Cell-free DNA screening blooms in expansion to low-risk pregnancies

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March 2017—Something about having the letters “DNA” in a test’s name may make the test seem like the last word, the key to a black-and-white, definitive diagnosis. That connotation has been problematic for cell-free DNA sequencing used for noninvasive prenatal testing, because the test is not intended or designed for diagnosis, but only for screening. It’s for that reason, in fact, that some maternal-fetal medicine specialists and clinical geneticists prefer to use the term “noninvasive prenatal screening,” with the acronym NIPS.

By either name, NIPS is on a roll. The cfDNA test, which detects common aneuploidies in chromosomes 13, 18, and 21 (Patau, Edwards, and Down syndromes), as well as sex chromosome aneuploidies, first became clinically and commercially available in the U.S. in 2011, says Diana W. Bianchi, MD, director of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. “Since then, it has been the fastest-growing genetic test in medical history. Well over 2 million women have undergone noninvasive prenatal screening worldwide—and that’s despite the fact that it’s not really considered standard of care yet; it’s considered an alternative option.”

Ted Snelgrove, chief business officer of the health technology company Counsyl, which offers a NIPS test, says the current growth in that testing is about 25 percent per year. “We anticipate the market in the U.S. to be between 1 million and 1.2 million tests in our sector.” He explains that projection by noting that of the 4.1 million pregnancies each year in the U.S. that come to term, about 3.3 million will be classified as average risk, while 800,000 will be classified as high risk. “Of the high-risk cohort, approximately 60 percent of them will receive NIPS. Of the average-risk cohort, we’ve estimated it’s closer to 18 percent.”

Snelgrove

The momentum for this kind of test volume is unusual, in Snelgrove’s view. “Usually, innovative tests like NIPS are introduced to physicians, who then offer it to patients, but in this case, patients seeking NIPS also drove rapid adoption. Doctors and insurers are just starting to come online with the idea of this screening for average-risk women. But the women themselves have been pursuing this.”

Counsyl was founded in 2007 to offer “clinically actionable genetic testing,” Snelgrove says, and added NIPS in late 2014 when it partnered with Illumina. “By and large, we’ve taken over their noninvasive prenatal testing. We’ve tweaked it to be a little more precise and to bring the cost down,” Snelgrove says.

Although NIPS-positive results must be confirmed by a diagnostic test like chorionic villus sampling or amniocentesis, retrospective studies have shown a significant decline in the number of these invasive procedures, particularly amniocentesis, during the period that NIPS has been available (Khalifeh A, et al. *Fetal Diagn Ther.* 2016;39[4]:292–296).

At the same time, the American College of Medical Genetics and Genomics notes, “Health care providers and patients have experienced marketing pressure, rapidly evolving professional practice guidelines, and confusion regarding the appropriate role of NIPS in prenatal practice.” Those forces helped spur the ACMG’s recent

publication (Gregg AR, et al. *Genet Med.* 2016;18[10]:1056–1065) of new standards for use of NIPS in the general obstetrical population (updating a 2013 statement on NIPS for high-risk pregnancies).

Dr. Bianchi's research is part of what has helped expand the use of NIPS in the general obstetrical population. She carried out a study in collaboration with Illumina (Bianchi DW, et al. *N Engl J Med.* 2014;370[9]:799–808) to address how sequencing of maternal plasma DNA compares with the standard of care, which is currently some variation of serum analyte or serum biochemical testing and nuchal translucency measurement through ultrasonography.

"We were asking: How does cfDNA compare to the standard of care in the same pregnant women? And what's the performance in a general obstetrical risk population? Our study showed that cfDNA performed significantly better than the standard of care in terms of positive predictive values: 63 percent in a low-risk population, compared with only 4.2 percent using current standard screening for all pregnant women," Dr. Bianchi says.

But the spike in orders for a test that has been available for barely five years came as a bit of a surprise to Dr. Bianchi. She credits social media, in part, for the increase. "It was almost a perfect storm. The test became available at more or less the same time that social media became active, so pregnant women were talking about it. In addition, this test, unlike prior genetic tests largely developed by academia and then transferred to industry, was intended for clinical use by the commercial sector from the very start. So there was the whole industry marketing piece as well."

