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**Abstract**

NIPT uptake in the general population has been rapidly increasing despite relatively low incidences of fetal aneuploidy in this cohort. This is further complicated by the inclusion of microdeletion syndromes, which have even lower positive predictive values (PPVs). This retrospective pilot study examines the performance and impact on patient decision making of a SNP-based NIPT in a general population cohort. A chart review was conducted of NIPT results from January 1, 2014 to August 24, 2015 at Montefiore Medical Center in the Bronx, NY. NIPT results were obtained for 3,747 samples. 1.33% of reports were high risk. One third of all high risk reports indicated a high risk for 22q11.2 deletion syndrome. 37.5% of women with high risk for 22q11.2DS elected diagnostic testing. None of the 5 whose diagnostic testing results were available were found to have an affected pregnancy. The total number of no call samples was 282 (7.53%). Cases in which the initial draw failed with low fetal fraction as a contributing factor had a redraw success rate of 28.75%. Differences in population characteristics can significantly impact the clinical utility of NIPT. The addition of conditions such as 22q11.2DS to NIPT panels will increase genetic counseling burden and complicate patient decision making. Overall, patients need to be aware that NIPT does not replace diagnostic testing, that PPVs differ significantly for microdeletion syndromes, and that redraw success is variable.

MeSH Keywords: prenatal diagnosis, fetal aneuploidy, cell-free DNA, noninvasive prenatal testing, noninvasive prenatal screening

## Introduction

Non-invasive prenatal testing (NIPT) has been available clinically since 2011 and has become a popular alternative or addition to traditional serum screening for chromosomal aneuploidy, boasting close to 100% accuracy and very high positive predictive values (PPV) for high-risk pregnant women (Taylor-Phillips et al., 2016). Although clinical validity and utility have been studied in the high risk population, there is less information available on utility for low risk women, despite increasing use of NIPT in the general obstetrical population. Some clinicians are hesitant to offer NIPT to a cohort with a relatively low incidence of fetal aneuploidy, as the test will have lower PPVs.

The recent inclusion of microdeletion syndromes on the NIPT panels offered by several companies has further complicated the use of this screen. Unlike the trisomies traditionally analyzed on NIPT, microdeletion syndromes are not associated with advanced maternal age. Therefore, microdeletion screening by NIPT is equally relevant for women under 35, who are considered to be at low risk to have a pregnancy affected with an aneuploidy. For both low and high risk populations, the low PPVs of microdeletion syndromes will lead to an increasing number of false positive results as more women are tested (Wapner et al., 2015). The low PPVs of microdeletion syndromes on NIPT is especially concerning as a few patients have been reported to have elected to terminate based solely on a positive NIPT result, without confirmatory testing (Weaver, 2013; Dobson et al., 2016).

This retrospective pilot study will explore the utility of NIPT including the microdeletion syndrome 22q11.2 deletion syndrome (22q11.2DS) in a general

population cohort, as measured by the effect on patient decision making. 22q11.2DS is the most common microdeletion syndrome in the general population (1 in 4,000) and clinically significant in that many affected individuals are born with serious congenital abnormalities, with most having mild to moderate cognitive impairments as well as a risk of psychiatric illness and immune dysfunction (Hacijhamdioglu et al., 2015). Early diagnosis is important as prompt treatment may improve prognoses (Botto et al., 2003).

Another aspect of NIPT that may affect the clinical utility of this screening tool in the general population is the impact of fetal fraction on test accuracy, including false positive and false negative rates. Fetal fraction is the relative amount of cell-free fetal DNA (cffDNA) to cell-free maternal DNA in maternal plasma. Low fetal fraction can affect the sensitivity and specificity of NIPT and decrease the likelihood that a result is obtainable on redraw (Levy et al., 2013). This characteristic of the screen may be especially important to consider in populations with high rates of obesity, since higher maternal weights are associated with lower fetal fractions (Rava et al., 2013; Ashoor et al., 2013; Kinnings et al., 2015). Thus, this pilot study will also examine the frequency of no call results with low fetal fraction and how this impacts the failure rate of the screen in a general population, low socioeconomic status cohort.

