48 First-trimester screening for aneuploidy

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INTRODUCTION

Advanced maternal age was the be all and end all of prenatal risk assessment for aneuploidy for nearly half a century (1–3). In virtually all countries, women above a given age cutoff were regarded as being at high enough risk of aneuploidy to warrant the costs and hazards of performing an invasive, diagnostic procedure. Over the past three decades, attempts have been made to refine the assessment of an individual woman's risk using biochemical and ultrasound markers within pregnancy. These have markedly improved the sensitivity (proportion of aneuploidy pregnancies at high risk, or detection rate) and specificity (proportion of unaffected pregnancies not at high risk) (3). Using a cutoff maternal age of 35, a 30% to 40% sensitivity and 90% to 95% specificity (or 5–10% false-positive rate) were the best available statistics throughout the 1970s and early 1980s (4).

Over the past 25 years, there have been multiple iterations of approaches that have improved the statistics of screening (2,3). Incorporation of these approaches has been haphazard with huge variability around the world and even within countries. Much has been written over the years describing the state of the art at given times and will not be repeated in extensive detail here for previous eras.

In 1984, Merkatz et al. published the association of low maternal serum alpha-fetoprotein (AFP) with an increased risk of aneuploidy in general (4), and Cuckle et al. confirmed that this holds for Down syndrome (5). In subsequent years, there was also an eventual understanding that the extent of AFP reduction differed according to the type of aneuploidy. For example, trisomy 18 has much lower values on average than Down syndrome (6).

Since AFP was already widely being used to screen for neural tube defects, at 16 to 18 weeks of gestation, it was relatively simple to include aneuploidy. This was done by the calculation of a likelihood ratio (LR) (proportion of aneuploidy pregnancies divided by proportion of unaffected pregnancies with the given AFP level) and using this to increase or decrease the maternal age-specific risk. The LR was derived from a Gaussian model of the AFP distributions in aneuploidy and unaffected pregnancies. The detailed practical mechanics of biochemical screening, such as adjustments for gestational age, race, diabetic status, multiple gestation status, and maternal weight have been published previously and will not be repeated here (2,3).

Maternal serum AFP screening for aneuploidy was widely adopted and had the potential to increase the detection rate, but it was inefficient. The optimal use of a biochemical or ultrasound marker is to screen all women regardless of age and to define high risk purely on the basis of the screening result. However, many clinicians did not consider a low-risk AFP result in an older woman as sufficient grounds for not offering

invasive testing. While the use of maternal serum AFP was a notable improvement over “how old are you,” it left much to be desired.

The situation changed in the late 1980s when the first highly discriminatory biochemical marker was discovered, namely human chorionic gonadotropin (hCG). The molecule is a hetero-dimer consisting of α and β subunits, which is not only present in maternal serum predominantly as the biologically active intact dimer, but 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. However, since 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 (3). Unfortunately, inconsistency in the terminology used to describe hCG assays has created confusion (sometimes deliberately) 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 (7).

Both intact (or total) hCG and free β -hCG are established markers of both Down syndrome and trisomy 18, being increased on average in the former and decreased in the latter type of aneuploidy (8,9).

MULTIPLE BIOCHEMICAL MARKERS

The discovery that hCG was a marker was quickly followed by another second-trimester marker, unconjugated estriol (uE_3), and later dimeric inhibin A (3,10). This gave the impetus in the 1990s, for the combination of multiple second-trimester maternal serum markers (3,10). As with AFP alone, an LR was calculated and used to modify the maternal age-specific risk. In this case, it was derived from a multivariate Gaussian model of the marker distributions taking into account the various correlations between markers.

Down syndrome detection rates increased to approximately 60% to 70% for a 5% false-positive rate (11–13). Table 1 shows the predicted detection rate for a fixed 1%, 3%, and 5% false positive using the principal combinations found in clinical practice (15).

While the switch over from AFP alone to two (*double test*), three (*triple test*), or four (*quadruple test*) marker combinations has gradually taken place in most countries, there have been continual disputes over the best combinations. There was essentially universal agreement that among the single markers in the second trimester, hCG, free β -hCG, and inhibin have the greatest discriminatory power. While uE_3 has similar discriminatory power to AFP, since the latter is already used for neural tube defect screening, it is always included as the second marker. There have been disputes over whether to include uE_3 as a third parameter. Some have claimed that the

Table 1 Biochemical Marker Combinations (Second Trimester): Predicted^a Detection Rate for a Given False-Positive Rate

