OBSTETRICS

Meta-analysis of first trimester Down syndrome screening studies: free β-human chorionic gonadotropin significantly outperforms intact human chorionic gonadotropin in a multimarker protocol

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OBJECTIVE: The purpose of this study was to compare free β and intact human chorionic gonadotropin in first trimester screening with pregnancy-associated plasma protein-A and nuchal translucency.

STUDY DESIGN: A Monte Carlo simulation trial was conducted based on a literature review of the PUBMED database (1966 to November 2005).

RESULTS: In younger patients (<35 years), detection of Down syndrome increased by 4, 5, 6, and 7 percentage points when free β was added to pregnancy-associated plasma protein-A and nuchal translucency compared with 0, 0, 2, and 4 percentage points for intact human chorionic gonadotropin at 9-12 weeks' gestation, respectively. In advanced maternal age patients (≥35), inclusion of free β-human chorionic gonadotropin reduced the false-positive rate by 2.5, 3.1, 3.8, and 4.4 percentage points compared with 0.1, 0.3, 1.0, and 2.2 percentage points for intact human chorionic gonadotropin at 9-12 weeks, respectively.

CONCLUSION: The results of our analysis suggest that in a first-trimester Down syndrome screening protocol free β-human chorionic gonadotropin achieves higher sensitivity and lower false-positive results than intact human chorionic gonadotropin . Moreover, intact human chorionic gonadotropin does not add substantially to screening performance until the end of the first trimester.

Key words: Down syndrome screening, first trimester, free β-human chorionic gonadotropin, intact human chorionic gonadotropin

Over the last 30 years, prenatal diagnosis has been performed by rationing diagnostic procedures to the highest risk patients. Initially, the estimation of risk was based solely on maternal age. Although the safety of diagnostic procedures has improved dramatically in these 3 decades, most physicians and patients would prefer that risk assessment be based on a more sophisticated approach beyond "How old are you?" Therefore, more precise estimates of patient specific risk based on clinical testing have evolved allowing patients to make better informed decisions.

First-trimester multiple marker Down syndrome screening that uses a combination of maternal serum free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), and nuchal translucency (NT) has been offered clinically for over 10 years.[1] First-trimester screening, with performance greater than that of second-trimester screening protocols, offers significant advantages, including earlier reassurance for the vast majority of patients and greater privacy and safety for patients who may decide to terminate an affected fetus.[2] There remains significant controversy as to the optimal combination of markers. The medical and economic implications of whichever markers or combinations of markers emerge as the mainstay of care are enormous. We therefore sought to determine the optimal approach between 2 debated and sometimes obfuscated models of how best to screen.

HCG is a heterodimer consisting of an alpha and beta subunit. It is present in maternal serum predominantly as the biologically active intact dimer, but it also exists to a much lesser degree as both the "free α" and "free β" subunits. Most modern hCG assays are actually nonspecific and measure both the intact dimer and the free β subunit of hCG. However, because the intact dimer is present in the maternal serum in a 200-fold molar excess relative to the free β subunit, these assays primarily reflect the intact hCG concentration.

Unfortunately, inconsistency in the terminology used to describe hCG assays (Table 1) has created confusion when comparing first-trimester screening studies. Intact hCG assays are often termed "total β" or simply "β-hCG" assays and some researchers have also described free β-hCG as "β-hCG."[3,4]

Most retrospective reports on biochemistry indicated that although the

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TABLE 1
Terminology to described assays for hCG related biochemical markers

Analyte	Subunit(s) measured	Synonyms or assay names
Intact hCG	α-β dimer	Total β-hCG Total β Total hCG β-βassay β-hCG α-β assay
Free β-hCG	β	Free β (Beta hCG) Free β subunit of hCG Free hCG β
Free α-hCG	α	Free α subunit Free α

free β subunit of hCG was a very effective marker for Down syndrome in the first trimester,[5-7] intact hCG was not.[8-11] As a result, the vast majority of prospective first-trimester screening studies have used the combination of free β-hCG, PAPP-A, and NT. The Serum, Urine, and Ultrasound Screening (SURUSS) study, however, suggested that, although free β-hCG is a better marker than intact hCG, there may be little difference in the overall screening results when included in a multiple marker protocol.[12] Although described as a prospective study with results based on 47,053 singleton pregnancies including 101 with Down syndrome, the SURUSS study is actually based on retrospective analysis of less than 500 samples. Further, we believe that a statistical correction to the model would have demonstrated the advantage of free β-hCG over intact hCG in first-trimester screening.[13] Regardless, the study has created some uncertainty and opened a debate in the United States about which protocol should be used in screening.[14]

