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INTEGRATED SCREENING FOR DOWN'S SYNDROME BASED ON TESTS PERFORMED DURING THE FIRST AND SECOND TRIMESTERS

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ABSTRACT

Background Both first-trimester screening and second-trimester screening for Down's syndrome are effective means of selecting women for chorionic-villus sampling or amniocentesis, but there is uncertainty about which screening method should be used in practice. We propose a new screening method in which measurements obtained during both trimesters are integrated to provide a single estimate of a woman's risk of having a pregnancy affected by Down's syndrome.

Methods We used data from published studies of various screening methods employed during the first and second trimesters. The first-trimester screening consisted of measurement of serum pregnancy-associated plasma protein A in 77 pregnancies affected by Down's syndrome and 383 unaffected pregnancies and measurements of nuchal translucency obtained by ultrasonography in 326 affected and 95,476 unaffected pregnancies. The second-trimester tests were various combinations of measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in 77 affected and 385 unaffected pregnancies.

Results When we used a risk of 1 in 120 or greater as the cutoff to define a positive result on the integrated screening test, the rate of detection of Down's syndrome was 85 percent, with a false positive rate of 0.9 percent. To achieve the same rate of detection, current screening tests would have higher false positive rates (5 to 22 percent). If the integrated test were to replace the triple test (measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin), currently used with a 5 percent false positive rate, for screening during the second trimester, the detection rate would be higher (85 percent vs. 69 percent), with a reduction of four fifths in the number of invasive diagnostic procedures and consequent losses of normal fetuses.

Conclusions The integrated test detects more cases of Down's syndrome with a much lower false positive rate than the best currently available test. (N Engl J Med 1999;341:461-7.)

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THE general approach to prenatal screening for Down's syndrome is to estimate a woman's risk of having an affected pregnancy on the basis of factors such as maternal age, serum concentrations of various analytes, and ultrasound measurements that have been found to be associated with Down's syndrome and are frequently referred to as screening markers for the disorder. Women with a risk above a specified level (e.g., a risk of ≥ 1 in 250) are classified as positive on screening. These women are then offered a diagnostic test — either amniocentesis or chorionic-villus sampling.

Screening for Down's syndrome in the second trimester of pregnancy, based on the concentrations of various markers in serum and maternal age, has become widely used in the past decade.^{1,2} Down's syndrome is associated with low maternal serum alpha-fetoprotein and unconjugated estriol concentrations and high maternal serum human chorionic gonadotropin and inhibin A concentrations. Measurements of the first three markers, in addition to age, constitute the widely used triple test; measurements of all four (with age) make up the quadruple test.^{2,3} In the first trimester, Down's syndrome is associated with high values for fetal nuchal translucency (measured by ultrasonography), high maternal serum concentrations of the free beta subunit of human chorionic gonadotropin, and low serum concentrations of pregnancy-associated plasma protein A. Nuchal translucency has been used either alone⁴ or in combination with the two serum markers (the combined test) in another screening protocol.⁵ Although the reliability of screening based on the serum markers is high, there is uncertainty about the reliability of the meas-

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urement of nuchal translucency; this uncertainty has led to debate about whether testing during the first trimester or the second is preferable.⁶⁻¹¹

With the current tests, 5 percent or more of screened women need to undergo amniocentesis in order for 60 to 80 percent of fetuses with Down's syndrome to be detected. Most women with positive screening tests have unaffected pregnancies. The false positive results, however, cause considerable anxiety, and about 0.9 in 100 women who undergo amniocentesis during the second trimester and 1.4 in 100 who undergo chorionic-villus sampling during the first trimester have miscarriages.² A screening test that had a rate of detection similar to those of the current tests but a markedly reduced rate of false positive results — thereby reducing the need for invasive diagnostic procedures — would be of great benefit. We evaluated a new screening method, which we call the "integrated test," that is designed to achieve this goal by integrating measurements of first- and second-trimester markers into a single test.

METHODS

We estimated the performance of prenatal screening for Down's syndrome on the basis of maternal age combined with published data on the distribution of several first- and second-trimester markers in pregnancies affected by and those not affected by Down's syndrome, as confirmed subsequently by chorionic-villus sampling, amniocentesis, or postnatal assessment. The estimates of the performance of first-trimester screening (at 10 to 13 weeks) were based on measurements of nuchal translucency in 326 fetuses affected by Down's syndrome^{4,5} and 95,476 unaffected fetuses^{5,12} and on measurements of serum pregnancy-associated plasma protein A and the free beta subunit of human chorionic gonadotropin in 77 affected pregnancies and 383 unaffected pregnancies.¹³ These estimates were corrected for the overestimation of the rate of detection by the measurement of nuchal translucency⁴ that resulted from the termination of pregnancies that would otherwise have ended in miscarriage.⁸ We did not, however, take into account the possibility that fetuses with Down's syndrome that is detected by screening are more likely to be aborted spontaneously than unaffected fetuses.

