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CELL-FREE FETAL DNA IN MATERNAL BLOOD

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Over the past decade, the world of medical genetics has expanded with myriad novel testing technologies and options for prenatal screening and diagnosis. A goal of these advancements has always been to reduce the number of invasive procedures and their associated risks for fetal loss. In October 2010, we reviewed the use of non-invasive sequential screening, which examines both nuchal translucency measurements and maternal serum analytes with a 90% detection rate for trisomy 18 and 21 using established risk estimate cutoffs. We now report on the latest advancement in non-invasive prenatal testing: circulating cell-free fetal DNA (cffDNA) in maternal blood, and present some preliminary recommendations regarding its role within the current screening paradigm.

cffDNA is DNA from the fetus (mostly placental) that circulates within maternal blood and represents about 5-10% of the cell-free DNA in the maternal circulation. Unlike intact fetal cells within maternal blood, which can persist for years after a pregnancy, cffDNA is cleared from the maternal circulation within hours after delivery. As such, fetal DNA detected during a pregnancy represents DNA from the current fetus.

The advent of cffDNA technology started in 1997 when Lo et al. reported the ability to detect cffDNA within the maternal circulation.[1] Since then, research has advanced several techniques to accurately detect and quantify cffDNA.[2,3,4] Current cffDNA technologies examine for differences in the relative quantities of fragments from specific fetal chromosomes (21, 18, 13, and, in some cases, X) within maternal plasma to distinguish affected fetuses from non-affected fetuses. At present, the two methods are known as 1) massively parallel shotgun sequencing (MPSS) and 2) digital analysis of selected regions assay in conjunction with the fetal-fraction optimized risk for trisomy evaluation algorithm (DANSR/FORTE). Technical differences between these methods may ultimately affect the efficiencies and costs of these respective assays. Two prospective studies demonstrating the use of these cffDNA technologies to non-invasively identify fetuses with the most common aneuploidies in high-risk populations have recently been published.

In early 2012, Bianchi et al. published an analysis of 532 prospectively-collected samples from high-risk pregnancies using MPSS and demonstrated sensitivity rates of 100% for trisomy 21 (89/89 cases detected), 97.2% for trisomy 18 (35/36 cases detected), 78.6% for trisomy 13 (11/14 cases detected), and 93.8% for monosomy X (15/16 cases detected).[5] The reported specificities for each of these aneuploidies were 100%; i.e., no false positive results.

More recently, Norton et al. published their analysis of 3,080 prospectively-collected samples from high-risk pregnancies using the DANSR/FORTE assay.[6] Using a 1% risk cutoff, they reported sensitivity rates of 100% for trisomy 21 (81/81 cases detected) and 97.4% for trisomy 18 (37/38 cases detected). Specificities were 99.97% (1 false positive) and 99.93% (2 false positives), respectively.

cffDNA testing has been examined only in high-risk populations of pregnant women; it has not been yet validated in the low-risk population.

A number of commercially available tests have recently been introduced into the clinical realm based on these technologies. It is important to emphasize that, at this point, cffDNA testing has been examined only in high-risk populations of pregnant women. Testing is currently available for women with:

- advanced maternal age;
- personal or family history of chromosome abnormality;
- fetal ultrasound with findings associated with an increased risk for aneuploidy; or
- positive screening test result.

cffDNA testing has not been yet validated in the low-risk population. Research trials investigating the validity of cffDNA testing in low-risk populations are on-going, including one in which Northwest Perinatal Center is participating.

Utilizing the aforementioned techniques, these tests boast detection rates of greater than 99% for trisomy 21 and 97% for trisomy 18. The advertised detection rates for trisomy 13 vary between the respective tests. In general, while cffDNA testing can be performed as early as 10 weeks' gestation, most labs recommend testing after 12 weeks to optimize cffDNA sample acquisition. Depending upon the test, results may be reported

either as positive/negative (MPSS method) or as a probability assessment (DANSR/FORTE method). At this time, amniocentesis or chorionic villus sampling is recommended for confirmation of positive test results.

