What Is the Importance of Second-Trimester "Soft Markers" for Trisomy 21 After an 11- to 14-Week Aneuploidy Screening Scan?

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Objectives—The purpose of this study was to evaluate the importance of second-trimester "soft markers" for trisomy 21 after an 11- to 14-week aneuploidy screening scan.

Methods—We conducted a retrospective cohort study of consecutive patients referred for measurement of the nuchal translucency (NT) as part of a screening protocol for aneuploidy. Patients who returned for an anatomic survey between 16 and 20 weeks' gestation were evaluated. The sonographic markers and anomalies associated with the detection of trisomy 21 in the second trimester were analyzed.

Results—There were 42 fetuses (0.4%) with trisomy 21 identified in the study cohort of 9692 patients. Trisomy 21 was suspected at the NT scan in 28 fetuses (67%) and at the second-trimester anatomic survey in 14 (33%). In fetuses first suspected of having trisomy 21 in the second trimester, 9 of 14 had normal anatomic survey results, and 5 of 14 had congenital malformations. All 14 fetuses had soft markers for aneuploidy. A thickened nuchal fold was identified in 5 of 9 fetuses with trisomy 21 and normal anatomic survey results, all of whom had an NT of less than 3.0 mm at the initial screening scan.

Conclusions—Second-trimester soft markers, especially a thickened nuchal fold, remain important observations in the detection of trisomy 21 by sonography among fetuses who have had first-trimester sonographic screening for aneuploidy.

Key Words—aneuploidy; first-trimester screening; nuchal fold; obstetric ultrasound; soft markers

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Abbreviations
NT, nuchal translucency

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In 2007, the American College of Obstetricians and Gynecologists recommended that all pregnant women be offered prenatal screening for aneuploidy.[1] Historically, risk assessment was based on second-trimester maternal serum screening along with genetic sonography to identify structural anomalies and "soft markers" for aneuploidy.[2–4] In the last decade, risk assessment has transitioned into the first trimester, in which sonographic metrics in conjunction with serum analytes are used to confer a patient-specific risk of aneuploidy.[1,5] Various paradigms for first-trimester or integrated first- and second-trimester screening have resulted in the detection of 85% to 98% of fetuses with trisomy 21, with a false-positive rate of 5%.[1] The question that remains is whether there is any importance to a soft marker for aneuploidy in the second-trimester fetus with normal anatomic survey results once a risk of Down syndrome has been established in the first trimester.

Materials and Methods

Institutional Review Board approval was obtained from the Partners Human Research Committee for this medical record study. The patients in this study were also included in another study that reports on the early detection of congenital malformations.[6] That study did not include an analysis of the soft markers for aneuploidy, which was the focus of this investigation. We conducted a retrospective cohort study of consecutive patients referred to a private ultrasound facility for sonographic measurement of the nuchal translucency (NT) and crown-rump length as part of a screening protocol for aneuploidy. Nasal bone evaluation was performed if requested by the referring obstetric provider. Patients were included in the study if there was at least 1 live fetus with a crown-rump length between 34 and 84 mm.

The first-trimester aneuploidy screening sonographic reports were reviewed to obtain the maternal age, fetal crown-rump length and NT measurements, and any additional anomalies. The nasal bone was recorded as "not requested" or if requested as "present," "absent," or "unable to obtain." In the case of an absent nasal bone, 2 consecutive evaluations, typically 1 week apart, were required to definitively report the nasal bone as absent. Patient race was not available. The type of aneuploidy screening protocol and subsequent pregnancy management were at the discretion of the referring provider.

