer's experience. Fetal position is also important, because intracardiac foci are best visualized when the cardiac apex is oriented toward the transducer.⁵⁴ Despite these variables, similar detection rates of EIF have been reported from different centers.²¹ As do many sonographic markers, EIF typically resolves regardless of the outcome.

In the first 2 sonographic reports of EIF and aneuploidy, Bromley et al¹⁶ detected EIF in 62 (4.7%) of 1312 control fetuses compared with 4 (18%) of 22 fetuses with trisomy 21, and Lehman et al⁵⁵ reported EIF in 39% of fetuses with trisomy 13 before 20 weeks. The current study confirms that EIF is associated with an increased risk of trisomy 21, although as an isolated finding, this association just reached statistical significance ($P = .046$). These data support a large number of other studies suggesting an association between EIF and trisomy 21, whereas some studies have failed to show this association.⁵⁶⁻⁵⁸

After exclusion of major abnormalities, EIF proved to be the single most common isolated finding in both fetuses with trisomy 21 (7.1%) and fetuses without abnormalities (3.9%). This is in general agreement with previous studies; a

prevalence of 3% to 7% has been reported among fetuses without abnormalities, with the largest prospective studies reporting a combined prevalence of 3.7%.²¹ The calculated LR for trisomy 21 (1.8; CI, 1–3.2) was slightly lower than suggested by previous studies (LR, 2.0–4.2).²¹ This may partly reflect our lower sensitivity of EIF for those fetuses evaluated retrospectively compared with those evaluated prospectively (17% versus 35.6%). The low LR (1.8) for EIF increases the risk above our threshold risk based on AAURA only for women 35 years or older. Also, Asian patients may not be at increased risk, because a higher prevalence of EIF in fetuses without abnormalities has been observed for these patients.⁵⁹

The risk of aneuploidy from isolated markers, including EIF, may be underestimated among low-risk patients because of incomplete ascertainment. Few patients with isolated EIF undergo chromosome analysis unless they are already considered at high risk. In one of the few studies to address this issue, Simpson and colleagues⁶⁰ evaluated 205 fetuses with isolated EIF from low-risk patients. Clinical follow-up was obtained by way of a standard questionnaire completed by the parents when the infant was 6 weeks old. Two infants (1%) proved to have aneuploidy (1 trisomy 21 and 1 unbalanced translocation).

On the other hand, the risk of EIF and other markers is probably overestimated in studies in which the fetal karyotype is known for all patients, because sonographic findings influence patient decision making. Many high-risk patients now wait for the results from the second-trimester sonography before deciding to undergo genetic amniocentesis, and high-risk patients are appropriately more likely to undergo genetic amniocentesis than low-risk patients on the basis of the same sonographic finding. To avoid this bias, we chose to include all consecutive patients who underwent second-trimester sonography in the current study. If we had restricted control fetuses only to those who had a known normal karyotype, then the frequency of EIF would have been substantially higher (174 [5.4%] of 3233) compared with all patients (3.9%). This effect was also suggested in other studies²¹ in which subjects were confined to those with a known fetal karyotype, which have also shown a higher prevalence of EIF.

The frequency of major or structural abnormalities (16.7%) observed in our series of fetuses with trisomy 21 is in agreement that of with Sohl et al,²⁸ who found major abnormalities in 16.4% of affected fetuses. A slightly higher frequency (21.8%) of structural abnormalities was found in a prior study at our own center, which did not exclude patients referred because of abnormal sonographic findings. Other studies^{50,61} that did not omit patients referred because of abnormal sonographic findings have also reported a higher rate of major abnormalities and would be expected to show a higher risk for individual markers.

The low detection of structural abnormalities reflects the low sensitivity of sonography for detection of cardiac defects among fetuses with trisomy 21 before 20 weeks. In the current series, cardiac defects were detected in 8% of affected fetuses, although it should be noted that the mean gestational age at the time of scanning was just 16.9 weeks. Even scanning at an optimal gestational age (24 weeks) under optimal conditions (dedicated fetal echocardiographic center), with inclusion of subtle ventricular septal defects and with prior knowledge of the fetal karyotype, Paladini and colleagues⁶² were able to detect heart defects in just more than half of fetuses with Down syndrome. Using less specific cardiac findings, such as right-left disproportion, pericardial effusion, and tricuspid regurgitation, DeVore⁶³ reported cardiac findings in 76% of fetuses with trisomy 21, but just 9% had an endocardial cushion defect. The mean gestational age for sonography in that study was also 18 weeks.