The introduction of any new testing modality typically raises questions regarding how it fits into the menu of screening and diagnostic tests in current use. At present, the American Congress of Obstetrics and Gynecology (ACOG) has yet to issue formal clinical guidelines regarding the use of this new technology. The International Society for Prenatal Diagnosis (ISPD) has broadly stated that "with suitable genetic counseling, MPS [massively parallel sequencing] can be helpful for women who may have been determined to be high risk by one of the previously recommended screening strategies."[7] As a result, the burden of the decisions on how to integrate this technology into practice has come to rest upon everyday clinicians, especially as more and more patients become aware of the availability of this new, non-invasive testing.

As with most aspects of medical care, a review of the benefits and limitations of any intervention is prudent. With regards to cffDNA testing, the benefits in the aforementioned high-risk population are that these tests 1) are non-invasive, 2) have high reported sensitivity and specificity, and 3) can be performed earlier in pregnancy than current methods of testing. The limitations are that they 1) only examine for a limited number of aneuploidies, as compared to the gold standards of CVS and amniocentesis, 2) are not currently covered by most insurance payers, 3) are not yet validated within the low-risk population, and 4) are not considered diagnostic. (See Table.)

Because of the complexities of the currently available options, early referral for genetic counseling is encouraged for women at a known increased risk – prior to 10 weeks' gestation, if possible.

At Northwest Perinatal Center, though we are excited about the potential uses of this new testing modality, we are very cognizant of its limitations. Given this, we currently discuss cffDNA testing as an option with patients in whom the test has been validated – specifically, only in women at increased risk. Given that it has not been validated in low-risk populations at this time, we do not believe that cffDNA testing is ready for use in the general population. While this may change as data emerge in the future, sequential screening continues to be the mainstay of aneuploidy screening for low-risk patients.

It is important to always keep in mind that all genetic testing is elective and requires a thorough discussion with the patient. Because of the complexities of the currently available options, early referral for genetic counseling is encouraged for women at a known increased risk (AMA, family history) – prior to 10 weeks' gestation, if possible. We have found that women who receive genetic counseling early in pregnancy benefit from the additional time to 1) understand the various testing options, 2) be thoughtful of their genetic testing decisions, and 3) contact their insurance providers.

The genetic counselors and perinatologists at Northwest Perinatal Center are available for any questions from health care providers and their patients regarding cffDNA testing or any other genetic testing. Please do not hesitate to contact us.

TABLE: PRENATAL TESTING OPTIONS FOR TRISOMY 21 AND OTHER FETAL ANEUPLOIDIES[8]

	cffDNA	Sequential Screening	CVS / Amniocentesis
Intended Population	currently only validated in women at increased risk: AMA, family history of aneuploidy, abnormal screening test, abnormal ultrasound	all women	women at increased risk: chromosomal abnormalities, known inherited disorders, ONTD
Gestational Age	10 weeks and over	11-20 weeks	**CVS:** 10-13 weeks; **amnio:** 15 weeks and over
Method	maternal blood	ultrasound, maternal blood	invasive procedure
Risks	none	none	**Miscarriage - CVS:** 0.5-1.0%; **amnio:** 0.2-0.5%
Detection Rate	**T21:** >99%; **T18:** 97-99%; **T13:** 79% (limited data)	**T21:** 80-95%; **T18:** 80-95%; **T13:** uncertain	**T21:** 99.99%; **T18:** 99.99%; **T13:** 99.99%
False-Positive Rate	<1%	3-5%	<1%
Turn Around Time	10-14 days	2-5 days	10-14 days
Results Reported As	positive/negative or numerical risk	numerical risk	normal/abnormal
Use with Multiples	**twins:** lab dependent; **triplets:** not validated	**twins:** validated; **triplets:** not validated	**twins:** validated; **triplets:** validated
Insurance Coverage	variable, limited	typically covered	typically covered
Cost to Insurance	variable, $800-2700	$850	$3500

OUR AUTHORS...

THOMAS LEE, MD

Dr. Lee attended Dartmouth College for his undergraduate studies and received his medical degree from the University of Pennsylvania School of Medicine. After finishing his residency in Obstetrics & Gynecology at the Reading Hospital and Medical Center, he completed his subspecialty training in Maternal-Fetal Medicine at Women & Infants' Hospital of Rhode Island/ Brown University, where his published research focused on Down syndrome and cell-free fetal DNA. He is board certified in both OB/GYN and maternal-fetal medicine.

