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Diagnostic Value of Digital Estimates of Trophoblastic Mosaicism in Genome-Wide Cell-Free Fetal DNA Screening

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Guest: Dr. Geert Martens is a board-certified clinical pathologist at the AZ Delta General Hospital in Belgium.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Beginning with the development of maternal serum screening for Down syndrome in the 1980s, researchers have continually worked to improve testing to maximize detection of chromosomal abnormalities while minimizing the number of false-positive results. The most recent advance, cell-free fetal DNA testing, offers vastly superior sensitivity and specificity relative to conventional screening for common aneuploidies, and also permits detection of rare abnormalities in other chromosomes. This comes at a price, though. Rare aneuploidies often are confined to placental tissue, while the fetus is unaffected. But typical cell-free DNA assays cannot distinguish between fetal and placental DNA. This means that inclusion of rare aneuploidies in prenatal screening can increase the false-positive rate, leading to increased performance of invasive procedures that could potentially harm the developing fetus.

A new research article, appearing in the October 2025 issue of *Clinical Chemistry*, describes a potential solution to this problem. Estimation of trophoblastic mosaicism, determining whether the aneuploidy is confined to the placenta or present in both placenta and fetus, can help inform post-test counseling, with the ultimate goal of reducing unnecessary invasive testing. In this podcast, we are joined by the article's senior author. Dr. Geert Martens is a board-certified clinical pathologist at the AZ Delta General Hospital in Belgium, specializing in molecular assays and liquid biopsy testing and oncology and prenatal screening. So, Dr. Martens, can you comment on the advantages of non-invasive prenatal testing, or NIPT, over conventional screening?

Geert Martens: So NIPT has two advantages. First of all, its sensitivity is much higher than the conventional biochemical screening or the old-school screening. NIPT detects trisomy 21 cases with a sensitivity of over 99%, much higher than the 90% sensitivity of old-school screening. But there is a second key advantage, and that's a huge deal. The false positivity rate

of NIPT is dramatically lower. Only 0.1% of all NIPT assays turn out false positive, which compares very well as compared to the 5% of old-school combined screening. Now, this is really critical because NIPT is a screening test. It is not a diagnostic test. So, any high-risk result that comes out of the NIPT needs to be confirmed with an invasive test such as amniocentesis. So, a lower false positive rate means that we can drastically reduce the number of women who need this procedure from about 5% to just one in thousands.

And so in countries like Belgium, where I work, and the Netherlands, both countries that adopted universal NIPT screening in first line, we've witnessed quite a dramatic drop in the number of amniocentesis procedures. And that's great because an amniocentesis is not a trivial procedure. It carries a risk for the mother and also the baby. It is also, of course, a source of lots of anxiety for the expectant parents, and it is a procedure that also has, of course, its financial cost for the system.

Bob Barrett: Now, your study uses a whole-genome NIPT method, which can look beyond the common trisomies and detect sex chromosome abnormalities and other rare anomalies. What is the impact of finding these additional issues? Does it truly improve screening for patients?

Geert Martens: Yeah, that's a great question, because expanding the scope of screening evidently has benefits, but it also entails quite some challenges. Let's start with some benefits. Our study was the first to systematically report on the screening of sex chromosome abnormalities. Some examples are the Turner syndrome in girls, and the Klinefelter syndrome in boys. And these are not rare because they occur roughly in one in thousands of pregnancies. And so we found that the test which we investigated performs quite well with a true positivity rate somewhere between 50 and 90%.

So while screening for these conditions, it remains a personal, ethical choice, but at least our data now show that the test is accurate enough to serve as a screening tool, and so any abnormal result is still, let's say, recommended to be followed by an amniocentesis, but at least this provides to the parents relevant information, allowing them, but also the doctors around them to prepare for such a child and provide supportive care during the childhood of this baby.

Now, there are some other chromosomal defects for which the genome-wide screening has clearly benefits, such as very small chromosome defects, and we call them microdeletions. And so we show that the test achieves an acceptable performance with around 40% of the abnormal NIPT results that are ultimately confirmed in the fetus. And so many of these microdeletions can have deleterious consequences for

the child. So, it is vital information allowing parents and doctors to prepare with, for instance, also the help of genetic counseling to see the possible impact of such an anomaly.