We know of no systematic reason for low detection of cardiac defects among fetuses with trisomy 21. All scans were conducted in a systematic way, over a sufficient period, by highly qualified sonographers and with direct physician supervision. We think that these results are representative of what other centers can expect in similar conditions, at a similar gestational age and with exclusion of referred patients.

Previous experience suggests that mild cerebral ventricular dilatation (lateral ventricle 10–15 mm) also increases the risk of trisomy 21.⁶⁴ Although some authors⁵ have categorized it as a major abnormality, we think it shares similar characteristics (nonspecific and often transient) with other minor markers. Because it is also uncommon and was not observed as an isolated finding, we did

not separately consider mild cerebral ventricular dilatation as a sonographic marker in this series.

A normal sonographic finding had an LR of 0.36. Therefore, a normal finding, when scanning is performed in a standardized way as described here, will decrease the risk of fetal Down syndrome approximately 64%. This result is in agreement with other studies suggesting that a normal sonographic finding decreases the risk of trisomy 21 in the range of 50% to 70%.^{6,30,35} This information is important, because many women otherwise considered at high risk on the basis of maternal age or biochemical screening may decide to avoid amniocentesis after a second-trimester "genetic sonogram."

Limitations of the current study should be acknowledged. Because genetic amniocentesis was not performed in all cases, patients with fetal Down syndrome may have been undetected. However, we included karyotypic data from both prenatal and postnatal samples to increase the ascertainment for trisomy 21. We think that the number of potential missed cases is small and would not change the conclusions of this study. It is reassuring to note that the number of fetuses with Down syndrome detected among low-risk patients ($n = 8$) was nearly identical to that expected by maternal age distribution during the time the control patients were examined (7.2 expected).

We attempted to minimize bias of sonographic findings by excluding cases referred because of sonographic abnormality. Nevertheless, patients were seen at a high-risk center, with nearly half of the control patients referred because of advanced maternal age or abnormal biochemical screening results (triple screen). Available evidence suggests that sonographic findings are independent of both maternal age and biochemical markers,^{37-39,49} further suggesting that sonographic assessment might be applicable to low-risk patients. However, caution should be exercised in applying these LRs to low-risk populations.

In conclusion, a single marker is commonly encountered for both fetuses with trisomy 21 (22.6%) and fetuses without abnormalities (11.3%). Four markers (nuchal thickening, echogenic bowel, shortened humerus, and EIF) were found to be statistically associated with trisomy 21, even as isolated findings. The degree of risk associated with each of the sonographic markers was calculated with LRs. This information can be integrated with the patient's a priori risk to provide an individual risk for both high- and low-risk patients.

References

1. Nyberg DA, Resta RG, Luthy DA, Hickok DE, Mahony BS, Hirsch JH. Prenatal sonographic findings of Down syndrome: review of 94 cases. *Obstet Gynecol* 1990; 76:370-377.
2. Benacerraf BR, Neuberger D, Bromley B, Frigoletto FD Jr. Sonographic scoring index for prenatal detection of chromosomal abnormalities. *J Ultrasound Med* 1992; 11:449-458.
3. Nicolaides KH, Shawwa L, Brizot M, Snijders RJ. Ultrasonographically detectable markers of fetal chromosomal defects. *Ultrasound Obstet Gynecol* 1993; 3:56-59.
4. Benacerraf BR, Nadel AS, Bromley B. Identification of second-trimester fetuses with autosomal trisomy by use of a sonographic scoring index. *Radiology* 1994; 193:135-140.
5. Vintzileos AM, Campbell WA, Guzman ER, Smulian JC, McLean DA, Ananth CV. Second-trimester ultrasound markers for detection of trisomy 21: which markers are best? *Obstet Gynecol* 1997; 89: 941-944.
6. Benacerraf BR. The second-trimester fetus with Down syndrome: detection using sonographic features. *Ultrasound Obstet Gynecol* 1996; 7:147-155.
7. Nyberg DA, Luthy DA, Resta RG, Nyberg BC, Williams MA. Age-adjusted ultrasound risk assessment for fetal Down's syndrome during the second trimester: description of the method and analysis of 142 cases. *Ultrasound Obstet Gynecol* 1998; 12: 8-14.
8. Benacerraf B, Frigoletto F, Laboda L. Sonographic diagnosis of Down syndrome in the second trimester. *Am J Obstet Gynecol* 1985; 153:49-52.
9. Benacerraf B, Gelman R, Frigoletto F. Sonographic identification of second-trimester fetuses with Down's syndrome. *N Engl J Med* 1987; 317: 1371-1376.
10. Gray DL, Crane JP. Optimal nuchal skin-fold thresholds based on gestational age for prenatal detection of Down syndrome. *Am J Obstet Gynecol* 1994; 171:1282-1286.
11. Nyberg DA, Dubinsky T, Resta RG, Mahony BS, Hickok DE, Luthy DA. Echogenic fetal bowel during the second trimester: clinical importance. *Radiology* 1993; 188:527-531.