## Background

*Accuracy of NIPT and Discordant Test Results*

Since the introduction of NIPT in the clinical setting in 2011, many groups have studied the validity of this screening modality in comparison to the pre-existing standard of care. Reported sensitivities and specificities of NIPT vary somewhat between different versions of the technology and different study populations, but overall NIPT has been shown to have much higher sensitivity and specificity than traditional screening methods (Bianchi et al., 2014). NIPT is known to be most accurate in detecting trisomy 21, with sensitivity and specificity reported to range from 98-100% (Bianchi et al., 2012; Palomaki et al., 2011). Somewhat lower accuracy has been described for trisomy 18, trisomy 13, and sex chromosome aneuploidies, but many investigators have shown sensitivities for these conditions to be higher than 90% and specificities for all conditions to be greater than 99% (Pergament et al., 2014; Nicolaides et al., 2014; Samango-Sprouse et al., 2013). A recent meta-analysis of the literature reported sensitivities of 99% for trisomy 21, 96.8% for trisomy 18, 92.1% for trisomy 13, 88.6% for monosomy X, and 93.8% for all other sex chromosome aneuploidies with specificity greater than 99% and false positive rates at 0.2% or below for all aneuploidies (Gil et al., 2014).

While the accuracy of NIPT offers an advantage over previous standard obstetrical screening, false positive and negative results continue to be a concern. This is an issue that has been garnering attention as NIPT is offered to increasing numbers of women, many of whom do not receive adequate information about the screen through pre- and post-test counseling (Leach et al., 2015). Studies have

validated high rates of sensitivity and specificity in both high and low risk cohorts, but few review positive predictive values (Dan et al., 2012; Pergament et al., 2014). This is of importance for clinicians as NIPT is being offered in increasing numbers to women in the low risk population, a group where PPVs are predicted to be lower based on a reduced baseline incidence of aneuploidy (ACOG, 2015; Gregg et al., 2013).

In a recent study comparing NIPT results to pregnancy outcomes in over 100,000 cases in China, the authors evaluated the overall efficacy of the screen to detect trisomies 21, 18, and 13. The study was performed on general population samples that did not categorize women by their risk status. Only 9 false negative results were observed. PPV was determined to be 92.19% for trisomy 21, 76.61% for trisomy 18, and 32.84% for trisomy 13 based on a total of 157 false positive results (Zhang et al., 2015).

In a study sponsored by Natera of close to 30,000 high and low risk cases, PPVs were reported to be 90.9% for trisomy 21, 93.1% for trisomy 18, 38.1% for trisomy 13 and 50% for monosomy X, based on cases with cytogenetic confirmation (Dar et al., 2014). Another study looking specifically at sex chromosome aneuploidies obtained a similar PPV of 54.17% (Yao et al., 2014). Positive predictive values mentioned in other studies vary widely and tend to have large confidence intervals due to smaller sample sizes (Bianchi et al., 2014; Neufeld-Kaiser et al., 2015).

Several studies have been done in follow up to discordant NIPT results in order to better understand the circumstances surrounding these false negative or

positive results. Conditions known to be responsible for causing discordant results are mosaicism, presence of a vanishing twin, maternal chromosome abnormalities, maternal malignancy, and technical errors (Grati et al., 2014).

Researchers have attempted to determine if there are underlying factors that can contribute to a false positive test result. Clinicians in New Jersey described three cases of false positive NIPT results for fetal sex chromosome aneuploidies (McNamara et al., 2015). In two of the cases, the mothers were found to be mosaic for Turner syndrome and in the third case the mother was known to be 47,XXX. In another study, investigators hypothesized that two false positive trisomy 18 results were actually due to benign maternal partial duplications of chromosome 18 (Snyder et al., 2015). Maternal cancer, usually appearing on NIPT analysis as multiple fetal aneuploidies, has also been implicated as a cause of discordant results (Bianchi et al., 2015).

In addition to aneuploidy, some labs have expanded NIPT to offer screening for common microdeletion syndromes. These include DiGeorge or 22q11.2 deletion, Cri-du-chat, Prader-Willi/Angelman, 1p36 deletion, and Wolf-Hirschhorn syndromes. Offerings vary from laboratory to laboratory. Although approximately 1.7% pregnancies have been shown to be affected by a clinically relevant microdeletion or microduplication (Wapner et al., 2012), the incidence of any one of these syndromes is very low in the general population, detection rates are lower relative to aneuploidies, and some laboratories leave these conditions off of reports unless the result is positive.

One study attempting to model the performance of NIPT (SNP-based) in detecting microdeletion syndromes showed a detection rate of 83.3% for 22q11.2DS and 45.5% overall (Wapner et al., 2015) but PPVs ranged from 3.8%-17% based on the incidence of the condition being analyzed. Of note, researchers in this study used artificial mixtures of DNA from mothers, affected children, and microdeletion cell lines in order to generate sufficient numbers of positive results to establish predicted detection rates.

