Noninvasive Means of Identifying Fetuses with Possible Down Syndrome: A Review

Cheryl Kubas MS
Genetic Counselor, Maternal Fetal Center, Florida Hospital, Orlando, Florida

Abstract

Women who are 35 years or older are offered invasive prenatal testing because of the increased risk of chromosomal abnormalities, especially Down syndrome. In an attempt to increase the number of Down syndrome fetuses being detected and decrease the number of invasive procedures being performed on pregnancies not affected with a chromosome abnormality, both biochemical and ultrasound screening methods are being studied and are summarized in this article. The ultrasound markers reviewed include increased nuchal thickness, increased nuchal lucency, shortened femur, shortened humerus, pyelectasis, hypoplastic ears, echogenic intracardiac focus, hypoplasia of the fifth middle phalanx, and echogenic bowel.

It is standard practice for any pregnant woman who will be 35 years or older at her due date to be offered prenatal testing through amniocentesis or CVS. This method of using maternal age identifies approximately 20% of fetuses with Down syndrome. The other 80% of fetuses are born to women under the age of 35 years. Due to the risk involved with amniocentesis and CVS, methods of screening low-risk women have been devised and are still being developed in an attempt to identify which of these women may be carrying a fetus with a chromosomal abnormality. Over the past 15 years, both biochemical and ultrasound markers have been studied to aid in identifying women who may be at higher risk for fetal aneuploidy. These women can then be offered prenatal testing if it is thought that the chance of having a chromosomal abnormality is significantly increased and higher than the risk involved with the procedure itself.

Additionally, because of the risk of miscarriage associated with invasive testing, some women either desire more information about their pregnancies before deciding whether to proceed with invasive testing or do not want any invasive testing at all. For these women, screening methods are of the greatest importance. This article will review the latest screening methods available. Because most pregnant women rely on their obstetricians, midwives, or obstetric nurses to provide them with information about these techniques, it is important that health care providers in the obstetric field be well informed about what screening tests are available and how effective they are at identifying at-risk pregnancies.

HISTORICAL BACKGROUND

The risk of a fetal chromosome abnormality is directly associated with maternal age. The most common of these chromosome abnormalities are trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome), and trisomy 13 (Patau syndrome). Because of the high intrauterine fetal loss rate associated with trisomies 13 and 18, Down syndrome is the most prevalent of these conditions seen at birth.

During the 1960s, amniocentesis was introduced for prenatal diagnosis of chromosomal abnormalities. Initially performed without the guidance of real-time ultrasound imaging, it was not readily accepted until the 1980s. At that time, amniocentesis was commonly recommended for advanced maternal age, a previous pregnancy complicated by a chromosome abnormality or neural tube defect, parental translocation, or a family history of a genetic disorder. Amniocenteses generally are performed using a 22-gauge spinal needle inserted abdominally into the amniotic sac under continuous ultrasonographic visualization.
An amniocentesis generally is performed at between 15 and 22 weeks, with the ideal timing being around 16 weeks' gestation. At this gestational age, it is considered a traditional or midtrimester amniocentesis. Numerous studies looking at the risks associated with midtrimester amniocentesis were performed in the 1980s. Hanson et al 5 noted in a review of 17 studies that the average rate for spontaneous fetal loss was 0.5%, with a range of 0.3% to 2.89%. According to the National Institute of Child Health and Development report of 1976, the rate for spontaneous abortion was not considered significantly higher for those women undergoing midtrimester amniocentesis compared to controls (3.3% versus 3.2%). Leschot et al 7 reported a loss rate of 0.5% within 3 weeks of the procedure, Tabor et al 8 reported a 1% increased loss rate 7 weeks after the procedure and Golbus et al 9 reported a 1.5% rate of loss. It also was noted that the fetal loss rate decreased with increasing physician experience. 7 Most experienced centers currently quote a loss rate of less than 0.5%.

In 1968, Mohr and Hahneman described transcervical chorionic villus sampling (CVS) as the first form of prenatal genetic testing capable of being performed during the first trimester. 10 Using an endoscope, they reported fetal loss rates as high as 50%. During the 1970s, Chinese investigators used CVS to determine fetal sex, but not until the 1980s—when ultrasound was used to accompany the procedure—did it become widely known and used. 11 After some refinement of the procedure, a transcervical CVS was performed using a catheter attached to a syringe that could be passed through the cervical canal into the uterine cavity to aspirate chorionic villi. 12 In 1986, the use of transabdominal CVS was described as an alternative to transcervical CVS to reduce the risk of bacterial contamination. 13 In 1986, the World Health Organization concluded that the incidence of fetal loss associated with CVS was no greater than 0.7%, and that the risk for complications decreased with physician experience. 14 In addition to the risk of miscarriage, there has been concern about an increased risk for transverse limb reduction defects and oromandibular hypogenesis. First reported by Firth et al 15 in 1991, others 16 , 17 confirmed the association in their own patients. Firth et al 18 later found that limb reduction defects were more common in women who had undergone CVS prior to 10 weeks' gestation. They hypothesized that the defects resulted from a disruption in normal vascular supply to the fetus due to damage to the uterine and placental blood vessels.

In addition to CVS and midtrimester amniocentesis, the option of early amniocentesis was first suggested in 1985 by Peakman at the 23rd Annual Somatic Cell Genetics Conference. 19 This procedure generally is performed between 12 and 14 weeks' gestation by obtaining 1 ml of amniotic fluid for each week of gestation. It is similar to the midtrimester amniocentesis with the exception of a higher rate of membrane tenting. 20 Membrane tenting occurs when fusion of the amnion and chorion has not occurred. When this occurs, penetration of the amniotic sac becomes very difficult. Viscarello et al 10 found the fetal loss rate to be 1.5% after an early amniocentesis in a review of six initial studies. It was further noted that the earlier the procedure was performed, the higher was the procedural loss rate.