Marker combination	False-positive rate		
	1%	3%	5%
AFP and free β-hCG	37%	53%	61%
AFP, free β-hCG, and uE ₃	42%	57%	65%
AFP, free β-hCG, uE ₃ , and inhibin	50%	64%	71%
AFP and intact hCG	33%	48%	56%
AFP, intact hCG, and uE ₃	39%	53%	60%
AFP, intact hCG, uE ₃ , and inhibin	46%	60%	67%

^aModeling with parameters in (14).

predicted marginal increase in detection rate cannot be achieved in practice. However, much of the prospective series literature did show the predicted benefit. Moreover, uE₃ is of value in the detection of trisomy 18, Smith–Lemli–Opitz syndrome, and placental sulfatase deficiency, where uE₃ levels are extremely low. Incidentally, levels are also slightly lowered in spina bifida and more so in anencephaly, but the changes are much less than that for AFP (16).

Like hCG, free β-hCG, and AFP, the levels of uE₃ and inhibin are altered on average in trisomy 18. For some of these markers, the direction of change is the same for both types of aneuploidy. Consequently, although the birth prevalence of trisomy 18 is 10-fold lower than Down syndrome, it is a relatively frequent incidental diagnosis in women having invasive testing following second-trimester screening. But in order to achieve a high detection for this disorder, it is necessary to calculate a separate risk specifically (8).

Despite overwhelming evidence by the early 1990s, and recommendations of national organizations such as the American College of Obstetricians and Gynecologists, that the multiple biochemical marker combinations discussed so far be offered, even by the millennium, still nearly 20% of patients in the United States who had screening were having AFP alone (3). Such slow uptake by practicing obstetricians is worrisome precedent for the incorporation of future test combinations.

Another long promising but yet fulfilled marker was the search for fetal cells in maternal circulation. Studies throughout the 1990s and early 2000s suggested that isolation and analysis of fetal cells might, in fact, become practical and useful as a screening test (17,18). Much of the 1980s and 1990s focused on ways to improve the efficacy of detection methods primarily centered on the need to increase the enrichment of fetal cells from the maternal blood circulation whose prevalence has been estimated to be approximately 1 in 10,000,000 cells with no clear likelihood of success (19). After the failure of the first lines of methodology in detecting fetal cells, modified approaches have emerged that are being evaluated for more precise identification and isolation of fetal cells.

A more recent line of research has been the detection of fetal cell-free DNA and RNA in the maternal circulation initially suggested by Lo et al. (20). There have been three generations, to date, of technologic approaches in this area. First, the concentration of these materials in maternal blood is approximately doubled in aneuploidy, and it was thought that

quantitative differences could be used as an additional marker. However, fetal DNA only makes up a small proportion of free DNA in maternal circulation such that the actual difference between a trisomic fetal contribution to overall maternal DNA levels is only a few percent. Second, Lo et al. proposed that using a placental-specific RNA such as PLAC-4 would allow a real 2:1 discrimination. His data suggested a 90% sensitivity for a 96% specificity. The methods were licensed to Sequenom whose unpublished investor presentations claimed 100% sensitivity for 99% sensitivity. Unfortunately these data were falsified, and the investigator has pleaded guilty to fraud charges setting back the approach immeasurably. Furthermore, RNA is very labile requiring stabilization within hours rendering problematic the use of the technique for mass screening. Third, the advent of the next-generation massive sequencing methods does have real scientific merit. There is significant potential for its use (21–26).

Amplification and molecular analysis of this circulating fetal product is already being used clinically for fetal gender and RhD determination. Other Mendelian diagnoses may probably follow in short order. It might eventually be used as a noninvasive method of prenatal diagnosis for aneuploidy, but much more research is still needed before this goal can be achieved in any reliable fashion. While the diagnosis of Mendelian disorders may be possible by fetal cells or cell-free DNA, it is very likely that aneuploidy detection will be seen as a screening test to modify risk as a predicate for invasive diagnostic procedures (26).

COMBINATION OF BIOCHEMICAL WITH ULTRASOUND MARKERS

Considerable work in the mid-1990s focused on the development of ultrasound markers to be used to modify the risk of aneuploidy. As early as 1985, Benacerraf et al. observed an increased nuchal skinfold during the second-trimester genetic ultrasound examination among aneuploid fetuses, but it was rarely used in routine screening (27). Nevertheless, in 1992, Nicolaides et al. reported on a related, but more discriminatory marker, nuchal translucency (NT) determined in the first trimester, which did enter routine practice, and results have now been reported on well over a million pregnancies scanned at 11 to 13 weeks of pregnancy (28). The literature on this development has been reviewed extensively elsewhere and will not be repeated here except to state that quality control of NT measurements is inferior to biochemical methods. Under-measurement of NT continues to be prevalent and lowers detection rates considerably (29,30). Instead, we concentrate on combinations between ultrasound NT and biochemical markers (*combined test*), which can be shown to yield a much higher detection rate than NT alone.

