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First-Trimester or Second-Trimester Screening, or Both, for Down's Syndrome

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ABSTRACT

BACKGROUND

It is uncertain how best to screen pregnant women for the presence of fetal Down's syndrome: to perform first-trimester screening, to perform second-trimester screening, or to use strategies incorporating measurements in both trimesters.

METHODS

Women with singleton pregnancies underwent first-trimester combined screening (measurement of nuchal translucency, pregnancy-associated plasma protein A [PAPP-A], and the free beta subunit of human chorionic gonadotropin at 10 weeks 3 days through 13 weeks 6 days of gestation) and second-trimester quadruple screening (measurement of alpha-fetoprotein, total human chorionic gonadotropin, unconjugated estriol, and inhibin A at 15 through 18 weeks of gestation). We compared the results of stepwise sequential screening (risk results provided after each test), fully integrated screening (single risk result provided), and serum integrated screening (identical to fully integrated screening, but without nuchal translucency).

RESULTS

First-trimester screening was performed in 38,167 patients; 117 had a fetus with Down's syndrome. At a 5 percent false positive rate, the rates of detection of Down's syndrome were as follows: with first-trimester combined screening, 87 percent, 85 percent, and 82 percent for measurements performed at 11, 12, and 13 weeks, respectively; with second-trimester quadruple screening, 81 percent; with stepwise sequential screening, 95 percent; with serum integrated screening, 88 percent; and with fully integrated screening with first-trimester measurements performed at 11 weeks, 96 percent. Paired comparisons found significant differences between the tests, except for the comparison between serum integrated screening and combined screening.

CONCLUSIONS

First-trimester combined screening at 11 weeks of gestation is better than second-trimester quadruple screening but at 13 weeks has results similar to second-trimester quadruple screening. Both stepwise sequential screening and fully integrated screening have high rates of detection of Down's syndrome, with low false positive rates.

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FIRST-TRIMESTER SCREENING FOR Down's syndrome that includes the use of ultrasonography to assess nuchal translucency has become widespread since its introduction by Nicolaides and colleagues in the early 1990s.¹⁻⁴ The largest U.S. study of first-trimester screening to date, involving 8514 pregnancies, reported a 79 percent detection rate at a 5 percent false positive rate.⁵ Second-trimester screening remains the most common approach to assessing the risk of Down's syndrome in the United States.⁶ When inhibin A is included in second-trimester quadruple screening, the estimated detection rate for Down's syndrome is 81 percent with a 5 percent false positive rate.⁷ However, little information is available on the comparative performance of these first- and second-trimester approaches. More complex options for risk assessment have also become available, including sequential screening (performance of screening tests at different times during pregnancy, with the results provided to the patient after each test) and integrated screening (performance of screening tests at different times during pregnancy, with a single result provided to the patient only after all tests have been completed).^{8,9}

Accurate comparison of the performance of different screening tests conducted at different times during pregnancy remains complex because of the bias that can arise from spontaneous pregnancy losses that may occur between the first-trimester and the second-trimester screenings. We conducted the First- and Second-Trimester Evaluation of Risk (FASTER) Trial with the goal of providing direct comparative data on currently available screening approaches to Down's syndrome from a large population followed prospectively.

METHODS

STUDY POPULATION

This study was conducted at 15 U.S. centers from October 1999 to December 2002. Institutional review board approval was obtained, and the participants gave written informed consent. The inclusion criteria were a maternal age of 16 years or older, pregnancy with a singleton live fetus, and a fetal crown-rump length of 36 to 79 mm (consistent with a gestational age of 10 weeks 3 days through 13 weeks 6 days at study entry).¹⁰ Women were excluded from the study if they had undergone prior measurement of nuchal translucency or if anen-

cephaly was diagnosed in the fetus. Patients whose fetuses had septated cystic hygroma were followed separately without contributing serum samples. The first-trimester risk was calculated from measurements of nuchal translucency and two serum markers, pregnancy-associated plasma protein A (PAPP-A) and the free beta subunit of human chorionic gonadotropin (*ff*hCG), together with maternal age. The patients returned at 15 to 18 weeks of gestation for second-trimester screening. At this time, a second-trimester risk was calculated from measurements of serum alpha-fetoprotein, total human chorionic gonadotropin (hCG), unconjugated estradiol, and inhibin A, together with maternal age.

