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LANDMARK STUDY

Cell-Free DNA Analysis for Down Syndrome Screening in the General Pregnancy Population

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OVERVIEW

To date, cell-free DNA (cfDNA) screening for fetal trisomy has been offered primarily to women at increased risk for fetal aneuploidy, but not to the general pregnancy population. In the April 1, 2015 online issue of the *New England Journal of Medicine*, Norton et al published the results of a large, prospective, multicenter, blinded study demonstrating that the Harmony test for risk assessment of trisomy 21 (Down syndrome) outperforms combined first trimester screening in the general pregnancy population.[1] This study supports the use of the Harmony test in any pregnancy, regardless of the age or risk of the patient.

INTRODUCTION

Since its clinical introduction in 2011, analysis of cell-free DNA (cfDNA) in maternal plasma has shown great promise in screening for trisomy 21, trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome). For trisomy 21, investigators have reported detection rates of >99% with false positive rates of <0.1%.[2] Some of the reports that were initially used to validate the technology relied on stored plasma samples from pregnancies with known outcomes or studied populations of women known to be at increased risk for aneuploidy based on their age or because of a high-risk standard screen. As a result, the American College of Obstetricians and Gynecologists supported the use of cfDNA screening for high-risk pregnancies, but specifically recommended against its use in the general pregnancy population, citing lack of validation data among women who are not at increased risk for aneuploidy.[3]

This paper in the *New England Journal of Medicine* presents the results of the most thorough evaluation of the Harmony test for trisomy in precisely this average-risk population to date. The Non-invasive Examination of Trisomy (NEXT) study enrolled 18,955 women in a prospective, multicenter, blinded comparison of the Harmony test with first trimester combined screening (FTS). In this large general screening population, cfDNA screening with Harmony for trisomy 21 had higher sensitivity (100% vs 79%), a substantially lower false positive rate (0.06% vs 5.4%), and a higher positive predictive value (PPV) than standard FTS.

A ROBUST, PROSPECTIVE, BLINDED STUDY

The NEXT study enrolled pregnant women presenting for routine serum aneuploidy screening in the first trimester. Each patient received FTS—by measurement of pregnancy-associated plasma protein A (PAPP-A), quantitative human chorionic gonadotropin (β-HCG), and nuchal translucency (NT)—from 35 collaborating centers in 6 countries over a 1-year period. Maternal blood was also sent to Ariosa Diagnostics for blinded cfDNA analysis with the Harmony Prenatal Test. The Harmony test uses targeted cfDNA analysis and employs a unique algorithm to provide an individualized probability (risk score) for certain trisomies. Patient care was managed with the results of the first trimester screening. cfDNA results were not disclosed to participants, their providers, or investigators.

Pregnancy and Newborn Follow-Up

An important feature of this study was that pregnancy outcomes were obtained from each patient included in the study by newborn examination or diagnostic testing. All the data collected were submitted to an independent data center and kept blinded from investigators until the study conclusion.

COMPARING PERFORMANCE OF SERUM SCREENING AND THE HARMONY TEST

This is the first and only study large enough to detect a statistically significant difference in the sensitivity of cfDNA testing

DISCLOSURE

Dr. Costa reports that she is on the advisory board and speakers' program for Ariosa Diagnostics, Inc.

The Harmony Prenatal Test is developed by Ariosa Diagnostics. Ariosa Diagnostics is a laboratory certified under the Clinical Laboratory Improvement Amendments (CLIA). As with other laboratory-developed tests, this testing service has not been cleared or approved by the US FDA or any other federal regulatory agencies. Non-invasive prenatal testing (NIPT) services based on cell-free DNA analyses are not diagnostic; high-risk results should be confirmed by diagnostic testing. Data have not been submitted to or evaluated by Federal regulatory agencies and the test is not for sale as an In Vitro Diagnostic (IVD) in the US or the EU.