Because of the risk of fetal loss associated with invasive prenatal testing by amniocentesis and CVS, some women are deciding against invasive prenatal testing entirely whereas others are combining the information they have received from various screening techniques (biochemical and ultrasound markers) before deciding whether to proceed with an amniocentesis. As a result, researchers are attempting to establish screening methods that will identify the highest percentage of fetuses with Down syndrome with the lowest false-positive rate.

This article will describe various markers currently employed for screening, both in isolation and in combination with other markers. Current biochemical screening methods including maternal serum alpha fetoprotein (MS-AFP) and double, triple, and quadruple screens will be reviewed. Some of the sonographic markers currently being studied and that will be described include increased nuchal thickness, shortened long bones, aplasia or hypoplasia of the fifth middle phalanx, intracardiac echogenic focus, echogenic bowel, pyelectasis, and hypoplastic ears.

**BIOCHEMICAL SCREENING**

**MS-AFP**

In 1984, Merkatz et al 1 and Cuckle et al 21 first described the use of MS-AFP levels for the screening of Down syndrome in women under the age of 35 years. They found that MS-AFP concentrations at
midtrimester were about 25% lower in pregnancies affected by Down syndrome. By using these measurements in combination with the women's age, they were able to identify approximately 20% of women under the age of 35 years carrying a fetus with Down syndrome with a 5% amniocentesis rate.

Double, triple, and quadruple screens

In 1992, Haddow et al reported on the use of three maternal serum markers. Using serum AFP, chorionic gonadotropin (hCG), and estriol concentrations in combination with maternal age, they identified 58% of fetuses with Down syndrome by offering amniocentesis to 3.8% of women. They found that the hCG levels were about two times higher than normal and estriol levels were 25% lower than normal in women carrying fetuses with Down syndrome.

Some laboratories are currently employing a fourth protein marker, inhibin A. Known as the quadruple screen, it was found that by screening for increased levels of hCG and inhibin A and decreased levels of AFP and estriol, approximately 70% to 80% of fetuses with Down syndrome could be identified with a false positive rate of 5%.

More recently, the triple and quadruple screens also have been used to screen for fetuses with trisomy 18. More than 60% of fetuses with trisomy 18 can be detected based on low levels of all proteins by offering amniocentesis to an additional 0.5% of women.

First trimester biochemical screens

Haddow et al looked at various biochemical markers in the first trimester and found that the detection rates for Down syndrome by protein marker were: 17% for AFP, 4% for estriol, 29% for hCG, 42% for pregnancy-associated protein A (PAPP-A). By combining PAPP-A, hCG, and maternal age, they were able to detect 63% of pregnancies with Down syndrome before 14 weeks' gestation.

ANATOMICAL MARKERS USED FOR ULTRASOUND SCREENING

Nuchal thickening

Among various signs used for diagnosing Down syndrome, it has been found that approximately 80% of newborns with Down syndrome have excess skin at the back of the neck. In 1985, Benacerraf et al first described “an abnormal amount of soft tissue or skin thickness” behind the fetal neck at the occiput in two of six cases of Down syndrome diagnosed by amniocentesis and only 1 of 898 controls. In 1985, Benacerraf et al added to their original paper with a total of 5 of 11 fetuses with Down syndrome being identified on the basis of increased nuchal fold. By measuring the nuchal folds of 303 fetuses undergoing amniocentesis, they concluded that between 15 and 20 weeks, the normal width of the nuchal fold was between 1 and 5 mm. Any measurement greater than this would be considered abnormal and warrant further investigation. Since that time, numerous studies have looked at the association between nuchal fold thickening and Down syndrome.

Buckshee et al developed nomograms of fetal nuchal fold thickness at 16 to 18 weeks, more than 18 to 20 weeks, and more than 20 to 22 weeks of 2.6 >= 0.2 mm, 3.8 >= 0.9 mm, and 4.0>=0.8 mm, respectively. They found one fetus with a nuchal fold thickness of 6 mm at 18 weeks that was subsequently diagnosed with Down syndrome. They measured nuchal fold thickness with the fetal head scanned in the transverse plane, which includes the thalamus, ventricles, and occipital tissue above the level of the cerebellum. The external surface of the occipital bone to the external surface of the overlying skin is measured. They noted that care must be taken to avoid angled planes or measuring below the level of the occipital bone to provide the most accurate measurement.

A study was conducted to look at varying nuchal fold thickness limits to determine the limit most effective for detecting the most fetuses with Down syndrome with a low false positive rate. Gray et al found that at 14 to 18 weeks, the optimum threshold was >=5 mm. This gave a sensitivity of 42% with a 2.9% positive screen rate. At 19 to 24 weeks, >= 6 mm was determined to be the optimum thickness with a sensitivity of 83% and positive screen rate of 3.7%. If they used a cutoff of 6 mm for 14 to 18 weeks, the
sensitivity dropped to 35%. Using two different cutoffs was determined to be the best technique for identifying fetuses with Down syndrome.

The sensitivity of nuchal fold thickening for Down syndrome ranged from 9% to 81%. By combining referenced studies (Table 1), an average of approximately 40% of fetuses with Down syndrome were found to have increased nuchal fold thickness. Toi et al. found a high false-positive rate and cautioned about relying on this sign as a sole indicator for Down syndrome. Itoh et al. found that in five of six cases of Down syndrome, nuchal fold thickening was transient. They speculated that the large disparity in sensitivities seen in different studies was due to this transiency. They recommended that measurements of nuchal fold thickness be made repetitively during the second trimester to improve the chance of detecting Down syndrome.