The four leading U.S. companies offering NIPS use slightly different technologies for their tests, all of which are laboratory developed; none are approved by the Food and Drug Administration. "Two are similar: Both Illumina and Sequenom use whole genome sequencing. Ariosa Diagnostics, which offers the Harmony test, uses a targeted approach on a microarray, and Natera's Panorama test uses targeted sequencing employing single nucleotide polymorphisms. But there are dramatic differences in test failure rates among the different technologies," she says.

Some NIPS laboratories now offer screening for microdeletions, which has much lower positive predictive values, Dr. Bianchi points out. "The PPVs are generally somewhere between zero to five percent for many of the conditions being tested. So there is a concern that all of the gains we've made with testing for the common whole chromosome aneuploidies might be lost with the low PPVs for copy-number variant testing." Nevertheless, she expects that NIPS will eventually become a first-tier screen for common chromosome aneuploidies, if costs come down and if insurance will cover it.

When NIPS first came on the scene, it was primarily offered to high-risk patients—women age 35 or older at time of delivery, who had a positive maternal serum screening result, an ultrasound examination suggestive of trisomy, or who had a previous child or fetus with a chromosomal abnormality. "That was the bulk of the patients, and certain test providers would even refuse a sample if it did not come from a high-risk patient," says Charles M. Strom, MD, PhD, vice president of genetics and genomics at Quest Diagnostics.

But about two years ago, "OB/GYNs began to demand the test become available for all patients because it's so much better than maternal serum screening in terms of its PPV. They had to deal with maternal anxiety that surrounds an abnormal serum screening result, which occurs in about five percent of women. Among these women, the PPV was about 10 percent," Dr. Strom says. "You end up doing a lot of amnios, and the amnios are mostly normal." With NIPS, the PPV can be up to 80 percent, he says, "so instead of doing 100 amnios and having only 10 positives, you do 100 and have 80 positives. Clinicians were much happier with that [DNA-based] test."

Much confusion surrounds the PPV numbers, however, and Dr. Strom decided he needed to initiate a study. "I felt that the public and physicians in general needed to be educated on the difference between false-positives and PPV so they wouldn't over-interpret the test, and I also wanted to see how the test was performing in the real world."

As use of NIPS expands to the low-risk population, that will change the PPV of the test, because PPV depends on test performance and the prevalence of the disorder, he explains. "So if you're doing the test on a high-risk

population, the prevalence will be highest; it will be lower in low risk. So you'd expect the PPV to decrease." Clinicians need to be aware of what PPV is and how it's calculated, he notes, so they can inform their patients.

Dr. Strom's study was published in 2015 as "Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases" (Wang JC, et al. *Genet Med.* 2015;17[3]:234–236). He reported finding significant discordance between conventional cytogenetics as a reference standard and positive NIPS findings. "I was not surprised at all by that finding, given the sensitivities and specificities of the assays. I have to say: This is a phenomenal test. It is performing at a very high level. It's just looking at things with a very low prevalence. And when you're doing that, even if you have specificities of more than 99.9 percent, your PPV is still not that good."

There are also biological reasons for the PPV to be low—in particular with trisomy 13. "There's a phenomenon called confined placental mosaicism where the placenta has trisomy 13 but the fetus is normal, so you will get a false-positive for trisomy 13 with NIPS, but it's not the fault of the test." This occurs fairly commonly, Dr. Strom says. "One thing we've learned over time with this new technology is that maternal variants can lead to false-positives."

Maternal microduplications can also affect test results. "We didn't know about them before we started doing NIPS," Dr. Strom says. "It turns out that women [as well as men] have duplications of parts of their chromosomes that have no effect on their health; they are just there as excess baggage, if you will—until they have NIPS, and if they have these duplications on chromosome 13, 18, or 21, it has the potential to create a false-positive. Ninety percent of circulating DNA is maternal, so if you just have a little bit of the chromosome mutated, it can be enough to skew the whole test to make it appear that she's carrying a fetus with a trisomy." But knowing about these maternal microduplications "allows you to increase the sensitivity/specificity of the test," he says.

These factors are all the more reason, he adds, why clinicians should take a cautious look at NIPS datasets and their sources to appreciate the subtleties of the testing. "Noninvasive prenatal testing is a wonderful test, but it is not perfect yet. It is screening, and the PPV is getting up there, but even at 98 percent, that means there is a two percent chance the fetus won't be affected, and that's what we need to emphasize."