In February of this year, researchers from Natera published a paper that looked at the efficacy of SNP-based NIPT testing for 22q11.2DS in a clinical setting (Gross et al., 2016). They examined 21,948 samples, of which 97 screened positive for 22q11.2DS. PPV was calculated to be 18% overall. For pregnancies considered to be high risk for the microdeletion before undergoing NIPT, or those that had associated ultrasound findings, the PPV was 89.9% (8/9) and for those considered to be low risk, or those without associated ultrasound findings, the PPV was 5.1% (2/39). Although an 18% PPV is higher than those reported in previous studies, the authors of this study acknowledge that some women may have been offered NIPT only after an associated ultrasound finding was discovered, which could potentially have skewed the data in favor of true positive results.

*Impact of NIPT Results on Patient Decision-Making*

While strict practice guidelines regarding the use of NIPT as a prenatal screening tool have yet to be published, the American College of Medical Genetics (2013), National Society of Genetic Counselors (2013), and American College of Obstetricians and Gynecologists (2015) have all released statements on the topic.

Each of the position statements raise concerns regarding the cost-effectiveness and clinical utility of NIPT in the general population. They also emphasize the need for pre and post-test counseling to ensure that women understand the accuracy and limitations of NIPT as a screening tool. The authors reiterate that NIPT is a screening tool that should be considered in conjunction with ultrasound findings and that diagnostic testing via CVS or amniocentesis should always be offered in the case of positive results before decisions are made about pregnancy management. ACOG (2015) and NSGC concur in their opinion that conventional screening methods should still be used as the standard of care in the low risk population. (Devers et al., 2013)

The reduced risk of NIPT relative to invasive diagnostic prenatal procedures seems to be increasing rates of NIPT uptake and decreasing the number of diagnostic procedures performed (Tiller et al., 2015). A retrospective study by Wax et al. found significantly increased rates of genetic counseling as well as decreased rates of diagnostic procedures (2015). Chetty et al. suggest that some women use NIPT rather than diagnostic testing after a positive first trimester screen (2013).

Multiple studies have described a decrease in the rates of CVS and amniocentesis after the introduction of NIPT (Larion et al., 2014; Tiller et al., 2015).. Although the sample size was small (n = 200), Tiller reported that 8% of women underwent a diagnostic procedure after a normal NIPT result. Reasons given by women for electing CVS or amniocentesis varied, including continued maternal anxiety about a chromosome abnormality, ultrasound findings, and positive biochemical screening results. Another study of women over 35 years of age

showed similar rates of uptake of invasive diagnostic procedures after a normal NIPT result, stating that 7% of women had amniocentesis or CVS subsequent to a negative noninvasive prenatal test compared to 60% of women who had a positive NIPT result (Pettit et al., 2014).

Concerns have been raised over women choosing to terminate based on NIPT results without confirming aneuploidy via diagnostic testing and karyotype analysis. A cohort of high risk patients was studied to determine uptake of diagnostic testing and termination rates after positive NIPT results (Dobson et al., 2016). They found that 64% of women underwent diagnostic testing after a high risk result and 67% of women overall chose to terminate after a positive result. Significantly, of the 67% of women who chose to terminate, 11 chose to do so without pursuing diagnostic testing. Of those who chose to terminate without confirmation of the NIPT result, 82% (9/11) had ultrasound findings. Additionally, 36% (4/11) of women who chose to terminate without diagnostic testing had not received post-test counseling (Dobson et al., 2016). Natera's 2016 study focusing on women who received high risk results for 22q11.2DS found that 50.5% (n=48) of women with these results elected diagnostic testing. Of the 11 women who were found to have true positive results on diagnostic testing, 2 elected termination.

*Fetal Fraction in NIPT*

Fetal fraction is the measurement of cell-free fetal DNA in relation to circulating cell-free maternal DNA in maternal plasma. It is used as a quality control measurement during NIPT to ensure that there is a sufficient amount of cffDNA represented in the sample to validate calls being made by the clinical laboratory.

Researchers are working to understand the factors that influence fetal fraction in an effort to reduce the number of samples in which results cannot be returned as well as to optimize testing algorithms. The cutoff for fetal fraction differs between laboratories, but is generally around 4%. The average fetal fraction at 10-13 weeks, or when NIPT is most often performed, is about 10-13% (Ashoor et al., 2013; Bianchi et al., 2014).

