Article:
Jennifer N. Dines, et al.
A Paradigm Shift: Considerations in Prenatal Cell-Free DNA Screening
J Appl Lab Med 2017;2:784-96.
http://jalm.aaccjnls.org/content/2/5/784

Guest: Dr. Tina Lockwood is Associate Professor and Director of the Genetics and Solid Tumor Diagnostics Laboratory in the Department of Laboratory Medicine at the University of Washington.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

The assessment of chromosomal abnormalities during early pregnancy has been a major focus of prenatal genetic screening. The development of reliable screening techniques has evolved significantly over the last 50 years and allows families and providers to optimize maternal and neonatal outcomes. Beginning with mid-trimester amniocentesis in the 1970s followed by chorionic villus sampling in the 1980s, prenatal genetic testing through invasive technique provided both benefits and risks.

These methods remain the gold standard for diagnostic testing, but less invasive methods are commonly used as first line screening tests. A number of complex multi maternal serum analyte options with or without fetal sonography were developed to improve the sensitivity and specificity of screening while maintaining a stable screen positive false rate of 5%.

Cell-free DNA analysis, also called non-invasive prenatal screening and IPS, has emerged as an alternative to the conventional analysis of maternal serum markers. Modern molecular techniques have now achieved sensitivity for the direct detection and analysis of placental DNA fragments that circulate in the maternal bloodstream and serve as a proxy for fetal aneuploidy.

This technology is transforming the field of prenatal screening and has significant implications for laboratories, patients, and physicians. Clinical practice guidelines for adaption of cell-free DNA analysis from relevant stakeholders such as the American Congress of Obstetricians and Gynecologists (ACOG) and the Board of the International Society for Prenatal Diagnosis (BISPD) are evolving as more is learned about its clinical performance.

In this podcast, we'll explore the impact cell-free DNA screening is having on prenatal genetic testing. "A Paradigm Shift: Considerations in Prenatal Cell-Free DNA Screening" was published in the March 2018 issue of JALM. The corresponding author is Dr. Tina Lockwood. Dr. Lockwood is Associate Professor and Director of the Genetics and Solid Tumor Diagnostics Laboratory in the Department of Laboratory Medicine at the University of Washington.

She's our guest for today's podcast. Welcome, Dr. Lockwood. The first question I have for you is this. Prenatal screening has been standard of care for decades. Why is there so much interest in this test?

Dr. Lockwood: It's a really good question and I really want to emphasize what you just said which is "prenatal screening." So, this is a very important concept to get across right from the beginning. This is a screening test, this is not a diagnostic test. And so when we say screening, we mean that we're applying this type of a test to an entire population of patients as opposed to only a subset of patients that are increased risk of developing the condition, in this case the prenatal aneuploidy.

So what has made this test so exciting to the community overall is that the detection rate is very high. So, with a single blood sample from the mom, we can perform this as early as nine weeks of a gestational age. So that's a very early time of pregnancy before we can do a lot of different ultrasound measurements. We can perform it as well through the entire, the end, of the pregnancy. So there is not a particular range of time that you can perform this test.

Another exciting piece of it is that the false positive rate is more than tenfold lower than what we have with maternal serum screening. That's a huge decrease when we think about applying this test to all pregnant women. So, a lot of women are not at increased risk of having an aneuploidy in their fetus and they would, with a high false positive rate, they would have to go on to do invasive diagnostic testing that carries risk, both to mom and to the pregnancy.

So, if we can decrease that, again, by more than tenfold, this is a huge advance in the field overall.

Randye Kaye: Wow, I can see why. That's great, and you already answered this next question a little, but I want to see if you wanted to elaborate on it at all. The current practice is that high risk pregnant patients are offered cell-free DNA prenatal screening. Do you think all pregnant patients should have this test?

Dr. Lockwood: I think all pregnant patients should be offered this test, absolutely. If you think about when we offered this test in 2011, it was the first commercial test that we were doing with this exciting new analyte called cell-free DNA. So, we hadn't used this before and it made sense that the industry overall, both laboratories and healthcare workers, sort of stepped into it slowly.