The estimates of the performance of second-trimester screening (at 14 to 22 weeks) were based on measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in a different study of 77 pregnancies affected by Down's syndrome and 385 unaffected pregnancies; these measurements were made on serum samples stored before first-trimester serum or ultrasound screening was introduced.¹⁴⁻¹⁶ The estimates of screening performance were modified on the basis of data on the improvement in determining the length of gestation with ultrasonography.¹⁷

The integrated test combines markers measured during both of the first two trimesters. The serum free beta subunit of human chorionic gonadotropin during the first trimester was excluded as a marker of Down's syndrome because of its expected high degree of correlation with serum total human chorionic gonadotropin during the second trimester. The results were similar when values for serum free beta subunit of human chorionic gonadotropin were included and those for serum total human chorionic gonadotropin were excluded.

All markers were expressed as multiples of the normal median for women with unaffected pregnancies at a given gestational age. A multivariate Gaussian model was fitted to the data on first-trimester and second-trimester markers in the affected and unaffected pregnancies, and the likelihood ratio was calculated. This ratio

TABLE 1. RATES OF DETECTION OF DOWN'S SYNDROME AT SPECIFIED FALSE POSITIVE RATES AND FALSE POSITIVE RATES AT SPECIFIED DETECTION RATES, ACCORDING TO THE TYPE OF SCREENING TEST.*

VARIABLE	SECOND TRIMESTER (14–22 WK)			FIRST TRIMESTER (10–13 WK)	FIRST AND SECOND TRIMESTERS
	DOUBLE TEST†	TRIPLE TEST‡	QUADRUPLE TEST§	COMBINED TEST¶	INTEGRATED TEST
	detection rate (%)				
False positive rate					
1%	35	46	54	72	85
3%	50	62	69	81	92
5%	59	69	76	85	94
7%	65	74	81	88	96
	false positive rate (%)				
Detection rate					
60%	5.4	2.7	1.6	0.2	0.03
70%	9.4	5.2	3.2	0.8	0.12
80%	16.5	10.2	6.6	2.6	0.45
90%	30.5	21.5	15.2	9.9	2.10

*All tests included maternal age and gestational age, estimated by ultrasonography.

†The double test includes measurements of serum alpha-fetoprotein and human chorionic gonadotropin.

‡The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin.

§The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A.

¶The combined test includes measurements of serum pregnancy-associated plasma protein A, the free beta subunit of human chorionic gonadotropin, and nuchal translucency (by ultrasonography).

||The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

was used to adjust the risk of having a pregnancy at a particular maternal age that, in the absence of screening, would result in a live-born infant with Down's syndrome. In Bayesian terms, the likelihood ratio was multiplied by the prior odds to calculate the posterior odds. The detailed methods of the calculation of multiples of the normal median and estimation of the statistical parameters used in the multivariate Gaussian distributions have been described previously,^{1,17} and the method of risk estimation has been empirically validated.¹⁸ The statistical parameters required to calculate the performance of the screening test have been reported previously.¹²⁻¹⁶ They include the coefficients for the correlation between markers within the same trimester, which are estimated separately in affected and normal pregnancies. Values for nuchal translucency and serum pregnancy-associated plasma protein A were not correlated with the second-trimester markers,^{19,20} a finding consistent with the absence of an association in the first trimester between the values for nuchal translucency and serum free beta subunit of human chorionic gonadotropin²¹⁻²³ and between the values for serum pregnancy-associated plasma protein A and those for other serum markers.¹³

We compared the performance of the integrated test in screening for Down's syndrome with that of the first-trimester and second-trimester screening tests by examining the detection rates for specified false positive rates and the false positive rates for specified detection rates of each test. The detection rate is the

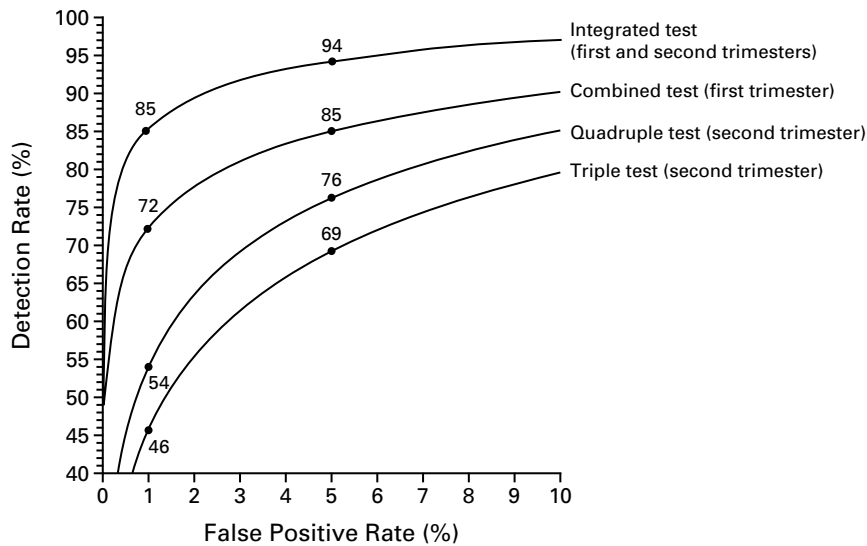


Figure 1. Rates of Detection of Down's Syndrome and False Positive Rates for Various Screening Tests.