Ultrasonography to assess nuchal translucency was performed according to a standardized protocol by specially trained ultrasonographers.⁴ A minimum of 20 minutes was reserved for the assessment, and transvaginal ultrasonography was used if necessary. The patient could return for a second evaluation if the initial attempt failed. All images were scored by a single reviewer at the main study center, and feedback was provided to the ultrasonographers. A random selection of 10 percent of images underwent additional review by an independent ultrasound quality-assurance committee. Median nuchal-translucency measurements and their standard deviations were monitored according to ultrasonographer and study site. Drift in these values triggered review of images and feedback to individual ultrasonographers.

ASSESSMENT OF RISK

Measurements of biochemical markers were converted into multiples of the median (MoM) for gestational age, adjusted for maternal weight and race or ethnicity. Nuchal-translucency MoM values were center-specific, and the mean of three measurements was used for calculation of risk. The risk of Down's syndrome was estimated by multiplying the maternal age-specific odds of the live birth of an infant affected by Down's syndrome¹¹ by the likelihood ratio obtained from the overlapping gaussian distributions of affected and unaffected pregnancies, as previously described.¹² These distributions were specified by using published statistical parameters.^{8,13} The distributions of nuchal-translucency measurements were based on all pregnancies, including those in which cystic hygromas were found. The patients were provided with two separate estimates of the risk of Down's syndrome, with cutoff

points chosen at the start of the trial; a positive result from first-trimester screening was defined as a risk at the end of pregnancy (40 weeks) of 1 in 150, and a positive result from second-trimester screening was defined as a risk at the end of pregnancy of 1 in 300. Because second-trimester screening was considered the standard of care, the risk cutoff point was chosen so that the rate of positive screening results was similar to that of current screening practice — that is, a rate of 5 percent, given the age distribution of pregnancies in the United States. The first-trimester risk cutoff point was chosen to yield a lower rate of positive screening results (2 to 3 percent) in order to ensure that the overall rate for the study population would not be excessive. The results were provided to the patients after all screening tests were complete, and patients with positive results from either first-trimester or second-trimester screening were offered formal genetic counseling and the option of amniocentesis for genetic analysis.

SCREENING TESTS

The following screening tests for fetal Down's syndrome were evaluated: measurement of first-trimester nuchal translucency alone; first-trimester serum screening alone (PAPP-A and β hCG were measured); first-trimester combined screening (nuchal translucency plus PAPP-A and β hCG); second-trimester quadruple screening (alpha-fetoprotein, total hCG, unconjugated estriol, and inhibin A); independent sequential screening (the results of combined screening were provided to the patient in the first trimester, and the results of quadruple screening in the second trimester, with both risks calculated independently); stepwise sequential screening (the results of combined screening were provided in the first trimester, and the results of quadruple screening in the second trimester; the risk in the second trimester was calculated with inclusion of the marker levels measured in the first trimester); serum integrated screening (PAPP-A was measured in the first trimester, and the results were not provided to the patient; quadruple markers were measured in the second trimester, and the risk in the second trimester was calculated with inclusion of the marker levels measured in the first trimester); and fully integrated screening (identical to serum integrated screening with the addition of first-trimester measurement of nuchal translucency). For all tests, the calculated risk took into account maternal age.

DATA COLLECTION

Research coordinators at each clinical site recorded information on patients by using a computerized tracking system to maximize the amount of data obtained. Copies of fetal and pediatric medical records were submitted for review by a single pediatric geneticist in all cases in which a possible fetal or neonatal medical problem was suspected, in all cases with a positive screening-test result but without karyotype results, and in a 10 percent random sample of all other cases in enrolled patients. Fetal chromosome status was determined by amniocentesis; by sampling neonatal cord blood in cases with a positive screening-test result in which the mother declined amniocentesis; or by tissue sampling in cases of spontaneous pregnancy loss, pregnancy termination, or stillbirth.