We have therefore conducted a meta-analysis based on the considerable body of available evidence to ascertain whether equivalent screening results could indeed be attained if intact hCG was substituted for free β-hCG in first-trimester screening with PAPP-A and NT.

Materials and Methods

A computerized search of English language studies, excluding review studies, from the PUBMED database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) up to November 2005 was performed. The initial search strategy used the following MESH terms: (1) Down syndrome, (2) trisomy, (3) pregnancy trimester first, (4) chorionic gonadotropin, (5) pregnancy-associated plasma protein A and 6. (#1 or #2) and #3 and (#4 or #5). A second search was conducted by using the following phrases instead of MESH terms: (1) "Down syndrome," (2) "trisomy," (3) "first trimester," (4) "chorionic gonadotropin," (5) "pregnancy-associated plasma protein A and 6" (#1 or #2) and #3 and (#4 or #5).

The initial search strategy yielded 154 studies, and the second strategy yielded 197 studies, of which 69 were unique to the second search. The 223 unique studies were reviewed and the data abstracted independently by 2 authors and compared. Differences in interpretation were resolved by consensus. To be included in the meta-analysis, the article had to contain maternal serum first-trimester free β-hCG, intact hCG, or PAPP-A data from Down syndrome pregnancies. In cases in which data included sample sets previously published, parameters were taken from the latest publication. There was no restriction on study design.

The literature search identified a total of 223 studies, of which 63 studies were excluded because they did not contain any new data or analysis of free β-hCG, intact hCG, or PAPP-A. Of the remaining 160 studies, 129 contained data on free β-hCG, 28 contained data on intact hCG, and 129 contained data on PAPP-A.

Of the 129 studies with data on free β-hCG, 25 were excluded because the data were included in other studies, 55 did not include Down syndrome cases, 22 did not supply distribution parameters or appropriate raw data to calculate distribution parameters, leaving 27 for the meta-analysis.[2,7,12,15-38] Of the 28 studies on intact hCG, 3 were excluded because the data were included in other studies, 3 did not include Down syndrome cases, 5 did not supply distribution parameters or appropriate raw data to calculate distribution parameters, leaving 17 for the meta-analysis.[7,11,12,23,24,27,30,33,35,36,39-45] Of the 129 articles on PAPP-A, 22 were excluded because the data were included in other studies, 58 did not include Down syndrome cases, 23 did not supply distribution parameters or appropriate raw data to calculate distribution parameters, leaving 26 for the meta-analysis.[2,7,12,15-26,28-34,37,41,46,47] In total, there were 37 studies included in the meta-analysis.

Distribution parameters for each analyte were recorded from each study. If parameters were not provided but raw data were available, statistical parameters were calculated from the raw data. Studies in which no distribution parameters or raw data were provided were excluded.

The parameters for the multivariate Gaussian distributions consist of a mean and SD for each marker and the correlation coefficients between each pair of markers. All parameters were based on $\log_{10}$ multiples of the median (MoM) values. The mean for each marker in the unaffected distribution was set equal to 0.0 (equivalent to $\log_{10}$ of 1 MoM). The mean for the Down syndrome distribution for each of the markers varies depending on the gestational age. The mean $\log_{10}$ MoM for the Down syndrome distribution was approximated by the log of the median. The overall median MoM value at each gestational age was calculated by determining the median of the observed median MoM values in each study after weighting each study by the number of Down syndrome

cases. The gestational-specific median MoM values were then regressed against gestational age, and the regression formula used to calculate the expected mean $\log_{10}$ MoM.