12. Nyberg DA, Resta RG, Luthy DA, et al. Echogenic bowel and Down's syndrome. *Ultrasound Obstet Gynecol* 1993; 3:330–333.
13. Muller F, Dommergues M, Aubry MC, et al. Hyperechogenic fetal bowel: an ultrasonographic marker for adverse fetal and neonatal outcome. *Am J Obstet Gynecol* 1995; 173:508–513.
14. Bromley B, Doubilet P, Frigoletto FD Jr, Krauss C, Estroff JA, Benacerraf BR. Is fetal hyperechogenic bowel on second-trimester sonogram an indication for amniocentesis? *Obstet Gynecol* 1994; 83:647–651.
15. Deren O, Mahoney MJ, Copel JA, Bahado-Singh RO. Subtle ultrasonographic anomalies: do they improve the Down syndrome detection rate? *Am J Obstet Gynecol* 1998; 178:441–445.
16. Bromley B, Lieberman E, Laboda L, Benacerraf BR. Echogenic intracardiac focus: a sonographic sign for fetal Down syndrome. *Obstet Gynecol* 1995; 86:998–1001.
17. Sepulveda W, Cullen S, Nicolaidis P, Hollingsworth J, Fisk NM. Echogenic foci in the fetal heart: a marker of chromosomal abnormality. *Br J Obstet Gynaecol* 1995; 102:490–492.
18. Vibhakar NI, Budorick NE, Scioscia AL, Harby LD, Mullen ML, Sklansky MS. Prevalence of aneuploidy with a cardiac intraventricular echogenic focus in an at-risk patient population. *J Ultrasound Med* 1999; 18:265–268.
19. Manning JE, Ragavendra N, Sayre J, et al. Significance of fetal intracardiac echogenic foci in relation to trisomy 21: a prospective sonographic study of high-risk pregnant women. *AJR Am J Roentgenol* 1998; 170:1083–1084.
20. Bromley B, Lieberman E, Shipp TD, Richardson M, Benacerraf BR. Significance of an echogenic intracardiac focus in fetuses at high and low risk for aneuploidy. *J Ultrasound Med* 1998; 17:127–131.
21. Winter TC, Anderson AM, Cheng EY, et al. The echogenic intracardiac focus in second trimester fetuses with trisomy 21: usefulness as a US marker. *Radiology* 2000; 216:450–456.
22. Benacerraf BR, Neuberg D, Frigoletto FD Jr. Humeral shortening in second-trimester fetuses with Down syndrome. *Obstet Gynecol* 1991; 77:223–227.
23. Nyberg DA, Resta RG, Luthy DA, Hickok DE, Williams MA. Humerus and femur length shortening in the detection of Down's syndrome. *Am J Obstet Gynecol* 1993; 168:534–539.
24. Benacerraf BR, Mandell J, Estroff JA, Harlow BL, Frigoletto FD Jr. Fetal pyelectasis: a possible association with Down syndrome. *Obstet Gynecol* 1990; 76:58–60.
25. Corteville JE, Dicke JM, Crane JP. Fetal pyelectasis and Down syndrome: is genetic amniocentesis warranted? *Obstet Gynecol* 1992; 79:770–772.
26. Stoll C, Dott B, Alembik Y, Roth MP. Evaluation of routine prenatal ultrasound examination in detecting fetal chromosomal abnormalities in a low risk population. *Hum Genet* 1993; 91:37–41.
27. Hill LM. The sonographic detection of trisomies 13, 18, and 21. *Clin Obstet Gynecol* 1996; 39:831–850.
28. Nadel AS, Bromley B, Frigoletto FD Jr, Benacerraf BR. Can the presumed risk of autosomal trisomy be decreased in fetuses of older women following a normal sonogram? *J Ultrasound Med* 1995; 14:297–302.
29. Vintzileos AM, Campbell WA, Rodis JF, Guzman ER, Smulian JC, Knuppel RA. The use of second-trimester genetic sonogram in guiding clinical management of patients at increased risk for fetal trisomy 21. *Obstet Gynecol* 1996; 87:948–952.
30. Sohl BD, Scioscia AL, Budorick NE, Moore TR. Utility of minor ultrasonographic markers in the prediction of abnormal fetal karyotype at a prenatal diagnostic center. *Am J Obstet Gynecol* 1999; 181:898–903.
31. Snijders RJM, Nicolaides KH. *Ultrasound Markers for Fetal Chromosomal Defects*. London, England: Parthenon Publishing; 1996.
32. Filly RA. Obstetrical sonography: the best way to terrify a pregnant woman. *J Ultrasound Med* 2000; 19:1–5.
33. Maclachlan N, Iskaros J, Chitty L. Ultrasound markers of fetal chromosomal abnormality: a survey of policies and practices in UK maternity ultrasound departments. *Ultrasound Obstet Gynecol* 2000; 15:387–390.
34. Drugan A, Johnson MP, Reichler A, Hume RF Jr, Itskovicz-Eldor J, Evans MI. Second-trimester minor ultrasound anomalies: impact on the risk of aneuploidy associated with advanced maternal age. *Obstet Gynecol* 1996; 88:203–206.