Table 1. Incidence of increased second trimester nuchal thickness in fetuses with Down syndrome

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Down syndrome fetuses with increased nuchal fold/</th>
<th>No. controls with increased nuchal fold/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. with Down syndrome</td>
<td>Total no. controls</td>
</tr>
<tr>
<td>Bahado-Singh, et al.</td>
<td>4/8 (50%)</td>
<td>9/643 (1.4%)</td>
</tr>
<tr>
<td>Benacerraf et al.</td>
<td>2/6 (33%)</td>
<td>1/896 (0.1%)</td>
</tr>
<tr>
<td>Benacerraf, et al.</td>
<td>5/11 (45%)</td>
<td>1/1693 (0.06%)</td>
</tr>
<tr>
<td>Benacerraf, et al.</td>
<td>12/28 (43%)</td>
<td></td>
</tr>
<tr>
<td>Benacerraf, et al.</td>
<td>22/32 (69%)</td>
<td>2/558 (0.34%)</td>
</tr>
<tr>
<td>Benacerraf, et al.</td>
<td>16/45 (36%)</td>
<td>0/106 (0%)</td>
</tr>
<tr>
<td>Bromley, et al.</td>
<td>27/53 (51%)</td>
<td></td>
</tr>
<tr>
<td>Crane, et al.</td>
<td>12/16 (75%)</td>
<td>35/3257 (1%)</td>
</tr>
<tr>
<td>Ginsberg, et al.</td>
<td>5/12 (41%)</td>
<td></td>
</tr>
<tr>
<td>Grandjean, et al.</td>
<td>17/44 (81%)</td>
<td>256/3264 (8.5%)</td>
</tr>
<tr>
<td>Hill, et al.</td>
<td>2/22 (9%)</td>
<td>0/286 (0%)</td>
</tr>
<tr>
<td>Itoh, et al.</td>
<td>6/8 (75%)</td>
<td></td>
</tr>
<tr>
<td>Lynch, et al.</td>
<td>5/9 (56%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Nyberg, et al.</td>
<td>7/68 (10%)</td>
<td></td>
</tr>
<tr>
<td>Perella, et al.</td>
<td>3/14 (21%)</td>
<td>12/128 (9%)</td>
</tr>
<tr>
<td>Toi, et al.</td>
<td>4/11 (36%)</td>
<td></td>
</tr>
<tr>
<td>Vintzileos, et al.</td>
<td>12/23 (63%)</td>
<td></td>
</tr>
</tbody>
</table>

| TOTALS | 150/371 (40%) | 316/10,902 (3%) |

1 Fraternal twin study.
2 Includes 2 with cystic hygromas.
3 Excludes 1982 and 1985 Benacerraf studies as the numbers are included in the 1987 study.

First trimester increased nuchal lucency

In 1990, Cullen et al. described the finding of cystic hygroma in the first trimester and its association with chromosome abnormalities. Since that time, others have described an association with what is currently called increased “nuchal lucency.” Pandya et al. prefer this description as it best describes this first trimester finding. During the second trimester, nuchalema or cystic hygroma are used to describe different findings of the fetal neck. Nuchal edema is described as a “subcutaneous accumulation of fluid, producing a characteristic tremor on ballottement of the fetal head” whereas cystic hygromas are “bilateral, septated cystic structures.”
Nicholaides et al 49 found that the prevalence of a nuchal translucency >=3 mm at 10 to 13 weeks was 4% in normal fetuses and 80% in trisomic fetuses. Nicolaides et al 49 found a 5-fold increase in trisomies with a 3 mm nuchal lucency and 24-fold increase at 4 mm and greater. Pandya et al 48 found that between 10 and 13 weeks’ gestation, a fetal nuchal lucency of 3 mm was associated with a four-fold increase in the maternal age risk for trisomies 21, 18, and 13. When the lucency was 4 mm or greater, there was a 29-fold increase. In addition, they found that when the nuchal lucency was 4 mm or more, there was a strong association with poor pregnancy outcome, regardless of fetal karyotype. In 1995, Pandya et al 51 adjusted these figures to 3-fold increase in trisomies with a 3 mm lucency, 18-fold increase with 4 mm lucency, 28-fold increase with a 5 mm lucency, and 36-fold increase at 6 mm and greater. Additionally, they found a nine-fold increase in Turner syndrome and an eight-fold increase in triploidy. The fetal loss rate was increased to 13% when the nuchal lucency was 5 mm and greater.

When a nuchal lucency above 3 mm was seen between 8 and 15 weeks, the chance of a chromosome abnormality was found to range from 12% 52 to 60% 53 with an average of 44% across studies (Table 2). Nadel et al 57 found a higher incidence in aneuploidy when hydrops accompanied the nuchal lucency or cystic hygroma. In 1998, Yagel et al 63 developed daily reference intervals for normal first trimester nuchal translucency. Using cutoff levels based on these intervals, they were able to obtain the same sensitivity for detecting fetuses with Down syndrome as using the standard 3 mm cutoff, but they found an increase in specificity from 87.9% to 94.6%. They concluded that using gestational age-specific nuchal fold measurement cutoffs could detect the same number of fetuses with Down syndrome with a lower rate of invasive testing. Of note, most researchers 47, 55, 56, 60 noted that in fetuses with increased nuchal lucency and a normal karyotype, the lucency resolved by 18 weeks' gestation.

