Clinical Chemistry

A New Era in Prenatal Diagnosis: The Use of Cell-Free Fetal DNA in Maternal Circulation for Detection of Chromosomal Aneuploidies

Article:
J. Shea, E. Diamandis, B. Hoffman, Y.M.D. Lo, J. Canick, and D. van den Boom.
A New Era in Prenatal Diagnosis: The Use of Cell-Free Fetal DNA in Maternal Circulation for Detection of Chromosomal Aneuploidies.
Clin Chem 2013;59:1151-1159.
http://www.clinchem.org/content/59/8/1151.extract

Guest:
Dr. Barry Hoffman is an Associate professor in the Department of Laboratory Medicine and Pathology at the University of Toronto and a Clinical Chemist at Toronto's Mount Sinai Hospital.

Bob Barrett: This is the podcast from *Clinical Chemistry;* I am Bob Barrett. Prenatal screening for chromosomal aneuploidies is a fundamental part of routine obstetric care in most countries.

Pregnant women identified as high risk based on the prenatal screen can then undergo invasive procedures such as amniocentesis to confirm the diagnosis. Unfortunately, a large number of women with unaffected pregnancies undergo invasive procedures, putting the fetus at unnecessary risk for miscarriage.

The discovery of fetal cell free DNA in plasma of pregnant women 14 years ago opened up the possibility of identifying chromosomal aneuploidies noninvasively through a single blood sample.

The August 2013 issue of *Clinical Chemistry* includes a Q & A feature on free fetal DNA in the maternal circulation and noninvasive prenatal testing. In this article, four leaders in the field of noninvasive prenatal diagnosis provide their opinion on this exciting advancement.

One of those participants was Dr. Barry Hoffman, an Associate Professor in the Department of Laboratory Medicine and Pathology at the University of Toronto, and a Clinical Chemist at Toronto's Mount Sinai Hospital, where he directs the hospital's regional laboratory prenatal screening program. He joins us today in this podcast.

Dr. Hoffman, can you briefly describe how next generation sequencing has been applied to the detection of chromosomal aneuploidies using cell free DNA circulating in the maternal blood during pregnancy?

Dr. Barry Hoffman: Sure Bob. The important point here to start with is that there is a fetal cell free DNA circulating in the maternal blood during pregnancy and that this fetal DNA is released

from apoptotic cells in the placenta. Importantly for the application to trisomy 21 and other chromosomal aneuploidies, the fetal DNA in the maternal circulation that has been released in the maternal circulation, reflects the chromosomal load in fetus itself.

So one could expect that when there is an extra copy of the 21st chromosome as is the case with trisomy 21 or Downs, for example, that there will be an increased amount of DNA from the 21st chromosome contributed by the fetus circulating in the mother's blood. Correspondingly, in conditions where there is only one copy of a chromosome in the fetus as is the case with Turner's syndrome which is a monosomy X, one could expect a decrement in the proportion of the X-chromosome in the circulating fetal DNA.

The circulating fetal DNA is present along with a larger proportion of the maternal cell free DNA. So it's not just an issue of assessing the load of the fetal DNA, but you have to tease the increment or decrement from the larger background contribution of the maternal DNA and this is now done with the new age technology, the so-called Next-Gen Sequencing which allows the parallel amplification of all these small little DNA fragments circulating in the maternal blood, both maternal and fetal, to determine the increment or decrement, again the expected amount, from a euploid fetus.

To do this one requires a very large number of sequencing reads, typically in the order of 5 to 8 million reads to be able to tease out, as I said, the increment or the decrement reliably to achieve a reliable measurement.

This is done in a number of ways. One approach is to use a massively parallel shotgun sequencing or random sequencing of all of the fragments present circulating in the maternal serum or plasma. Then to use informatics to assess the load or the count of the chromosome of interest, by mapping the sequences back to the reference DNA or reference genome.

Another approach is to use targeted amplification sequencing only of the chromosomes of interest if one is restricting the test, to let's say, trisomy 21 and another related aneuploidies such as 18 and 13 and some of the sex chromosomal aneuploidies as well--only amplifies those, only sequence those--and then be able to do the bioinformatics workup to match these sequences back to the referenced genome to determine the actual counts and then the relative proportions and determine exactly if in fact the fetus is affected with a trisomy or a monosomy condition.

This has worked extremely well and it leads to a very reliable test. In fact what it amounts to is non-invasive karyotyping particularly of the chromosomes that have been targeted with the workup. The buzz and the interest in this next-gen approach is that it leads to tests that have accuracies greater than 99% for a number of the established trisomy, such as trisomy 21 and 18, and with false positive rates well, well below 1%. This is an astounding performance relative to current screening paradigms which use surrogate biochemical markers in conjunction with phonographic images and imaging markers, and finally the maternal age to determine a risk, where typically the best of these current strategies can achieve is about 90% detection for a 3% to 5% positive rate.

So clearly 99 plus percent protection with false positive rates well under 1% clearly exceed what can be achieved. Even with the very best current strategies with additional markers being added with additional photographic markers as well, one can achieve at best 95% for about 2% to 3% positive rate. So this new paradigm clearly outshines and outperforms the current screening strategies.