SDs of the unaffected and Down syndrome distributions for each of the markers were determined by taking a weighted average of the variances (the square of the SD) from each of the studies, in which the weights were equal to 1 less than the number of cases. When fewer than 10 cases were studied, SDs and covariances were excluded. If raw data were used to calculate the SD, the SD was calculated by subtracting the 10th percentile from the 90th percentile of the $\log_{10}$ MoM distribution and dividing by 2.563. In some studies, the interquartile range divided by 1.34898 was used if no other data were available. Because the mean level in Down syndrome is based on a regression equation, and the observed SDs were in many cases determined without accounting for the regression effect, the Down syndrome SD was adjusted. The statistical appendix describes how the adjustment factor was determined. The correlation coefficients were determined from covariances and SDs. The covariances were determined by taking a weighted average of the observed covariances from each study, in which the weights were equal to 1 less than the number of cases. Correlation coefficients were then determined by dividing the covariance by the SD of each of the markers. For Down syndrome parameters, the adjusted SD was used in the last step. The parameters for NT were taken from a meta-analysis by Cuckle et al.[48] A correlation of 0 was used for each of the biochemical analytes with NT.

False-positive and detection rates were determined with the use of Monte Carlo simulation trials that use multivariate Gaussian distributions of log-MoM values for Down syndrome and unaffected pregnancies based on meta-analysis. After parameters were determined, in-house C language software on a SCO Open-Server 5.05 operating system (SCO Group, Lindon, UT) was used to invoke a random number generator to create sets of 100,000 simulated MoM values for free β-hCG, intact hCG, PAPP-A, and NT for unaffected and Down syndrome affected pregnancies. Likelihood ratios were determined for each simulated set. On the basis of a given risk cutoff, it was determined whether the given set would produce a risk that would be screen positive or screen negative for each maternal age from 15-49 years. From these results, age-specific false-positive and detection rates were determined. A weighted average of age-specific rates was calculated to determine overall false-positive and detection rates. The weights were taken from the percentage of total live births born to mothers at each given maternal age in the United States in the year 2000 (Vital Statistics of the United States, 2000, Volume I, Natality, http://www.cdc.gov/nchs/datawh/statab/unpubd/natality/natab2000.htm) and the risk of Down syndrome. The maternal age-related risk of Down syndrome was determined by using the formula of Cuckle.[49]

To determine whether the difference in screening performance was significant when free β-hCG was used instead of intact hCG, 100 separate simulations of the meta-analysis were performed. In each simulation, 37 studies were randomly selected with replacement from the 37 studies used in the final analysis. Distribution parameters were then calculated based on this random selection of studies and detection, false-negative and false-positive rates determined as described earlier, except that the calculations were based on 10,000 datasets instead of 100,000 data sets. The *P* value equaled the number of times out of the 100 simulations that the performance of intact hCG was greater than or equal to that of free β-hCG.

TABLE 2
Detection rates at a 5% false-positive rate in published studies in which free β-hCG, intact hCG, and PAPP-A were analyzed in the same sample set

		Gestational wks					
Publication	Protocol	9	10	11	12	13	Overall
Macintosh et al[42]							*
Wald et al[†7]	Free β-hCG/PAPP-A						62
	Intact hCG/PAPP-A						54
Haddow et al[‡33]	Free β-hCG/PAPP-A						71
	Intact hCG/PAPP-A						68
Cassals et al[‡30]	Free β-hCG/PAPP-A						58
	Intact hCG/PAPP-A						56
Wald et al[†12]	Free β-hCG/PAPP-A		74	70	67	67	69
	Intact hCG/PAPP-A		68	63	63	67	64
Spencer et al[†23,24]	Free β-hCG/PAPP-A	71	69	67	65	63	66
	Intact hCG/PAPP-A	62	58	56	56	56	56

All results based on modeling.

* Data not provided.

† Data provided in listed publication.

‡ Data calculated from statistical parameters provided in listed publication.