### Table 2. Studies of increased nuchal lucency in the first trimester (8 to 15 weeks)

<table>
<thead>
<tr>
<th>Study</th>
<th>No. chromosome abn/No. increased nuchal lucency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brambati, et al. 58</td>
<td>(18.6%)</td>
</tr>
<tr>
<td>Cullen, et al. 47</td>
<td>15/29 (52%)</td>
</tr>
<tr>
<td>Johnson, et al. 53</td>
<td>41/68 (60%)</td>
</tr>
<tr>
<td>Nadel, et al. 57</td>
<td>12/26 (46%)</td>
</tr>
<tr>
<td>Nicholaides, et al. 49</td>
<td>18/51 (35%)</td>
</tr>
<tr>
<td>Podobnik, et al. 50</td>
<td>17/35 (49%)</td>
</tr>
<tr>
<td>Reyners, et al. 52</td>
<td>5/41 (12%)</td>
</tr>
<tr>
<td>Salvodelli, et al. 54</td>
<td>15/28 (54%)</td>
</tr>
<tr>
<td>Shulman, et al. 55</td>
<td>15/32 (47%)</td>
</tr>
<tr>
<td>Szabo, et al. 59</td>
<td>43/96 (45%)</td>
</tr>
<tr>
<td>van Vugt, et al. 61</td>
<td>47/102 (46%)</td>
</tr>
<tr>
<td>Van Zalen-Sprock, et al. 6b</td>
<td>7/22 (32%)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>235/530 (44%)</td>
</tr>
</tbody>
</table>

By taking the maternal age-related chance for trisomy 21 and multiplying it by a likelihood ratio depending on the deviation from normal nuchal-translucency thickness, Snijders et al 62 developed a method of risk assessment. Using their method, they assigned a risk of 1 in 300 or higher for a chromosome abnormality to 8.3% of normal pregnancies, 82.2% of pregnancies with trisomy 21, and 77.9% of pregnancies with other chromosome abnormalities.

Shortened long bones
Femur length. It has long been known that individuals with Down syndrome have short stature and that their legs and arms appear disproportionately short as compared with the trunk. Using this knowledge, it was surmised that measurements of fetal limb length by ultrasound might provide a method to identify fetuses with Down syndrome prenatally.

In 1987, Lockwood et al described the assessment of biparietal diameter, femur length, and biparietal diameter/femur length ratio in 55 fetuses with Down syndrome as compared to 544 controls. They found no significant difference in biparietal diameter between Down syndrome fetuses and controls, although the Down syndrome fetuses had significantly shorter femurs. Of greatest statistical significance was the difference in biparietal diameter and femur length ratio. Using a cutoff of 1.5 SD above the normal population, they were able to identify 51% to 70% of Down syndrome fetuses at two different centers, with a false-positive rate of 7% and 4.6%, respectively.

Other researchers have used a different method for determining a short femur length. By using a reference table of normal femur lengths and dividing the measured femur length by this value, they defined a femur length as being short when the ratio was less than or equal to 0.91. Dicke et al and Lynch et al compared the effectiveness of biparietal diameter to femur length versus measured to expected femur length ratios. Dicke et al found the biparietal diameter to femur length ratio to be the most effective in detecting fetuses with Down syndrome by 18% versus 15%. Lynch et al studied nine sets of fraternal twins where one twin had Down syndrome and found the measured to expected femur length ratio to be more effective at determining the fetuses with Down syndrome with a 55% detection rate versus 22% by the biparietal diameter to femur length ratio. They also noted that 44% of the normal twins had short femurs as determined by this method versus 11%. Due to these discrepancies, most researchers use whichever method they have found to be most effective in their own studies. The sensitivity of femur length shortening for the detection of Down syndrome has been shown to range from 14% to 72%, with an average of approximately 45% based on referenced studies (Table 3).

Table 3. Studies of shortened femur length
Humerus length Fitzsimmons et al. \textsuperscript{74} took measurements of limb lengths in 31 Down syndrome fetuses and 174 controls, postmortem, to determine which bone lengths were the best predictors of Down syndrome. They found that by using a cutoff of 2 SDs below the norm in the 32 fetuses with Down syndrome, 3 had short femurs, 4 had short tibias, 5 had short fibulas, 9 had short humeri, 10 had short radii, and 7 had short ulnas. They concluded that the upper extremity bones were shorter than normal more often than were the bones of the lower extremity.

Rodis et al. \textsuperscript{70} studied 11 fetuses with Down syndrome between 15 and 22 weeks’ gestation and compared them with 1,890 controls with regard to femoral and humerall lengths. Seven of 11 fetuses with Down syndrome had humerus lengths below the fifth percentile (64%), whereas only 2 of 11 (18%) had a femur length below the fifth percentile. Of the controls, 95 of 1,890 normal fetuses had a femur length less than the fifth percentile and 95 of 1,890 had a humerus length less than the fifth percentile. They concluded that the length of the humerus had a greater sensitivity than femur length in detecting cases of Down syndrome.

The best criteria for determining shortened humerus length was found to be comparing the measured humerus length to the expected humerus length and considering a ratio of less or equal to 0.90 \textsuperscript{31, 71, 75} or 0.89 \textsuperscript{68} to be abnormal. Romentsch et al. \textsuperscript{71} found a shortened humerus in 12 of 43 (28%) of Down syndrome fetuses and a shortened femur in only 8 of 43 (19%) of Down syndrome fetuses. Benacerraf et al. \textsuperscript{75} also found a higher percentage of fetuses with Down syndrome with shortened humeri (12 of 24) than femurs (10 of 24).

Nyberg et al. \textsuperscript{68} compared both femur and humerus lengths of 45 fetuses with Down syndrome to 942 controls with normal karyotypes and found that although an equal number (11 of 45) fetuses with Down syndrome had short femurs and humeri, only 4% to 5% of controls had a short femur or humerus. They concluded that the finding of either a shortened humerus or femur increased the fetal risk of Down syndrome and the finding of both a shortened femur and humerus further increased the risk of Down syndrome.