Dr. Lee has been with Northwest Perinatal Center since 2002. While he thoroughly enjoys taking care of all high-risk pregnancies, he has special interest in the fields of diabetes in pregnancy and preterm delivery. Dr. Lee currently serves as the Managing Partner for Northwest Perinatal Center and as the chairperson of the Health Information Management Committee at Women's Healthcare Associates.

WENDY BUSCH, MS, CGC

Wendy attended Oregon State University for her undergraduate degree in community health. She went on to receive her Master of Science in Human Genetics at Sarah Lawrence College in New York.

Wendy has worked in the field of medical genetics for 28 years, including 10 years at Legacy Emanuel Hospital in its Department of Medical Genetics. She has served as a genetic counselor and the genetic clinical coordinator at Northwest Perinatal Center since joining the practice in 1994.

Wendy has been published in the *American Journal of Medical Genetics* and *Prenatal Diagnosis*. She has presented at the National Society of Genetic Counselors and the American Society of Human Genetics. She is certified in Genetic Counseling by the American Board of Medical Genetics and is a founding member of the American Board of Genetic Counseling.

INTRODUCING...

BARBRA M. FISHER, MD, PHD

Dr. Fisher attended the University of California, San Diego as an undergraduate, receiving her bachelor's degree in chemistry/biochemistry. She completed her doctorate in biochemistry at the University of Wisconsin-Madison before attending the Medical College of Wisconsin. She completed her OB/GYN residency at the University of Utah Medical Center in Salt Lake City. She was a general OB/GYN working for the University of Utah for several years before going on to complete her maternal-fetal medicine fellowship at the University of Colorado School of Medicine. Dr. Fisher is board certified in OB/GYN and board-eligible in maternal-fetal medicine.

MEREDITH K. WILLIAMS, MD

Dr. Williams attended the University of Michigan, receiving her bachelor's degree before going on to the Johns Hopkins University School of Medicine in Maryland. She returned to Ann Arbor for her OB/GYN residency and completed her subspecialty training in maternal-fetal medicine at the University of California at Davis. After, she served on the faculty at the Indiana University School of Medicine. Dr. Williams is board certified in OB/GYN and maternal-fetal medicine.

Citations

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7. ISPD RAPID RESPONSE STATEMENT: Prenatal Detection of Down Syndrome using Massively Parallel Sequencing (MPS): a rapid response statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, 24 October 2011.
8. Modified from NCHPEG+NSGC, Non-invasive prenatal testing (NIPT) factsheet. 2012

IN THIS ISSUE:

CELL-FREE FETAL DNA IN MATERNAL BLOOD

The latest development in prenatal screening for certain aneuploidies offers many promising advantages, but comes with some limitations, too. This issue explains the technology, studies to date and potential clinical applications.

NEW MATERNAL-FETAL MEDICINE SPECIALISTS

Barbra M. Fisher, MD, PhD and Meredith K. Williams, MD join Northwest Perinatal Center.

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The clinicians of NORTHWEST PERINATAL CENTER specialize in complete medical services for the highest risk pregnancies. We are located in Portland, Oregon in the Peterkort medical offices near Providence St. Vincent Medical Center. We provide comprehensive high-risk obstetrical care that includes:

- Pre-pregnancy counseling
- Genetic counseling
- Prenatal screening for chromosomal abnormalities
- Ultrasound
- Prenatal diagnosis
- Amniocentesis
- Chorionic villus sampling
- Management of complicated pregnancies, such as:
  - recurrent miscarriages or stillbirths
  - multifetal pregnancies
  - hypertension/high blood pressure
  - diabetes
  - premature birth
  - Rh disease
  - fetal complications

When you refer a high-risk patient to Northwest Perinatal Center, you can be confident that your patient is cared for by an experienced and compassionate team of clinicians. Care options are designed to fit your preferences and your patient's individual needs, including one-time consultations, patient continuing co-management, or complete assumption of care by our clinicians. We also offer immediate in-hospital consultations and complete care for maternal transports. We use state-of-the-art technology in supporting the evaluation and care of your patient, and are committed to prompt follow-up in what are frequently stressful circumstances.

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