Bob Barrett: What's the turnaround time for this method, and when during pregnancy should this be performed?

Dr. Barry Hoffman: So the turnaround time of commercially available Next-Gen Sequencing based test that detect trisomy are typically of the order of 1 to 2 weeks. Somewhat longer in fact than the two to four day delay routinely achieved by traditional current screens carried out in the first or second trimester of pregnancy.

This NGS or Next-Gen Sequencing throughput depends on how well the testing lab has automated, simplified, and efficiently meshed from end-to-end discrete steps required to generate a clinical results such as target selection, library construction, template preparation sequencing, data processing, and interpretation.

Other interrelated factors that bear on the throughput include the inherent speed of the core sequencing technology employed, the number of multiplex sample that can be combined in a single run typically with a larger alumina platforms, 150 to 200 patient genomes are measured in a single run so one can multiplex a significant number of patient samples and the quality and length of the sequencing reads and in turn impact on the required depth of coverage and alignment efficiency with the genome.

Although, the absolute and relative amount of fetal DNA in the circulating cell free DNA of the mother is last really in

gestation published studies have shown that a NIPD can reliably detect fetal trisomies as early as the tenth week of gestation. That's well in the first trimester. Such sampling in the late first trimester fits nicely with elements of current obstetrical practice and the emerging concept of a late first trimester routine. The first possible visit of the pregnancy in which the fetus is assessed for anatomic anomalies by ultrasound, screend for a trisomies either by traditional current screening paradigms or the new NIPD analysis of cell free DNA, or both, carried out in a contingent fashion. Finally, the fetus is assessed for the likelihood of early onset preeclampsia occurring prior to 34 weeks of gestation.

Bob Barrett: Now, is this test commercially available now, and if so how expensive is it?

Dr. Barry Hoffman: This test is offered by four vendors currently in North America. It's been available since October 2011 offered by Sequenom Center for Molecular Medicine. Other vendors have introduced the test. Verinata Health introduced the test in March of 2012. Ariosa Diagnostics, Inc. began offering its test and its version in May of 2012. Finally, Natera Incorporated offered their panorama test at the end of 2012 in December.

The out-of-pocket payment to women who opt for this test ranges significantly, actually, from $500 to about $1700 depending on the vendor. The vendors may opt to charge payers or insurance companies considerably more for the test. But the cost to women range from $500 to $1700 out-of-pocket costs.

Bob Barrett: Doctor, what are the impediments to implementing this test now to replace the current prenatal maternal serum screening tests?

Dr. Barry Hoffman: There is no question that this non-invasive prenatal analysis of fetal cell free DNA in the mother's blood is a far superior test than traditional trisomy screen, that uses a biochemical and imaging biomarkers. As I alluded to earlier, the new technology gives detection rate in excess of 99% with false positive rates well below 1%.

However, the new technology is also considerably more expensive and thus: better tests, but more costly. It can be expected that those who can afford to pay for the molecular testing either privately or through insurance will seek it as their initial test. However, it is unlikely that government-funded prenatal screening programs, as in Canada, will have the funds to pay for the universal application of molecular testing as a first-tier test to all women undergoing prenatal screening, but ignoring this new test also leads to testing inequity and unequal access to quality screening, and both

of these are anathema to government-funded health care programs.

So a cost-neutral solution to this dilemma is a contingent testing paradigm whereby traditional screening is carried out first and molecular testing is offered only to the screen positives with the risk cut point selected of the first-tier screen, the traditional screen, so that the savings from pure costly invasive procedures and a less genetic counseling offset of the increased cost of the molecular testing.

The performance of such contingent screening in terms of the positive rate approaches that of molecular testing alone. Unfortunately, the false negative rate is somewhat higher because there is a false negative rate of about 10% to 15% associated with the fear of first-tier testing and those non-positive screens would not be sent on to the second tier molecular testing or the NIPD testing.

In addition the delay in a final result in a two-tier system should be acceptable in terms of turnaround time, if due attention is paid to speeding the initial screen positive samples to the second stage of testing. In Ontario, preliminary financial analysis has shown that contingent screening is cost-neutral when the positive rate of the traditional initial screen is set at about 5% and the positive rate cut point of the initial tier test can be increased and liberalized as the cost of the molecular testing decreases.

Bob Barrett: And what's the position of the International Society for Prenatal Diagnosis and other authorities on this new testing paradigm?

Dr. Barry Hoffman: A number of International bodies have released position papers, practice guidelines, comments related to this exciting new development, this new technology. These include the American College of Obstetricians and Gynecologists in a 2012 statement, the National Society of Genetic Counselors in a 2012 statement, the California Technology assessment form in 2012, the International Society for Prenatal Diagnosis in an early 2013 statement, and finally the Society of Obstetricians and Gynecologists of Canada, their genetics committee, in an technical update issued in February 2013.