The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin in the second trimester. The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester. The combined test includes measurements of serum pregnancy-associated plasma protein A, free beta subunit of human chorionic gonadotropin, and nuchal translucency in the first trimester. The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

proportion of affected pregnancies with a positive test result, also called sensitivity; the false positive rate is the proportion of unaffected pregnancies with a positive result, equivalent to 1 minus the specificity. The methods used to estimate the risk of Down's syndrome and the performance of screening have been described in detail elsewhere.^{24,25} Estimates of the number of unaffected fetuses that were lost as a result of amniocentesis or chorionic-villus sampling were obtained from a review of randomized trials (which produced values of 0.9 percent and 1.4 percent, respectively).²

RESULTS

The performance of second-trimester screening alone, first-trimester screening alone, and the integrated test that incorporated measurements from both trimesters is shown in Table 1. At a 5 percent false positive rate, the estimated rate of detection with the integrated test was 94 percent, greater than that with the most effective second-trimester test (quadruple test, 76 percent) or first-trimester test (combined test, 85 percent). At a 1 percent false positive rate, the estimated rate of detection for the integrated test was 85 percent (54 percent and 72 percent for the quadruple and combined tests, respectively). The integrated test detected at least as many affected pregnancies at a 1 percent false positive rate as either first-trimester or second-trimester screening alone at a 5 percent false positive rate. The extent to which the integrated test is

TABLE 2. RATES OF DETECTION OF DOWN'S SYNDROME AT SPECIFIED FALSE POSITIVE RATES AND FALSE POSITIVE RATES AT SPECIFIED DETECTION RATES FOR THE INTEGRATED TEST AND THREE VARIATIONS.*

VARIABLE	INTEGRATED TEST†	VARIATIONS OF INTEGRATED TEST		
		WITHOUT SERUM INHIBIN A	WITHOUT NUCHAL TRANSLUCENCY	WITHOUT SERUM INHIBIN A AND NUCHAL TRANSLUCENCY
detection rate (%)				
False positive rate				
1%	85	82	66	60
3%	92	90	79	74
5%	94	92	85	80
7%	96	94	88	84
false positive rate (%)				
Detection rate				
60%	0.03	0.05	0.6	1.0
70%	0.12	0.20	1.4	2.2
80%	0.45	0.73	3.2	5.0
90%	2.10	3.3	8.8	12.6

*All tests included maternal age and gestational age, estimated by ultrasonography.

†The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

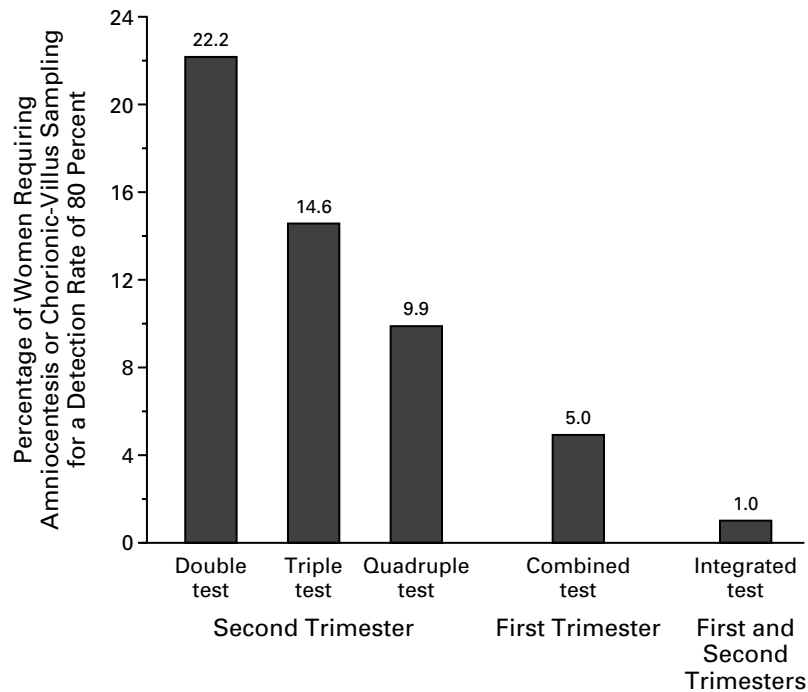


Figure 2. Percentage of Screened Women Who Would Need to Undergo Amniocentesis or Chorionic-Villous Sampling in Order for 80 Percent of the Pregnancies Affected by Down's Syndrome to Be Detected, According to Type of Screening Test.

