Serum versus cell-free DNA screening

THE ISSUE: Sequential prenatal screening (combined serum and ultrasound testing) for aneuploidy detection is now offered routinely. Women identified as screen-positive (high risk) are then offered invasive testing for definitive diagnosis. Since 2011, cell-free (cf)DNA sequencing has become available and is now recommended as an intermediate option for screen-positive women who decline invasive testing. With the anticipation of further cfDNA price decreases, it is timely to consider the benefits and harms of offering cfDNA for primary screening as an alternative to sequential screening. When faced with selecting a screening option, women will benefit from receiving reliable information for each test, including the detection and false-positive rates, availability, consequences of a positive or negative test, and associated harms. This debate focuses on the comparison of disorders identified and associated detection rates for sequential vs cfDNA screening in the general pregnancy population.

Where have all the trisomies gone?

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In a recent publication, Norton et al[1] claim that prenatal screening based on a combination of serum and ultrasound measurements will identify more chromosome abnormalities than will next-generation sequencing of circulating cell-free (cf)DNA. This finding seems counter-intuitive. On the basis of our analysis of this publication and previous relevant studies by this group,[2,3] we conclude that the claim is incorrect and have identified methodologic and interpretive issues that merit consideration. This is not a new or isolated instance of a high-profile publication of a cohort study containing overestimated detection rates of Down syndrome.[4] Determining an accurate detection rate based on observed cases in which karyotypes were not obtained on all patients requires paying strict attention to potential biases, especially relating to ascertainment.[5-9] Screening recommendations from one professional society place emphasis on the need to account for ascertainment bias in reporting observed detection rates of Down syndrome.[10] Detection rates for cfDNA testing generally are

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Women should decide which conditions matter

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Traditional prenatal screening, by the use of a combination of serum and ultrasound markers, has been the standard approach to prenatal screening for many years. Incremental improvements have led to the current model, which includes first- and second-trimester serum analytes combined with measurement of first-trimester nuchal translucency. This screening test provides numeric risk assessment for trisomies 21 and 18, as well as for neural tube defects. Approximately 5% of pregnancies are flagged as screen positive, and it has been recognized that "false-positive" screening results indicate risk for a host of other fetal and obstetric abnormalities or adverse outcomes.[1-3] Cell-free DNA (cfDNA) screening, in contrast, is a far more precise test for a limited number of aneuploidies. Although clearly a better test for trisomy 21, this approach does not provide any information regarding risk for rare aneuploidies, obstetric complications, open fetal defects, or other fetal structural abnormalities. Furthermore, although the false-positive rate is very low, the exclusion of a large

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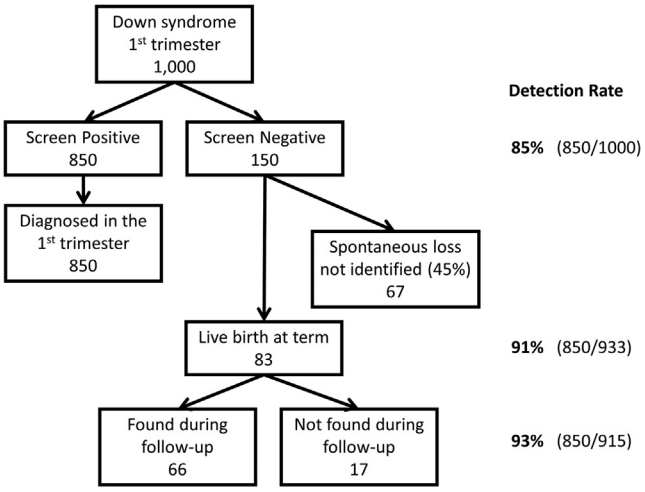
Dr Palomaki (*continued*)

based on studies in which karyotypes were performed in all of the pregnancies.