All of these bodies acknowledge that the striking potential of this new technology and these new testings in the field of aneuploidy, and all essentially accept that clinical studies have validated its use in high risk women and high risk women for aneuploidies particularly in now in this specific case of Down would include those with a history of prior pregnancy with trisomy, positive traditional screening test, prenatal balanced Robertsonian translocations with an

increased risk of fetal trisomy 13 or 21, advance maternal age greater than 35 years, or with those with the fetal ultrasound findings with increased risk of aneuploidy.

So all accept that the test has been validated in high-risk women and accept its use and even recommend its use under such circumstances. A number of the statements point out that perhaps the test is still better suited as a second-tier test as opposed to a first-tier universally applied test.

Also, and importantly, all these statements indicate that the test, although it performs exceptionally well, in fact strikingly well, should not be considered to be a diagnostic test and women should not act irrevocably on the result of this noninvasive prenatal testing that results. In fact, that all women should undergo genetic counseling following a positive test result and be offered traditional invasive diagnostic testing--amniocentesis or chorionic villus sampling--to confirm the diagnosis. This is very, very important. It is not yet considered to be diagnostic, but merely is a very, very good screening test.

Bob Barrett: Has this technology been applicant to other chromosomal aneuploidies aside from trisomy 21?

Dr. Barry Hoffman: Yes, indeed. In fact, the same technology has been applied and in fact is available commercially to detect trisomy 18 and 13 as well as the number of the sex chromosome aneuploidies such a turner which is monosomy-X or Klinefelter's which is an additional copy of X say in a male XXY.

Also, on the horizon and as already showcased in a number of preliminary studies published in the literature, the same technology with a few twists and optimizations can be applied to detecting sub-chromosomal deletions and duplications and in addition can be applied to a single-nucleotide polymorphisms or SNP analysis.

So the currently available offerings which include the common trisomies of 21, 18, and 13 along with the sex chromosomal aneuploidies is just the tip of the iceberg and the companies are consistently increasing of the range of the testing that they offered to include additional conditions. So this chromosomal creep and DNA creep I think will be an expected development here as the companies strive to increase their market share and differentiate themselves one from the other.

Bob Barrett: Can this technology be applied to twins or in vitro fertilization pregnancies?

Dr. Barry Hoffman: Multiple gestation is currently a contraindication for the non-invasive prenatal testing of cell-free fetal DNA in the maternal plasma and is specifically ruled out by ultrasound examination prior to the physician ordering the test.

When twins are detected, an alternative is traditional screening including biochemical and imaging biomarkers even though it is well established that the performance of the traditional screening in multiple gestation is considerably poor than in singleton pregnancies.

However, there is no a priori reason why the molecular analysis of circulating cell-free DNA to detect and increment in the proportion of fetal trisomy 21 in the maternal plasma could not be applied to multiple gestation. Doubtedly, validating the test in such pregnancies will be hampered by the rarity of affected fetuses and complexities related to mono and di zygosity.

But when there is adequate fetal DNA relative to the maternal background there should be no problem with carrying out the analysis to determine the proportion of DNA from each fetus. It is possible in fact to determine in dichorionic pregnancy or dizygotic pregnancies the load from each fetus separately and to then be able to rule on the presence of trisomy in each.

In terms of in vitro pregnancies, there is no a priori reason why these are not suitable for the new non-invasive prenatal testing technologies. These need to be validated with increased numbers of IVF pregnancies in the literature, but there is no a priori reason why these are not appropriate and early studies such as the Melissa Study show that in a limited number of such patients the results were perfectly acceptable.

The caveat here is that those test that also use the maternal age as part of the risk calculation the prior test odds of having a trisomy would need to incorporate the maternal aid, the individual giving the donor egg in IVF pregnancy if someone else has in fact donated the egg. But other than this, the application should be straightforward and no problems are foreseen.

Bob Barrett: Finally, doctor, two recent publications have shown that NIPD has the potential to noninvasively determine the entire fetal genome from maternal blood. What implications do you foresee rising from this discovery and how will this build upon current clinical practices?

Dr. Barry Hoffman: Targeting the molecular analysis of fetal cell free DNA in the maternal circulation to a specific anomaly or even a limited set of anomalies all with known adverse clinical outcomes is

reasonably straightforward in terms of current clinical practice and ethical norms. But as the number of anomalies included in the molecular testing increases so does the difficultly of coherently and ethically presenting the findings to the ordering physician, the genetic counselor, the parents, eventually to the fetus after birth, and the related kindred.

But noninvasively sequencing the entire fetal genome is the ultimate open-ended test unquestionably an exciting technical tour de force, but utterly devastating even as a proof of concept insofar as applying the information clinically will require an entirely new ethical educational technical and physician delivery framework, none of which is currently in place.

It will take time, resources, new insights, and in fact much hard work before the potential NGS or Next-Gen Sequencing at the level of the entire genome can be fully realized in the clinic.

Bob Barrett: Dr. Barry Hoffman is an Associate professor in the Department of Laboratory Medicine and Pathology at the University of Toronto and a Clinical Chemist at Toronto's Mount Sinai Hospital where he directs the hospital's regional laboratory prenatal screening program. Dr. Hoffman is been our guest in this podcast from *Clinical Chemistry.*

I am Bob Barrett. Thanks for listening.