<table>
<thead>
<tr>
<th>Study</th>
<th>Method of femur measurement*</th>
<th>No. Down syndrome fetuses with short femur/Total no. Down syndrome fetuses</th>
<th>No. control fetuses with short femur/Total no. control fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benacerraf, et al.\textsuperscript{30}</td>
<td>1</td>
<td>23/32 (72%)</td>
<td>63/588 (11%)</td>
</tr>
<tr>
<td>Benacerraf, et al.\textsuperscript{31}</td>
<td>2</td>
<td>20/45 (44%)</td>
<td>4/106 (4%)</td>
</tr>
<tr>
<td>Bromley, et al.\textsuperscript{32}</td>
<td>2</td>
<td>25/53 (47%)</td>
<td>1/45 (2%)</td>
</tr>
<tr>
<td>Brumfield, et al.\textsuperscript{73}</td>
<td>1</td>
<td>6/15 (40%)</td>
<td>1/45 (2%)</td>
</tr>
<tr>
<td>Dicke, et al.\textsuperscript{72}</td>
<td>1</td>
<td>(18%)</td>
<td></td>
</tr>
<tr>
<td>Ginsberg, et al.\textsuperscript{39}</td>
<td>1</td>
<td>5/11 (46%)</td>
<td></td>
</tr>
<tr>
<td>Lockwood, et al.\textsuperscript{65}</td>
<td>1</td>
<td>18/35 (51%) (Ctr. 1)</td>
<td>26/349 (7%)</td>
</tr>
<tr>
<td>Lynch, et al.\textsuperscript{35}</td>
<td>1</td>
<td>2/9 (22%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>(fraternal twin study)</td>
<td>2</td>
<td>5/9 (55%)</td>
<td>4/9 (44%)</td>
</tr>
<tr>
<td>Nyberg, et al.\textsuperscript{67}</td>
<td>2</td>
<td>7/49 (14%)</td>
<td>35/572 (6%)</td>
</tr>
<tr>
<td>Nyberg, et al.\textsuperscript{68}</td>
<td>2</td>
<td>11/45 (24%)</td>
<td>44/350 (5%)</td>
</tr>
<tr>
<td>Rodis, et al.\textsuperscript{70}</td>
<td>2</td>
<td>2/11 (18%)</td>
<td></td>
</tr>
<tr>
<td>Romentsch, et al.\textsuperscript{71}</td>
<td>2</td>
<td>(19%)</td>
<td></td>
</tr>
<tr>
<td>Vintzileos, et al.\textsuperscript{41}</td>
<td>2</td>
<td>7/23 (24.1%)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{*1} = biparietal diameter/femur length $\geq$ 1.55 SD and 2 = measured femur length/expected femur length $\leq$ 0.91.

Humerus length Fitzsimmons et al. \textsuperscript{74} took measurements of limb lengths in 31 Down syndrome fetuses and 174 controls, postmortem, to determine which bone lengths were the best predictors of Down syndrome. They found that by using a cutoff of 2 SDs below the norm in the 32 fetuses with Down syndrome, 3 had short femurs, 4 had short tibias, 5 had short fibulas, 9 had short humeri, 10 had short radii, and 7 had short ulnas. They concluded that the upper extremity bones were shorter than normal more often than were the bones of the lower extremity.

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The best criteria for determining shortened humerus length was found to be comparing the measured humerus length to the expected humerus length and considering a ratio of less or equal to 0.90 \textsuperscript{31, 71, 75} or 0.89 \textsuperscript{68} to be abnormal. Romentsch et al. \textsuperscript{71} found a shortened humerus in 12 of 43 (28%) of Down syndrome fetuses and a shortened femur in only 8 of 43 (19%) of Down syndrome fetuses. Benacerraf et al. \textsuperscript{75} also found a higher percentage of fetuses with Down syndrome with shortened humeri (12 of 24) than femurs (10 of 24).

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syndrome by 11-fold. The sensitivity of shortened humeri for the detection of fetuses with Down syndrome ranges from 24% to 64% with an average of 41% based on referenced studies (Table 4).

Table 4. Studies of shortened humerus length

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Down syndrome fetuses with short humerus/Total no. Down syndrome fetuses</th>
<th>No. control fetuses with short humerus/Total no. control fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benacerraf et al.</td>
<td>12/24 (50%)</td>
<td>25/400 (6%)</td>
</tr>
<tr>
<td>Benacerraf et al.</td>
<td>17/32 (53%)</td>
<td>34/588 (6%)</td>
</tr>
<tr>
<td>Benacerraf et al.</td>
<td>20/37 (54%)</td>
<td>3/84 (4%)</td>
</tr>
<tr>
<td>Bromley, et al.</td>
<td>19/46 (41%)</td>
<td>43/350 (5%)</td>
</tr>
<tr>
<td>Nyberg, et al.</td>
<td>11/45 (24%)</td>
<td>7/11 (64%)</td>
</tr>
<tr>
<td>Rodis, et al.</td>
<td>12/43 (28%)</td>
<td>10/23 (48%)</td>
</tr>
<tr>
<td>Vintzileos, et al.</td>
<td>---------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>TOTALS</td>
<td>108/261 (41%)</td>
<td>105/1422 (7%)</td>
</tr>
</tbody>
</table>

Pyelectasis

Fetal renal pyelectasis is a fairly common finding on prenatal ultrasound. It is defined as an anteroposterior diameter of the renal pelvis of 4 mm or greater between 15 and 20 weeks' gestation, 5 mm or greater between 20 and 30 weeks' gestation, and 7 mm or greater between 30 and 40 weeks, although below 10 mm is not usually associated with significant renal abnormalities at birth. It is also known that fetuses with chromosomal abnormalities have a higher incidence of abnormalities of the genitourinary tract. Wickstrom et al defined pyelectasis as >=4 mm before 33 weeks gestation and >=7 mm after 33 weeks. Using this definition, 60% of fetuses with pyelectasis were found to have postnatal complications and 3 of 82 had Down syndrome.