Completeness of ascertainment was assessed by calculating the expected number of cases of Down's syndrome from the maternal age distribution of the enrollees and recent age-specific birth prevalence data.¹⁴ On the basis of these data, 112 cases of Down's syndrome were expected in the second trimester; we identified 117 cases, suggesting that all cases were probably identified.

STATISTICAL ANALYSIS

Screening performance was based on the maternal age-specific risk of having an affected live-born child, corrected to early mid-trimester to allow for loss of fetuses with Down's syndrome from this time until term,¹¹ and applied to the U.S. standard population of births for 1999.¹⁴ MoM values for each pregnancy were calculated by dividing the observed marker concentration by the median value for unaffected pregnancies with the same fetal crown-rump length. The first trimester was not treated as a single time period, because MoM values of the markers in affected pregnancies change linearly with gestational age. Confidence intervals for the estimates of screening performance of the combined, quadruple, fully integrated, serum integrated, and stepwise sequential testing strategies were derived by bootstrapping with 1000 Down's syndrome dataset replications. These confidence intervals give the range of values within which the true screening performances are likely to lie. To compare screening performances of different strategies, the difference between pairs of tests was determined for each dataset replication and the 95 percent confidence intervals of these differences were calculated.

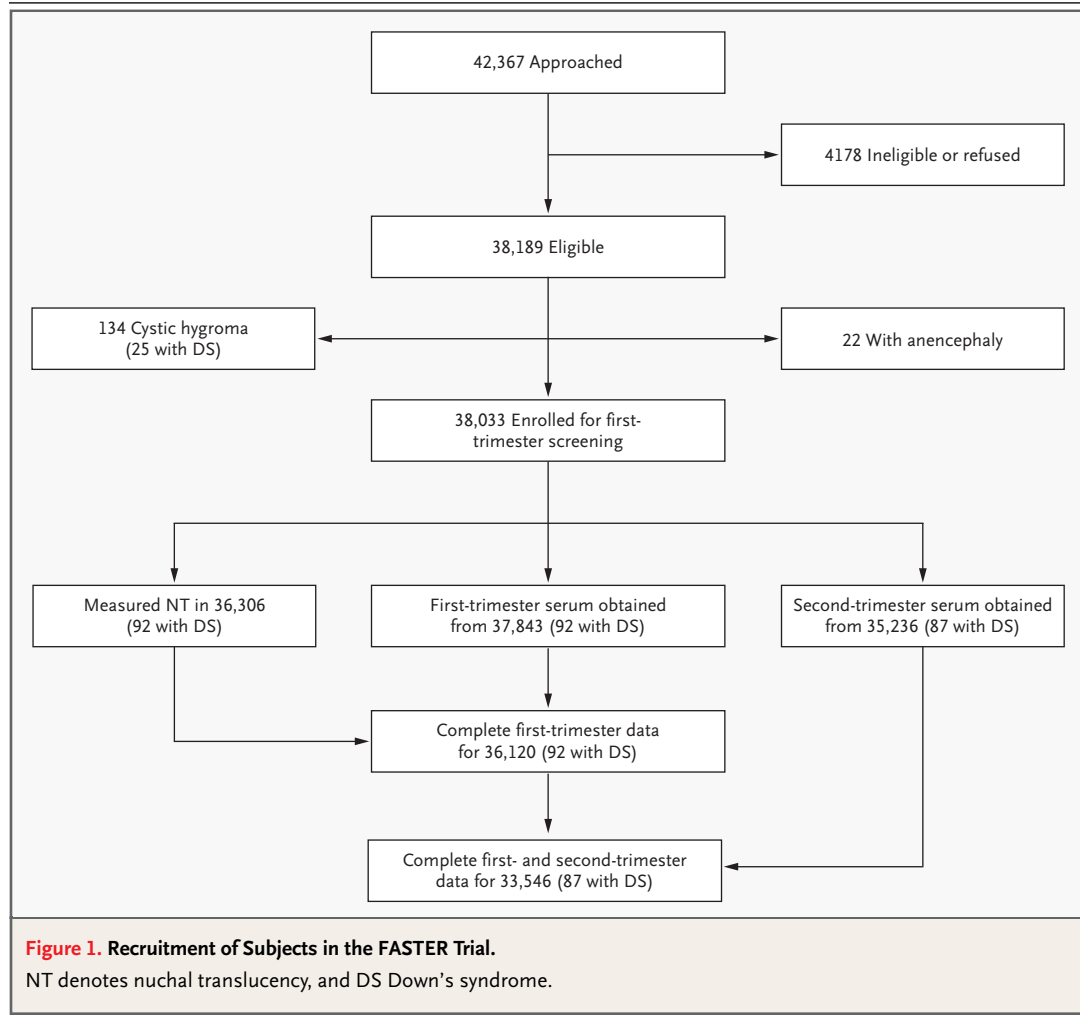
An independent replication of the data analysis was performed by the Foundation for Blood Research, Scarborough, Maine, and the results were reported to the data-monitoring committee, which was independent of the FASTER Trial consortium. These results were consistent with those of the primary analysis.