A 2013 study by Ariosa Diagnostics looked at over 22,000 pregnancies and examined the dynamics of fetal fraction throughout gestation (Wang et al., 2013). They found that 1.9% of samples drawn after 10 weeks gestation failed to produce results because they had less than 4% fetal fraction, and that sample failure was more likely to occur in samples drawn at earlier gestations and in individuals with higher maternal weight. Additionally, they concluded that on average, fetal fraction increases 0.1% per week between 10 and 21 weeks gestation and 1% per week after 21 weeks. The inverse association between fetal fraction and maternal weight may be due to a dilution effect from an increased circulatory volume (Haghiac et al., 2012). It is also hypothesized that maternal weight causes a decrease in fetal fraction because adipose tissue turnover is known to be accelerated in obese women leading to an increase in circulation of maternal cell free DNA (Haghiac et al., 2012). Other studies have subsequently confirmed the relationships between fetal fraction and maternal weight as well as gestational age (Rava et al., 2013; Ashoor et al., 2013; Kinnings et al., 2015).

In a large retrospective study of over 23,000 NIPT samples taken from pregnancies with male fetuses, researchers in China assessed the effects of

maternal and fetal characteristics on the amount of circulating cell free fetal DNA (Zhou et al., 2015). They considered pre-gestational diabetes, hyperthyroidism, pre-existing maternal hypertension, maternal BMI, hepatitis B status, fetal aneuploidies and twin versus singleton pregnancies. They concluded that trisomy 21 and lower maternal body mass index (BMI) were associated with higher fetal fraction, while trisomy 18 and pre-existing maternal hypertension were associated with lower than average fetal fraction. Other factors did not show significant correlations.

Another study examining only the relationship between fetal aneuploidy and fetal fraction looked at fetal fractions in women 10-23 weeks gestation to determine if fetal karyotype affected cffDNA and maternal cell-free DNA ratios. They found that trisomy 21 pregnancies had higher than average fetal fractions, while trisomy 18, trisomy 13, and monosomy X pregnancies had lower than average fetal fractions (Rava et al., 2013). Pergament et al.'s reported in 2014 that in cases of fetal aneuploidy, results were not returned 16% of the time due to low fetal fraction, as opposed to approximately 5% of cases without aneuploidy.

Beyond maternal characteristics and fetal aneuploidy, researchers have begun to explore less static factors in an effort to reduce the rate at which individuals will fail to receive an NIPT result due to low fetal fraction. Early studies have indicated a potential relationship between low fetal fraction and fetal characteristics such as crown-rump length, maternal ethnicity and the level of other pregnancy-related analytes such as pregnancy associated plasma protein A (Papp-A) (Ashoor et al, 2013). The potential association between low fetal fraction and aneuploidy or

other complications of pregnancy have raised the question of whether or not low fetal fraction is itself grounds for concern.

In addition to the characteristics examined by Ashoor's group, one study looked at the effect of exercise on fetal fraction. They demonstrated that fetal fraction is lower when maternal blood samples are taken immediately after physical activity, but then return to normal by 30 minutes post-exercise (Schlütter et al., 2014).