Patients who returned to our imaging laboratory at Diagnostic Ultrasound Associates, PC, for a fetal anatomic survey were imaged in accordance with the American Institute of Ultrasound in Medicine guideline for second-trimester sonography with additional compulsory imaging of the great vessels, orbits including lenses, and nasal bone as required by our office protocols.[7] The criteria for identifying a soft marker in our laboratory have been previously reported.[2,3] Imagers were rarely aware of the results of aneuploidy screening at the time of the anatomic survey. For most fetuses with trisomy 21 who were scanned in the second trimester, numeric aneuploidy screening results were obtained after the anatomic scan by retrospective review of the medical record. Pathologic findings and pregnancy outcomes were obtained by review of the medical records for patients with an NT of 3.0 mm or greater, an absent nasal bone, a nuchal fold of 5.0 mm or greater, or a structural anomaly. A list of abnormal karyotypes originating from amniocentesis performed at our facility and outcome reports from our quality assurance program were cross-referenced with the patients in this study cohort.

Results

A total of 9692 fetuses were evaluated for an NT measurement. The mean maternal age was 32.9 years (SD, 4.4 years). The median crown-rump length at the time of the NT scan was 59 mm (range, 34–84 mm). The mean and median NT measurements were each 1.6 mm (SD, 0.6 mm; range, 0.5–13 mm). Nasal bone evaluation was requested in 4527 fetuses, as obstetric provider requests for nasal bone evaluation in the first trimester increased over the study period from 1.9% in 2008 to 85% in 2012.

An anatomic evaluation at Diagnostic Ultrasound Associates was performed on 8968 of the 9692 fetuses (92.5%) between 16 and 20 weeks. A normal anatomic structural survey was reported in 8843 of 8968 (98.6%). Soft markers were identified in 1272 of 8843 (14.2%) of those with normal structural survey results. Most (95%) of those with soft markers had a single marker; 4% had 2 markers; and 1% had 3 or more markers (Table 1).

There were 42 fetuses (0.4% [1/230]) with trisomy 21 identified in the study cohort. Down syndrome was suspected based on sonographic findings at the time of the NT scan in 28 fetuses (67%) and at the second-trimester anatomy scan in 14 (33%; Table 2).

Of the 28 fetuses with trisomy 21 and sonographic findings in the first trimester, all had an NT of 3.0 mm or greater. First-trimester nasal bone evaluation had been requested in 15 of 28 fetuses, and the nasal bone was absent in 9 of 15 (60%) and present in 6. Termination of pregnancy was elected before an anatomic survey in 26 of 28 fetuses

Table 1. Trisomy 21 Cases by Markers Among 8843 Fetuses With Normal Anatomic Survey Results between 16 and 20 Weeks' Gestation

Parameter	Total	Trisomy 21
Total fetuses	8843	9
Any marker	1272	9
Nuchal fold ≥5 mm	12	5
Absent/small nasal bone	15	2
Echogenic intracardiac focus	697	4
Short femur/humerus	16	2
Short femur	44	0
Short humerus	7	1
Pyelectasis	239	3
Hyperechoic bowel	49	0
Choroid plexus cysts	311	1
No. of markers		
1	1206	2 (0.2)
2	51	4 (7.8)
3	12	2 (16.7)
4	3	1 (33.3)

Values in parentheses are percentages.

(93%) with trisomy 21 identified in the first trimester. One fetus with both an NT of 3.0 mm or greater and an absent nasal bone in the first trimester had an anatomic survey in the second trimester at our facility and was found to have a ventricular septal defect and several markers and was subsequently terminated. The other fetus with an NT of 3.0 mm or greater did not return for second-trimester imaging and had a diagnosis of trisomy 21 after birth.