Isolated Sonographic Markers for Down Syndrome in the Second Trimester

35. Winter TC, Uhrich SB, Souter VL, Nyberg DA. The "genetic sonogram": comparison of the index scoring system with the age-adjusted US risk assessment. *Radiology* 2000; 215:775–782.
36. Bromley B, Lieberman E, Benacerraf BR. The incorporation of maternal age into the sonographic scoring index for the detection at 14–20 weeks of fetuses with Down's syndrome. *Ultrasound Obstet Gynecol* 1997; 10:321–324.
37. Nyberg DA, Luthy DA, Cheng EY, Sheley RC, Resta RG, Williams MA. Role of prenatal ultrasound in women with positive screen for Down syndrome based on maternal serum markers. *Am J Obstet Gynecol* 1995; 173:1030–1035.
38. Drugan A, Reichler A, Bronstein M, Johnson MP, Sokol RJ, Evans MI. Abnormal biochemical serum screening versus 2nd-trimester ultrasound-detected minor anomalies as predictors of aneuploidy in low-risk patients. *Fetal Diagn Ther* 1996; 11:301–305.
39. Verdin SM, Economides DL. The role of ultrasonographic markers for trisomy 21 in women with positive serum biochemistry. *Br J Obstet Gynaecol* 1998; 105:63–67.
40. Smith-Bindman R, Hosmer W, Feldstein VA, Deeks JJ, Goldberg JD. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. *JAMA* 2001; 285:1044–1055.
41. Borrell A, Costa D, Martinez JM, et al. Early midtrimester fetal nuchal thickness: effectiveness as a marker for Down syndrome. *Obstet Gynecol* 1996; 175:45–49.
42. Locatelli A, Piccoli MG, Vergani P, et al. Critical appraisal of the use of nuchal fold thickness measurements for the prediction of Down syndrome. *Am J Obstet Gynecol* 2000; 182:192–197.
43. Bahado-Singh RO, Oz UA, Kovanci E, et al. Gestational age standardized nuchal thickness values for estimating mid-trimester Down's syndrome risk. *J Matern Fetal Med* 1999; 8:37–43.
44. Tannirandorn Y, Manotaya S, Uerpairojkit B, Tanawattanacharoen S, Charoenvidhya D, Phaosavasdi S. Cut-off criteria for second-trimester nuchal skinfold thickness for prenatal detection of Down syndrome in a Thai population. *Int J Gynaecol Obstet* 1999; 65:137–141.
45. Vincoff NS, Callen PW, Smith-Bindman R, Goldstein RB. Effect of ultrasound transducer frequency on the appearance of the fetal bowel. *J Ultrasound Med* 1999; 18:799–803.
46. Hill LM, Fries J, Hecker J, Grzybek P. Second-trimester echogenic small bowel: an increased risk for adverse perinatal outcome. *Prenat Diagn* 1994; 14:845–850.
47. Slotnick RN, Abuhamad AZ. Prognostic implications of fetal echogenic bowel. *Lancet* 1996; 13: 347:85–87.
48. Rodis JF, Vintzileos AM, Fleming AD, et al. Comparison of humerus length with femur length in fetuses with Down syndrome. *Am J Obstet Gynecol* 1991; 165:1051–1056.
49. LaFollette L, Filly RA, Anderson R, Golbus MS. Fetal femur length to detect trisomy 21. A reappraisal. *J Ultrasound Med* 1989; 8:657–660.