RESULTS

A total of 42,367 patients were approached for enrollment (Fig. 1). The demographic characteristics of the 38,033 patients enrolled are summarized in Table 1. Data on pregnancy and pediatric outcomes were obtained in 36,837 cases (97 percent). One hundred two approved ultrasonographers performed all nuchal-translucency evaluations. The ultrasonographer failed to obtain an adequate nuchal-translucency image in 1727 cases (4.5 percent),

and in a further 974 cases (2.6 percent) the images were rejected at central review. Adequate nuchal-translucency measurements were therefore obtained in 35,332 cases (92.9 percent). Complete first- and second-trimester screening data were available for 33,459 unaffected pregnancies and 87 pregnancies affected by Down's syndrome. There were 117 cases of Down's syndrome in the population of 38,167 patients (38,033 enrolled patients plus 134 patients whose fetuses had cystic hygromas). Of the 117 cases of Down's syndrome, 25 were in the cystic-hygroma subgroup and 92 occurred among the 38,033 pregnancies described in this report.

Table 2 summarizes the performance of first- and second-trimester screening, by counting the number of detected and false positive cases above the risk cutoff levels used. Table 3 presents the median MoM values in pregnancies affected by Down's



syndrome for individual markers at each week during the first trimester and the detection rates for each marker used alone. The median MoM values in affected pregnancies are not constant from 11 through 13 weeks of gestation, so that the performance of tests that include measurement of nuchal translucency and PAPP-A declines, and the performance of tests that include measurement of $\text{f}\beta\text{hCG}$ improves, over this time period.

The second-trimester median MoM values for markers in affected pregnancies were 0.74 for alpha-fetoprotein (95 percent confidence interval, 0.67 to 0.82), 1.79 for hCG (95 percent confidence interval, 1.59 to 2.01), 0.61 for unconjugated estriol (95 percent confidence interval, 0.55 to 0.67), and 1.98 for inhibin A (95 percent confidence interval, 1.74 to 2.26). The observed median MoM value of 0.61 for unconjugated estriol was substantially lower than almost all previously published estimates.¹⁵ In a meta-analysis of 733 pregnancies affected by Down's syndrome, the median MoM value for unconjugated estriol was 0.72 (95 percent confidence interval, 0.68 to 0.75).¹⁵ The effect of this unexpected finding in our study would be improved rates of detection of Down's syndrome, at a 5 percent false positive rate, of 86 percent (instead of 81 percent) for quadruple screening and 78 percent (instead of 69 percent) for triple screening. The median MoM value of 0.61 for unconjugated estriol is likely to be an outlying low result that would tend to produce an overestimation of second-trimester screening performance, since the 95 percent confidence in-

Table 1. Demographic Characteristics of the 38,033 Enrolled Patients.