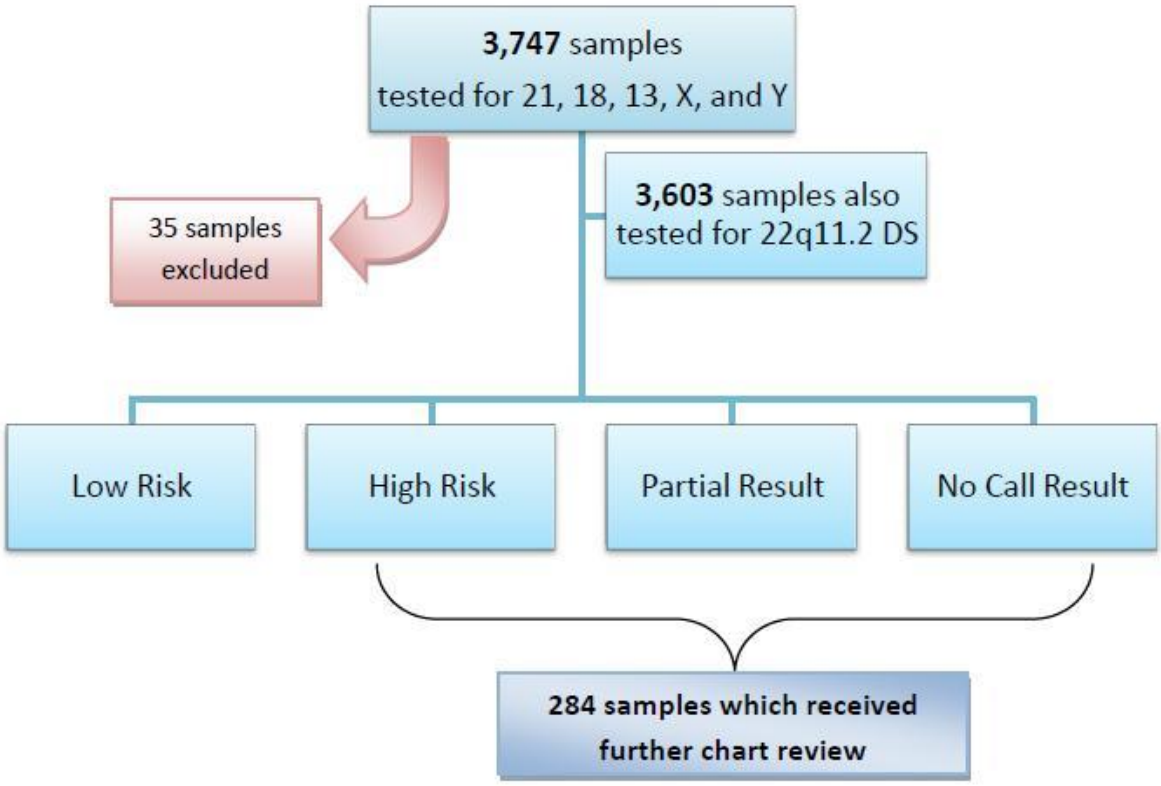
When NIPT fails with low fetal fraction as a contributing factor, laboratories often request an additional sample from the patient to rerun the test. Rescue rates of samples with insufficient fetal fraction at the first blood draw were reported by one group to be higher than 50%, even with the second blood draw taking place within 10 days of the first (Kinnings et al., 2015). Similar results were also seen by the group from Ariosa, who showed a 56% recovery rate redraw at an average of 3-4 weeks after the original blood draw (Zhou et al., 2015).

## Methods

### *Study Design*

This study is a retrospective chart review of patients at Montefiore Medical Center in the Bronx, NY. The study was approved by the Albert Einstein College of Medicine Institutional Review Board (#2013-2888) as well as the Sarah Lawrence College Institutional Review Board (#00009775) under exempt status.

*Study Sample*




The sample consisted of 3,747 blood samples drawn at Montefiore Medical Center from January 1, 2014 to August 24, 2015 on which SNP-based NIPT was performed. During this time, all pregnant women were offered NIPT regardless of their risk for a fetal chromosome abnormality. All 3,747 samples were analyzed for aneuploidy on chromosomes 21, 18, 13, X and Y. 3,603 of these samples were also analyzed for the 22q11.2 microdeletion as it was added to reports from May 7, 2014 onwards. 27 samples were initially excluded due to inadequate blood volume. The samples were then categorized by type of result: Low Risk, High Risk, Partial Result, and No Call. No Call results, which are referred to by the laboratory as “no result”,

were further categorized by the reason for test failure. An additional 8 samples were excluded due to obtaining a twin/triploidy result and/or being conceived via in-vitro fertilization. All women who received a high risk, partial, or no call report made up a cohort for which further data was collected (n = 284).

*Data Collection and Measures*

Data collected from NIPT reports included the test result, fetal fraction, maternal age at delivery, maternal weight, and gestational age at the time of sample collection. Additional information from each patient's electronic medical record was gathered if available. This consisted of maternal health conditions, past and current pregnancy complications, ultrasound findings, prenatal screening results, diagnostic testing results, and pregnancy outcomes.

For certain analyses, the cohort was divided into two sub-cohorts indicative of a patient's pre-NIPT risk. High-risk *a priori* status is determined by a set of standards that varies between hospitals, but tends to include factors such as maternal age, medical and family history, abnormal ultrasound findings, and positive maternal serum screening results (Benn et al., 2013). In this study, "low *a priori* risk" for fetal aneuploidy is defined as maternal age < 35 with no reported "high risk" indications (ultrasound abnormalities, positive screening tests, and relevant family history). These criteria are consistent with those used by the NSGC and ACOG in their position statements (2015).

