ORIGINAL ARTICLE

Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population

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ABSTRACT

Objective Evaluate noninvasive prenatal testing (NIPT) with cell-free DNA as a screening method for trisomies 21, 18, and 13 in an obstetrical clinical practice setting.

Methods Observational study of pregnant women who underwent prenatal screening for fetal trisomy from 30 July 2012 to 1 December 2012. NIPT was offered to all patients in addition to first trimester combined screening (FTS).

Results The cohort included 289 women with mean age of 32.3 years (range: 17.8–42.0) who underwent testing at 13.0 gestational age weeks (range: 10.1–20.7). NIPT results were provided for 98.6% of patients at a mean reporting time of 9.3 calendar days. With NIPT, all patients had a risk less than 1:10 000 for trisomy 21, 18, or 13. With FTS, 4.5% of patients had screening results indicating an increased risk for trisomy 21. One patient who had an elevated trisomy 21 risk with FTS elected to have an amniocentesis, which revealed a euploid fetus.

Conclusions NIPT has the potential to be a highly effective screening method as a standard test for risk assessment of fetal trisomies 21, 18, and 13 in general pregnant populations. © 2013 John Wiley & Sons, Ltd.

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INTRODUCTION

Noninvasive prenatal testing (NIPT) with cell-free DNA (cfDNA) for fetal trisomy risk assessment has been shown to be both highly sensitive and highly specific across numerous studies.[1–10] NIPT detects greater than 99% of trisomy 21 cases. Trisomy 18 and trisomy 13 have slightly lower detection rates. False positive rates for NIPT using cfDNA can be as low as less than 0.1% for each trisomy.[1–3,11] Conventional first trimester combined screening (FTS) with serum markers and nuchal translucency measurement generally have detection rates of 85–90% with an inherent false positive rate set at 5%.[12,13]

NIPT using directed cfDNA analysis methods provides a risk assessment for fetal trisomy 21, 18, and 13.[4,5] This screening test can be performed at any time after 10 weeks and is not restricted to 'windows of time' during a pregnancy. The test does require that the fraction of fetal DNA in the plasma be greater than 4% and is not yet validated for use in pregnancies with multiple fetuses. A recent clinical study in a general screening population of 2049 patients correctly classified ten trisomy cases (8 trisomy 21 and 2 trisomy 18) at a combined false positive rate of 0.1%.[2] The study was retrospective and relied upon the analysis of archived frozen plasma samples.

We report the experience of implementing NIPT prospectively in clinical practice for a general screening population. The practice setting is a privately owned clinic that provides general obstetrical and gynecological services to a diverse population in the greater Atlanta, Georgia area.

METHODS

Data from patients between 30 July 2012 and 1 December 2012 were included in this study. Upon Institutional Review Board approval, a retrospective chart review was conducted to collect clinical data on those patients who had undergone NIPT.

As is the standard of care at Obstetrics and Gynecology of Atlanta (Atlanta, GA), all women are offered prenatal testing for aneuploidy. The obstetric provider offers general counseling regarding the pregnancy as well as genetic counseling at the initial clinic visit. Patients are advised of their risk of aneuploidy and the various screening and diagnostic tests available to them. They are provided with literature support from NTD labs and Ariosa Diagnostics as well as internal literature from the clinic. During the study period, patients were advised of their eligibility for the Harmony™ Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA) if they had a singleton pregnancy. As the Harmony test

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was a new technology, patients were still offered the conventional *First Trimester Screen* test (NTD Labs, Melville, NY) that consisted of serum measurement of pregnancy associated plasma protein-A (PAPP-A) and free ß-human chorionic gonadotrophin (ß-hCG) along with nuchal translucency measurement. If they chose NIPT testing in addition to first trimester combined screening; testing was performed at the same time as the gestational requirement of >10 weeks.

For NIPT, up to 20 ml of whole blood was collected via standard venipuncture into two Cell Free DNA BCT™ tubes (Streck, Omaha, NE), and then samples were sent at ambient temperature without any further processing via courier for analysis. A healthcare provider from the clinic reported the test results to each patient.

Descriptive data are presented in mean values with standard deviations and in numbers and percentages for categorical variables.

RESULTS

Between 30 July 2012 and 1 December 2012, 289 patient samples were drawn for prenatal testing with NIPT. Maternal characteristics are depicted in Table 1. The mean maternal age was 32.3 years (range: 17.8–42.0) and the mean gestational age at time of NIPT was 13.0 weeks (range: 10.1–20.7). The mean maternal weight was 67.7 kg (range: 46.3–125.6).

Of the 289 patients who opted for NIPT, one sample was received greater than 5 days from the time of blood draw and was excluded as it did not meet laboratory acceptance criteria. Of the remaining 288 patient samples tested, 98.6% (284 of 288) were given a test result whereas 1.4% (4 of 288) failed sample quality control criteria and did not yield a test result. Of the four patient samples that did not yield a test result, two of the samples were from the same patient. The mean time from blood draw to patient test result was 9.3 calendar days (range 7–18) whereby transit time of blood draw to laboratory receipt of the patient sample was on average 2.2 calendar days (range 2–4). Figure 1 shows the distribution of time from blood draw to test result report in calendar days.