Dr. Gregg: "If the aim in any screening program is to have improved or maximal sensitivity, specificity, and positive and negative predictive value regardless of the population it's applied to, NIPS fits the bill because it works for both high-risk and low-risk patients."

Anthony Gregg, MD, president-elect of the American College of Medical Genetics and Genomics, believes use of NIPS for the general obstetrical population has been fully justified. "I can tell you if the aim in any screening program is to have improved or maximal sensitivity, specificity, and positive and negative predictive value

regardless of the population it's applied to, NIPS fits the bill because it works for both high-risk and low-risk patients," says Dr. Gregg, a professor in and chief of the Division of Maternal-Fetal Medicine, University of Florida. He also is finding he has many fellow advocates. "I think NIPS is increasingly recognized as the most efficient way to offer screening for the kinds of conditions that, at least right now, people are interested in having screening tests for."

Those conditions include not only trisomies 13, 18, and 21 but also sex chromosome aneuploidies. "Admittedly, patients are using this technology to help them identify gender at a very early age, before ultrasound does," Dr. Gregg says. "There are cases of sex chromosome aneuploidy for which it's possible that early information to families may allow for earlier treatment, for clinical observations, and for subsequent interventions they wouldn't otherwise have the opportunity to experience. But many of the conditions identified have a variable phenotype. So not all of them are relevant.

"That's why the ACMG suggests that patients engage in a careful conversation with their provider before simply checking the 'X''Y' box for gender determination," Dr. Gregg adds. This is one of the recommendations contained in a new policy statement the ACMG issued last year to address the expanding use of NIPS in the general obstetrical population (Gregg AR, et al. *Genet Med.* 2016;18[10]:1–5).

Microdeletions and microduplications were also addressed in the ACMG policy statement—in particular, a common microdeletion called DiGeorge syndrome on chromosome 22. NIPS, Dr. Gregg says, like all screening, requires careful discussion before checking all the possibilities the test can uncover. "Screening is voluntary. The patient can opt out of 22, DiGeorge syndrome, and other microduplication analyses. They can opt out of the sex chromosome analysis. So they have available to them opportunities to either learn more or not learn more, and of course they can walk away and not learn anything at all."

The specialty organizations, including the ACMG, the American College of Obstetricians and Gynecologists, and the Society for Maternal-Fetal Medicine, stress that patients should not rely solely on results from a NIPS test, that a positive screen must be confirmed with a diagnostic test. "On the other hand, if we look at the screening test that this one nudges off the table—the serum-ultrasound test in the first trimester or the ultrasound test in the second trimester—it's very clear that when this test is positive and when it's negative, it's much more likely to be a fact than with the other tests," Dr. Gregg says. "For example, NIPS has a 10-fold lower false-positive rate [.05 percent] and detection rate above 98 percent for trisomy 21 and trisomy 18."

The organizations also agree that all pregnant patients, not just high-risk patients, should be offered the diagnostic standard of care, which is chorionic villus sampling or amniocentesis before 20 weeks. Another standard contained in the ACMG policy statement is clear reporting of the metrics the organization believes are important: sensitivity, specificity, the percent fetal fraction, and positive and negative predictive value for those tests that were used.

"Most patients are very much interested in having screening tests and having the best possible technology applied to them. Statistically, most patients are negative," Dr. Gregg notes. "But it can get very difficult when results come back in, and how to convey information around results—what are next steps, and what a test result really means for that particular patient—is where medical knowledge really needs to ramp up a few degrees."

The general recommendation by a few groups is to interpret a "no-call" as a positive screening test, and offer diagnostic testing, not a repeat blood draw, Dr. Gregg says. "'No-calls' due to low fetal fraction occur more often in morbidly obese patients and when the sample is obtained too early in gestation. This can occur because of uncertain pregnancy dating. Samples should be obtained after nine or 10 weeks."

Arecently completed study performed by researchers at Women and Infants Hospital in Providence, RI, demonstrated that NIPS, once it is made available to average-risk pregnant women, can have a striking uptake rate. The laboratory at the hospital has been a leader in prenatal screening since the 1980s, and it provides serum/ultrasound screening to most pregnant women in Rhode Island and in many other states, says Geralyn Lambert-Messerlian, PhD, a professor of pathology and laboratory medicine at Brown University. Her work in the

U.S., focused on bringing the hormone inhibin A into prenatal screening, is behind many of the protocols now used. “We were involved in the groundwork that led to prenatal screening today,” she says.