Characteristic	No. of Patients	No. of Fetuses with Down's Syndrome	Percent of Total Patients
Maternal age at expected date of delivery*			
<35 Yr	29,834	28	78.4
≥35 Yr	8,199	64	21.6
Maternal race or ethnic group†			
White	25,459	65	66.9
Hispanic	8,607	17	22.6
Black	2,031	5	5.3
Asian	1,556	4	4.1
Other	380	1	1.0
Gestational age of fetus at first-trimester screening			
10 wk 3 days to 10 wk 6 days	1,345	0	3.5
11 wk 0 days to 11 wk 6 days	8,583	19	22.6
12 wk 0 days to 12 wk 6 days	17,052	44	44.8
13 wk 0 days to 13 wk 6 days	11,053	29	29.1

* The mean (±SD) maternal age at the expected date of delivery was 30.1±5.8 years.
 † Race or ethnic group was self-reported.

tervals of our observed values and the meta-analysis values do not overlap, whereas our other results are all consistent with published values. Our subsequent results are therefore based on the pooled median MoM of 0.72 for unconjugated estriol obtained from a meta-analysis.¹⁵

Table 4 shows the estimated performance of a

Table 2. Directly Observed Performance Characteristics of First- and Second-Trimester Screening Tests for Down's Syndrome.

Screening Test	Risk Cutoff	Detection Rate* <i>percent (no. positive/total no.)</i>	False Positive Rate <i>percent</i>
First-trimester combined screening			
Hygroma not included	1:150	77 (71/92)	3.2
Hygroma included	1:150	82 (96/117)	3.2
First-trimester combined screening			
Hygroma not included	1:300	82 (75/92)	5.6
Hygroma included	1:300	86 (100/117)	5.6
Second-trimester quadruple screening	1:300	85 (74/87)	8.5
Sequential screening in both trimesters†	1:150 for 1st trimester 1:300 for 2nd trimester	94 (82/87)	11

* The detection rate is subject to bias, because an unknown proportion of fetuses with hygroma might have been spontaneously aborted before the second trimester, when most cases of Down's syndrome were ascertained.

† The detection rate is based on a positive result from either the first-trimester combined screening at a risk cutoff of 1 in 150 or the second-trimester quadruple screening at a risk cutoff of 1 in 300, with both screening results being calculated independently.

Table 3. Multiple of the Median (MoM) Values for First-Trimester Levels of Markers in Pregnancies Affected by Down's Syndrome and Estimated Detection Rates for a 5 Percent False Positive Rate.*

Marker	No. of Completed Weeks of Gestation		
	11	12	13
	<i>median MoM value</i>		
Nuchal translucency			
Estimated†	2.13	1.91	1.71
Observed (95% CI)	2.14 (1.58–2.91)	2.26 (1.80–2.84)	1.43 (1.06–1.95)
PAPP-A			
Estimated†	0.42	0.47	0.53
Observed (95% CI)	0.31 (0.18–0.52)	0.46 (0.36–0.59)	0.74 (0.51–1.08)
fβhCG			
Estimated†	1.89	2.05	2.23
Observed (95% CI)	2.08 (1.16–3.70)	1.79 (1.21–2.66)	2.42 (1.52–3.85)
	<i>estimated detection rate (percent)‡</i>		
Nuchal translucency	63	60	55
PAPP-A	51	44	37
fβhCG	22	25	29

* CI denotes confidence interval, PAPP-A pregnancy-associated plasma protein A, and fβhCG the free beta subunit of human chorionic gonadotropin.

† The estimated MoM values were derived from regression of the value of each marker against gestational age.

‡ The detection rates were estimated without the use of maternal age.

variety of screening approaches, applied to the 1999 U.S. distribution of maternal ages (mean age, 27.1 years, with 13.2 percent 35 years of age or older).¹⁴ First-trimester serum screening and nuchal-translucency measurement perform similarly, but the combination of both is superior for detecting Down's syndrome at 11 to 13 weeks of gestation. Serum integrated screening performs similarly to first-trimester combined screening yet does not require nuchal-translucency measurement. Fully integrated screening (including measurement of nuchal translucency) yields the highest detection rates with the lowest false positive rates as compared with other forms of screening. Quadruple screening performs better than triple screening (measurement of alpha-fetoprotein, hCG, and unconjugated estriol), with both lower false positive rates and higher detection rates. The detection rates at various false positive rates and the false positive rates at various detection rates are summarized in Table 4.