Of the 284 patient test results with NIPT, all had a trisomy 21, 18, and 13 risk score of <0.01% and were therefore classified as low risk for trisomy. The average fetal fraction for patient samples was 12.4% (Table 1). FTS results were available for 267 of the 284 patients, and 12 patients (4.5%) were screen positive with FTS using a risk cutoff of 1 : 311, which was the recommended cutoff from the testing laboratory. The distribution of the highest trisomy risk score for each patient sample with FTS and NIPT are shown in Figure 2a and Figure 2b, respectively. One of the 289 patients underwent invasive testing and was found to have a euploid fetus. This patient had a trisomy risk of less than 0.01% with NIPT but a one in five risk of trisomy 21 with FTS.

Table 1 **Maternal characteristics of patients undergoing testing**

Characteristic	Values
Maternal mean age (years) ± SD (range)	32.3 ± 4.7 (17.8–42.0)
Gestational mean age (weeks) ± SD (range)	13.0 ± 1.5 (10.1–20.7)
Maternal mean weight (kg) ± SD (range)	67.7 ± 14.4 (46.3–125.6)
Maternal mean height (cm) ± SD (range)	164.7 ± 6.2 (147.3–185.4)
Fetal mean fraction (%) ± SD (range)	12.4 ± 4.5 (2.9–37.6)
Ethnicity, *n* (%)	
Caucasian	218 (75%)
African American	39 (13%)
Asian	27 (9%)
Hispanic	5 (2%)

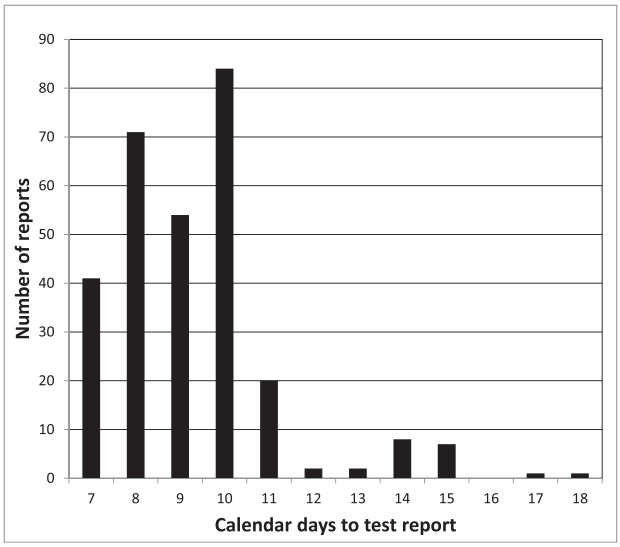

Figure 1 **Reporting time for NIPT in calendar days from time of blood draw**

DISCUSSION

This study summarizes the outcomes of our clinical use of NIPT in a general screening population. A test result was obtained in 98.6% of patients tested at an average of 9.3 calendar days (7 business days). Four patients did not receive a test result, with one because of delays in laboratory receipt of the blood sample. Of the samples that failed quality control criteria, the patients associated with these samples had a higher maternal weight (97–112 kg) as compared to those who received results. Previous studies have demonstrated that higher maternal weight is negatively correlated with fetal fraction. Adequate fetal fraction, which is currently defined as 4% or greater, is the principal requirement for satisfactory NIPT screening.[14] In our patient cohort, we also saw a negative correlation between maternal weight and fetal fraction (data not shown).

All patients who received an NIPT result received test scores of less than 0.01% for trisomies 21, 18, and 13. Of those patients who had conventional FTS, the screen positive rate was 4.5%. Standard clinical practice at Obstetrics and Gynecology of Atlanta prior to instituting concurrent NIPT would be to recommend invasive testing for these screen positive patients. By incorporating NIPT results into clinical practice, only one patient underwent invasive testing. This patient received discordant results between the two