RESULTS

Table 2 shows a list of 6 studies[7,12,23,24,30,33,42] in which free β-hCG, intact hCG, and PAPP-A were analyzed in the same sample set. Of the 6 studies, 5 studies showed that the combination of free β-hCG and PAPP-A had higher detection than intact hCG and PAP-P-A, and 1 study did not provide enough information to determine detection rates. Of the 5 studies showing improved performance the, improved performance ranged from 2% to 10%. Two of the 5 studies provided detection rates by gestational week. In both studies, the performance of free β-hCG relative to intact hCG when each was combined with

TABLE 3
Univariate detection rates of free β-hCG, intact hCG, and PAPP-A by gestational age at a 5% false-positive rate

	Gestational age (wk)			
Analyte	**9**	**10**	**11**	**12**
Free β-hCG	19%	21%	24%	28%
Intact hCG	1%	3%	6%	12%
PAPP-A	57%	49%	41%	33%

Results are without maternal age. For free β and intact hCG, numbers represent percentage of cases above the 95th percentile. For PAPP-A, numbers represent percentage of cases below the 5th percentile. Data based on statistical parameters from supplementary data (Table 3A-D available online only).

PAPP-A was greatest at the earlier gestational weeks. In a third study,[33] the median MoM at 9-11 weeks of free β-hCG (2.31) was nearly twice that of intact hCG (1.38).

The Gaussian distribution parameters for free β-hCG, intact hCG, and PAPP-A are shown in the didactic data along with a listing of the data from the individual studies. The univariate detection rate of each marker without maternal age from 9-12 weeks' gestation is shown in Table 3. For free β-hCG and intact hCG, the detection efficiencies increase with gestational age; whereas for PAPP-A, the detection efficiency decreases. Overall, the data suggest that free β-hCG detects 3 times as many cases across the gestational age range of 9-12 weeks as intact hCG. There were more than 10-fold more cases detected at the earliest gestations (ie, 9-10 weeks).

At a fixed 5% false-positive rate, when the blood sample was drawn at 9, 10, 11, and 12 weeks, inclusion of free β-hCG into the PAPP-A/NT protocol reduced the false-negative rate by approximately 26%, 29%, 33%, 35%, respectively (Table 4). Inclusion of intact hCG into the PAPP-A/NT protocol reduced the false-negative rate by 0%, 5%, 12%, and 21% when the blood sample was drawn at 9, 10, 11, 12 weeks, respectively (Table 4). Averaging across weeks, there was a 31% reduction in the false-negative rate with free β-hCG and a 9% reduction in the false-negative rate with intact hCG ($P = .01$, ie, in only 1 of 100 simulations was the false-negative rate with intact hCG reduced more than with free β-hCG).

At a fixed 90% detection rate, inclusion of free β-hCG into a PAPP-A/NT protocol reduced the false-positive rate by 41%, 45%, 48%, and 52% at 9, 10, 11, and 12 weeks, respectively (Table 5). Inclusion of intact hCG into the PAPP-A/NT protocol reduced the false-positive rate by 2%, 8%, 20%, and 37% at 9, 10, 11, and 12 weeks, respectively (Table 5). Averaging across weeks, there was a 47% reduction in the false-positive rate with free β-hCG and a 17% reduction in the false-positive rate with intact hCG ($P = .01$).

To assess potential bias of these results, we repeated the simulation 100 times by randomly selecting studies with replacement from the 37 studies in the meta-analysis and then recalculating all of the results. This analysis indicated that at a fixed 5% rate the average reduction in the false-negative rate was 28% (bias −3% compared with presented results) for free β-hCG and 12% (bias 3% compared with presented results) for intact hCG. Similarly, at a fixed 90% detection rate the average reduction in the false-positive rate was 44% (bias of −3% compared with presented results) with free β-hCG and 20% with intact hCG (bias of 3% compared with presented results).

In clinical practice, Down syndrome screening is usually performed with a risk cutoff equal to that of a 35-year-old patient. By using the clinically relevant 35-year-old cutoff (1 in 384 at term) in younger patients, the addition of free β-hCG into the PAPP-A and NT protocol had a greater impact on detection compared with intact hCG. In younger patients, free β-hCG increased the detection rate by 4, 5, 6, and 7 percentage points at 9-12 weeks, respectively, whereas intact hCG only increased the detection rate by 0, 0, 2, and 4 percentage points at 9-12 weeks, respectively (didactic data). On an annual basis in the

TABLE 4
Reduction in the false-negative rate of first-trimester Down syndrome screening when free β-hCG or intact hCG is added to PAPP-A/NT at a fixed 5% false-positive rate