To compare the performance of different screening tests, it is not appropriate to rely on the 95 percent confidence intervals surrounding the point estimates of performance of the main screening tests, as shown in Table 4. Therefore, the performance of different screening tests was compared on the basis

of many samplings from the study population. These comparisons showed that, at false positive rates of 1 percent or 5 percent, the detection rates were significantly different for the various testing strategies, except for the serum integrated and combined-screening tests, for which the detection rates were not significantly different (Table 5).

Subgroup analyses were performed of data from women 35 years of age or older and from those younger than 35 years. For women 35 or older, first-trimester combined screening had a detection rate of 95 percent at a false positive rate of 22 percent, as compared with a detection rate of 92 percent at a false positive rate of 13 percent for second-trimester quadruple screening and a detection rate of 91 percent at a false positive rate of 2.0 percent for integrated screening (with first-trimester markers measured at 11 weeks). For women under 35, first-trimester combined screening had a detection rate of 75 percent at a 5.0 percent false positive rate, as compared with a detection rate of 77 percent at a 2.3 percent false positive rate for second-trimester quadruple screening and a detection rate of 77 percent at a 0.4 percent false positive rate for integrated screening.

Another option is stepwise sequential screen-

Table 5. Differences in False Positive Rates for a Given Detection Rate, and Differences in Detection Rates for a Given False Positive Rate for Specified Pairs of Screening Tests.*

Screening Test	Percent Detection Rate			Percent False Positive Rate	
	75	85	95	1	5
	percentage points of difference between false positive rates (95% CI)			percentage points of difference between detection rates (95% CI)	
Combined — 11 vs. 12 wk†	-0.2 (-0.6 to 0.0)	-1.0 (-1.9 to -0.3)	-3.7 (-5.4 to -2.3)	1.5 (0.1 to 2.6)	1.8 (1.1 to 2.5)
Combined — 11 vs. 13 wk†	-1.1 (-2.1 to -0.4)	-3.1 (-4.9 to -1.6)	-8.1 (-12 to -5.2)	6.1 (3.8 to 8.5)	4.9 (3.3 to 6.5)
Combined — 12 vs. 13 wk†	-0.8 (-1.5 to -0.4)	-2.1 (-3.1 to -1.3)	-4.4 (-6.2 to -2.7)	4.6 (3.6 to 6.0)	3.1 (2.2 to 4.0)
Nuchal translucency alone vs. combined†	-6.9 (-10 to -2.6)	-16 (-23 to -9.1)	-38 (-49 to -29)	19 (14 to 28)	17 (12 to 24)
Serum only vs. combined†‡	-5.9 (-8.7 to -3.2)	-12 (-16 to -6.9)	-24 (-33 to -15)	23 (17 to 30)	17 (11 to 21)
Combined† vs. quadruple§	-1.9 (-6.0 to -0.6)	-3.5 (-12 to -0.3)	-4.4 (-22 to 6.9)	13 (5.0 to 29)	6.5 (0.0 to 18)
Serum integrated vs. combined†¶	0.0 (-0.8 to 1.6)	-0.2 (-2.6 to 3.8)	-2.7 (-12 to 9.8)	0.2 (-12 to 7.2)	0.5 (-7.4 to 5.8)
Fully integrated vs. combined†¶	-1.0 (-2.0 to -0.4)	-3.1 (-5.7 to -1.4)	-14 (-22 to -6.4)	15 (3.3 to 19)	8.6 (4.5 to 12)
Quadruple vs. triple	-3.9 (-7.0 to -2.3)	-6.3 (-12 to -3.3)	-9.5 (-19 to -3.1)	16 (7.7 to 22)	11 (5.8 to 17)
Serum integrated vs. quadruple	-1.9 (-4.8 to -1.3)	-3.7 (-8.7 to -2.3)	-7.1 (-14 to -3.6)	13 (10 to 19)	7.0 (4.6 to 12)
Fully integrated vs. quadruple	-3.0 (-6.8 to -1.9)	-6.7 (-14.2 to -4.1)	-18 (-34 to -11)	28 (23 to 38)	15 (11 to 24)
Fully integrated vs. serum integrated	-1.0 (-2.3 to -0.5)	-2.9 (-6.3 to -1.6)	-11 (-20 to -6.2)	15 (10 to 22)	8.1 (4.9 to 14)

* A 95 percent confidence interval that does not include zero suggests a significant difference between the results of the two screening tests. Significant differences were found for all pairs of tests in the table, except for the serum integrated test versus the combined test. The first-trimester markers for the combined and fully integrated tests were measured at 11 weeks of gestation, except where otherwise stated. CI denotes confidence interval.