The hospital’s “DNAFirst” study arose from that experience. “When we learned about cell-free DNA technology and what it promised to do in prenatal screening, we were very interested in learning more about it. Professional societies were recommending that cfDNA only be used for high-risk women; they had concerns about how cfDNA would work in the general population. So we decided to study exactly that.”

Many things are different about the general obstetrical population, Dr. Messerlian notes. “One of the first is that high-risk women often have extensive conversations with specially trained genetic counselors about their risk of aneuploidies and test performance, while the general-population patients are most often seen by primary care obstetrical providers in a busy environment. There is not time for as much detail in the conversations about aneuploidy risk and screening.”

“We called the study DNAFirst,” says Glenn Palomaki, PhD, “because DNA is the first screening test women will be offered for Down syndrome, trisomy 18, and other common trisomies.” Dr. Palomaki, assistant director of the Division of Medical Screening and Special Testing at Women and Infants Hospital, was a speaker in a recent AACC webinar on cell-free DNA-based prenatal screening in the general pregnancy population.

“DNAFirst was a demonstration project. As far as we know, it’s the first study of cfDNA in a true general pregnant population where the results were used in clinical decision-making and education was provided by primary obstetrical care providers,” Dr. Palomaki said in the webinar. “DNAFirst testing was offered at no charge to the patients or their insurance companies so we could simulate what it might be like screening a couple of years from now, when the price may have dropped and/or insurers are covering it for testing in the general population. This would be similar to the current status for those doctors or midwives who are offering integrated or combined screening now. The concept was ‘Let’s switch over to cfDNA as a primary screen and we’ll see how it works in the real world.’” When approached by the DNAFirst project staff, five of the seven large practices in Rhode Island agreed to participate in offering NIPS to their general obstetrical patients.

The result was an across-the-board 18 percent increase in screening, Dr. Palomaki said. “Ninety-one percent of women chose optional trisomy testing, which included reporting of the fetal sex. We heard from women and providers that they liked learning the fetal sex early—at 11, 12, or 13 weeks—instead of waiting for an ultrasound.” In addition, “for the common trisomies 21, 18, and 13, the test worked quite well. It had high predictive value and a false-positive rate of only four pregnancies out of 2,681, or less than two per thousand” (Palomaki GE, et al. *Genet Med.* Epub ahead of print Jan. 12, 2017. doi:10.1038/gim.2016.194). So he believes that “we now finally have brand-new evidence that primary obstetrical providers in the U.S. can offer cfDNA to the general population as part of routine practice and they are reasonably happy doing it.”

Says Dr. Messerlian: “We found that patients were able to learn about the benefits and limits of the test through their primary care providers. Even though the obstetricians may not be at the same level of expertise as genetic counselors, they were as good at educating patients about the basics of cfDNA screening as they were with conventional serum screening, if not better.”

Another discovery was that women were eager to accept cfDNA screening even more than serum testing, and they seemed to understand the test a little better, Dr. Messerlian says. “I think in part it’s the simplicity of it. Sequential serum screening is very complicated—you have to do an ultrasound and have a couple of blood draws—while cfDNA is one time, one blood sample.”

Whether the positive results of the study will carry through to clinical practice remains to be seen. “We’ll see as time goes on what the actual uptake is. And a lot of that is complicated by the cost of the test and lack of insurance reimbursement.” Insurers typically reimburse for the test for high-risk women but are only now starting to expand to the general population. “That hadn’t been true until 2016, when ACOG and ACMG issued guidelines that were more liberal regarding testing of low-risk women,” Dr. Messerlian says.

Until the coverage increases, even though many pregnant women are starting to ask for cfDNA, she expects many women will choose serum screening, which is less expensive than cfDNA, so they don't have to pay for testing out of pocket.

Dr. Messerlian

Dr. Messerlian believes clinicians understand the cfDNA test has high sensitivity as well as high specificity. But one difference between the available assays does create a disadvantage for cfDNA: a test failure rate that ranges from one percent to five percent. "That's really where the companies differ. Some have higher failure rates than others, and much of that is inherent to their method." Some women also need to have both serum testing and cfDNA testing. "For example, cfDNA testing doesn't screen for neural tube defects. And so patients who want such screening have to come back for us to draw another blood sample to look for neural tube defects in the second trimester."