GA at draw	GA at U/S	Detection rate (false-negative rate)			Reduction in false-negative rate	
		PA/NT	+ Intact hCG	+ Free β	+ Intact hCG	+Free β
9	11	89 (11)	89 (11)	92 (8)	0%	−27%
9	12	88 (12)	88 (12)	91 (9)	0%	−25%
9	13	85 (15)	85 (15)	89 (11)	0%	−27%
9	**Avg**	**87 (13)**	**87 (13)**	**91 (9)**	**0%**	**−26%**
10	11	87 (13)	88 (12)	91 (9)	-8%	−25%
10	12	85 (15)	86 (14)	90 (10)	−7%	−33%
10	13	83 (17)	83 (17)	88 (12)	0%	−29%
10	**Avg**	**85 (15)**	**86 (14)**	**90 (10)**	**−4%**	**−29%**
11	11	85 (15)	87 (13)	90 (10)	−13%	−33%
11	12	83 (17)	85 (15)	89 (11)	−12%	−35%
11	13	80 (20)	82 (18)	86 (14)	−10%	−30%
11	**Avg**	**83 (17)**	**85 (15)**	**88 (12)**	**−12%**	**−33%**
12	11	83 (17)	87 (13)	89 (11)	−24%	−35%
12	12	81 (19)	85 (15)	88 (12)	−21%	−37%
12	13	78 (22)	82 (18)	85 (15)	−18%	−32%
12	**Avg**	**81 (19)**	**85 (15)**	**87 (13)**	**−21%**	**−35%**

Avg, average; *GA*, gestational age; *U/S*, ultrasound
The bolded numbers represent the average of weeks 9, 10, 11, and 12.

TABLE 5
Reduction in the false-positive rate of first-trimester Down syndrome screening when free β-hCG or intact hCG is added to PAPP-A/NT at a fixed 90% detection rate

GA at draw	GA at U/S	False-positive rate			% Reduction in false-positive rate	
		PA/NT	+ Intact hCG	+ Free β	+ Intact hCG	+ Free β
9	11	5.9	5.8	3.5	−2%	−41%
9	12	7.1	6.9	4.1	−3%	−42%
9	13	9.3	9.1	5.5	−2%	−41%
9	**Avg**	**7.4**	**7.3**	**4.4**	**−2%**	**−41%**
10	11	7.8	7.2	4.3	−8%	−45%
10	12	9.4	8.7	5.1	−7%	−46%
10	13	12.3	11.3	6.8	−8%	−45%
10	**Avg**	**9.8**	**9.1**	**5.4**	**−8%**	**−45%**
11	11	9.9	8	5.1	−19%	−48%
11	12	12.1	9.6	6.2	−21%	−49%
11	13	15.6	12.5	8.1	−20%	−48%
11	**Avg**	**12.5**	**10.0**	**6.5**	**−20%**	**−48%**
12	11	12.3	7.8	5.9	−37%	−52%
12	12	14.9	9.4	7.1	−37%	−52%
12	13	19.3	12.3	9.3	−36%	−52%
12	**Avg**	**15.5**	**9.8**	**7.4**	**−37%**	**−52%**

The bolded numbers represent the average of weeks 9, 10, 11, 12.

United States, assuming 50% of all patients are screened in the first trimester, free β-hCG will detect an additional 67, 84, 102, and 122 cases when blood is drawn at 9-12 weeks, respectively, whereas intact hCG would detect only an additional 3, 12, 35, and 75 cases, respectively (didactic data and Figure 1).

In patients 35 years of age and older, free β-hCG had a greater impact on false-positive rate compared with intact hCG when added to the PAPP-A/NT protocol. In older patients, compared with the PAPP-A/NT protocol, free β-hCG decreased the false-positive rate by 2.5, 3.1, 3.8, and 4.4 percentage points at 9-12 weeks, respectively, whereas intact hCG decreased the false-positive rate by only 0.1, 0.3, 1.0, and 2.2 percentage points at 9-12 weeks, respectively. Assuming 50% of all patients in the United States are screened in the first trimester and a 1% loss rate for chorionic villi sampling (CVS), free β-hCG would reduce the number of iatrogenic losses relative to the PAPP-A and NT protocol alone by 69, 85, 102, and 118 on an annual basis when blood is drawn at 9 to 12 weeks, respectively, while intact hCG would reduce the number of iatrogenic losses by 2, 9, 28, and 59, respectively (didactic material and Figure 2).