I also talked about downsides. And so, this is where it gets complicated. There is one specific category of chromosomal abnormalities that is very problematic. And these are called the rare autosomal trisomies, or RAT. Basically, this means a trisomy of any other chromosome than chromosome 21, 18, and 13. And so while each of these rare autosomal trisomies on itself is rare, together as a group, they are quite common. About three in thousand women who get a genome-wide NIPT will receive such a high-risk result. And so to put that in perspective, that is just as common as a high-risk result for Down syndrome. So, if we systematically report these findings, this has quite a huge impact.

And so now what is specifically the problem of these rare autosomal trisomies? Our study just confirmed what some other large studies, like the Dutch TRIDENT-2 study, have shown. That is that only 6% of these rare autosomal trisomies are actually confirmed in the fetus. So the other 94% of the cases, the anomaly is not confirmed in the fetus, although it is present and limited to the placenta. So that means that if we send every woman with a rare autosomal trisomy result to amniocentesis, we are causing quite a lot of stress, cost, and potential harm with a limited benefit.

So our proposal, and discussed in this *Clinical Chemistry* study, is to be smarter about this. Based on the pooled data from our study and previous large-scale studies, we can now just distinguish between low-risk and high-risk rare autosomal trisomies. And so we recommend that only the high-risk ones that are frequently confirmed in the fetus, like a trisomy of chromosome 2, 12, 14, 15, 16, or 22, should automatically trigger an amniocentesis. For all the other rare autosomal trisomies, we would, let's say, consider a more conservative approach with a more intensified ultrasound monitoring of the growth of the baby, but without necessarily performing an amniotic puncture.

Bob Barrett: Doctor, your study's central finding revolves around what you call digital estimates of trophoblastic mosaicism. This sounds technical. Can you break that down for our listeners and explain how this new approach can make NIPT better?

Geert Martens: Yeah, that's indeed the key novelty, I think, of our paper. And indeed, at first it sounds quite complex, but the core idea is actually quite straightforward. So, if you think about the placenta, it's a fascinating organ. It is the interface between the growing baby and the mother, and it is a complete new organ that in no time, during the first trimester of the pregnancy, develops from nothing. And so it is an organ with

a lot of cell growth, a lot of cell division. And as a consequence of that, it is prone to accumulate sometimes like small genetic errors in these cells. Now, coming back to that problematic category of the rare autosomal trisomies, when we know that more than 90% of these anomalies are not confirmed in the fetus, that does not mean that the NIPT test is analytically wrong.

What is going on is that these anomalies are only present in these fast-growing placental trophoblastic cells. This is something that is called confined placental mosaicism. So the anomaly is there. It is in the placenta and it is correctly identified by the NIPT, but luckily it is not present in the fetus, right? So NIPT generates a high-risk result and the amniocentesis shows a normal baby. Until now, we just kind of simply categorized NIPT result as high risk or low risk. But the technology has evolved. And so in our study, we go deeper into a novel metric that we call the mosaic ratio, or a digital estimation of trophoblastic mosaicism.

So what does this mean? In our study and the technology that we used looks at the strength of the abnormal signal and it compares this to the total amount of placental DNA in the blood of the mother, something that we call the fetal fraction. So if you make the proportion between these two, the NIPT can estimate which proportion of the placental cells are affected by any given trisomy. And it is precisely this estimate that is called the trophoblastic mosaic ratio. And so this concept of a mosaic ratio has been around for quite some time, but until now, it wasn't exactly clear whether it had any real diagnostic value. And so that's precisely the key novelty of our study using a very well-powered, very homogeneous cohort in our BELNIPT study that was performed on 32,175 consecutive pregnancies, we were able to find the answer.

And so what we find was quiet, let's say, surprising and groundbreaking. And we found that when less than 50% of the placental cells were affected by any anomaly, so a mosaic ratio below 0.5, that the risk for the fetus being affected was zero. And this kind of held for all types of anomalies, so it appeared rather a universal rule. It was the case for the common trisomies like the Down syndrome, but also for the Turner syndrome, for microduplications, and most importantly, also for these very annoying rare autosomal trisomies.

