Aneuploidy screening: Newer noninvasive test gains traction

Favorable results from the 2 studies reviewed here have prompted ACOG to recommend that cell-free DNA screening be discussed with all pregnant patients.

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PRACTICE CHANGER

Discuss cell-free DNA testing when offering fetal aneuploidy screening to pregnant women.[1,2]

STRENGTH OF RECOMMENDATION

A: Based on multiple large, multi-center cohort studies.

Bianchi DW, Parker RL, Wentworth J, et al; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370:799-808.[1]

Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med*. 2015;372:1589-1597.[2]

ILLUSTRATIVE CASE

A 28-year-old gravida 2, para 1001 at 10 weeks gestation presents to your clinic for a routine first-trimester prenatal visit. Her first child has no known chromosomal abnormalities and she has no family history of aneuploidy. She asks you which tests are available to screen her fetus for chromosomal abnormalities.

Pregnant women have traditionally been offered some combination of serum biomarkers and nuchal translucency to assess the risk of fetal aneuploidy. Cell-free DNA testing (cfDNA) is a form of noninvasive prenatal testing that uses maternal serum samples to conduct massively parallel sequencing of cell-free fetal DNA fragments. It has been offered to pregnant women as a screening test to detect fetal chromosomal abnormalities since 2011 after multiple clinical studies found high sensitivities, specificities, and negative predictive values (NPVs) for detecting aneuploidy.[3-6] However until 2015, practice guidelines from the American Congress of Obstetricians and Gynecologists (ACOG) recommended that standard aneuploidy screening or diagnostic testing be offered to all pregnant women and cfDNA be reserved for women with pregnancies at high risk for aneuploidy (strength of recommendation: B).[7]

CARE (Comparison of Aneuploidy Risk Evaluation) and NEXT (Noninvasive Examination of Trisomy) are 2 large studies that compared cfDNA and standard aneuploidy screening methods in pregnant women at low risk for fetal aneuploidy. Based on new data from these and other studies, ACOG and the Society for Maternal-Fetal Medicine (SMFM) released a new consensus statement in June 2015 that addressed the use of cfDNA in the general obstetric population. The 2 groups still recommend conventional first- and second-trimester screening by serum chemical biomarkers and nuchal translucency as the first-line approach for low-risk women who want to pursue aneuploidy screening; however, they also recommend that the risks and benefits of cfDNA should be discussed with all patients.[8]

STUDY SUMMARIES

CARE was a prospective, blinded, multicenter (21 US sites across 14 states) study that compared the aneuploidy detection rates of cfDNA to those of standard screening. Stan-

cfDNA can't detect neural tube or ventral wall defects, so women who choose this method should be offered maternal serum alpha-fetoprotein or ultrasound evaluation.

dard aneuploidy screening included assays of first- or second-trimester serum biomarkers with or without fetal nuchal translucency measurement.

This study enrolled 2042 pregnant patients ages 18 to 49 (mean: 29.6 years) with singleton pregnancies. The population was racially and ethnically diverse (65% white, 22% black, 11% Hispanic, 7% Asian). This study included women with diabetes mellitus, thyroid disorders, and other comorbidities. cfDNA testing was done on 1909 maternal blood samples for trisomy 21 and 1905 for trisomy 18.

cfDNA and standard aneuploidy screening results were compared to pregnancy outcomes. The presence of aneuploidy was determined by physician-documented newborn physical exam (97%) or karyotype analysis (3%). In both live and non-live births, the incidence of trisomy 21 was 5 of 1909 cases (0.3%) and the incidence of trisomy 18 was 2 of 1905 cases (0.1%).

The NPV of cfDNA in this study was 100% (95% confidence interval, 99.8%-100%) for both trisomy 21 and trisomy 18. The positive predictive value (PPV) was higher with cfDNA compared to standard screening (45.5% vs 4.2% for trisomy 21 and 40% vs 8.3% for trisomy 18). This means that approximately 1 in 25 women with a positive standard aneuploidy screen actually has aneuploidy. In contrast, nearly one in 2 women with a positive cfDNA result has aneuploidy.

Similarly, false positive rates with cfDNA were significantly lower than those with standard screening. For trisomy 21, the cfDNA false positive rate was 0.3% compared to 3.6% for standard screening ($P<.001$); for trisomy 18, the cfDNA false positive rate was 0.2% compared to 0.6% for standard screening ($P=.03$).

NEXT was a prospective, blinded cohort study that compared cfDNA testing with standard first-trimester screening (with measurements of nuchal translucency and serum biochemical analysis) in a routine prenatal population at 35 centers in 6 countries.

