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### Noninvasive Prenatal Testing: Navigating Through Changing Currents

#### Announcer:

This is CME on ReachMD. The following activity, titled *Noninvasive Prenatal Testing:*

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Your faculty is Dr. Lee Shulman, professor of obstetrics and gynecology and reproductive genetics at the Feinberg School of Medicine at Northwestern University in Chicago, Illinois.

#### LEE SHULMAN:

Hello, everyone, and welcome to this presentation on noninvasive prenatal testing and navigating through changing currents.

So, for today's objectives, we're going to look at a variety of guidelines that have been produced over the last two to three years that discuss how to and in which situations do we use circulating cell-free nucleic acid technology or NIPT (noninvasive prenatal testing) to screen women for fetal chromosome abnormalities. We're going to talk about the benefits and disadvantages of conventional or traditional fetal chromosome aneuploidy screening tests, and, in that way, compare them to current molecular-based technologies, also look at the evolving role and increasing use of NIPT in a general-risk or a non-low-risk population, and really hopefully give you some pointers on how to incorporate this technology into your practice.

Let's go over an overview of prenatal aneuploidy screening. First and foremost, several years ago, the American College of Obstetricians and Gynecologists released several guidelines that talked about the importance of offering both screening and diagnostic options to your patients, and so when we talk about diagnostic testing, we're talking about amniocentesis and CVS. I hate using the term "gold standard," but clearly these two tests provide us with a thorough, comprehensive, and accurate assessment of fetal chromosomes as well as other genomic and Mendelian disorders. Screening does not provide us with that comprehensive nature. Screening is a risk adjustment process, and I think one of the issues that a lot of clinicians have is that they frequently conflate screening and diagnostics. They're not. A screening test will never tell us normal or abnormal. A screening test will give us an adjusted risk, maybe a very low adjusted risk, maybe a very high adjusted risk, but an adjusted risk notwithstanding, and clearly all of the professional societies recommend that, particularly in a woman who has a positive screening result, be offered some sort of diagnostic testing.

So, when it comes to screening, we have lots of options, lots of algorithms. The more conventional, traditional algorithms look at serum biomarkers like alpha-fetoprotein, beta hCG, also ultrasound markers like nuchal translucency measurements. More recent screening algorithms incorporate, again, circulating cell-free nucleic acid technology or NIPT,

Despite the perception that diagnostic testing carries with it a marked increased risk for fetal loss, in fact, both CVS and amniocentesis, when done by trained individuals, carries a very small negligible risk of pregnancy loss, and then we have, again, first and second and even third trimester diagnostic tests. Chorionic villus sampling can be performed in the late first trimester, amniocentesis in the second and third trimesters. As I mentioned earlier, screening tests assess risk, adjust risk, and primarily identify pregnancies at increased risk of the common chromosome abnormalities, trisomy 21 or Down syndrome, 18, and 13, and in some instances, some of the tests also evaluate for sex chromosome aneuploidies and provide a sex prediction, and as you all know, there are not only first and second trimester screening tests but combinations of tests that involve first and second trimester measurements. We recognize, I think, all of us who provide obstetrical care, that as a woman gets older, her risk of having a pregnancy affected with a chromosome abnormality

increases. However, despite that very well recognized association, it's important to understand that all pregnant women are at risk for fetal chromosome abnormalities and, in fact, 80% of births that occur in women do occur in women under the age of 35, and, in fact, the majority of children who are born with Down syndrome, trisomy 21, are born to women who are under the age of 35.

, amniocentesis has been available since the 1970s. It's classically performed between 15 and 20 weeks. Initially, maternal age was really the major determining factor. However, there have always been others from an increased risk for neural tube defects by history or by serum screening with maternal serum alpha-fetoprotein testing, ultrasound abnormality suggestive of fetal chromosome abnormality, as well as other genetic disorders like cystic fibrosis, sickle cell disease, Tay-Sachs, etc. Again, once serum screening became available in the 1980s, abnormal serum screening outcomes became an indication and perhaps became the most common indication for considering amniocentesis. , I mentioned procedure risks of being low. Procedure-related miscarriage rates are somewhere, again, in most busy centers between 1 in 1,000 to 1 in 1,600 over the baseline. Obviously, we can get some leakage of fluid membrane rupture, but in the vast majority of cases, this usually seals on its own without intervention, and a very, very small risk of infection and bleeding.

