gw webinar

CELL-FREE DNA TESTING
FOR FETAL ANEUPLOIDY: LESSONS LEARNED AND IMPLICATIONS FOR CLINICAL PRACTICE

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A **GenomeWeb/Roche** White Paper

This white paper is based on a webinar presentation by Dr. Kypros Nicolaides, Professor of Fetal Medicine at King's College London, in which he provided a broad overview of prenatal screening for fetal aneuploidies, with an exploration of how cell-free DNA testing is influencing the field.

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HISTORICAL PERSPECTIVE

Dr. Nicolaides began with a historical look at screening for Down syndrome, noting that the field has seen "major advances every ten years" for the last 60 years. The late 1990s were notable for the development of the first trimester combined screening test, which demonstrated an ability to screen for trisomies 18 and 13 in addition to trisomy 21.

"So suddenly from an ideology, a philosophy, a process of thinking around screening for Down's," the field had to adapt to "the potential of diagnosing other chromosomal abnormalities," he said.

Dr. Nicolaides described the emergence of noninvasive cell-free DNA testing in the early 2010s as a "new era" in the field. A "big breakthrough" came in 2012 when his team used the Ariosa Harmony non-invasive prenatal test on 2,000 samples from routinely tested women and found "complete separation" in the results between eight cases of Down syndrome and nearly 2,000 cases that did not have Down syndrome.[1] The same was true for trisomy 18 cases in the study cohort.

This finding demonstrated two things, Dr. Nicolaides said. The first was that the test was applicable to a routine screening population, not just high-risk pregnancies, and the second was that it could be offered at the eleventh to thirteenth week of pregnancy.

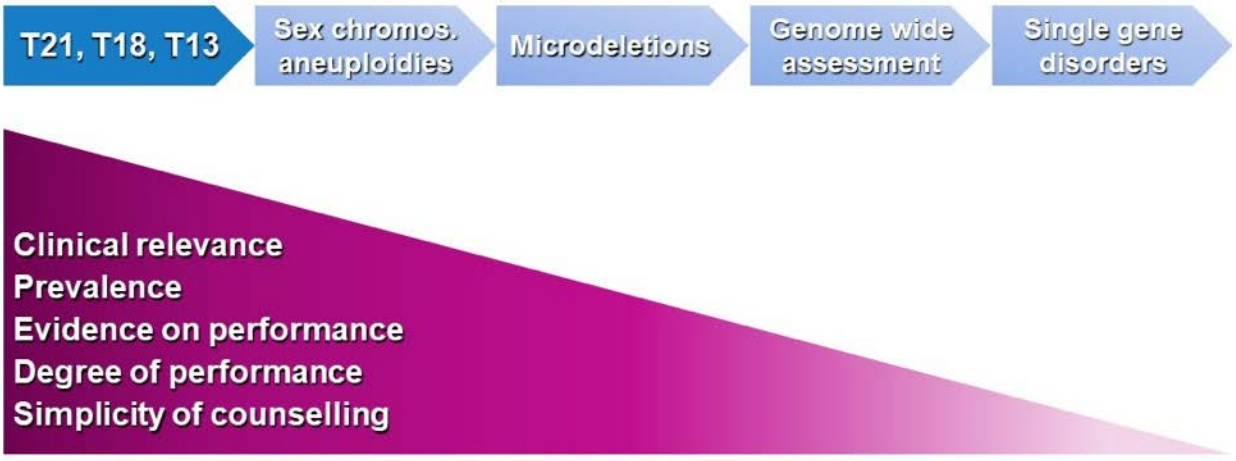
LESSONS LEARNED

Dr. Nicolaides discussed a range of learnings from the use of cell-free DNA testing in clinical care in the past decade, including an understanding of the biological factors that can influence results, possible implementation models, the importance of fetal fraction, and application in twin pregnancies.

While extensive evidence has shown that the detection rate for Down syndrome with cell-free DNA testing with the Harmony test is consistently more than 99 percent, with a false positive rate of less than 0.1 percent, Dr. Nicolaides noted that confined placental mosaicism can lead to discordant results. Confined placental mosaicism is seen more frequently in trisomy 13 and trisomy 18 than trisomy 21, and this should be taken into account when evaluating a pregnancy with a positive cell-free DNA result.[2]

In practice, he said, this means the likelihood of confined placental mosaicism and presence or absence of ultrasound findings can guide the decision of what type of confirmatory procedure to perform after a positive cell-free DNA test. For example, a pregnancy with normal appearing ultrasound and a positive cell-free DNA result for trisomy 13 will not likely benefit from chorionic villus sampling (CVS), because confined placental mosaicism is suspected. Instead, amniocentesis should be considered. On the other hand, a normal appearing ultrasound and positive cell-free DNA result for trisomy 21 is less likely to be due to confined placental mosaicism so a CVS should be considered.