Benacerraf et al. studied 7,400 women of which 210 had fetal pyelectasis (54 unilateral and 156 bilateral). Seven of the 210 (3.3%) had Down syndrome. Retrospectively, they found that of 44 fetuses with Down syndrome over a 5-year period, 25% had pyelectasis. Others have found a similar detection rate. Bromley et al detected 23% of fetuses with Down syndrome after the finding of pyelectasis, Vintzileos et al detected 26%, Nyberg et al found a rate of 17%, and a later study by Benacerraf et al detected 24%. Overall, it was found that although the presence of pyelectasis only slightly increases the risk of Down syndrome, approximately one fourth of fetuses with Down syndrome will have this finding.

Ear measurements

Hall found that 60% of newborns with trisomy 21 had dysplastic ears with regard to length, width, shape, and position. Birnholz et al described the use of ultrasound to evaluate fetal ear measurements and found that fetuses with chromosomal abnormalities had ears that were shorter by 1.5 SD or more.

Shimizu et al speculated that fetal ear measurements might be useful in predicting fetuses with Down syndrome. They developed nomograms of fetal ear widths and length from 18 to 42 weeks and found that ear width-length ratios remained constant throughout pregnancy. Awrrad et al studied 418 fetuses, including 4 with trisomy 21 and 6 with trisomy 18 and found that a measured-to-expected ear length ratio of less than 0.8 was 75% sensitive in detecting Down syndrome fetuses and was 98.8% specific (only 5 of 408 controls had a ratio less than 0.8). Gill et al confirmed a variation in fetal ear
measurements in trisomy 21 versus fetuses with normal chromosomes by studying formalin preserved fetuses in the second trimester; however, they did not consider this finding to be diagnostically useful due to the wide range of normal variation in the sizes.

Echogenic intracardiac focus

An echogenic intracardiac focus (EIF) generally is considered to be a normal variant and is observed in about 5% of fetuses undergoing second trimester ultrasound. An EIF is considered to be present when the echogenicity in the region of the papillary muscle in either ventricle is comparable to bone. When an EIF was present, it was located in the left ventricle 91% of the time, in the right ventricle in 7.5% of fetuses, and in 1.5% of fetuses, it was present in both ventricles.

Roberts et al found an intracardiac focus in 16% of fetuses with Down syndrome, 39% with trisomy 13, and only 2% of control fetuses on autopsy. Bromley et al studied 1,334 women of 66 (4.9%) had an EIF. Four of these fetuses (6%) were subsequently diagnosed with Down syndrome. Of 22 fetuses with Down syndrome, 4 (18%) had an EIF whereas 62 of 1,312 (4.7%) fetuses without trisomy 21 had an EIF. In a later study, Bromley et al found that 16 of 53 (30%) of fetuses with Down syndrome had an intracardiac echogenic focus. Lehman et al studied fetuses with trisomy 13 and found an EIF present in 30% of cases on fetal ultrasound. A subsequent study by Bromley et al found a 4.8% incidence of aneuploidy in 290 fetuses with an EIF.

Hypoplasia of the fifth middle phalanx

Hypoplasia or aplasia of the middle phalanx of the fifth digit is known to occur in approximately 60% of individuals with Down syndrome. Benacerraf et al examined the fifth phalanx to determine if such a finding could be used prenatally to identify fetuses with Down syndrome. They found hypoplasia to be present in four of five fetuses with Down syndrome between 17 and 20 weeks' gestation. In addition, the fifth digit was more likely to be curved inward (clinodactyly) in fetuses with Down syndrome.

Benacerraf et al defined a shortened fifth middle phalanx as a ratio of the length of middle phalanx of the fifth digit to the middle phalanx of the fourth digit less than or equal to 0.70. They found that the average among controls was 0.85 and 0.59 among fetuses with Down syndrome. Using a cutoff of 0.70, they were able to detect six of eight (75%) of fetuses with Down syndrome. Using this criterion, Vintzileos et al were able to detect 3 of 23 fetuses (19%) with Down syndrome.

Echogenic bowel

The presence of a hyperechoic mass in the fetal abdomen most often represents bowel when ascites is not present. It is considered to be hyperechoic when its echogenicity is similar to that of surrounding bone. In most cases, this is found to be a normal variant, it has also been described in association with chromosome abnormalities, cystic fibrosis, intrauterine growth retardation, intrauterine infections, and other anomalies. Nyberg et al prospectively diagnosed echogenic bowel in 7% of second trimester fetuses with Down syndrome. Scioscia et al reviewed 22 cases of fetal echogenic bowel and found that 5 cases had trisomy 21 and 1 case had trisomy 18, resulting in 27% fetuses with echogenic bowel being aneuploid.