*Data Analysis*

All statistical tests were performed using GraphPad InStat version 3.10 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. A $P < 0.05$ was considered statistically significant.

Mean, median, standard deviation, and ranges were calculated for patient and sample characteristics. The Mann-Whitney test was used to examine differences in characteristics between the "low *a priori* risk" and "high *a priori* risk" sub-cohorts. Linear correlation analysis was used to determine the relationships between gestational age and fetal fraction as well as the days elapsed between redraws and the change in fetal fraction.

*Patient Demographics*

According to Montefiore's 2014-2017 Community Services Plan, over 85% of hospital discharges are residents of Bronx County in New York State. The Bronx is the United States' poorest urban county with a poverty rate of 31.5% (vs. 14.8% nation-wide) as well as the highest unemployment rate at 11.9% (U.S. Census Bureau, 2014). The 2012 U.S. Census indicated that the Bronx is ethnically diverse, with its population categorized as 54.3% Hispanic, 33.2% African-American, 10.6% White, 3.7% Asian, and 3.3% other. The health status of Montefiore's population has higher than average rates of mortality, asthma, diabetes, and obesity among its patients (Montefiore Hospital, 2013).

## Results

*Patient and Sample Characteristics*

The patient and sample characteristics for the 284 reviewed samples are detailed in Table 1. Mean maternal age at time of delivery was 31.4 years and the mean gestational age at sample draw was 97.1 days or 13 weeks 6 days. Only the first draw samples were considered in this table. Table 2 compares the mean maternal age, gestational age at first sample draw, maternal weight, and fetal fraction of the "high *a priori* risk" and "low *a priori* risk" sub-cohorts.

**Table 1:** Cohort Characteristics

| **Maternal Age (years)** | *Cohort (n = 284)* |
|---|---|
| Mean | 31.4±6.2 |
| Median | 31.0 |
| Range | 15.0 - 44.0 |
| **Gestational Age (days)** | |
| Mean | 97.1±19.2 |
| Median | 90.0 |
| Range | 67.0 - 193.0 |
| **Maternal Weight (lbs)** | |
| Mean | 193.2±54.4 |
| Median | 181.0 |
| Range | 92.0 - 378.0 |
| **Fetal Fraction (%)** | |
| Mean | 5.1±3.3 |
| Median | 3.8 |
| Range | 1.0 - 18.7 |

**Table 2**: Sub-cohort Comparisons

| | *Women with high a priori risk (n = 143)* | *Women with low a priori risk (n = 141)* |
|---|---|---|
| **Maternal Age (years)** | | |
| Mean | 34.8±5.8 | 27.6±4.1 |
| Median | 36.0 | 28.0 |
| Range | 19.0 - 44.0 | 15.0 - 34.0 |
| **Gestational Age (days)** | | |
| Mean | 101.3±23.4 | 93.1±13.0 |
| Median | 91.0 | 89.0 |
| Range | 67.0 - 193.0 | 67.0 - 150.0 |
| **Maternal Weight (lbs)** | | |
| Mean | 193.1±52.4 | 194.1±55.2 |
| Median | 185.0 | 181.0 |
| Range | 92.0 - 348.0 | 98.0 - 375.0 |
| **Fetal Fraction (%)** | | |
| Mean | 5.6±3.3 | 4.6±3.3 |
| Median | 4.5 | 3.3 |
| Range | 1.5 - 17.1 | 1.0 - 18.7 |

For Maternal Age, the medians differed significantly ($p < 0.0001$).
For Gestational Age (first draw), the medians differed significantly ($p = 0.0132$).
For Maternal Weight, the medians did not differ significantly ($p = 0.9516$).
For Fetal Fraction, the medians differed significantly ($p = 0.0004$).

*High Risk Results*

A breakdown of samples that received high risk NIPT results are listed in Table 3. Overall, 1.33% (n=48) of reports were high risk. One third (16 out of 48) of all high risk reports reported indicated a high risk for 22q11.2 DS. Of women who received a result indicating a high risk for 22q11.2 DS, 37.5% elected diagnostic testing via amniocentesis or CVS. None of the 5 whose diagnostic testing results

were available were found to have an affected pregnancy. Positive predictive values could not be calculated due to constraints on the sample size. Table 4 summarizes the number of high risk NIPT results stratified by sub-cohort (high or low a priori risk), or the pre-NIPT risk of the patients.