- The 93% detection rate reported for Down syndrome serum/ultrasound screening is an overestimate. This rate was derived by the use of data from a cohort of 452,901 women enrolled in the California Screening Program.[2] The 1184 screen-positive cases detected in the first or second trimesters were divided by the total number of Down syndrome cases identified (1184 screen positive + 91 screen negative). In that analysis, the authors correctly determined that 245 Down syndrome pregnancies could not be accounted for (using reliable projections of expected Down syndrome births based on the maternal age distribution) but then ignored this information. It is surprising that the authors did not take this finding into account in either the previous or present publication.[3]

FIGURE

A flowchart representing a hypothetical cohort of 1000 Down syndrome pregnancies screened in the first trimester



A flowchart representing a hypothetical cohort of 1000 Down syndrome pregnancies screened in the first trimester by a test with a "true" detection rate of 85%, demonstrating how greater detection rates can be computed incorrectly by not accounting for ascertainment biases. Of the 1000 cases, 850 are screen positive and 150 cases are screen negative (85% detection rate). Of the 150 screen-negative cases, however, an estimated 45% (67) will be spontaneously lost, are unlikely to be karyotyped, and will not be counted as cases. This results in 83 live born cases. If the detection rate were computed directly with only the 933 identified cases, it would be overestimated at 91% (850/(850 + 83)). This, however, assumes that all screen negative and live born cases will be identified. If, for example, 20% of live born screen negative cases (17) were not identified during follow-up, the detection rate would be even more inflated at 93% (850/(850 + 17)).

Palomaki. Serum versus cell-free DNA screening. Am J Obstet Gynecol 2016.

Dr Norton (*continued*)

number of patients as the result of failed tests or the presence of other fetal abnormalities introduces bias in evaluating test performance.[4-6] We whole-heartedly agree with Palomaki et al[7] that "It is critical that groups reporting screening performance of any prenatal test carefully consider the impact of bias on the estimated detection rates, so that data used for counseling and policy-making will be accurate."

In a recent publication, our group compared the detection of chromosomal abnormalities based on traditional sequential screening, as conducted in the California Prenatal Screening Program, with predicted performance outcomes had cfDNA screening been instead used as a primary screening test in this same cohort.[8] We had recognized that sequential screening identifies many women as having "false-positive" results for trisomy 18 and/or 21 who are then found to have a fetus affected by a different disorder. Arguably, these tests are not "false" positive. We also wished to consider the implications of the careful curation of the included populations that has been characteristic of studies of cfDNA screening, in which a large number of women are excluded from analysis because of failed tests; these failed tests are somewhat comparable to "false-positive" serum results because they indicate an increased risk of adverse outcomes.[6] The goal of our study was therefore to broaden the discussion regarding the relative benefits and limitations of each approach.

Traditional sequential screening and cfDNA screening produce different types of information, although both are screening tests for fetal abnormalities. Sequential screening is a very broad test that provides information about many fetal conditions, and therefore has better detection if the denominator is "all chromosome abnormalities" or "all birth defects." It therefore may be a better test for women who are at low risk for aneuploidy but at average risk for a wide range of conditions. cfDNA, on the other hand, is a very precise test for 3 aneuploidies. It has a better detection rate if the denominator used is "all cases of trisomy 21" and may be a more appropriate test for women in whom this single condition is the primary concern. These women, however, need to be informed of the limited number of conditions this test detects and also need to be offered additional screening for other birth defects. The availability of 2 screening tests for trisomy 21, each with different benefits and harms, has led to vigorous debate as to which of these tests, separately or in combination, in which patients, should be used.

This debate is reminiscent of the discussions that occurred 20 years ago regarding the appropriate role of rapid aneuploidy testing with fluorescence in situ hybridization for trisomies 13, 18, 21, and the sex chromosomes, and whether full karyotyping was needed. As pointed out by Caine et al[9] in a 2005 issue of *The Lancet*, "Replacement of full karyotyping with rapid testing for trisomies 13, 18, and 21 after a positive screen for Down's syndrome will result in