The double test includes measurements of serum alpha-fetoprotein and human chorionic gonadotropin in the second trimester. The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin in the second trimester. The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A. The combined test includes measurements of serum pregnancy-associated plasma protein A, the free beta subunit of human chorionic gonadotropin, and nuchal translucency in the first trimester. The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

better than the best first-trimester or second-trimester test (the combined test and the quadruple test, respectively) and the triple test is shown in Figure 1. At a 1 percent false positive rate, the rate of detection was 85 percent, as compared with 46 percent for the triple test. The steep early rise in the rate of detection with the integrated test reflects both high rates of detection and low rates of false positive results.

Table 2 shows the performance of the integrated test without the measurement of nuchal translucency (because some centers may not have experience with this procedure) and serum inhibin A values (because some centers may not use this marker). Despite the loss of performance, a comparison of the data in Tables 1 and 2 shows that if the measurement of either nuchal translucency or serum inhibin A were omitted it would still be of benefit to integrate first- and second-trimester markers into a single screening test. If it is not possible to measure nuchal

translucency, ultrasonography should still be used to date the pregnancy — by measuring, for example, crown-rump length — in order to maximize the performance of the integrated screening test (since the serum concentration of pregnancy-associated plasma protein A increases by about 35 percent per week during the first trimester).

The percentages of screened women who would require an invasive diagnostic procedure and karyotypic analysis in order for 80 percent of pregnancies affected by Down's syndrome to be detected are shown in Figure 2, according to the screening test used. The percentage is the positive rate, including both true and false positives, in the numerator and all pregnancies screened in the denominator. The percentages decreased from 22.2 percent with the double test to 1 percent with the integrated test. For each test, Table 3 shows the risk cutoff needed to achieve a detection rate of 85 percent, and the estimated odds of an affected infant's being born for

TABLE 3. ODDS OF DELIVERING AN INFANT WITH DOWN'S SYNDROME AT TERM AMONG WOMEN WITH POSITIVE TESTS AND NUMBERS OF PROCEDURE-RELATED LOSSES OF UNAFFECTED FETUSES ACCORDING TO THE SCREENING TEST USED.*

SCREENING TEST	RISK CUTOFF	FALSE POSITIVE RATE (%)	ODDS OF DELIVERING AN INFANT WITH DOWN'S SYNDROME AT TERM IF TEST IS POSITIVE	NO. OF PROCEDURE-RELATED LOSSES OF UNAFFECTED FETUSES	
				FOR EVERY 100 PREGNANCIES FOUND BY THE TEST TO BE AFFECTED BY DOWN'S SYNDROME	FOR EVERY 100,000 WOMEN SCREENED
First and second trimesters					
Integrated test†	1 in 120	0.9	1:9	8	8
Variations of integrated test					
Without serum inhibin A	1 in 190	1.5	1:13	12	13
Without nuchal translucency	1 in 410	5.2	1:47	42	47
Without nuchal translucency and serum inhibin A	1 in 560	7.7	1:70	63	69
First trimester					
Combined test‡	1 in 540	4.9	1:45	61	66
Second trimester					
Quadruple test§	1 in 630	9.8	1:88	79	88
Triple test¶	1 in 830	14.5	1:131	118	130
Double test	1 in 1040	22.1	1:200	180	199

*All tests included maternal age and gestational age, estimated by ultrasonography.

†The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

‡The combined test includes measurements of serum pregnancy-associated plasma protein A, free beta subunit of human chorionic gonadotropin, and nuchal translucency.

§The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A.

¶The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin.

||The double test includes measurements of serum alpha-fetoprotein and human chorionic gonadotropin.

women with a positive result. The odds (the ratio of the number affected to the number unaffected) for the integrated test is 1:9, much higher than that for the first-trimester combined test (1:45) or for the best second-trimester test (1:88). There was also a substantial reduction in the number of unaffected fetuses lost as a result of amniocentesis or chorionic-villus sampling; 8 unaffected fetuses would have been lost per 100 pregnancies found by the integrated test to be affected, as compared with 61 per 100 and 79 per 100 with the best first-trimester and second-trimester tests, respectively.

To achieve a detection rate of 85 percent with the integrated test, the risk cutoff would be set at 1 in 120 (Table 3), a level at which the false positive rate would be 0.9 percent. Using the best test in the second trimester (the quadruple test) or in the first trimester (the combined test), a much lower cutoff (1 in 630 or 1 in 540, respectively) would be needed

to achieve a similar detection rate, and the false positive rates would be much higher (9.8 percent and 4.9 percent). At a 5 percent false positive rate, the detection rate of the integrated test would be 94 percent, but the risk cutoff required to achieve this rate (1 in 940) may be regarded as too low to be clinically acceptable.