So, we wanted to start with patients who were at increased risk such as women who are over 35 years of age when they're becoming pregnant. They're at increased risk of having a baby that has a chromosomal aberration like a trisomy 21, Down syndrome. In those patients, it made sense to start this testing where they're at increased risk.

But we've performed this test on millions of pregnant women now, in a fairly short period of time, just a few years since it was originally offered. And we have really great evidence, both the sum of what commercial laboratories have reported back into the literature, so all of these women that have been screened, so we have that data. Over 200,000 women have been screened where we see the outcome of the pregnancy. So, we really know if that baby had a trisomy or not, and we see the positive predictive value of this test is over 96% for patients looking at Down syndrome.

That's a huge number, when we think about maternal serum screening where it's much, much lower than that—more than an order of magnitude lower than that. It's a great advance to the field and again, we're ready at this point to expand this testing from what we call high-risk pregnancies to a general population pregnancy.

Randye Kaye: Okay. I can certainly see why. There have been numerous case reports about false positive and false negative cell-free DNA prenatal results. So what do you think physicians and laboratorians should understand about the limitations of cell-free DNA prenatal screens?

Dr. Lockwood: The biggest limitation that everyone needs to be aware of is what we're measuring in this test. So we're using cell-free DNA, which are these small little DNA fragments that we find in maternal circulation. They come both from mom and they come from the baby. Actually, the vast majority of what we find in mom's circulation is from the turnover of mom's blood cells. That's a normal turnover of what we see there. Interestingly, the DNA that we measure from baby is actually from the placenta, so it's not directly derived from the baby.

So, any disruptions we have to mom's normal cell-free DNA production, such as if mom has cancer that she might not

know about, or if there's any sort of an acute injury, that can alter the cell-free DNA accuracy for this testing. Similarly, if there is any change in the placenta's complement of chromosomes versus what we find in the baby. So if there's trisomy in the placenta only and not in the baby, that can cause a false positive result.

In the end, I think the most important thing for everyone to be aware of is that there needs to be good communication with the laboratory that's performing in the test. This is just like all other areas of laboratory medicine. If the result doesn't make sense in the clinical context, we need to contact the laboratory and have a dialogue.

Randye Kaye: Okay. That makes a lot of sense. What can the community expect from future research and cell-free DNA related to prenatal testing. Will new tests make existing screening methods and diagnostic tests obsolete, do you think?

Dr. Lockwood: There are certainly some screening tests that provide information that you can't get from the current state of cell-free DNA and some of that is because of the analyte that we're using which is, again, it's derived from the placenta, it's not derived from the baby. But I think the word that we should apply to this area, cell-free DNA prenatal aneuploidy screening, is more.

We're going to see more conditions that are going to be screened. So, going from a whole chromosome to pieces of a chromosome such as microdeletions and microduplications, we're already doing that. We'll see that get adapted more and more as time goes on and we have more evidence. We'll also see that we're adding into this prenatal screening area single gene disorders.

Some labs again are already doing this, things like screening for cystic fibrosis or achondroplasia. So, from this maternal sample, you can see if the baby has these Mendelian disorders. I envision a future where we would actually be screening mom and dad. Do some molecular profiling for mom and dad and that would determine what disorders the baby is at risk for and cell-free DNA screening could be used to actually see if the baby carries these specific alleles that are present in mom and dad.

This is a very exciting development in prenatal medicine and I'm confident that we'll see more and more changes, adding more and more testing in the very near future. It'll be a continuously expanding area.

Randye Kaye: Very, very exciting. Thank you so much for joining us today.

Dr. Lockwood: Thank you very much for having me.

Randye Kaye: That was Dr. Tina Lockwood from the University of Washington, talking about "A Paradigm Shift: Considerations in Prenatal Cell-Free DNA Screening" from the March 2018 issue of JALM. Thanks for tuning in for JALM Talk. See you next time and don't forget to submit something for us to talk about.