FIGURE 1
Additional Down syndrome cases detected annually when intact hCG (open box) or free β-hCG (Δ) are added into the PAPP-A and NT protocol in patients younger than 35 years

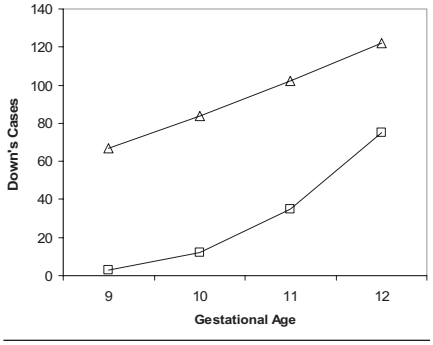
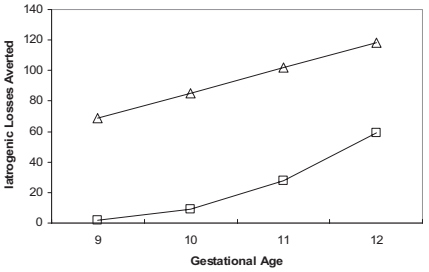


FIGURE 2
Iatrogenic losses averted when intact hCG (open box) or free β-hCG (Δ) are added into the PAPP-A and NT protocol in patients 35 years or older



This meta-analysis suggests that in a first-trimester Down syndrome screening protocol free β-hCG achieves higher sensitivity and lower false-positives than intact hCG.

At 13 weeks' gestation, at a fixed 5% false-positive rate, free β-hCG reduced the false-negative rate by 34% compared with 32% for intact hCG, whereas at a fixed 90% detection rate, free β-hCG reduced the false-positive rate by 56% compared with 53% with intact hCG when either marker was added into the PAPP-A and NT protocol.

Comment

Hallahan et al[40] demonstrated in a direct comparison that free β-hCG was superior to intact hCG as individual markers in 7 studies in which both free β-hCG and intact hCG were measured in both samples. In this analysis we evaluated 6 studies[7,12,23,24,30,33,42] in which free β-hCG, intact hCG, and PAPP-A as well were evaluated in the same dataset. The data demonstrate that the combination of free β-hCG and PAPP-A is superior to intact hCG and PAPP-A with improved detection rates ranging from 2% to 10%. In the 2 studies that evaluated data at individual gestational ages, the improvement with free β-hCG was greatest at earlier gestational ages. In a third study,[33] the median MoM at 9-11 weeks of free β-hCG (2.31) was nearly twice that of intact hCG (1.38).

Multiple studies have shown first-trimester screening that used free β-hCG, PAPP-A, and NT can achieve detection of 85% to 90% of Down syndrome cases at an approximate 5% false-positive rate. With detection rates this high, by using a fixed 5% false-positive rate to assess the incremental increases in detection efficiency of each marker will always make it appear that the last marker added is not very effective. This will tend to mask the differences of individual markers when compared in multimarker protocol. However, evaluating the incremental reduction in the false-negative rate (ie, the percentage of cases detected that were missed by the other markers) is a much more accurate and appropriate assessment of the last added marker.

We have evaluated adding free β-hCG vs intact hCG into a multiple marker Down syndrome screening protocol that already included PAPP-A and NT in the first trimester of pregnancy. By using a fixed 5% false-positive rate, inclusion of free β-hCG reduced the overall false-negative rate by 31%. This is greater than the 23% reduction in the false-negative rate that inhibin adds to second-trimester Quad testing.[50] Inclusion of intact hCG reduced the overall false-negative rate by only 9%. Overall free β-hCG lowered the false-negative rate more than 3 times as much as intact hCG. In other words, free β-hCG detected 3 times the number of additional Down syndrome cases as intact hCG when included with PAPP-A/NT.