The most widely studied first-trimester biochemical markers are pregnancy-associated plasma protein (PAPP)-A and free β-hCG. The discriminatory power of PAPP-A decreases with gestation, as does NT, while that of free β-hCG increases with gestation. First-trimester multiple-marker Down syndrome screening using a combination of the biochemical and ultrasound markers has been offered clinically for nearly 15 years (3). Most centers perform the NT scan at 11½ to 12½ weeks since the NT is easier to visualize than at earlier and some fetal structural abnormalities can be seen, while by

Table 2 Nuchal Translucency with or Without Biochemical Marker Combinations (First Trimester): Predicted^a Detection Rate for a Given False-Positive Rate

Marker combination	Gestation (weeks)		False-positive rate		
	Serum	NT	1%	3%	5%
NT alone	-	11	64%	73%	77%
	-	12	62%	71%	75%
	-	13	57%	66%	71%
PAPP-A, free β -hCG, and NT	10	11	74%	83%	87%
	11	11	74%	83%	87%
	11	12	73%	82%	86%
	12	12	72%	80%	84%
	12	13	68%	77%	82%
	13	13	66%	75%	80%

^aModeling with parameters in (14).

Table 3 Predicted^a Detection Rate for a 5% False-Positive Rate when Adding Either Intact or Free β -hCG to a PAPP-A and NT Protocol, According to Gestation when Blood Drawn (NT at 11–12 Weeks)

Serum (weeks)	Intact hCG	Free β -hCG
9	80%	84%
10	78%	83%
11	75%	81%
12	77%	80%

^aModeling with parameters in (7).

13 weeks the discriminatory power is reduced. To maximize the effectiveness of the biochemical markers and to ensure that the risk is calculated and immediately available at the same time, the woman attends for the ultrasound examination, while the blood sample may be drawn 1 to 2 weeks before the scheduled scan date, ideally at 10 weeks of gestation (14,31,32).

Table 2 shows the predicted detection rate for a fixed 1%, 3%, and 5% false positive using the marker combinations at different gestations and for comparison the results for NT alone. As before, the predictions are based on statistical modeling using published marker parameters derived by meta-analysis (14). The addition of biochemical markers leads to a substantial increase in the detection rate, which is considerably higher than that for the second-trimester combinations. PAPP-A levels are also altered on average in trisomy 18. Since for all the first-trimester markers the direction of change is the same for Down syndrome and trisomy 18, unlike in the second trimester, there is little value in a separate risk calculation.

First-trimester screening not only has a yield greater than that of the second-trimester screening protocols, but also 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 (32). There is no disagreement over the utility of combining first-trimester biochemical markers and NT, but there remains controversy over the use of free β -hCG rather than intact (or total) hCG in the combination in the United States but not the rest of the world. The medical and economic implications of this choice in the United States are

enormous, and we therefore dwell on this otherwise relatively minor point.

Most retrospective reports on biochemistry indicated that while free β -hCG was a very effective marker for Down syndrome in the first trimester, intact hCG was not (3,7). Indeed, the discriminatory power of intact hCG is extremely low at 10 weeks and does not even reach that of the weakest second-trimester markers until 12 weeks (10). Consequently, the vast majority of prospective first-trimester screening studies have used free β -hCG in combination with PAPP-A, and NT. The Serum, Urine, and Ultrasound Screening Study tested both hCG isoforms and suggested that free β -hCG is a more discriminatory first-trimester marker than intact hCG, but there may be little difference in the overall screening performance when included in a multiple marker protocol (10). Although often described as a prospective study with results on more than 47,000 pregnancies, the biochemical results are actually based on retrospective analysis of less than 500 samples. Furthermore, we believe that a statistical correction to the analysis would have demonstrated the advantage of free β -hCG even when combined with other markers (7). Nevertheless, the study has created some uncertainty and opened a debate in the United States about which isoform of hCG to use in first-trimester screening.

This prompted us to address the issue using a meta-analysis based on a considerable body of available evidence to predict the consequence of adding either free β -hCG or intact hCG to a PAPP-A and NT protocol; the methodology was described extensively in the paper (7). At a fixed 5% false-positive rate, when the blood sample was drawn at 9, 10, 11, and 12 weeks, adding free β -hCG reduced the false-negative rate by 26%, 29%, 33%, and 35%, respectively, compared with reductions of 0%, 5%, 12%, and 21%, respectively, adding intact hCG. Averaging across weeks, the reductions were 31% and 9%, a statistically significant difference ($p = 0.01$). Similarly, at a fixed 90% detection rate, adding free β -hCG reduced the false-positive rate by 41%, 45%, 48%, and 52% compared with 2%, 8%, 20%, and 37% for intact hCG, averages of 47% and 17% ($p = 0.01$). Overall multivariate detection rates averaged a 3% to 6% improvement using free β as opposed to total hCG, and maximal detection was achieved by blood drawn at 9 to 10 weeks with NT at 11 to 12 weeks (Table 3) (7).