In practice, this would entail that as soon as you observe in the NIPT metrics that less than 50% of the placenta is affected, that you would no longer have to recommend an amniocentesis. And in our data sets with whole-genome screening, this would lead to 60% avoidance of unnecessary amniocentesis. And so this is truly a game changer. And so we are so proud of this result because it means that we can

provide a much better post-test counseling. And we hope this way that we can alleviate most of the anxieties that couples face after receiving an abnormal screening result. Of course, at this stage, we still have to be cautious. We have to gather more information before we can formally amend the guidelines. But if we achieve that, it would be a massive step forward.

Bob Barrett: It does sound incredibly promising, but is this mosaic ratio something that only your lab can calculate, or can your results be generalized to other NIPT assays?

Geert Martens: Yeah, that's a critical point that was also addressed in the editorial that accompanied our paper. The specific term "mosaic ratio" is a metric that was part of the Illumina VeriSeq assay that was used in our laboratory. However, our study also provides a roadmap for other labs that, for instance, are using a laboratory-developed whole-genome assay or alternative commercial methods. So at least we showed that by using standard open source bioinformatic tools, any lab performing whole-genome NIPT can calculate a metric that is very similar as the mosaic ratio. Essentially, it involves dividing the statistical score of the abnormality by the measured fetal fraction.

And evidently, any lab that tries to copy such a mosaic ratio by open-source tools will have to do some kind of validation, but we demonstrate that the diagnostic value of such an open-source estimate of trophoblastic mosaicism is as good as the one in the commercial method that we use. So yes, we do believe that our findings are broadly generalizable, and we really hope that other NIPT labs worldwide will follow our example.

Bob Barrett: Finally, Dr. Martens, every researcher hopes their work makes real-world impact. What would be the ideal outcome of your study, and what's next? What are the next steps?

Geert Martens: So yeah, we hope, of course, that our paper reaches a very broad audience, not only scientists and doctors, but also health officials, that this can help drive adoption of NIPT, but also the patients themselves. And so we're not waiting. We have already implemented some of these findings in our clinic. For instance, when we have a screening result of a rare autosomal trisomy of chromosome 7, we have already changed our guidelines. We do not longer perform an amniocentesis, and we just do a more intensified ultrasound monitoring. For the common trisomies 21, 18, 13, the high-risk RAT, we still, certainly out of safety, even in case of low-grade mosaic cases, we still perform an amniocentesis for the time being.

So, a first real impact of our findings is that it transformed what we call the post-test counseling. This is the waiting period, typically when patients get the results around week 12 of pregnancy, of three weeks up to week 15 when an amniocentesis is performed. This is a time for the expectant parents that they have quite a lot of stress. And so, when our data show that the abnormality is a low-grade mosaicism, we give that information during the counseling to the gynecologist, to the patients, just to kind of reduce their anxiety. And thus far, we get very positive feedback of these patients and it is perceived as something that really reduces the stress of that, of receiving an abnormal NIPT result.

Now, the ultimate goal, of course, is to go far beyond this and actually change the guidelines. The next step is to build an international consensus between providers of NIPT and come together. First, we will have to aggregate large data sets, clearly detail on which populations the NIPT was performed, a more general population, low risk, or a more high-risk population. And based on the aggregation of all of these data, we should clearly make a new consensus guidelines on which anomalies will not require an amniocentesis. And then second, also hopefully integrate the concept of mosaic ratio into this practice. And so, yeah, for me, these are very exciting times. And so, yeah, throughout all of these efforts, our main focus remains on the expectant couple. And so changing guidelines takes time. But if our study can help safeguard the power of genome-wide screening while preventing 60% of unnecessary amniocentesis, I will be a very happy doctor indeed.

Bob Barrett: That was Dr. Geert Martens from AZ Delta General Hospital in Belgium. He wrote a new research article in the October 2025 issue of *Clinical Chemistry,* discussing estimation of trophoblastic mosaicism to reduce false positive cell-free DNA screening results. He's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.