Prenatal screening for fetal aneuploidy: time to examine where we are and where we are going

Joseph R. Biggio Jr, MD

Aneuploidy is a major cause of perinatal loss and long-term childhood morbidity. Since the first report of an association between low maternal serum alpha-fetoprotein and fetal aneuploidy in 1984,[1] the sensitivity and specificity of screening techniques have improved markedly. Different combinations of screening metrics have been examined over the years, but ultimately the First and Second Trimester Evaluation of Risk Study provided the best evidence on the performance of various strategies.[2] Based on these results, the American College of Obstetricians and Gynecologists recommended that the best screening test for aneuploidy uses a combination of first (ultrasound measurement of nuchal translucency and serum pregnancy-associated plasma protein A) and second trimester (serum α-fetoprotein, human chorionic gonadotropin, unconjugated E_3, and dimeric inhibin-A) analytes. These combined tests are able to achieve a detection rate for trisomy 21 of approximately 92% at a screen-positive rate of 5%.[3] However, multiple marker screening is still relatively nonspecific, despite the improvements in test sensitivity. Depending on the screening strategy and the age of the population and its risk, the positive predictive value (PPV) of an abnormal screen for trisomy 21 remains low in the 2-6% range.[4]

Although improvements in screening began with attempts to isolate fetal cells in the maternal circulation, small fragments of "fetal" DNA (confirmed by the identification of Y-chromosome material) were isolated, dramatically changing the paradigm for enhanced development of this screening modality.[5-7] The Human Genome Project, which advanced bioinformatics, and high-throughput next generation sequencing technologies facilitated the use of fetal DNA into a practical screening strategy.[8,9] In 2011, the first study to demonstrate the ability of this technique to identify trisomy 21 in prospectively collected samples was published.[10] Shortly thereafter, what initially was termed *noninvasive prenatal testing* became commercially available in the United States. Since then, laboratories that use different analytic and statistical platforms have introduced screening tests based on the isolation of fetal DNA from the maternal circulation.[10-14] Although the platform details differ from 1 laboratory to another, overall sensitivity and specificity among the different fetal DNA screening tests appear to be similar.[15]

Initial validation studies of DNA-based screening for aneuploidy were conducted in high-risk populations with a prevalence of aneuploidy of at least 1 in 50 (eg, patients with advanced maternal age, abnormal serum screen, abnormal ultrasound scanning results, a previous affected fetus, or a balanced translocation. Although the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine endorsed the use of *fetal DNA-based screening* in 2012, the endorsement was limited to singleton pregnancies in women from these high-risk groups.[16]

We have learned a considerable amount about the performance of DNA-based screening for aneuploidy since its initial introduction to clinical practice only 4 years ago. First, although the testing frequently was referred to as fetal DNA testing, the isolated DNA fragments are actually from placental trophoblasts.[17] Although this may seem a matter of semantics, possible explanations for some abnormal screening results include confined placental mosaicism and vanishing twin.[18,19] For this reason, many have adopted the term *cell-free DNA screening* in favor of noninvasive prenatal testing (and noninvasive prenatal screening) because conventional serum screening is noninvasive as well. Second, we have learned that not every woman will receive an interpretable result and that those who fail to receive a result are at increased risk for fetal aneuploidy.[20] An inconclusive result can be due to problems with DNA sequencing or analysis or a low fetal fraction (the proportion of DNA of placental origin compared with the total amount of DNA isolated from the maternal plasma sample).[20-22] The fetal fraction is affected by a number of factors that include gestational age, obesity, and the presence of fetal aneuploidy. Not only does fetal fraction in the sample affect the ability to obtain a result, but also it may affect its accuracy.[23] Whether an inconclusive result is treated as screen positive or screen negative affects the overall detection rate (sensitivity) and false-positive rate (specificity) of the test. Typically, however, women with inconclusive results have been excluded from studies of test characteristics of cell-free DNA.

More recently, the performance of cell-free DNA screening for aneuploidy in an unselected lower risk obstetric population has been reported, which allows a better understanding

From the Department of Obstetrics and Gynecology, Center for Women's Reproductive Health, University of Alabama at Birmingham, Birmingham, AL.

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Corresponding author: Joseph R. Biggio Jr, MD. jbiggio@uab.edu

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of its performance in these women.[11,21,24-26] Although the sensitivity and specificity of cell-free DNA screening in the general population appear similar to that found in higher risk groups, given the lower prevalence of aneuploidy, the PPV is appreciably lower than in the high-risk women in whom this screening was investigated initially. For example, the PPV for a 40-year-old woman with an abnormal cell-free DNA screening result for trisomy 21 is 87%, but the corresponding value for a 25-year-old woman with the same result is only 33%. There are similar decrements in PPV for other aneuploidies, with the PPV decreasing even further for increasingly rare conditions.[27] Although the PPV for cell-free DNA exceeds that for conventional screening tests for all of the common aneuploidies, more recent data stress the importance of a clear understanding of screening test characteristics and the need for diagnostic testing before changes in pregnancy management, (eg, termination).