A more recent test, one that is done in the first trimester, is chorionic villus sampling. It's been available since the 1980s. It's again performed in the late first trimester, early second trimester. It has the same indications as the amniocentesis except for the neural tube defect indication. We typically do this one of two ways depending on where the placenta is located, either transabdominally for an anterior placenta, or a transcervical placement of a catheter for a posterior placed placenta, and again, the risks are about the same, 1 in 500, 1 in 1,000 over the baseline. The reason why this seems to be slightly higher, , is not because of the procedure-related risk but rather because when you do a procedure on a 10-11 week pregnancy, the a priori baseline risk of fetal loss, of miscarriage, is higher than when a procedure is performed at 15 or 16 weeks when the a priori or baseline risk is considerably less. So, when, in fact, we do look at CVS and amniocentesis, there was, absolutely no statistical difference between CVS and amniocentesis loss rates, and I would say for those centers that are able to perform both and perform a lot of both procedures, you would likely find no difference in the risk of loss after CVS compared to after amniocentesis.

we're going to start talking about screening strategies, that there're an awful lot of algorithms out there. We have first trimester screening, either by biomarkers, with or without nuchal translucency measurement. We've got second trimester measurement with quad test or even the Penta test, looking at biomarkers, and we're able to combine measurements in the first and second trimester, whether it's a fully integrated screen, a serum integrated screen, a sequential screen. depending on the laboratory that's available to you, and what you're most comfortable in performing, these tests do provide a higher level of detection and a lower level of what people call a false positive, meaning a positive screen not associated with a chromosome abnormality, and then we have what we call a contingent screen where you reflexed a second trimester screening of your first trimester screen is not clearly positive or negative, so lots of options there. Again, it's probably best to discuss this with, first, your laboratory that may be providing this service to you as well as maternal-fetal or genetic professionals that you may be referring patients to.

So, let's take a look at trisomy 21. When we take a look at the oldest of the screening, meaning the second trimester multi-marker screen, whether it's a triple screen or a quad screen, we see that the detection rate for trisomy 21, and that's really critical to understand, we're talking solely about trisomy 21, or Down syndrome. We are not talking about all chromosome abnormalities, we are not talking about fetal anomalies, and far too often, physicians and patients not so much confuse but talk about trisomy 21 as though, if that's negative, everything's going to be fine. Trisomy 21 is but one chromosome abnormality that can affect a fetus and a newborn. It is not a statement that discusses all chromosome abnormalities, all fetal abnormalities. It is specific to trisomy 21 and trisomy 21 alone. So, the conventional testing developed in the 1980s detected about 80% of fetuses with Down syndrome. It did that by offering patients, 5% of patients, an amniocentesis, again, the screen positive rate, and the non-detection rate was 19%.

. The sequential screen provides a 95% detection rate with a screen positive rate also about 5%; the contingent, a slightly lower detection rate but a considerably lower screen positive rate. So, we have been able to use conventional biomarkers to markedly improve the ability to detect fetuses with trisomy 21. However, to state again, that this is solely related to trisomy 21 and no other fetal abnormalities.

So, what are the limitations with conventional prenatal screening algorithms? You know, I will say that the biggest limitation is a lack of understanding by our patients about what this screening does and, more importantly, what it doesn't do, and because of that, the need for appropriate counseling by obstetrical providers or a genetics or maternal-fetal specialist to ensure that the patient is choosing a test that they understand what information is going to be provided and really what information is not going to be provided. Again, the accuracy of screening outcomes, a lot of times clinicians confuse positive predictive value with sensitivity. A 95% detection rate of fetal Down syndrome, that's not a 95% positive predictive value, and, in fact, the positive predictive value with these more conventional algorithms in the range of about 3-4%, meaning only 3-4% of positive screens are going to be associated with an actual case of fetal trisomy 21.

So, let's spend most of the time that we have left on talking about cell-free nucleic acid or NIPT.

this is isolated cell-free nucleic acid from maternal blood. It is mostly derived from the placenta,. Even though the detection rates for fetal trisomy 21 are over 99% with very low false positive rates, those same numbers don't apply to the other chromosomes that can be

evaluated by NIPT, , NIPT is not as comprehensive or as accurate as a diagnostic test is for other chromosome abnormalities, other genomic abnormalities, , NIPT is not right now applicable for the evaluation of Mendelian or genetic disorders like cystic fibrosis, etc. .

I will start off by saying there has never been a head-to-head trial that has evaluated these particular technologies, so I am not going to be able to answer for you which technology is better than the other. Just suffice it to say that, based on which product you may be using, the risk assessment is likely going to be different than another laboratory's product.

So, we've got sequencing or what used to be called massive parallel sequencing, SNP or single-nucleotide polymorphism, or a microarray-based analysis, the detection rates, the so-called false positive rates in the published information on these technologies, are similar, what's important, instead of going through the technological differences, is really to understand what this technology does. It, in a sense, does not evaluate fetal DNA.