Trisomy 21 Detection in the Second Trimester

Fourteen fetuses with trisomy 21 had had an NT of less than 3.0 mm at the first-trimester evaluation. First-trimester nasal bone evaluation had been requested in 7 of these 14 fetuses and was present in all 7. These fetuses were first sonographically suspected as having trisomy 21 at the time of the anatomic survey. Nine of those 14 (64%) had normal anatomic survey results but had at least 1 soft marker for aneuploidy. Of these 9 fetuses, 5 had a thickened nuchal fold; 2 had an absent or a small nasal bone; 4 had an echogenic intracardiac focus; 3 had pyelectasis; 3 had short long bones; and 1 had a choroid plexus cyst. Two, 3, and 4 markers were seen in 4, 2, and 1 fetuses with trisomy 21, respectively. Two fetuses with trisomy 21 had an isolated soft marker: 1 had an echogenic intracardiac focus, and 1 had an absent nasal bone. The details of the first- and second-trimester sonographic findings for these 9 fetuses are shown in Table 3. Risk estimates for trisomy 21 were not known to the sonologist at the time of the anatomic survey except in 2 cases: 1 fetus was referred for an "abnormal screen"; however, the numeric risk estimate was not known; and the other fetus had a diagnosis of trisomy 21 based on a karyotype from chorionic villous sampling. This fetus was the only one with a known karyotypic diagnosis of trisomy 21 at the time of the scan. The remaining 7 fetuses with trisomy 21 were referred without specific aneuploidy risk estimates. Table 3 shows the indications for the scans, listing the information available to the sonologist before performing the sonographic examinations and the risk estimates for aneuploidy retrieved by review of the medical records after the anatomic survey. The screening results were abnormal in 5 of 9 fetuses with trisomy 21. Three fetuses were considered at low risk for trisomy 21, and 1 had not had a maternal serum analyte analysis performed and had no numeric risk estimate for aneuploidy.

Table 2. Criteria for Sonographic Suspicion of Trisomy 21 by Trimester

Parameter	Trisomy 21
Total	42
Suspected in 1st trimester	28 (67)
Suspected in 2nd trimester	14 (33)
1st trimester	
NT ≥3.0	28 (67)
Nasal bone evaluation requested	15
Absent nasal bone in 1st trimester	9
Absent nasal bone in 1st trimester, NT <3.0 mm	0
Anomaly at 11- to 14-wk scan	6
2nd trimester (16–20 wk)	
Any 2nd-trimester anatomic scan	15
Normal anatomic survey with marker	9
Anomaly at anatomic survey	6
Any anomaly (1st or 2nd trimester)	12

Values in parentheses are percentages.

Table 3. Sonographic Features of 9 Fetuses With Trisomy 21 and Normal Second-Trimester Anatomic Survey Results Who Were First Suspected of Having Aneuploidy Based on Second-Trimester Sonography

MA, y	CRL, mm	NT, mm	1st-Trimester NB	Indication for 16–20-wk Scan	GA, wk	2nd-Trimester Soft Markers	Screen Type	Screening Results[a]
41	55	2.0	NR	Trisomy 21[b]	18	Absent NB	SST	1 in 2
32	49	1.4	NR	Survey	18	NF/PYEL	SST	1 in 370
38	55	2.9	PRES	Survey	18	NF/PYEL	SST	1 in 2
37	60	2.3	NR	Survey	19	NF/PYEL, short H	Combined	1 in 75
37	54	1.4	PRES	Survey	18	EIF, small NB	Combined	1 in 3181
35	49	2.6	NR	Abnormal screen	16	NF, EIF, short F/H	SST	1 in 20
34	68	2.4	PRES	Survey	19	NF, short F/H,	SST	1 in 120
39	60	2.0	PRES	Amniocentesis, AMA	16	EIF	None	None
36	56	2.5	PRES	Survey	17	EIF, CPC	Combined	1 in 944

AMA indicates advanced maternal age; CPC, choroid plexus cyst; CRL, crown-rump length; EIF, echogenic intracardiac focus; F, femur; GA, gestational age at anatomic survey; H, humerus; MA, maternal age at NT scan; NB, nasal bone; NF, nuchal fold of 5 mm or greater; NR, not requested; PRES, present; PYEL, pyelectasis; and SST, serial sequential test.

[a]Screening results known after imaging.

[b]Karyotype known at the time of imaging.