The results of any meta-analysis may be subjected to underlying bias in any of the individual studies. One possible source of bias is that some of the studies were from prospective interventive studies, which could be subject to viability bias (ie, the ability of the test to detect cases that would be destined to miscarry). Indeed, it has been observed that low free β-hCG, low PAPP-A, and increased NT are associated with spontaneous abortions in chromosomally normal fetuses.[51] If cases of Down syndrome that were destined to miscarry also tended to have low free β-hCG, low PAPP-A, and increased NT this bias would thus underestimate the benefit of free β-hCG and overestimate the benefit of PAPP-A and NT in detecting Down syndrome. Therefore, the overall benefit of adding free β-hCG to PAPP-A/NT may be even larger that that observed in this analysis.

A second source of bias that meta-analysis is subject to is reporting or publication bias. In other words, positive results are more likely to be published than negative results. Second-trimester multiple marker Down syndrome screening is commonly used with intact hCG. As a result, there would have been great interest and benefit to include intact hCG in a first-trimester screening protocol. However, as initial studies on intact hCG as a first-trimester marker of Down syndrome were negative,[8-11] it is possible that other negative studies on intact hCG were underreported. If so, such studies would further validate our conclusions.

The Spencer study undoubtedly has a significant impact on the overall meta-analysis because it represents a significant number of Down cases. The 9-13 week free β-hCG medians in the meta-analysis were 1.67, 1.77, 1.87, 1.98, and 2.10 compared with 1.55, 1.74, 1.91, 2.05, and 2.16 with the Spencer study.[23,24] For intact hCG, the 9-13 week medians in the meta-analysis were 0.89, 1.04, 1.21, 1.41, and 1.65 compared with 0.83, 1.02, 1.23, 1.43, and 1.61 with the Spencer study.[23,24] However, any apparent undue weighting of the Spencer study is only because it includes data from several studies that would have otherwise been included in this meta-analysis.

Historically, maternal serum Down syndrome screening has been performed in patients younger than 35 years in an effort to detect cases in an otherwise low-risk population. More recently, screening has also been applied to patients older than 35 years in an effort to reduce the number of invasive procedures. The results of this meta-analysis indicate that when using the clinically relevant 35-year-old risk cutoff, free β-hCG as compared with intact hCG will increase the detection rate in patients younger than 35 years and lower the false-positive rate in patients 35 years and older. These improvements in performance optimize the performance of screening to achieve the most number of detected cases for the fewest patients classified as "increased risk," which is the primary goal of any screening program. As such, there are significant advantages in terms of reduced cost, reduced iatrogenic losses, and the ability to screen as early as possible.

A significant advantage of first-trimester screening is the ability to diagnose Down syndrome before the time that the patient is noticeably pregnant and has begun the transition from "being pregnant" to "having a baby." Inherent in this process is the ability to perform diagnostic tests during this early stage of pregnancy. Typically, such diagnostic testing is performed by the end of the 12th week of gestation. As a result, the inability of intact hCG to improve screening performance before this period makes it inappropriate to use intact hCG in first-trimester Down syndrome screening.

There is general consensus in the literature that free β-hCG is a superior marker to intact hCG when evaluated individually, and the debate has been about whether the benefits of free β-hCG are diluted when it is used with other markers. Our data from an extensive meta-analysis demonstrate that free β-hCG significantly improves the performance of first-trimester screening with PAPP-A/NT by increasing the sensitivity, lowering the false-positive rate, and achieving earlier diagnosis than intact hCG. It is time to declare this debate finished and move on to nationwide implementation of the optimal protocol. ■

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APPENDIX
Calculation of Down syndrome SDs

The difference between the total SD and the within-gestation SD was determined by using the formula: Difference = (Total SD)^2 − (Within GA SD)^2. By using the study of Spencer, total SD equaled .2858, .2526, and .3027 while within GA SD equaled 0.2787, 0.2238, 0.2822 for free β-hCG, intact hCG, and PAPP-A, respectively.

For individual studies, the total SD was used. If this parameter was not available but the within GA SD was available, the total SD was estimated by the following formula: Total SD = ((Within GA SD)^2 + Difference)^0.5.

The overall total SD was then adjusted to get an estimate of the within GA SD using the following formula: Overall within GA SD = ((overall Total SD)^2 − Diff)^0.5. The overall within GA SD parameter was then used in the calculation of false-positive and detection rates.

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