† The combined test in the first trimester consists of measurement of nuchal translucency, pregnancy-associated plasma protein A (PAPP-A), and the free beta subunit of human chorionic gonadotropin (fβhCG).

‡ The serum-only test consists of measurement of PAPP-A and fβhCG.

§ The quadruple test consists of measurement of alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester.

¶ The serum integrated test consists of measurement of PAPP-A in the first trimester and alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester. The fully integrated test consists of measurement of nuchal translucency and PAPP-A in the first trimester and alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester.

ing, in which patients undergo first-trimester combined screening with the results provided immediately and those with positive tests are offered chorionic villus sampling. Patients with negative tests return at 15 weeks so that the quadruple markers can be measured, and a new risk estimate is provided that combines the results of measurement of the first-trimester and the second-trimester markers. Setting a 2.5 percent false positive rate for each screening component in this model results in an estimated detection rate of Down's syndrome of 95 percent (95 percent confidence interval, 91 to 97 percent), at an overall false positive rate of 4.9 percent. At the same 95 percent detection rate, the false positive rate for fully integrated screening was 4.0 percent (the 95 percent confidence interval for the difference between stepwise sequential and fully integrated screening is 0.1 to 1.3 percent).

DISCUSSION

The FASTER Trial was designed to compare, in a single population, first-trimester screening for Down's syndrome with second-trimester screening (the current standard of care) and with screening in both trimesters. Our results demonstrate that first-trimester screening for Down's syndrome is highly effective, but combinations of measurements of markers from both the first and the second trimesters yield higher detection rates and lower false positive rates.

We found that using both nuchal translucency and serum markers in the first trimester is more effective in screening for Down's syndrome than using either alone. At 11 weeks of gestation, adding PAPP-A and fβhCG determinations to measurement of nuchal translucency increases the detection rate of Down's syndrome from 70 percent to

87 percent, at a 5 percent false positive rate (Table 4). The differences observed between combined screening and measurement of either nuchal translucency or serum markers alone are clinically significant and support the use of first-trimester combined screening for risk assessment. The only exception may be in the case of multiple gestations (which were excluded from the present study), in which serum markers are difficult to interpret and nuchal-translucency measurements may allow for fetus-specific risk calculation.

Although the effectiveness of screening by measurement of *f* β hCG appeared to improve between 11 and 13 weeks, the effectiveness of screening by measurement of nuchal translucency or PAPP-A declined over this interval, so that screening at 11 weeks resulted in better detection rates overall. Other screening programs that use first-trimester markers, such as integrated or sequential screening, will also be subject to degradation in performance if the first-trimester component is delayed until 13 weeks. Estimates of risk based on gestational age-specific measurements will be more accurate than estimates based on measurements taken during the period from 11 through 13 weeks as a whole.

Ultrasonography for the measurement of nuchal translucency can be a difficult technique to perform consistently well, as evidenced by the 7 percent rate of failed or suboptimal imaging in our study. A recent U.S. study suggested a rate of failure to obtain an image of only 0.5 percent, but no data were provided on image quality.⁵ However, the detection rate of Down's syndrome by measurement of nuchal translucency appeared lower than in the present study (79 percent, at a 5 percent false positive rate).⁵ This suggests that quality assurance, as performed by us, may contribute to improved screening performance.

Second-trimester quadruple screening had a higher false positive rate than first-trimester combined screening performed at 11 or 12 weeks. The estimated performance based on week-specific measurements indicated an advantage of combined screening over quadruple screening if the first-trimester measurements are obtained at 11 weeks, but not if they are obtained later.