50. Bahado-Singh RO, Oz AU, Kovanci E, et al. New Down syndrome screening algorithm: ultrasonographic biometry and multiple serum markers combined with maternal age. *Am J Obstet Gynecol* 1998; 179:1627–1631.
51. Vintzileos AM, Egan JFX. Adjusting the risk for trisomy 21 on the basis of second-trimester ultrasonography. *Am J Obstet Gynecol* 1995; 172: 837–844.
52. Snijders RJ, Sebire NJ, Faria M, Patel F, Nicolaidis KH. Fetal mild hydronephrosis and chromosomal defects: relation to maternal age and gestation. *Fetal Diagn Ther* 1995; 10:349–355.
53. Wickstrom EA, Thangavelu M, Parilla BV, Tamura RK, Sabbagha RE. A prospective study of the association between isolated fetal pyelectasis and chromosomal abnormality. *Obstet Gynecol* 1996; 88:379–382.
54. Ranzini AC, McLean DA, Sharma S, Ananth CV. Fetal intracardiac echogenic foci: does visualization depend on the orientation of the four-chamber view? *J Ultrasound Med* 1999; 18(suppl):S:108.
55. Lehman CD, Nyberg DA, Winter TC III, Kapur RP, Resta RG, Luthy DA. Trisomy 13 syndrome: prenatal US findings in a review of 33 cases. *Radiology* 1995; 194:217–222.
56. How HY, Villafane J, Parihus RR, Spinnato JA. Small hyperechoic foci of the fetal cardiac ventricle: a benign sonographic finding? *Ultrasound Obstet Gynecol* 1994; 4:205–207.
57. Achiron R, Lipitz S, Gabbay U, Yagel S. Prenatal ultrasonographic diagnosis of fetal heart echogenic

- foci: no correlation with Down syndrome. *Obstet Gynecol* 1997; 89:945–948.
58. Dildy GA, Judd VE, Clark SL. Prospective evaluation of the antenatal incidence and postnatal significance of the fetal echogenic cardiac focus: a case-control study. *Am J Obstet Gynecol* 1996; 175: 1008–1012.
 59. Shipp TD, Bromley B, Lieberman E, Benacerraf BR. The frequency of fetal echogenic intracardiac foci with respect to maternal race. *Ultrasound Obstet Gynecol* 2000; 15:460–462.
 60. Simpson JM, Cook A, Sharland G. The significance of echogenic foci in the fetal heart: a prospective study of 228 cases. *Ultrasound Obstet Gynecol* 1996; 8:225–228.
 61. Drugan A, Bronshtein M, Itskovitz-Eldor J, Johnson MP, Evans MI. Ontogeny of isolated ultrasound markers for fetal aneuploidy. *Fetal Diagn Ther* 1999; 14:266–269.
 62. Paladini D, Tartaglioine A, Agangi A, Teodoro A, Forleo F, Borghese A, Marinelli P. The association between congenital heart disease and Down syndrome in prenatal life. *Ultrasound Obstet Gynecol* 2000; 15:104–108.
 63. DeVore GR. Trisomy 21: 91% detection rate using second-trimester ultrasound markers. *Ultrasound Obstet Gynecol* 2000; 16:133–141.
 64. Mahony BS, Nyberg DA, Hirsch JH, et al. Mild idiopathic lateral cerebral ventricular dilatation in utero: sonographic evaluation. *Radiology* 1988; 169: 715–721.