The reduction in the false positive rate with the integrated test is particularly evident for older women (Table 4). Among women 35 years of age or older, the false positive rate of the integrated test was only 3.3 percent (with a risk cutoff of 1 in 120), as compared with 19 percent for the triple test (with the usual risk cutoff of 1 in 250), with a gain in detection (92 percent vs. 88 percent). For every 100,000 women 35 years of age or older who were screened, only 30 unaffected fetuses would be lost because of diagnostic procedures with the integrated test, as compared with 171 with the triple test.

TABLE 4. RATES OF DETECTION OF DOWN'S SYNDROME AND FALSE POSITIVE RATES ACCORDING TO MATERNAL AGE AND SCREENING TEST.*

MATERNAL AGE	DOUBLE TEST†		TRIPLE TEST‡		QUADRUPLE TEST§		INTEGRATED TEST¶	
	DETECTION RATE	FALSE POSITIVE RATE	DETECTION RATE	FALSE POSITIVE RATE	DETECTION RATE	FALSE POSITIVE RATE	DETECTION RATE	FALSE POSITIVE RATE
	percent							
15–34 yr	46	4.0	58	3.7	69	4.1	81	0.7
≥35 yr	86	24	88	19	91	17	92	3.3
≥15 yr	61	5.6	69	4.9	77	5.2	85	0.9

*All tests included maternal age and gestational age, estimated by ultrasonography. The risk cutoff levels were as follows: double and triple tests, 1 in 250; quadruple test, 1 in 300; and integrated test, 1 in 120.

†The double test includes measurements of serum alpha-fetoprotein and human chorionic gonadotropin.

‡The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin.

§The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A.

¶The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A in the second trimester.

DISCUSSION

Prenatal screening for Down's syndrome is usually performed by testing of maternal serum when the woman is between 14 and 22 weeks pregnant. Depending on the serum markers used, this method yields detection rates of 59 to 76 percent at a false positive rate of 5 percent; the combination of serum testing and ultrasonography at 10 to 13 weeks yields a detection rate of about 85 percent with a false positive rate of 5 percent (Table 1). The integrated test can achieve a similarly high detection rate but at a much lower false positive rate (0.9 percent). Consequently the need for amniocentesis or chorionic-villus sampling is reduced by four fifths, with a similar reduction in the loss of unaffected fetuses. The fact that the results of screening would not be available for an additional few weeks may be seen as a disadvantage, but any such disadvantage is outweighed by the substantial increase in safety.

The estimates presented here are based on direct observations in large studies of first-trimester and second-trimester screening and validated statistical methods. Among women in established screening programs, who were grouped according to their level of risk as determined by the triple or quadruple test, the predicted risk in each group was close to the observed prevalence of Down's syndrome.^{18,26,27} Because the same method of risk estimation was used to calculate the screening performance of the integrated test and because it was based on similar numbers of affected pregnancies, there is no reason to believe that our estimates of performance for the integrated test are any less valid than those for the tests currently available.

The integrated test takes advantage of the fact that different screening markers discriminate between the presence and absence of Down's syndrome at different times in pregnancy. For example, measurements of serum pregnancy-associated plasma protein A are useful only before 14 weeks, and those of inhibin A only after 14 weeks.² The low false positive rate of the integrated test would not be possible if first-trimester and second-trimester screenings were conducted independently; also, confusion would arise from giving women different estimates of risk at different stages of pregnancy. We examined whether women with extreme values for positive first-trimester screening results (based on increased nuchal translucency and older maternal age, with or without low values for serum pregnancy-associated plasma protein A) had so high a risk that their fetuses were affected by Down's syndrome that a diagnostic test should be offered without waiting to carry out tests in the second trimester. This was not the case; even a high initial risk estimate may be substantially reduced and the screening result may become negative with the integrated test.

An indication of the effectiveness of screening and prenatal diagnosis with the integrated test is shown in Tables 1 and 3. For example, at an 85 percent rate of detection, the use of the integrated test instead of the triple test would obviate the need for amniocentesis in 13.6 of every 100 women with unaffected pregnancies (a false positive rate of 14.5 minus a rate of 0.9). The triple test is usually used with a 5 percent false positive rate; the integrated test with a cutoff of 1 in 120 would detect more affected pregnan-

cies (85 percent vs. 59 percent) and would have only about one fifth the proportion of false positive results (0.9 percent vs. 5 percent).

The integrated test, through the use of information collected during both trimesters, makes screening and prenatal diagnosis much safer and more effective than other methods currently available. There is a further advantage over first-trimester screening alone in that amniocentesis, which is somewhat more accurate and safer than chorionic-villus sampling,² would be the diagnostic test used. Implementation of the integrated test would require that women seek prenatal care between 10 and 13 weeks of gestation and return within 5 weeks.