In our study, the first-trimester results were not released until the completion of second-trimester screening so as to allow an unbiased comparison of the two approaches. Since fetuses with septated cystic hygroma are at particularly high risk for fetal

aneuploidy, patients with this finding were immediately informed and offered chorionic villus sampling, and they were not included in our calculation of risks.¹⁶ Thus, our estimates of screening performance apply only to pregnancies without cystic hygromas.

Measurement of a combination of markers in both the first and the second trimesters provides the best screening performance. We studied the performance of two types of integrated screening (involving measurement of markers at different gestational ages, but provision of a single result after all testing is complete)⁸: the fully integrated model, which incorporates first-trimester nuchal-translucency measurements, and the serum integrated model, which does not. A single prospective nested case-control study from Europe found Down's syndrome detection rates of 94 percent for fully integrated screening and 87 percent for serum integrated screening, at a 5 percent false positive rate.^{7,17} In the current study, fully integrated screening performed significantly better than either first-trimester combined screening or second-trimester quadruple screening alone. Serum integrated screening performed similarly to first-trimester combined screening and may be a useful alternative in situations in which staff appropriately trained in assessing nuchal translucency are not available. The differences between screening tests were less apparent if the false positive rate was set at 5 percent (as has been commonly adopted) rather than 1 percent, because the detection rates of all the tests are relatively high.

A major disadvantage of integrated screening is that it precludes the performance of chorionic villus sampling for early definitive diagnosis. With independent sequential screening, first-trimester combined-screening results are provided immediately, and women with positive results may choose to undergo chorionic villus sampling. Women with negative results return for quadruple screening, the results of which are interpreted without reference to the first-trimester results. Our results indicate a high false positive rate (11 percent, for a 94 percent detection rate) and reduced accuracy with such a strategy and thus suggest that it should not be used.

Stepwise sequential screening, in contrast, keeps the false positive rate low and provides early results to women with a positive test, but it combines the results of both the first-trimester and the second-trimester measurements into a final second-trimes-

ter risk assessment. With first-trimester combined screening at 11 weeks, and a false positive rate of each component set at 2.5 percent, stepwise sequential screening resulted in a high detection rate of Down's syndrome, similar to that obtained by fully integrated screening, although with a slightly higher false positive rate. The sequential approach described here is simply one example of sequential testing. (Setting different false positive rates would result in different yields.) Further research is needed to determine the most effective method of sequential screening and to compare it with other screening programs.

In conclusion, when there is appropriate quality control for measurement of nuchal translucency, first-trimester combined screening is a powerful tool for the detection of Down's syndrome. Stepwise sequential screening and fully integrated screening are both associated with high detection rates and acceptable false positive rates; the advantage of earlier diagnosis associated with sequential screening must be weighed against the lower false

positive rate obtained with integrated screening. Consideration of the costs associated with different strategies and of patient preferences will help guide the choice between these approaches.

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Drs. Canick and Wald hold international and U.S. patents for unconjugated estriol as a marker in prenatal screening for Down's syndrome (for example, U.S. patents 5506150 and 5605843, issued on April 9, 1996, and February 25, 1997, respectively). Dr. Nyberg reports having received lecture fees from GeneCare. Dr. Timor-Tritsch reports having received lecture fees from General Electric Medical Ultrasound and having received ultrasound equipment support from Philips Ultrasound. Dr. Bianchi reports holding equity ownership in, and receiving grant support from, Living Microsystems, and also reports having received lecture fees from Ross Products. Dr. Lambert-Messerlian reports having served as a consultant to Diagnostic Systems Laboratories. Dr. Wald holds patents for the integrated screening test for Down's syndrome using first- and second-trimester markers together as a single test (integrated screening) (for example, U.S. patent 6573103, issued on June 3, 2003). Dr. Wald is also a director of Logical Medical Systems, which makes Alpha, the interpretative software used for the Down's syndrome risk calculation in FASTER, and he is a director of Intema, which licenses the integrated screening test. Dr. Canick reports having served as a consultant to, and having received grant support from, Diagnostic Systems Laboratories.

APPENDIX

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