The study by Norton et al[28] compares the detection of chromosome abnormalities in the general obstetric population using sequential screening to the modeled detection of cell-free DNA screening for both common chromosomal abnormalities and all aneuploidies using parameters from published peer-reviewed literature. Importantly, the authors adjusted detection rates based on the frequency of "no result" reporting for each individual abnormality, instead of using only detection rates for women who were given a conclusive result. In addition, the investigators examined performance under 2 different management strategies: (1) treat "no result" as screen negative and (2) treat "no result" as screen positive. Conventional sequential screening allowed detection of nearly 82% of all chromosome abnormalities. In contrast, the modeled detection rate of cell-free DNA for all chromosome abnormalities was significantly lower, regardless of whether "no result" cases were considered screen negative or screen positive (71% and 77%), respectively. Moreover, treating the "no result" cases as screen positive raised the overall screen positive rate for cell-free DNA screening to approximately 4.1%, compared with 5% for sequential screening. Cell-free DNA detected mores cases of trisomy 21 and some sex chromosome aneuploidies, but fewer cases of trisomy 18, than sequential screening. Performance was similar for trisomy 13 and 45,X. The greatest benefit to sequential screening was its ability to identify 54% of rare chromosome abnormalities that are not currently detected with cell-free DNA screening. Interestingly, when these rare abnormalities are excluded, the detection rates for the common autosomal and sex chromosome aneuploidies are similar for cell-free DNA and sequential screening (71% vs 69%, respectively).

Although the modeled screening performance represents an aggregate of detection, false-positive, and "no result" rates for laboratories with published validation data and does not account for laboratory variation, these findings are important and should cause all obstetrics providers to contemplate the current and future directions of fetal aneuploidy screening. There is little debate that cell-free DNA screening can be a useful and accurate screening test for common autosomal and sex chromosome aneuploidies in singleton gestations. However, the issue becomes more convoluted when its (in)ability to detect other chromosome abnormalities is considered. Although the initial concept of screening focused on the detection of trisomy 21, this has evolved as the ability to detect other chromosomal abnormalities with the same panel of analytes expanded. The nonspecific nature of conventional screening tests for aneuploidy allowed for the serendipitous identification of a number of chromosome abnormalities not initially targeted by screening, which included the sex chromosome abnormalities, duplications, deletions, and other structural rearrangements. In fact, Norton et al[29] previously reported that, in higher risk women with an abnormal sequential screen, there remains a 2% residual risk of a chromosome abnormality, even with a negative cell-free DNA screening result. In the current study, because the prevalence of the common aneuploidies is lower and the relative proportion of rare abnormalities is higher in a younger population, the residual risk is even higher, approximately 2.6%.

With this in mind, obstetrics care providers should ask, "What is the real purpose and scope of screening for aneuploidy?" Is it to identify pregnancies that are at increased risk for a finite number of conditions that are relatively well-known and associated with distinct phenotypes, or is it to identify pregnancies that are at increased risk for any number of conditions that could be associated with adverse perinatal or childhood outcomes? If the ultimate goal is to deliver the most information and reassurance to pregnant women, then current cell-free DNA screening strategies may not be the best approach. Moreover, when we consider that the earliest form of prenatal screening was for neural tube defect detection, it is important to consider how such screening will be incorporated into the evolution of prenatal screening approaches.

Although there has been emphasis on the reported low false-positive rate of cell-free DNA screening and the potential reduction in the need for diagnostic testing, as noted earlier, these estimates often were based only on women who received a result.[30] Given the data from Pergament et al[20] regarding the risk of aneuploidy in the setting of a "no result," women who do not receive a result need further follow-up evaluation and counseling and consideration for diagnostic testing. When the patients with "no result" are incorporated into measures of screening performance, the specificity of cell-free DNA screening declines and approaches that of conventional screening.[27]

The present report by Norton et al[28] highlights important concepts that can better inform our counseling and medical decision-making. However, to truly assess the nuances and range of differences in screening performance between cell-free DNA and conventional approaches, which include factors that affect individual patient variation, large prospective study cohort studies in the general obstetric population are needed. This comprehensive data will allow an unbiased assessment of screening performance for the wide array of abnormalities that may be detected. Given the rapidity with which cell-free DNA screening is evolving,

obtaining these performance data as soon as possible is of considerable urgency. ■

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