Test failure rates and the biases of cfDNA testing are faults cited in a recent "Point/Counterpoint" article in the November 2016 issue of the *American Journal of Obstetrics & Gynecology*. On one side are Drs. Messerlian and Palomaki and James Haddow, MD, all of the Department of Pathology and Laboratory Medicine, Women and Infants Hospital; on the other side are Mary Norton, MD, of the UCSF Department of Obstetrics, Gynecology, and Reproductive Sciences, and Miriam Kuppermann, PhD, of the UCSF Department of Epidemiology and Biostatistics. Drs. Norton and Kuppermann draw a contrast between sequential testing, "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,'" and cfDNA, which they contend is a "a very precise test for 3 aneuploidies" that requires that women be offered additional screening for other birth defects. The Women and Infants authors disagree that serum-ultrasound screening can detect more chromosome abnormalities than cfDNA, and they also point out that all chromosome abnormalities are not of equal health significance.

Aside from pockets of people who are holding on to traditional first and second trimester screening, there is little debate within the ACMG itself about the value of the screening, Dr. Gregg says. "And my understanding is that a large number of third-party payers are recognizing this in an average-risk population, not just a high-risk population. The test has the potential to reduce imaging costs in the second trimester, it has the potential to reduce anxiety, and it has the potential to reduce the number of invasive tests performed because of the lower false-positive rates. So increasingly we're seeing that third-party payers are recognizing this in the context of clinical utility."

Ted Snelgrove of Counsyl describes other important factors in the U.S. system of reimbursement for clinical laboratory testing that will soon affect the uptake of NIPS. Over 40 percent of pregnancies are to women who are under Medicaid, he says. "So Medicaid is the largest single payer in the system for pregnancy in general, and obviously they're seeing a lot of claims for genetic screening."

There is tremendous variability now across the country in Medicaid policy coverage and payment rates. "Some states have no policy at all, and pay zero. Some have very generous policies that pay over $600 or $700. It varies a lot because Medicaid doesn't have its own pricing mechanisms. At the national level, Medicaid has always relied on Medicare for pricing, and Medicaid pays 80 percent of the Medicare payment."

Last year, Snelgrove says, Medicaid asked Medicare to price noninvasive prenatal screening, and Medicare set the price around $800. "So if Medicaid uses the guidance from Medicare as its baseline across the country, we could

see pricing for NIPS become a lot more standardized. I'm anticipating that over the next year we should see a lot less variability."

Coverage provided by the other big payers, mostly private health plans, is evolving. "Reimbursement for this screening has been a bit of a moving target with these insurance plans because it's so new," Snelgrove says. "It just popped up a few years ago, and they're still figuring some of this out." Large plans now cover average-risk pregnant women to some extent, he says, but several payers are reviewing their policies and are likely to make changes in the next year to broaden coverage for screening.

The shift to the average-risk market has created economies for the diagnostics companies, Snelgrove says. "The opportunity to speak to all women who are becoming pregnant simplifies the marketing at some level, because basically the testing is the same. There was only a sort of artificial distinction before between high and low risk." Speaking with doctors is also simpler, he adds. "We are spending less time trying to get them to understand risk stratification and more time talking about what the results mean and how to interpret the results." That the NIPS tests are noninvasive has "absolutely" brought a new level of interest, he says.

Whichever type of testing is offered, prenatal testing in general remains a sensitive topic, Dr. Messerlian says. "However, I think people need to keep in mind that it's an optional test for women, and not every woman chooses to have it. Women who have that information about their pregnancy make a lot of different choices. Not everybody terminates the pregnancy; it can often be helpful to know you are carrying an affected child in order to prepare the home or the care of the child." In the U.S., Dr. Bianchi says, 40 percent of women carrying fetuses diagnosed with Down syndrome choose to continue their pregnancies.

The sequencing technology employed in cfDNA testing has a number of potential uses in many areas of health care, Dr. Messerlian says, including cancer, transplantation and in vitro fertilization protocols, and research she is conducting is exploring those possibilities. At the moment, cfDNA isn't mainstream clinical care yet, but "I think ongoing research will expand not only the use of this technology in prenatal care but also in many areas of health." [hr]

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