**Table 3:** NIPT High Risk Results and Pregnancy Outcomes

| High Risk Result | # | % of High Risk Results | % of Total (3,603) | Amnio or CVS | POC | Consistent with Diagnostic Test Result | TOP | SAB | Term | N/A |
|---|---|---|---|---|---|---|---|---|---|---|
| **22q11.2 DS:** | 16 | 33.33 | 0.44% | 6 | 0 | 0 of 5* | 0 | 0 | 11 | 5 |
| **Monosomy X:** | 8 | 16.67 | 0.22% | 3 | 0 | 2 of 3 | 1 | 1 | 6 | 0 |
| **Trisomy 13:** | 8 | 16.67 | 0.22% | 6 | 0 | 2 of 6 | 3 | 0 | 4 | 2 |
| **Trisomy 18:** | 6 | 12.50 | 0.17% | 2 | 2 | 3 of 3* | 3 | 3 | 1 | 0 |
| **Trisomy 21:** | 5 | 10.42 | 0.14% | 4 | 0 | 3 of 4 | 3 | 1 | 2 | 1 |
| **XXY:** | 3 | 6.25 | 0.08% | 2 | 0 | 1 of 2 | 1 | 0 | 1 | 1 |
| **XXX:** | 1 | 2.08 | 0.03% | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| **XYY:** | 1 | 2.08 | 0.03% | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Total** | 48 | | 1.33% | 23 | 2 | 11 of 24 | 11 | 5 | 26 | 10 |

* One CVS sample failed to yield a result in each of these categories.

**Table 4:** High Risk NIPT Results Stratified by Sub-cohort

| Sub-cohort | Total | High Risk NIPT Results: | 22q | MX | T13 | T18 | T21 | XXY | XXX | XYY | % of Sub-cohort with HR Results |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Women with High a priori Risk** | 143 | 33 | 8 | 7 | 6 | 6 | 4 | 1 | 1 | 0 | **23.08%** |
| **Women with Low a priori Risk** | 141 | 15 | 8 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | **10.64%** |

*Decision Making*

The breakdown of all high risk NIPT results by pregnancy outcome and diagnostic testing uptake is detailed in Table 3. Of the 48 high risk NIPT results,

47.9% (n=23) elected to have a diagnostic procedure (amniocentesis or CVS), 41.7% (n=20) declined diagnostic testing and the remaining patients experienced a miscarriage or intrauterine fetal demise. Over 9% (22 of 236) of patients who received a no call or partial result on NIPT also elected to have diagnostic testing. Of those with no call reports who subsequently chose diagnostic testing, 68.2% (15 patients) had received abnormal ultrasound findings, other abnormal prenatal screening results (such as first trimester or quad screens), or had relevant family history, while 31.8% (7 patients) had not.

Overall, 8 patients elected to terminate their pregnancies after aneuploidy was confirmed via diagnostic testing and 2 patients elected to terminate their pregnancies without confirmation via diagnostic testing. In both cases, significant ultrasound anomalies were present. 6 patients who received a high risk NIPT result and had abnormal findings on ultrasound did not opt for termination.

*No Call Samples*

Result category was determined for 3,747 samples (low risk, high risk, partial result, no call). The total number of no call samples was 282, or 7.53% of total samples. The majority of no call reports occurred on a patient's first blood draw with 213 (5.68%) of no call samples represented in this category. The remaining 1.84% (69 samples) of no call reports occurred when the sample was a redraw on a patient with a previous no call or partial result report. Table 5 displays the number of no call reports on first draw by category as listed on the reports. Laboratory processing and low fetal fraction each represented 31.92% of all no call reports. The third most

common explanation was a low fetal fraction in combination with analytical factors, representing 20.66% of reports.

**Table 5:** No Call Report Categories, Redraw Uptake, and Redraw Success Rates for No Call Samples

| No Call Report Types (1st Draw only) | Percent of Total (out of 213 no call reports) | Redraw Uptake by Category | Success Rate of Redraw[g] | Fail Rate of Redraw[h] | Partial Result Rate of Redraw[i] |
|---|---|---|---|---|---|
| **DNA Pattern[a]** | 8.92 (19) | 10.53 (2/20) | 0.00 (0/2) | 100.00 (2/2) | 0.00 (0/2) |
| **Laboratory Processing[b]** | 31.92 (68) | 69.12 (44/68) | 88.64 (39/44) | 4.55 (2/44) | 6.82 (3/44) |
| **Low ff[c]** | 31.92 (68) | 73.53 (50/68) | 10.00 (5/50) | 84.00 (42/50) | 6.00 (3/50) |
| **Low ff with analytic factors[d]** | 20.66 (44) | 68.18 (30/44) | 60.00 (18/30) | 30.00 (9/30) | 10.00 (3/30) |
| **Quality Metrics[e]** | 6.57 (14) | 50 (7/14) | 14.29 (1/7) | 85.71 (6/7) | 0.00 (0/7) |
| **Uninformative maternal/fetal DNA pattern[f]** | 0.47 (1) | 00 (1/1) | 100.00 (1/1) | 0.00 (0/1) | 0.00 (0/1) |
| **Totals** | 100.00 (213) | 67.87 (131/193)* | 48.09 (63/131)* | 45.04(59/131)* | 6.08 (9/131)* |