Bromley et al found the incidence of echogenic bowel in pregnancy to be 0.6%. Of 50 fetuses with echogenic bowel, 29 (58%) were normal at delivery, 8 (16%) were aneuploid. Six (12%) of the fetuses had Down syndrome, one had trisomy 13, and one had Turner syndrome. Eight of the fetuses with echogenic bowel were growth retarded (of which three ended in intrauterine or neonatal demise) and five were unknown. The fetuses with chromosome abnormalities also were found to have other anomalies present. A total of 48 fetuses were determined to have Down syndrome, giving a 12.5% detection rate on the basis of echogenic bowel alone. A similar study by Dicke et al found a 3.3% incidence of aneuploidy with echogenic fetal bowel. Nyberg et al found echogenic bowel in 5 of 94 (5%) fetuses with Down syndrome. Bromley et al found echogenic bowel present in 13 of 53 (25%) and Benacerraf et al in 7 of 45 (16%) of fetuses with Down syndrome.
Slotnick et al considered the determination of echogenic bowel to be too subjective because sonodensity was not quantified. They devised an ultrasonic grading system to quantify echogenicity. They assigned normal fetal bowel a score of 0. By adjusting the ultrasonic time gain setting to compare the echogenicity of the fetal bowel to the iliac crest, they assigned a score of 1 to bowel that was echogenic, but less than that of the iliac crest. A score of 2 was assigned if the echogenicities were the same and a score of 3 when the bowel echogenicity was greater than that of the iliac crest. They classified 40 fetuses as grade 1, 81 as grade 2 and 24 as grade 3. None of the grade 1 fetuses were found to be aneuploid or have cystic fibrosis. Of the grade 2 fetuses, two had trisomy 21 and two had cystic fibrosis. Of the 24 fetuses with grade 3 echogenic bowel, 6 had trisomy 21 and 5 had cystic fibrosis.

Sepulveda et al suggested that the cause of increased echogenicity may be due to: 1) the swallowing of amniotic fluid after an intra-amniotic bleed or gastrointestinal bleed due to thrombocytopenia resulting in hypercellular meconium or 2) mesenteric ischemia and impaired bowel motility. They suggest that echogenic bowel is seen more often in fetuses with Down syndrome because of abnormal placentation and subsequent intra-amniotic bleeding or growth retardation. Growth retardation can be caused by mesenteric ischemia. Sickler et al described the case of a newborn with trisomy 21 who died at 2 days. Autopsy revealed calcified meconium in the bowel. They described a mechanism similar to that seen in fetuses with cystic fibrosis. Because of an absence in pancreatic enzymes and high protein content of the meconium, viscosity is increased, which leads to inspissation of meconium. In fetuses with Down syndrome, decreased levels of intestinal microvillar enzymes have been found in amniotic fluid, similar to that seen in cystic fibrosis. Brock et al assimilates this finding to constipation in utero.

Using a combination of ultrasound markers

In an effort to identify the highest percentage of fetuses with Down syndrome, a combination of ultrasound markers often are used for screening. As new markers are studied and their potential value in identifying fetuses with Down syndrome assessed, they often become added to a “check list” of ultrasound findings to be ruled out. This type of ultrasound has come to be called a targeted or “genetic sonogram.”

Benacerraf et al found that 75% of Down syndrome fetuses could be identified when either an increased nuchal fold or shortened femurs were present. This carried a specificity of 98%. Ginsberg et al found that when either femur length or nuchal thickening was present, the sensitivity was 81% with a specificity of 93%.

Perella et al also studied these two markers, but wanted to compare fetuses with both markers present to ones with either one or the other marker present. They concluded that the femur length alone was the best indicator for Down syndrome because 9% of their control population had increased nuchal thickness. When nuchal thickening was studied in isolation or in combination with the presence of shortened femur length, the false-positive rate was considered to be too high. When the criteria required that both a shortened femur and increased nuchal thickness be present, the sensitivity was 45.5% for Down syndrome. Benacerraf et al also studied the use of humerus length in combination with nuchal thickening and found the same sensitivity (75%) as with femur length and nuchal thickening. Overall, these studies demonstrated that the presence of either an increased nuchal fold or decreased femur length as a criterion for amniocentesis yielded a higher detection rate for Down syndrome than either the triple screen or maternal age.

Because of the increasing number of ultrasound markers being studied, Benacerraf et al developed a weighted scoring system. The more specific findings for Down syndrome receive a higher score than findings that carry a high rate of center variability (i.e., long bone length and pyelectasis) or are found at a higher incidence in normal pregnancies (i.e., echogenic bowel). In 1992, they established the following method of scoring: an increased nuchal fold or major structural anomaly received 2 points each. A short femur, short humerus, or pyelectasis received 1 point each. Using this scoring system, a score of 2 or more detected 81% of Down syndrome fetuses and 4% of controls. In 1994, Benacerraf et al added to their scoring system the presence of hyperechoic bowel and choroid plexus cysts, with each receiving 1 point. Using this modified scoring system, 73% of fetuses with Down syndrome had a score of 2 or more and 80% had a score of 1 or more, with specificities of 96% and 90%, respectively. The use of choroid
plexus cysts as a screening method for Down syndrome is questionable because only 2% of fetuses with Down syndrome and 2% of controls had choroid plexus cysts. In a study by Verdin et al, the use of choroid plexus cysts as markers was not found to be effective for the detection of Down syndrome. None of their fetuses with Down syndrome were found to have a choroid plexus cyst, whereas 27 fetuses with a normal karyotype had choroid plexus cysts. Benacerraf et al did note that 46% of fetuses with trisomy 18 had choroid plexus cysts and their use in screening for aneuploidies should still be considered, although the majority of fetuses with trisomy 18 would likely have other abnormal findings present.

In 1997, Bromley et al modified the scoring system by the addition of an intracardiac echogenic focus and advanced maternal age. The presence of an echogenic focus was associated with a score of 1, a maternal age of 35 to 40 years was associated with a score of 1, and an age of greater than 40 years with a score of 2. Using a score of 2 or more, they were able to identify 75% of fetuses with Down syndrome with a 6% false positive rate. Using a score of 1 or more, they identified 87% with a false positive rate of 27%.

Nyberg et al found that they could detect 50% of fetuses with Down syndrome with a false positive rate of 10% when increased nuchal thickness, echogenic bowel, cerebral ventricular dilatation, pyelectasis, or short femurs were present. They determined that the presence of a marker on ultrasound increased the risk of Down syndrome by 5.6-fold, whereas a “negative” ultrasound reduced the risk by 45%.