Figure 1



Implementation of cell-free DNA analysis into clinical care varies by country, he said. Due to concerns with the cost of the test, many European countries have adopted a contingent screening model, where women initially have first trimester combined screening and cell-free DNA testing is offered only to those pregnancies found to have a risk above a defined cut-off. The cut-off is decided regionally and can range from one in 100 to one in 1,000. A lower cutoff will allow more women to have cell-free DNA analysis, so the detection rate of the screening program will be higher, but at a higher cost to the health care system. Belgium and the Netherlands, meanwhile, are currently offering cell-free DNA testing as a first-line screening to all pregnant women.

Dr. Nicolaides explained that fetal fraction — the proportion of cell-free DNA in the mother's blood that comes from the placenta — is an important factor influencing confidence in the test result. The lower the fetal fraction, the less accurate cell-free DNA testing is at distinguishing a trisomy from an unaffected pregnancy. This is why a laboratory may not issue a result in the case of low fetal fraction.

"The most common reason for having a failed result is that the fetal fraction is very low," he said. This happens more frequently in pregnancies with trisomy 18 or 13, higher maternal weight, or cases of severe placental insufficiency, but not in pregnancies with Down syndrome. Thus, it is the "completely wrong way of thinking" to recommend that a failed result requires follow-up by invasive testing.

In the case of a failed test, he said the next step should be an ultrasound. "If everything looks completely normal, then you don't do an invasive test purely because the cell-free DNA test has failed." Instead, he suggested, you repeat the cell-free DNA test, since more than half of cases will get a result.

Dr. Nicolaides explained that the cell-free DNA testing failure rate is considerably higher in twin pregnancies than in singletons. His team found that this higher failure rate "is not because they are twins, but because a much higher proportion of twins are conceived by [*in vitro* fertilization] and for some reason, in IVF pregnancies we have a much higher risk of having a failed result."

He has conducted several studies evaluating cell-free DNA in twin pregnancies and recently concluded that cell-free DNA screening for trisomy 21 in twin pregnancies has a similar detection rate to that in singletons, with a similar false positive rate.[3] But pregnancies with vanishing twins are an exception, he said.

"Unfortunately, in these cases, you cannot get a reliable result because the cell-free DNA from the [demised fetus] will continue to send into the maternal circulation cell-free DNA for several weeks." In these cases, he said, "you just do the combined test. You don't do the cell-free DNA test."

IS MORE BETTER?

Cell-free DNA testing has the capability to detect many more chromosomal abnormalities than trisomies, Dr. Nicolaides noted, asking "Is that good or is that bad?"

In the case of trisomy 21, he said, "we know what the condition is, we know exactly what the performance of the test is, we know how common it is, we know what the people decide around this, we know how to counsel women, and we think women understand what you tell them."

But as screening tests detect an increasing number of other conditions, "the evidence becomes weaker and weaker, both in terms of the clinical relevance of the conditions that we are looking for and our understanding on the performance of the test," he said. *(See Figure 1)*

> Dr. Nicolaides said he feels it is "completely wrong in clinical medicine to adopt the ideology of 'more is better.' I strongly believe that the more, the worse, if the 'more' have not been properly clinically validated."

For example, in the case of microdeletions and duplications, cell-free DNA testing is "pretty awful" because it misses 90 percent of the clinically significant microdeletions. In this scenario, a woman "comes along to us to share in the joy that she's pregnant and her baby looks all right, and we start terrorizing her. We start talking to them about abnormalities that she didn't want to think about, and then we offer them tests that at the very best will address ten percent of the microdeletions." In many of these cases, the women end up having an invasive test. "So in the name of introducing a non-invasive test, we will increase the rate of invasive tests."

He also cautions against premature adoption of another area where cell-free DNA testing has been suggested — the detection of maternal malignancies. "We are nowhere near being sure about … how to manage such results, and what is the health benefit for women that have this test," he said.■

1. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 2012;207:374.e1-6.

2. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2017;50:302-314.

3. Gil MM, Galeva S, Jani J, Konstantinidou L, Akolekar R, Plana MN, Nicolaides KH. Screening for trisomies by cfDNA testing of maternal blood in twin pregnancy: update of The Fetal Medicine Foundation results and meta-analysis. *Ultrasound Obstet Gynecol* 2019; 53:734-742.