Canick et al. (33) carried out a secondary analysis of data from the First and Second Trimester Evaluation of Risk (FASTER) trial (34) and found that while the sensitivity of free β -hCG was notably higher than that for total hCG, there was only a 1% detection rate difference in combination with PAPP-A and NT and it was not statistically significant. The authors concluded that the choice of the third marker was not critical. The analysis was not based on the entire 38,000 patients in the trial, but only on statistical modeling with total hCG and free β -hCG parameters derived by retrospectively assaying stored serum samples from 79 Down syndrome and 395 unaffected pregnancies (31).

Canick’s conclusions regarding screening policy are unwarranted for three reasons. First, the standard deviation of free β -hCG was much wider than generally found elsewhere, possibly due the specific reagents used to retrospectively assay the samples. The standard deviation among unaffected pregnancies, compared with values from all the

recent large published prospective first-trimester series, and that from our own experience is shown in Table 4. This will inevitably have led to the underestimation of effectiveness for free β -hCG. Moreover, the retrospective testing was done over a short time period, reducing the standard deviation compared with routine practice. Hence, the discrepancy with routine practice is even greater. Second, decisions regarding screening policy need to take account of the totality of available evidence. Modeling with parameters derived by meta-analysis from series including several hundred Down syndrome pregnancies indicates a 2% detection advantage for free β -hCG (7,14). The failure of a relatively small study to find a statistically significant effect is insufficient grounds for changing policy. Third, at the high detection rates achievable by first-trimester marker combinations, a 2% increment in detection is not small. Each marginal increase in detection is achieved by a large increase in the false-positive rate. Had the current study estimated the false-positive rate for a fixed 90% detection rate, as we have done above, this would have been apparent. Therefore, on the basis of all available evidence, there is no good reason for health planners to consider substituting intact hCG for free β -hCG in first-trimester protocols.

SEQUENTIAL SCREENING METHODS

In the last few years, considerable discussion of screening policy has been focused on carrying out screening in both the first and second trimesters, in sequence. In general, this achieves a higher yield than screening within trimester. Three types of sequential policy have received attention. The first to be proposed was a form of nondisclosure sequential screening using first-trimester PAPP-A and NT together with

second-trimester AFP, uE₃, free β -hCG or intact hCG, and inhibin (*integrated test*). Risks are not used clinically until all markers have been tested. The proponents of such “integrated” screening argue that higher sensitivities can be achieved and therefore justify the nondisclosure. However, many clinicians in the United States and elsewhere feel that it is simply not acceptable under local culture and ethical beliefs to withhold potentially serious screening results for a month when the odds of substantial change are minimal. Such an approach also has the substantial disadvantage that there is no early diagnosis or reassurance. Also there may be practical difficulties such as a considerable proportion of patients who do not present for the second half of the screening evaluation. There are also profound ethical concerns over nondisclosure of potentially considerably increased risks that could be addressed in the first trimester with its considerable advantages of privacy for the patient in difficult reproductive decisions. A second approach (*step-wise test*) begins with first-trimester PAPP-A, free β -hCG or intact hCG, and NT; those with low risk have second-trimester AFP, uE₃, free β -hCG or intact hCG, and inhibin; their risk is estimated from all seven markers. It is important to use a high first-trimester cutoff than with nonsequential screening, otherwise the overall false-positive rate will be too high. And it is essential to use all seven markers together when calculating the final risk. It is invalid to ignore the first-trimester markers at this stage although many practitioners are doing so because they do not have access to the appropriate risk calculation software. This policy restores some first-trimester diagnosis.

A third policy, more efficient than the other types, is called the *contingent test*. This begins with first-trimester PAPP-A, free β -hCG or intact hCG, and NT. Women with very high risk are offered immediate invasive prenatal diagnosis and only those with borderline risks are offered second-trimester AFP, uE₃, free β -hCG or intact hCG, and inhibin; their risk is estimated from all seven markers. The borderline is chosen so that a large proportion of women have early assurance. This group has such a low risk that it is very unlikely that further markers will lead to a final high-risk result.