TABLE 1 NEXT Study Test Performance for Trisomy 21 by Screening Method

	Standard Screening	Harmony	Harmony	Harmony
Cohort	Primary cohort (n=15,841)		Women aged <35 years (n=11,994)	Low risk by standard screen (n=14,957)
Sensitivity	78.9%	100.0%	100.0%	100.0%
Specificity	94.6%	99.9%	99.9%	99.9%
PPV	3.4%	80.9%	76.0%	50.0%
NPV	99.9%	100.0%	100.0%	100.0%

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

compared with FTS in a general pregnancy population. After exclusions, the primary cohort consisted of 15,841 women. The average age of women in the analysis group was 31 years (range, 18-48 years). The gestational age of all pregnancies at enrollment was between 10 and 14 weeks. Overall, there were 38 cases of trisomy 21. The Harmony test identified all 38 cases (100% sensitivity) with a false positive rate of 0.06% (9/15,803) while FTS identified 30 cases (78.9% sensitivity) with a false positive rate of 5.4% (854/15,803). This translates into a PPV of 80.9% for Harmony compared with 3.4% for FTS (**TABLE 1**).

The Lowest Risk Sub-Group

Of the overall study group of 15,841 women, 76% (11,994) were aged <35 years and thus considered "low-risk" based on this historical classification of risk. In this "low-risk" group, Harmony correctly classified all trisomy 21 cases (19 of 19) with 6 false positive results, giving a PPV of 76.0%.

Trisomies 13 and 18

For trisomy 18, the false positive rate was 0.01% for Harmony compared with 0.31% for FTS; for trisomy 13, Harmony had a false positive rate of 0.02%, compared with 0.25% for standard screening. Of the 10 trisomy 18 cases in the cohort, Harmony and FTS detected 9 and 8 cases, respectively. In the group of 11,185 patient samples analyzed for trisomy 13, there were 2 cases of trisomy 13. Both were detected by Harmony; 1 was detected by FTS. These numbers are too small to determine sensitivity; however, the high specificity compared with serum screening was clearly demonstrated.

Prenatal Tests With No Result

Of 16,329 otherwise eligible patients, 308 (2%) were excluded because of failure to obtain FTS results and 488 (3%) were excluded for failure to obtain a cfDNA result. Further evaluation of the latter group revealed a higher median maternal weight in tests with insufficient fetal cfDNA for analysis, a finding consistent with previous research.[4] A higher prevalence of fetal aneuploidy also was observed in the group with no cfDNA results. This finding, also reported previously, has not been as systematically investigated.[5] In light of this observation, it is important that patients who do not receive a result be offered re-draw for cell-free DNA screening or other follow-up testing depending on the broader clinical picture.

STUDY CONCLUSIONS: THE HARMONY TEST IS A BETTER PRIMARY SCREENING TEST FOR TRISOMY 21 THAN CONVENTIONAL SERUM-BASED TESTING

In the short history of cfDNA analysis for clinical use, no other study has achieved the power of this study in terms of size and overall design. With 15,841 pregnancies in the primary analysis cohort, this is the largest direct comparison of cfDNA screening to serum-based screening in the general pregnancy population.

Motivated by experience and the growing body of supportive evidence, many clinicians have concluded that all pregnant women deserve access to cfDNA technology, regardless of risk status.[6] This study provides rigorously obtained data supporting this practice.

The obstetric community must still address important issues inherent in the widespread use of cfDNA technology, such as patient access, the role of first-trimester ultrasound in a screening paradigm, the challenge of properly educating patients about their testing options, and the appropriate algorithm to employ when cfDNA results are not obtained on the first blood sample. However, this trial has definitively answered affirmatively the unresolved question of whether the Harmony test performs as well in average-risk patients as it does in high-risk patients.

SUMMARY

The NEXT study demonstrates the superior performance of the Harmony test compared with standard screening for trisomy 21 in the general pregnancy population. The sensitivity of Harmony was significantly higher with a false positive rate 90-fold lower than standard screening. The PPV for Harmony was 80.9% for cfDNA, compared with 3.4% for standard screening.

REFERENCES

1. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy [published online ahead of print April 1, 2015]. *N Engl J Med*. doi: 10.1056/NEJMoa1407349.
2. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(3):249–266.
3. American College of Obstetricians and Gynecologist Committee on Genetics. Committee opinion no. 545: noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol*. 2012;120(6):1532–1534.
4. Ashoor G, Poon L, Syngelaki A, Mosimann B, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: effect of maternal and fetal factors. *Fetal Diagn Ther*. 2012;31(4):237–243.
5. Pergament E, Cuckle H, Zimmermann B, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol*. 2014;124(2 Pt 1):210–218.
6. Dondorp W, de Wert Guido, Bombard Y, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening [published online ahead of print March 18, 2015]. *Eur J Hum Genet*. doi:10.1038/ejhg.2015.57.