What it's looking for is r changes in the percentage of particular chromosome-specific nucleic acid, and so the best example here is, in fact, looking for Down syndrome, trisomy 21, in the fetus, and what you have to sort of embrace in order to best understand this is that a man, a woman, who has two chromosome 21s has approximately a 1.38% of their nucleic acid being 21-specific, and what these technologies do and what they're able to identify is if that woman, who herself has two chromosome 21s, is carrying a fetus with three chromosome 21s, then the percentage of 21-specific nucleic acid in her bloodstream, of which 10%, and this is the first thing you need to embrace, 10% of the cell-free nucleic acid in her bloodstream is fetal in nature, that her percentage of 21-specific, if she's carrying a fetus with trisomy 21, jumps from 1.38% to 1.44%, and that's a very small difference in percent that is now able to be consistently and accurately evaluated through the technological advances, There are slight differences in what each test offers as far as a clinical assessment. Some will do twins, some won't do twins. Some will evaluate the pregnancy for triploidy, and again, some of them offer microdeletion testing. Again, this is an important thing for you to discuss with your partners in your office, with your maternal-fetal specialists and genetic specialists that you are using in consultation for your patients, and really what kind of service do these companies provide your office. All of these issues go into your decision on who do you use as an NIPT provider.

we find very similar capabilities of these technologies to evaluate the pregnancies for trisomy 21, 18, and 13. Now, most of that early work were in women who were 35 and over or women who had an abnormal ultrasound. Clearly, that is a relatively small percentage of the overall obstetrical population. Mary Norton, in 2015, did the first study looking at NIPT in low-risk patients, and what she found is, while clearly NIPT in low-risk patients provided considerably better detection rates than conventional testing, , as a woman gets younger, her risk gets less, and the positive predictive value goes down, not because it doesn't work as well, again, positive predictive value is a mathematical process, and, in fact, if you are evaluating a population with a lower likelihood or a lower frequency of these problems, then missing one is going to have a more profound impact on positive predictive value than, say, in a higher risk population.

but regardless of that, NIPT clearly performs better than traditional screening in detecting the most common chromosome abnormalities with a considerably lower "positive screen rate" than we find with conventional assays, but with that, again, clearly issues with NIPT. It is a screening test. What will this screening test offer information to your patients? What won't it reveal to your patients? when I give this talk, I make the following statement, is that NIPT screening algorithms are never wrong. the reason why I say that is, let's say you get an NIPT result, although you'll never get an NIPT result of this number, but let's say it adjusted the risk for fetal Down syndrome in Mrs. Smith to 1 in 1,000,000, and it turns out that her pregnancy does have Down syndrome. Well, clearly the NIPT was wrong, and the answer to that is, no, the NIPT was not wrong. NIPT provides a numerator and a denominator. There was still a 1 in 1,000,000 risk. Unfortunately, Mrs. Smith was that 1, and while the prediction of her Down syndrome risk was not accurate, the screening outcome was not wrong. If your patients want something to know whether their fetus is normal, has a normal chromosome complement or an abnormal chromosome complement, there is no screening test that will provide that information to your patient. Only a diagnostic test can provide that kind of information to your patient. So, clearly, counseling is needed so that patients understand what they're doing before they get it done and what is the implication of the result afterwards.

What is the role of nuchal translucency screening? at our center, we do not routinely offer nuchal translucency screening to women having NIPT primarily because the nuchal translucency measurement is no longer part of the Down screening algorithm, but I still believe that there's great importance of an early ultrasound assessment looking for multifetal pregnancies, fetal demise, gross abnormalities even in the early stage. We talk about the extent of NIPT screening, and it's again important that patients know that this is solely related to the common chromosome abnormalities and nothing else, and, again, the role of soft markers, how does NIPT evaluate those pregnancies, multiple gestations. Some of the labs offer twin assessment; some don't. Clearly, I would recommend that a triplet pregnancy or higher not be offered this or any other screening algorithm.

how do we integrate this? we look at limitations, and the limitations are when we take a look at reported chromosome abnormalities, while trisomy 21 in that late first trimester pregnancy is the most common, there are others that are not evaluated with even the more expansive screening algorithm products that are out there, so, again, understanding, and hoping the patients understand, the limitations of this screening approach. Clearly, fewer limitations than we had with more conventional screening assays. Again, this is placental tissue and not fetal tissue, so we have all the issues that we have recognized with CVS. While they don't typically impact clinical assessment of CVS, they can wreak some havoc here because we don't have the filter that we have the CVS. We don't have a

cytopathologist or a cytogeneticist evaluating that chorionic villus tissue. So, confined placental mosaicism, demised twin, I think it's important to recognize the relatively high frequency, I don't want to say high, but especially amongst sex chromosome positive results with NIPT, of there being maternal mosaic abnormalities, obviously not enough to prevent pregnancy, but it may be impacting placenta or even the fetus herself/himself, and then maternal malignancy has been detected with this technology.