In the United States, the use of the integrated test instead of the triple test for prenatal screening for Down's syndrome would detect about 800 more affected pregnancies and save about 1400 unaffected fetuses from being lost as a result of amniocentesis or chorionic-villus sampling each year if all women identified as being at high risk underwent either of these diagnostic tests (the numbers would be proportionately lower if fewer women elected to be tested). In England and Wales, the corresponding numbers would be about 160 and 280.

REFERENCES

1. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;297:883-7. [Erratum, *BMJ* 1988;297:1029.]
2. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *J Med Screen* 1997;4:181-246. [Erratum, *J Med Screen* 1998;5:110, 166.]
3. Haddow JE, Palomaki GE, Knight GJ, Foster DL, Neveux LM. Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A. *J Med Screen* 1998;5:115-9.
4. Pandya PP, Snijders RJM, Johnson SP, De Lourdes Brizot M, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. *Br J Obstet Gynaecol* 1995;102:957-62.
5. Wald NJ, Hackshaw A. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn* 1997;17:821-9.
6. Nielson JP. Assessment of fetal nuchal translucency test for Down's syndrome. *Lancet* 1997;350:754-5.
7. Nicolaides KH, Sebire NJ, Snijders RJM, Johnson S. Down's syndrome screening in the UK. *Lancet* 1996;347:906-7.
8. Hackshaw AK, Wald NJ, Haddow JE. Down's syndrome screening with nuchal translucency. *Lancet* 1996;348:1740.
9. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. *Lancet* 1998;352:343-6.
10. Haddow JE. Antenatal screening for Down's syndrome: where are we and where next? *Lancet* 1998;352:336-7.
11. Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. Screening of maternal serum for fetal Down's syndrome in the first trimester. *N Engl J Med* 1998;338:955-61.
12. Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998;18:519-23.
13. Wald NJ, George L, Smith D, Densem JW, Petterson K. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. *Br J Obstet Gynaecol* 1996;103:407-12.
14. Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. *Prenat Diagn* 1996;16:143-53. [Erratum, *Prenat Diagn* 1997;17:285-90.]
15. Wald NJ, Densem JW, George L, et al. Inhibin-A in Down's syndrome pregnancies: revised estimate of standard deviation. *Prenat Diagn* 1997;17:285-90.
16. Wald NJ, Densem JW, Smith D, Klee GG. Four marker serum screening for Down's syndrome. *Prenat Diagn* 1994;14:707-16.
17. Wald NJ, Cuckle HS, Densem JW, Kennard A, Smith D. Maternal serum screening for Down's syndrome: the effect of routine ultrasound scan determination of gestational age and adjustment for maternal weight. *Br J Obstet Gynaecol* 1992;99:144-9.
18. Wald NJ, Hackshaw AK, Huttly W, Kennard A. Empirical validation of risk screening for Down's syndrome. *J Med Screen* 1996;3:185-7.
19. De Biasio P, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. First trimester screening for Down's syndrome using nuchal translucency measurement with free β -hCG and PAPP-A between 10 and 13 weeks of pregnancy: the combined test. *Prenat Diagn* 1999;19:360-3.
20. Lam YH, Lee CP, Sin SY, Wong HS, Tang R, Tang MHY. First trimester nuchal translucency and second trimester serum screening for fetal Down's syndrome. *Ultrasound Obstet Gynecol* 1998;12:Suppl 1:A62. abstract.
21. Brizot ML, Snijders RJM, Bersinger NA, Kuhn P, Nicolaides KH. Maternal serum pregnancy-associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstet Gynecol* 1994;6:918-22.
22. Brizot ML, Snijders RJM, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. *Br J Obstet Gynaecol* 1995;102:127-32.
23. Noble PL, Ab raha HD, Snijders RJM, Sherwood R, Nicolaides KH. Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free β -hCG and fetal nuchal translucency thickness. *Ultrasound Obstet Gynecol* 1995;6:390-5.
24. Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol* 1987;94:387-402.
25. Royston P, Thompson SG. Model-based screening by risk with application to Down's syndrome. *Stat Med* 1992;11:257-68.
26. Canick JA, Rish S. The accuracy of assigned risks in maternal serum screening. *Prenat Diagn* 1998;18:413-5.
27. Onda T, Tanaka T, Takeda O, et al. Agreement between predicted risk and prevalence of Down syndrome in second-trimester triple-marker screening in Japan. *Prenat Diagn* 1998;18:956-8.

CORRECTION

Integrated Screening for Down's Syndrome

To the Editor: Wald et al. (Aug. 12 issue)¹ describe a protocol for screening for Down's syndrome that is based on tests performed during both the first and second trimesters of pregnancy. However, their estimates are based on data from multiple studies, some rather small. Most important, they fail to consider the advantages of a diagnosis in the first trimester.