Table 5 shows the predicted detection rate for a fixed 1%, 3%, and 5% false positive for different sequential policies, as before based on statistical modeling using published marker parameters derived by meta-analysis. In the current predictions, the first-trimester very high cutoff for step-wise and contingent is set to obtain an early detection rate of 70%, and the very low cutoff for contingent screening is set so that 85%

Table 4 Standard Deviation (SD) of First-Trimester Free β -hCG in Different Series of Unaffected Pregnancies			
Series	Reagents	#	SD ^b
Krantz et al., 2002	NTDLabs	10,106	0.222
Crossley et al., 2002	Kryptor	17,229	0.269
Muller et al., 2003	Delfia	5634	0.269
Spencer et al., 2003	Kryptor and Delfia	31,773	0.261
Rozenberg et al., 2006	Delfia	10,632	0.257
Author's experience ^a	Delfia	28,276	0.266
Weighted average ^c	Delfia, Kryptor, and NTDLabs	103,646	0.260
Canick et al., 2006	DSL	395	0.307
Delfia, Perkin-Elmer Life Sciences, and Kryptor, Brahms. ^a Routine screening tests carried out in Leeds, UK. ^b Log ₁₀ units. ^c Weighted by the number of pregnancies. Abbreviation: DSL, Diagnostic Systems Laboratories.			

Table 5 Sequential Screening Policies: Predicted ^a Detection Rate for a Given False-Positive Rate					
First-trimester combination ^b with second-trimester AFP, free β -hCG, uE ₃ , inhibin	Early detection rate	Second-trimester tests	False-positive rate		
			1%	3%	5%
Integrated test					
PAPP-A and NT	0%	100%	86%	92%	94%
Step-wise test					
PAPP-A, free β -hCG, and NT	66% ^c	99%	86%	93%	95%
Contingent test					
PAPP-A, free β -hCG, and NT	66% ^c	12% ^d	86%	91%	92%
^a Modeling with parameters in (14). ^b Biochemistry and NT at 10 and 11 weeks. ^c 1 in 50 (term) cutoff. ^d 1 in 1500 (term) cutoff.					

of women have early assurance. The prediction is that the contingent test is extremely efficient, achieving comparable detection rates with the other types of sequential screening but requiring second-trimester tests on just a small proportion of women. The tabulated combinations use free β -hCG, but similar analyses with intact (or total) hCG, tested in the late first trimester and/or the second trimester, reach the same conclusion.

Sequential screening can also be carried out within trimester. One contingent approach suggested by Nicolaides et al. has the potential to further increase detection while completing all screening within the first trimester (35). It is proposed that women with borderline risk based on PAPP-A, free β -hCG, and NT are offered a more specialist scan to determine, among other things nasal bone, and reassess the risk. Nasal bone hypoplasia is a very powerful marker of aneuploidy but requires appropriate training not generally available. Another approach might be to measure additional biochemical markers in a very early serum sample at about 8 weeks of gestation in addition to the 10-week sample; one possible very early marker is ADAM12 (36).

SECOND-TRIMESTER REVISITED

Despite the clear advantages of beginning the screening process in the first trimester, some women do not present until later in pregnancy. If biochemical screening alone, even with the *quadruple test*, is regarded as inadequate, there is the option of combining information on the serum markers and ultrasound results to improve detection. The results of the genetic sonogram can be used to modify the risk from the biochemical markers. Provided an algorithm is used that uses a separate LR for the presence *and absence* of each soft marker, the detection rate can be increased by 5% to 10% (37). Moreover, there are new second-trimester ultrasound markers such as prenatal translucency, which are even more discriminatory than the traditional soft markers and can by themselves increase detection by about 15% (38).

CONCLUSIONS

Over the past 35 years, there has been a steady evolution of biochemical and also biophysical parameters used to adjust the risk that a woman is carrying a fetus with aneuploidy. At every step, there has been controversy as to the best methods to employ in this endeavor. We have laid out many of the proposals, and our conclusions are that as of 2011:

1. A combined test in the first trimester can yield a very high detection rate for an acceptable false-positive rate.
2. Second-trimester multiple marker biochemical screening yields a much lower detection rate and imposes a considerable emotional burden in requiring women to be very visibly pregnant, feel the baby moving, and have to undergo second-trimester termination methods if an abnormality is found and the women choose to end the pregnancy.
3. Sequential screening in both trimesters yields even higher detection rates, and the most efficient methods is the contingent test. Centers with appropriate training and experience of newer ultrasound markers such as nasal bone hypoplasia

could consider carrying out the contingent test within the first trimester.

Although comprehensive national data are not available, there is a continuing shift toward the first trimester. Only the exact components and timing remain to be determined.

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