There were 5 fetuses newly suspected of having aneuploidy based on major anatomic abnormalities at the time of the structural survey, 2 of whom were members of a dichorionic twin pair in which the other twin was normal. Numeric screening results were known from the indications for the scans in 2 fetuses before anatomic imaging, and the remaining 3 were referred for a structural survey without aneuploidy screening information. These 5 fetuses had a variety of soft markers in addition to the structural anomaly, and screening results confirmed after imaging were abnormal in 3. One fetus who was part of a twin pair had no numeric risk estimate for aneuploidy calculated. The other fetus (also one of a twin pair) had a numeric risk estimate for aneuploidy that was considered low (Table 4).

Second-Trimester Nuchal Fold and Nasal Bone Ossification

Among the 1272 fetuses with normal anatomic survey results and soft markers, 12 had a nuchal fold of 5 mm or greater, and 15 had an absent nasal bone. Down syndrome was diagnosed in 5 of 12 fetuses (41.7%) with a nuchal fold of 5 mm or greater and in 2 of 15 (13.3%) with an absent or small nasal bone (Table 1). In fetuses with a nuchal fold of 5 mm greater on the second-trimester scan, 8 of 12 (66.7%) had an NT of less than 3.0 mm at the first-trimester screen, including all 5 fetuses with trisomy 21.

The fetal nasal bone was absent in the first trimester in 41 of 4527 fetuses (0.9%) for whom an evaluation had been requested. The aneuploidy status was available by karyotype or neonatal examination in 37 of 41 fetuses (90%) with an absent nasal bone, and the karyotype was abnormal in 14 of 37 (38%). Nine of the 14 (64%) had trisomy 21.

Twenty-two of the 41 fetuses with an absent nasal bone at the time of the 11- to 14-week scan had an anatomic survey at our facility in the second trimester, 1 of whom had trisomy 21. The nasal bone could be evaluated in 21 of 22 fetuses in the second trimester. The nasal bone had ossified and was present at the second-trimester anatomic scan in 9 of 21 (43%). The nasal bone remained absent in 12 of 21 (57%), including the single fetus with trisomy 21. One fetus did not have the nasal bone appearance reported at the second-trimester anatomic scan because of the fetal position.

Twenty fetuses had no evidence of a structural anomaly on the anatomic scan, and 2 had structural anomalies. The fetus with trisomy 21 had a ventricular septal defect as well as an absent nasal bone and a thickened nuchal fold. The other fetus had postaxial polydactyly noted at the anatomic scan, but the nasal bone had ossified and was normal.

Overall, 36 of 42 fetuses (86%) with trisomy 21 were terminated. The termination rates were 96% when trisomy 21 was suspected sonographically in the first trimester and 64% when it was suspected sonographically in the second trimester.

Discussion

The second-trimester anatomic scan in conjunction with the identification of soft markers is a routine part of obstetric care and until recently was the primary method of risk assessment for aneuploidy. In the last decade, the option of aneuploidy screening using sonographic metrics and serum analytes has shifted into the first trimester, successfully detecting most fetuses with trisomy 21.[1] The second-

Table 4. Structural Anomalies and Soft Markers in 5 Fetuses With Trisomy 21 That Was First Sonographically Identified in the Second Trimester

MA, y	CRL, mm	NT, mm	1st-Trimester NB	Indication for 16–20-wk Scan	GA, wk	Anomaly	2nd-Trimester Soft Markers	Screen Type	Screening Results[a]
44	54	2.1	NR	Abnormal screen, 1:21	19	VSD	NF, EIF, small NB, short F/H	SST	1 in 2
36	53	1.5	NR	Abnormal screen, 1:2	16	AVC	Small NB	SST	1 in 2
40	74	2.3	NR	Survey	16	TOF	Absent NB, PYEL	None, twin	None
41	58	2.2	PRES	Amniocentesis, AMA	16	AVC, MVM	PYEL, short F/H	SST	1 in 25
33	57	2.2	PRES	Survey	17	MVM, VSD, small stomach	PYEL, short H	Combined 1st-trimester, twin	1 in 7601

AVC indicates atrioventricular canal; MVM, mild ventriculomegaly; TOF, tetralogy of Fallot; and VSD, ventricular septal defect. Other abbreviations are as in Table 3.