Chorionic-villus sampling in the first trimester and amniocentesis in the second trimester are equally effective and safe procedures with similar rates of procedure-induced loss at experienced centers.^{2,3} Total loss rates after chorionic-villus sampling are higher because of the higher rate of spontaneous abortion at the earlier gestational age when sampling is performed. The advantages of diagnostic testing in the first trimester are the inherently greater privacy at the time of the diagnosis and the greater safety of termination of the pregnancy (when desired) if the results are abnormal, as well as the earlier reassurance if the results are normal. The option of making the diagnosis in the first trimester is eliminated by combined first- and second-trimester screening.

A woman's approach to prenatal diagnosis is a personal one. Screening tests need to be sufficiently sensitive and specific to provide meaningful guidance, but a protocol that maximizes the results by delaying the diagnosis will not be satisfactory to most women. In a recent study of a first-trimester screening protocol involving the use of nuchal translucency and biochemical markers, the sensitivity for the detection of Down's syndrome was 89 percent, with a 5 percent false positive rate.⁴ The approach of informing women of the risks on the basis of the results of screening in the first trimester, although leading to a slightly higher frequency of invasive testing, allows a couple to choose immediate testing by chorionic-villus sampling or to await additional, noninvasive evaluation in the second trimester.

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References

1. Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N Engl J Med* 1999;341:461-467.
2. Canadian Collaborative CVS-Amniocentesis Clinical Trial Group. Multicentre randomised clinical trial of chorion villus sampling and amniocentesis. *Lancet* 1989;1:1-6.
3. Rhoads GG, Jackson LG, Schlesselman SE, et al. The safety and

efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Engl J Med* 1989;320:609-617.

4. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999;13:231-237.

To the Editor: As practicing clinicians, we question not only the delay necessitated by combining first- and second-trimester screening for Down's syndrome, but also the method of screening. The proposed first-trimester tests¹ have been criticized both by many biochemistry laboratories in the United Kingdom² and by a well-known ultrasound screening department,³ and both groups consider the estimated first-trimester detection rates optimistic.

There is little reported evidence on sequential changes in maternal serum markers. A combination of first- and second-trimester results must be based on the assumption that markers that are abnormal in the first trimester will also be abnormal in the second trimester; conversely, normal markers will be normal in both trimesters. We studied 187 normal pregnant women in whom the duration of gestation was determined by ultrasonography at four-week intervals starting between day 45 and day 76 of gestation and ending between day 128 and day 180. At each visit, we measured serum pregnancy-associated plasma protein A, alpha-fetoprotein, unconjugated estriol, and total and free beta human chorionic gonadotropin. Despite adequate correction for gestational age on a population basis, we found that the multiple of the median value sometimes changed by as much as 1 and that in any one woman, the multiple of the median value could increase, decrease, increase and decrease, or remain roughly constant during gestation. Therefore, risk estimates calculated at different times varied; depending on the combinations of tests used (with two or three analytes), the discordance rate ranged from 12.3 percent to 25.7 percent. The effect of combining two biochemical measurements and one ultrasound measurement in the first trimester with four biochemical measurements in the second trimester cannot be estimated.

Consequently, we believe it is impossible to design a multistage test with the use of population-based values from independent assessments in different trimesters. Furthermore, it is impossible to estimate the benefits of such a test — that is, whether it will identify more cases of Down's syndrome or will simply increase the false positive rate with no actual diagnostic gain.

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References

1. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn* 1997;17:821-829.
2. Reynolds TM, Dunstan F, Nix B, et al. Response to Wald, N.J., Hackshaw, A.K. (1997). *Prenat Diagn* 1998;18:511-515.
3. Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998;18:519-521.

To the Editor: The editorial by Copel and Bahado-Singh¹ on prenatal screening for Down's syndrome raises troubling questions about the often-overlooked distinction between investigational tools and clinical practice. In assessing the role of sequential first- and second-trimester screening for Down's syndrome, they endorse clinical implementation of a method of screening that should still be considered investigational.

First-trimester screening for Down's syndrome with the use of measurements of nuchal translucency has been evaluated by several groups outside the United States. The rates of detection of Down's syndrome ranged from 29 percent to 91 percent.² The results of first-trimester biochemical screening have been much more consistent with rates of detection ranging from 55 percent to 63 percent.³ However, the combination of first-trimester biochemical screening and measurement of nuchal translucency has never been subjected to rigorous evaluation in a single large group of pregnant women. After evaluating all the available data, the Committee on Genetics of the American College of Obstetricians and Gynecologists recently concluded that first-trimester measurement of nuchal translucency, with or without serum testing, remains an investigational procedure and is not recommended for routine clinical use.⁴

The editorial is confusing in that the authors initially state that the best way to combine screening tests should be determined by measurements in a single group of women, but they later say that they currently recommend both first- and second-trimester screening.¹ What data are Copel and Bahado-Singh using to justify the implementation of such a combined method of screening? What criteria are they using to define an increased risk of Down's syndrome in the first trimester, and from what published data are such risks derived?