There are some companies who talk about fetal fraction as though it is the most important aspect of this. There's a lot of debate with that. Some companies use fetal fraction as an arbiter whether or not they're going to release a result. Some other companies do not. Clearly, the higher the fetal fraction, the easier it is to get an accurate assessment, and fetal fraction, if too low, may result in either an uninformative or an incorrect assessment.

So, when we do have that no call, the one thing we recognized early on was that these pregnancies, for whatever reason, were associated with a much higher risk for fetal chromosome abnormalities. Mary Norton had a six-fold increase for aneuploidy. Gene Pergament, in his earlier study, had a two-and-a-half-fold increase risk for aneuploidy amongst pregnancies with an uninterpretable or no call NIPT, and when we take a look at the Kaiser experience, we again find a considerable increased risk for chromosome abnormality. over 4,000 pregnancies assessed. No results were in 2.5%. 63 were redrawn. There were still 26 no call, and of all the no call, there was no final result in 65 of the over 4,000 initial patients, , we again see a 14% risk of chromosome abnormality in these 65. That's an inordinately high number considering that the risk of chromosome abnormality in a woman who is 35 years old is 0.5%, so a very, very high number of chromosome abnormalities in pregnancies in which a no call or a no final result was obtained by NIPT. I mentioned earlier the issue of positive predictive value, the clinical utility. It is important to un-confuse ourselves about this.

conventional screening algorithms have approximately a 3-4% positive predictive value. NIPT has about an 80%, on average, positive predictive value for trisomy 21, about a 60% positive predictive value for trisomy 18, about 40-50% for trisomy 13, and about somewhere between 10 and 25% for X and Y chromosome abnormalities, and obviously with positive predictive value, the lower the prevalence in the population, again the lower the positive predictive value, not because it doesn't work as well, just because it is a lower prevalence and, therefore, a higher percentage lost with any one or two cases that are not accurately assessed.

Clearly, NIPT far outperforms conventional screening protocols in high-risk, in low-risk, in all-risk patients. It is always a better assessment for the common chromosome abnormalities than any of the first or second or combination screening algorithms that I mentioned earlier. Now, we have guidance from our professional societies. The ACOG-SMFM Practice Bulletin #163 that was published in 2016. , basically talking about how this is screening; it is a risk adjustment process; a negative test does not ensure an unaffected pregnancy; a positive test should be referred for further counseling and be offered diagnostic testing; what they stated here, "The no call result should prompt genetic counseling and an offer of diagnostic testing," and again, "Cell-free DNA does not replace the accuracy in diagnostic precision of prenatal diagnose testing," and, "For now, routine screening for microdeletions should not be performed," mostly because we really don't have good clinical validation studies for these particular assays. This was reaffirmed in 2018, and added to that was that NIPT may be used in low-risk populations understanding its limitations and the continued lack of recommendation for use for evaluating fetuses with microdeletions.

The American College of Medical Genetics went a little bit further, saying that it could replace conventional screening. The College of Medical Genetics does not recommend screening for chromosome abnormalities other than 13, 18, 21 or to screen for genome-wide CNVs (copy number variants) microdeletions, and again, they recommended NIPT results indicated a high-risk or a screen positive result, not normal, not using the words normal or abnormal.

Again, false positive results are a possibility. It's not diagnostic, and again, no call due to fetal fraction should be offered further counseling and diagnostic testing, and it's somewhat of a difficulty for us. We deal with a high number of patients who are obese or morbidly obese, and clearly fetal fraction goes down in cases of obese women, so there is that challenge. What I would strongly recommend is that if you're going to redraw it, fine. Don't try a different laboratory. And if the recall is a no call, a third draw is not going to help things out at that point. I mentioned briefly how we have gone forward in the field of cell-free nucleic acid. We were the second site in the world to offer it clinically in November of 2011. At that point, it was just screening for trisomy 21. we're now looking at the capability, of screening for genomic deletions or duplications anywhere from seven megabases or larger. So, this technology continues to expand. Work continues in trying to apply it to not only further applications in the prenatal and obstetrical population but potentially looking at its applications in the nonpregnant, in the male population, whether it be cancer or other disease detection, For at risk of repeating myself again, clearly NIPT far outperforms older conventional assays. That doesn't mean we get rid of those older assays. Cost, application, etc., all has a role in what patients choose. Clearly, we can use NIPT in low-risk patients. Whether or not insurance will cover it is really based on each individual patient. NIPT is not a substitute for diagnostic testing. First trimester ultrasound remains important for diagnosing fetal structural abnormalities and other genetic disorders. Patients should be informed of all their options and their advantages and disadvantages. In the case of an ultrasound abnormality, most of my colleagues, and myself included, strongly encourage the offering of diagnostic testing. NIPT offers great potential, but like with everything that we do in our practices, we need good validation studies. We have them for the common chromosome abnormalities for some of the newer applications that are starting to be offered.

Announcer:

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