In 1996, Vintzileos et al studied 573 women undergoing genetic amniocentesis for advanced maternal age, abnormal biochemical screen, or both. The markers they examined included shortened long bones, major structural anomalies, pyelectasis, increased nuchal thickness, echogenic bowel, choroid plexus cysts, hypoplastic fifth middle phalanx, increased space between the first and second toes, and single umbilical artery. Of 14 fetuses with Down syndrome, 12 of 14 had two or more markers, 1 of 14 had one marker, and 1 of 14 had no markers for a sensitivity of 93% when using the criteria of one or more markers. The amniocentesis rate was less than 20%.

Verdin et al was able to detect 82% of fetuses with Down syndrome using decreased femur length, echogenic bowel, pyelectasis, major structural defects, or choroid plexus cysts. Their false-positive rate was 10%.

In an effort to reduce the false positive rate, Vintzileos et al investigated which markers were the most effective diagnostically. Using multivariate logistic regression, they found that the presence of an increased nuchal fold, short humerus, or pyelectasis yielded a sensitivity of 87% with a false positive rate of 7%. The markers they studied included shortened femur, shortened humerus, increased nuchal thickness, pyelectasis, echogenic bowel, choroid plexus cysts, hypoplastic fifth middle phalanx, wide space between the first and second toes, and two-vessel cord.

Deren et al studied the value of subtle findings in conjunction with a structural anomaly, shortened long bone length, and increased nuchal thickness. They found that the presence of either clinodactyly or an echogenic bowel as a marker increased the sensitivity for Down syndrome from 53% to 63%. They did not find isolated pyelectasis to be a significant predictor of Down syndrome.

The above studies looked at ways of increasing a woman's risk for Down syndrome based on ultrasound findings. Nadel et al studied the effectiveness of a normal appearing ultrasound at decreasing the risk of Down syndrome for a woman with an elevated risk due to maternal age or biochemical screen. Using Bayes' theorem, and considering a score of 1 or more on the “scoring system” to be abnormal (which was done to maximize sensitivity), they showed that a 42-year-old could reduce her risk compared to that of a 35-year-old. For a woman between the ages of 35 and 41 years with a normal ultrasound, this could result in decreased rate of amniocentesis. By using this method, Nadel et al were able to identify 86% of fetuses with Down syndrome in women previously considered to be at high risk. Bahado-Singh et al performed a similar study and developed a table demonstrating decreased risk after a normal ultrasound. After a normal ultrasound, they found that the risk of a 39-year-old would drop to 1 in 292, whereas that of a 40-year-old would still be considered increased for Down syndrome.
Vintzileos et al 41 used the sensitivities and specificities of various ultrasound markers reported in the literature between 1983 and 1993 in conjunction with maternal age to develop a chart of adjusted risks. They determined that with a normal ultrasound, the risk of Down syndrome dropped below that of a 35-year-old for all ages up to 46 years. They concluded that the use of a “genetic sonogram” in experienced hands may be used to adjust the maternal age or biochemical marker risk for Down syndrome—this would reduce the number of women who would be candidates for amniocentesis that would, in turn, reduce the procedure-related loss rate of chromosomally normal fetuses.

For women considering prenatal testing, there are important questions they should ask when considering how to proceed.

* Do I want diagnostic testing that will tell me whether or not the fetus has Down syndrome with greater than 99% accuracy?
* Would I consider terminating the pregnancy if the fetus had Down syndrome (or another chromosome abnormality)?
* When I weigh the risk of prenatal testing against the information that the test will provide me, which carries the most weight? (Do I want to know almost definitively if the fetus has Down syndrome to enable me to prepare for the birth?)
* What is the expertise of the sonographer or physician who will be performing my ultrasound and evaluation?

Some women decide to proceed directly with invasive prenatal testing, whereas others opt to proceed with ultrasound and/or biochemical screenings to obtain more information about their pregnancy. Some do this to aid them in making a decision about invasive testing, others would not consider invasive testing under any circumstances because of the risk involved, but would still like to have as much risk free information as possible about their pregnancy.

With the flood of information and new studies being published every month, it is not always easy to sift through the different studies to determine which are most pertinent or to keep current with the latest information. Additionally, many patients have access to the Internet, which can provide them with a vast array of information, some of which may not be correct. For this reason, the task of keeping patients well informed can be very difficult for the health care provider. This review attempts to summarize the latest information available on alternatives or adjuncts to invasive prenatal testing through amniocentesis or CVS for women at increased risk to be carrying a fetus with Down syndrome. As methods for screening pregnancies and rates for detecting Down syndrome fetuses vary greatly from study to study, it is impossible to make a simple statement summarizing the findings of all pertinent studies. More studies are needed to better assess which screening techniques will detect the most pregnancies with a chromosome abnormality.

Overall, for women who are at increased risk for carrying a fetus with Down syndrome because of advanced maternal age or a biochemical screen, prenatal testing via amniocentesis or CVS should be discussed. Both CVS and amniocentesis provide the only means of diagnosing a chromosome abnormality prenatally and are considered greater than 99% accurate. The availability of an ultrasound screening for Down syndrome also should be discussed, and the option of how to proceed left to the woman. The autonomy of a woman considering her options should be of utmost importance to the professionals providing their services, and it is the responsibility of these professionals to do their best to ensure that patients are as well informed as possible about available options. Depending on the practice where the woman is seen, this information may be provided by a genetic counselor, physician, nurse, nurse practitioner, midwife, or, ideally, by a combination of these professionals.

As the number of tests available during pregnancy continues to grow, many practitioners will undoubtedly rely more and more on nurses to help explain these tests and their results to pregnant women and their families. It will, therefore, be of ever increasing importance for nurses to keep abreast of the latest studies and findings.

REFERENCES