[a]Screening results known after imaging.

trimester anatomic scan remains the primary method for identifying fetal abnormalities, and there is no doubt that fetuses with structural anomalies are at an increased risk for karyotypic and other genetic abnormalities.[8] In fetuses with normal second-trimester anatomic survey results, a quandary exists as to the clinical importance of a soft marker, especially if the fetus has previously been screened for aneuploidy in the first trimester. Some authors have reported an increased detection rate of Down syndrome, and others caution about a decrease in the detection rate and false reassurance. Furthermore, screen-positive rates have been reported to both increase and decrease.[9–15]

Our study is unique in that it addresses the sonographic features of trisomy 21 first noted in the second trimester after first-trimester sonographic assessment using the NT measurement and nasal bone evaluation (if requested). In this study, 28 of 42 fetuses (67%) with trisomy 21 were identified in the first trimester on the basis of NT measurement alone, a rate comparable to other studies.[1,16] Aneuploidy screening results were rarely known to the sonologist at the time of the 16- to 20-week anatomic scan, which detected the remaining 14 of 42 fetuses (33%) with trisomy 21 based on anomalies and soft markers, the most notable being a thickened nuchal fold. A thick nuchal fold was observed in 56% of fetuses with trisomy 21 who had normal anatomic survey results. This finding supports the nuchal fold as a critical marker in the sonographic detection of aneuploidy, even in a prescreened population, as all of these fetuses had an NT of less than 3 mm. This finding is also supported by a report from Finland demonstrating that the NT measurement is the most important factor leading to negative first-trimester combined screening results for Down syndrome.[17]

Aagaard-Tillery et al[14] evaluated the use of genetic sonography after Down syndrome screening and demonstrated that the sensitivity for detection of Down syndrome increased by 1% to 9%, depending on the type of initial screening. In that study, a thickened nuchal fold carried the highest likelihood ratio for Down syndrome.

Our overall detection rate for soft markers (14.2%) among fetuses with normal structural survey results is comparable to what has previously been reported.[2] A limitation of our study is that the detection rate for second-trimester soft markers in fetuses with trisomy 21 is not directly comparable to other studies, as it was not logistically feasible to methodically review the medical records of each fetus with normal sonographic results. We therefore used the karyotype information from our quality assurance program to cross-reference fetuses with trisomy 21 who had been scanned in our laboratory with this study cohort. It is possible that a fetus with trisomy 21 who had an NT of less than 3 mm and did not return for an anatomic survey or had structurally normal second-trimester scan results in our laboratory could have been missed in this analysis. The contribution of a small or an absent nasal bone was hampered by the absence of data concerning patient race.

In this population of fetuses with trisomy 21 imaged in the second trimester, 5 of 14 (36%) had anatomic abnormalities, and all 5 had cardiac defects. This rate is slightly higher than the 26% that we observed in the last decade in an unscreened population and may have been due to improved imaging quality.[2]

Although 67% of fetuses with Down syndrome were identified sonographically in the first trimester, this number improves when the sonographic findings are used in combination with serum analytes to obtain optimal screening detection rates. Taking into account the first-trimester sonographic examination as well as serum screening information, 4 of 42 fetuses (9.5%) with Down syndrome were considered "low risk" until the second-trimester anatomic scan. This number is in agreement with prior studies reporting an 85% detection rate for the combined screen.[1] Of these 4 fetuses with Down syndrome, 1 had a major anomaly, and 3 had a normal anatomic survey with soft markers.

Our experience with second-trimester genetic sonography in a sonographically prescreened population underscores the importance of a thickened nuchal fold, especially in a fetus with normal anatomic survey results. The observation of this marker should prompt genetic counseling and consideration of further evaluation.

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