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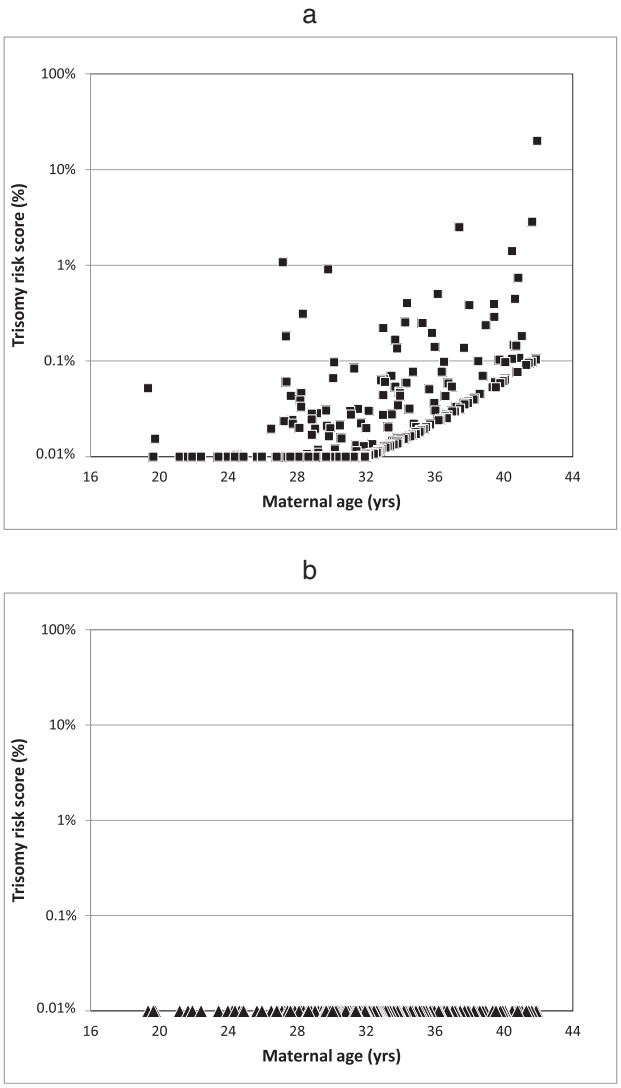

Figure 2 (a) Risk scores ($n = 267$) with first trimester combined screening (serum PAPP-A, serum ß-hCG, and nuchal translucency) based on maternal age. (b) Risk scores ($n = 287$) with NIPT based on maternal age. Risk scores are plotted on a logarithmic scale

screening tests. Conventional FTS provided a high risk for fetal trisomy 21 result. Invasive testing showed this result to be a false positive result. Screening with NIPT correctly classified the patient as low risk for trisomy 21.

NIPT can be performed as early as 10 weeks of gestation and has a reported performance of improved detection and lower false positive rate than the conventional screening. Despite excellent detection and low false positive rates, NIPT using cfDNA should still be regarded as a high performance screening test, rather than a diagnostic, as published data demonstrate less than 100% sensitivity and specificity. The American Congress of Obstetrics and Gynecology and Society of Maternal–Fetal Medicine recently issued a joint statement highlighting the role of NIPT as a screen and not diagnostic.[15] They also recommended the use of NIPT in high risk pregnancies but not in low risk pregnancies, although the latest clinical data to support use in a general screening population was not included in their evaluation.[2,6,16]

The clinical use of NIPT in a general screening population allows for equal access to a technology that can detect more trisomy cases, avoid false positive results resulting in unnecessary costly and invasive diagnostic testing, and provide clearer risk scores as compared with conventional prenatal screening tests. The wide spectrum of risk score values seen with conventional screening can lead to patient confusion and the need for extensive counseling by healthcare providers. In our study cohort, we had no high risk (*screen positive*) results with NIPT, whereas we had 4.5% of patients test *screen positive* with FTS. Those who test *screen positive* are subjected to extensive counseling and as a result, often undergo invasive tests: chorionic villus sampling or amniocentesis. As the vast majority of *screen positive* results are false positives, such follow-on invasive testing creates undue anxiety for patients, increases costs to the medical system, and places euploid fetuses at risk for procedure related miscarriage. Although serum markers may provide information on other pregnancy complications such as intrauterine growth restriction, such findings are incidental and not the primary reason for screening.[17]

As a result of the initial experience with NIPT, Obstetrics and Gynecology of Atlanta now offers NIPT as a first-line screening test to patients with singleton pregnancies of at least 10 weeks gestational age. Patients are scheduled for a follow-up visit 2 weeks after their NIPT to review their results and receive counseling. If at the 2-week follow-up visit an NIPT result is not available, patients are advised to undergo conventional FTS.

A limitation of this study is that clinical outcomes of pregnancy to assess the accuracy of screening test results are not yet available. Although this study involved a moderate number of patients from a single center, we believe the findings can be translated to other obstetrical practices. Although current NIPT tests almost exclusively evaluate for trisomy 21, 18, and 13, additional work is underway to expand to evaluate other conditions. The clinical utility of any broadened screening to include conditions not currently offered will need to be tempered by the required increase in patient and provider education, as well as a need to address the cumulative increase in false positive results that will happen as more conditions are tested. All of this should be considered before introduction into clinical care.

CONCLUSION

Noninvasive prenatal testing (NIPT) with cfDNA appears to have some distinct advantages as a screening tool for trisomies 21, 18, and 13 in singleton pregnancies. NIPT can be performed at any point after 10 weeks and has superior detection rates and substantively lower false positive rates as compared with conventional prenatal screening methods. Broader NIPT use will depend on affordability of testing and even with broader adoption, ultrasound evaluation will still be important to assess for fetal viability, presence of multiples, and evaluation of structural and genetic conditions.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Noninvasive prenatal testing (NIPT) with cell-free DNA has been shown to be highly accurate for detection of fetal autosomal trisomies.

WHAT DOES THIS STUDY ADD?

- This study shows the clinical use of NIPT for trisomies 21, 18, and 13 in a general screening population in the US, including a comparison against first trimester combined screening.

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