Until further data are available, we endorse the opinion of the American College of Obstetricians and Gynecologists and strongly caution physicians and patients that combined first-trimester screening should not be performed or acted on in clinical practice apart from research protocols. The standard of care in the United States for screening for

Down's syndrome continues to be measurements of multiple serum markers in the second trimester, with consideration of maternal age.

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References

1. Copel JA, Bahado-Singh RO. Prenatal screening for Down's syndrome – a search for the family's values. *N Engl J Med* 1999;341:521-522.
2. Stewart TL, Malone FD. First trimester screening for aneuploidy: nuchal translucency sonography. *Semin Perinatol* 1999;23:369-381.
3. Canick JA, Kellner LH. First trimester screening for aneuploidy: serum biochemical markers. *Semin Perinatol* 1999;23:359-368.
4. First-trimester screening for fetal anomalies with nuchal translucency: ACOG committee opinion no. 223. Washington, D.C.: American College of Obstetricians and Gynecologists, October 1999.

The authors reply:

To the Editor: Jenkins and Wapner question the size of the studies used in our analysis. We used three principal data sets, each based on at least 77 pregnancies with Down's syndrome, from a total of about 150,000 pregnancies. Jenkins and Wapner are correct in suggesting that, all other things being equal, offering a screening test for Down's syndrome early in pregnancy is better than offering one later. But all things are not equal. The integrated test is considerably better than first-trimester screening alone. With the same detection rate of 85 percent, there is about an 80 percent reduction in the false positive rate and therefore an 80 percent reduction in the loss of unaffected fetuses even if, as judged by Jenkins and Wapner, chorionic-villus sampling and amniocentesis are equally effective and safe.

Reynolds et al. express an unjustified concern about screening methods. Their criticisms of first-trimester biochemical screening were addressed,¹ and the estimates from other studies of first-trimester biochemical screening were similar to ours.² We used data on nuchal translucency and Down's syndrome that were reported by Nicolaides et al.³ — data that Reynolds et al. cite in their letter and thus accept as authoritative. Other studies indicate that our estimates of the performance of screening in the first trimester, with the combined use of

biochemical measurements and nuchal translucency, are accurate.^{4,5} The integrated test is not based on the assumption that markers that are abnormal in the first trimester will also be abnormal in the second trimester. The strength of the integrated test rests on the fact that this is not the case.

We agree with Malone et al. that the results of screening with measurements of nuchal translucency have been variable, largely because of variations in the accuracy of the measurement.

The potential value of the integrated test in increasing the effectiveness of screening, particularly by reducing the associated anxiety and fetal loss, is large and should not be lost sight of in discussions about the different components of the test.

Unfortunately, there was an editorial error in our article. The error concerns Figure 2, which shows the percentage of women screened who would need to undergo amniocentesis or chorionic-villus sampling in order for 85 percent of the pregnancies affected by Down's syndrome to be detected. In the figure as printed, 80 percent appears instead of 85 percent. The error was made on the vertical axis of the figure, in the figure title, and in the 10th line in the right-hand column on page 464.

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References

1. Wald NJ, Hackshaw AK. Authors' reply. *Prenat Diagn* 1998;18:515-519.
2. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *J Med Screen* 1997;4:181-246. [Erratum, *J Med Screen* 1998;5:110, 166.]
3. Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998;18:519-521.
4. de Graaf IM, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, van Lith JM. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenat Diagn* 1999;19:458-462.
5. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free β -chorionic gonadotropin and pregnancy-associated plasma protein-A. *Obstet Gynecol* 1999;13:231-237.

To the Editor: We regret any confusion caused by our editorial. The recommendations that we made were those that we might currently

provide to a family member who was pregnant, using an empirical approach based on careful appraisal of the available data. We believe that we were clear in our call for a study with a better design than that of Wald et al. Two studies are currently being undertaken nationally to investigate the very questions raised by Malone et al. Nevertheless, the use of measurements of nuchal translucency to screen for Down's syndrome is entering the clinical realm in the United States as increasing numbers of women undergo fetal ultrasonography in the first trimester. This use is based on a large body of evidence from studies involving close to 100,000 women and over 300 cases of Down's syndrome.¹ A legitimate question is whether the centralization, rigorous standards, and ongoing quality control required to yield the high screening performance reported by Nicolaides and colleagues can be duplicated in the United States.

The question at hand is what to do during the two to four years it will take to confirm or refute the existing reports. We believe that with the reported high correlation between abnormal nuchal translucency and Down's syndrome and the preponderance of studies supporting this claim, it is legitimate to discuss this test and to offer it to interested couples.

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References

1. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multi-centre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. *Lancet* 1998;352:343-346.