This study enrolled 18,955 women ages 18 to 48 (mean: 31 years) who underwent traditional first-trimester screening and cfDNA testing. Eligible patients included pregnant women with a singleton pregnancy with a gestational age between 10 and 14.3 weeks. Prenatal screening results were compared to newborn outcomes using a documented newborn physical examination and, if performed, results of genetic testing. For women who had a miscarriage or stillbirth or chose to terminate the pregnancy, outcomes were determined by diagnostic genetic testing.

The primary outcome was the area under the receiver-operating-characteristic (ROC) curve for trisomy 21. Area under the ROC curve is a measure of a diagnostic test's accuracy that plots sensitivity against 1-specificity; <.700 is considered a poor test, whereas 1.00 is a perfect test. A secondary analysis evaluated cfDNA testing in low-risk women (ages <35 years).

The area under the ROC curve was 0.999 for cfDNA compared with 0.958 for standard screening ($P=.001$). For diagnosis of trisomy 21, cfDNA had a higher PPV than standard testing (80.9% vs 3.4%; $P<.001$) and a lower false positive rate (0.06% vs 5.4%; $P<.001$). These findings were consistent in the secondary analysis of low-risk women.

Both the CARE and NEXT trials also evaluated cfDNA testing vs standard screening for diagnosis of trisomy 13 and 18 and found higher PPVs and lower false positive rates for cfDNA compared with traditional screening.

WHAT'S NEW

Previously, cfDNA was recommended only for women with high-risk pregnancies. The new data demonstrate that cfDNA has substantially better PPVs and lower false positive rates than standard fetal aneuploidy screening for the general obstetrical population.

So while conventional screening tests remain the most appropriate methods for aneuploidy detection in the general obstetrical population, according to ACOG and SMFM, the 2 groups now recommend that all screening options—including cfDNA—be discussed with every woman. Any woman may choose cfDNA but should be counseled about the risks and benefits.[8]

CAVEATS

Both the CARE and NEXT studies had limitations. They compared cfDNA testing with first- or second-trimester screening and did not evaluate integrated screening methods (sequential first- and second-trimester biomarkers plus first-trimester nuchal translucency), which have a slightly higher sensitivity and specificity than first-trimester screening alone.

Multiple companies offer cfDNA, and the test is not subject to Food and Drug Administration approval. The CARE and NEXT studies used tests from companies that provided funding for these studies and employ several of the study authors.

Although cfDNA has increased specificity compared to standard screening, there have been case reports of false negative results. Further testing has shown that such false negative results could be caused by mosaicism in either the fetus and/or placenta, vanishing twins, or maternal malignancies.[8-10]

In the CARE and NEXT trials, cfDNA produced no results in 0.9% and 3% of women, respectively. Patients for whom cfDNA testing yields no results have higher rates of aneuploidy, and therefore require further diagnostic testing.

Because the prevalence of aneuploidy is lower in the general obstetric population than it is among women whose pregnancies are at high risk for aneuploidy, the PPV of cfDNA testing is also lower in the general obstetric population. This means that there are more false positive results for women at lower risk for aneuploidy. Therefore, it is imperative that women with positive cfDNA tests receive follow-up diagnostic testing such as chorionic villus sampling or amniocentesis before making a decision about termination.

All commercially available cfDNA tests have high sensitivity and specificity for trisomy 21, 18, and 13. Some offer testing for sex chromosome abnormalities and microdeletions. However, current cfDNA testing methods are unable to detect up to 17% of other clinically significant chromosomal abnormalities,[11] and cfDNA cannot detect neural tube or ventral wall defects. Therefore, ACOG and SMFM recommend that women who choose cfDNA as their aneuploidy screening method should also be offered maternal serum alpha-fetoprotein or ultrasound evaluation.

CHALLENGES TO IMPLEMENTATION

cfDNA testing is validated only for singleton pregnancies. Physicians should obtain a baseline fetal ultrasound to confirm the number of fetuses, gestational age, and viability before ordering cfDNA to ensure it is the most appropriate screening test. This may add to the overall number of early pregnancy ultrasounds conducted.

Counseling patients about aneuploidy screening options is time-consuming, and requires discussion of the limitations of each screening method and caution that a negative cfDNA result does not guarantee an unaffected fetus, nor does a positive result guarantee an affected fetus. However, aneuploidy screening is well within the scope of care for family physicians who provide prenatal care, and referral to genetic specialists is not necessary or recommended.

Some patients may request cfDNA in order to facilitate earlier identification of fetal sex. In such cases, physicians should advise patients that cfDNA testing also assesses trisomy risk. Patients who do not wish to assess their risk for aneuploidy should not receive cfDNA testing.

Finally, while cfDNA is routinely recommended for women with pregnancies considered at high risk for aneuploidy, many insurance companies do not cover the cost of cfDNA for women with low-risk pregnancies, and the test may cost up to $1,700.[12] The overall cost-effectiveness of cfDNA for aneuploidy screening in low-risk women is unknown. JFP

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