Results are listed as percentages with number of samples included in parentheses. **a.** Reported as "No results due to DNA pattern that cannot be interpreted by this assay. A repeat specimen is not indicated." **b.** Reported as "Laboratory processing of this specimen could not yield results; therefore, submission of a repeat specimen is required for testing." **c.** Reported as "No results. Fetal fraction was below the threshold for analysis." **d.** Reported as "No results due to borderline low fetal fraction in combination with other analytic factors likely specific to this sample." **e.** Listed on the report as "Unable to report. This sample was processed and does not meet quality metrics" **f.** Reported as "No results due to uninformative (unmatching) maternal/fetal DNA patterns. A repeat specimen is not indicated." **g.** Success rate is determined by the number of women that received a result on all chromosomes being tested and 22q11.2 deletion syndrome upon redraw after receiving a no call report from their initial blood draw (all individuals in this category received low risk results upon redraw). **h.** Fail rate is determined by the number of women who received a no call report upon redraw after receiving a no call report from their initial blood draw. **i.** Partial rate is determined by the number of women who received a partial result upon redraw after initially receiving a no call report from their initial blood draw. *The DNA pattern and uninformative maternal/fetal DNA pattern categories were excluded from this calculation as the laboratory did not request redraw on these samples.

*Redraw Uptake and Success Rates*

Uptake of NIPT redraw after receipt of a no call report on an initial blood draw is shown in Table 5. The overall redraw rate across all no call report types was 67.87%. Amongst categories for which a redraw was indicated, success was highest for those indicated as laboratory processing error (88.64%) and second highest for those indicated as low fetal fraction and analytical factors (60%). Those that received a no call report due to low fetal fraction or quality metrics had much

lower success rates at redraw (10% and 14.29%, respectively). Cases in which the initial draw failed with low fetal fraction as a contributing factor (low ff and low ff with analytic factors) had a redraw success rate of 28.75% (23 out of 80 women). If partial results are considered as successful redraws the success rate was 36.25% (29 out of 80 women). If only women 220 pounds or less are considered, redraw success rates were 37.5% (15 out of 40 women) for full results and 42.5% (17 out of 40 women) when partial results are included.

*Redraw Samples and FF Change*

For women who received no call reports after their initial blood draw with low fetal fraction indicated as a cause (categories: low fetal fraction and low fetal fraction with analytical factors) and chose to have a redraw, changes in fetal fraction were compared between the initial blood draw and the redraw as depicted in Table 6. Fetal fraction increased on average by 0.564% (0.458% in the low fetal fraction group and 0.746% in the low fetal fraction with analytic factors group). Fetal fraction increased between blood draws in 67.11% of samples (51 out 76 redraws) and decreased in 32.89% (25 out of 76 redraws) with an average time between initial draw and redraw of 16.10 days.

For all samples that had redraw data available, a significant positive correlation was found between the number of days from the first to second draws and the change in fetal fraction % ($r = 0.2472$, $P = 0.0175$). Overall, there was a significant positive correlation between gestational age and fetal fraction % ($r = 0.2294$, $P = 0.0010$).

**Table 6**: Changes in Fetal Fraction between Initial Blood Draw and Redraw

| Result of 1st Draw | Total # Women Who Elected Redraw | Average change in ff between 1st and 2nd draw (%) | % of Women Positive Change[a] in ff between draws | % Women with a Negative Change[b] in ff between draws | Avg. Days to Redraw |
|---|---|---|---|---|---|
| **Low ff** | 48 | +0.458 | 68.75 (33/48) | 31.25 (15/48) | 15.92 |
| **Low ff and analytic factors** | 28 | +0.746 | 64.29 (18/28) | 35.71 (10/28) | 16.28 |
| **Totals** | 76 | +0.564 | 67.11 (51/76) | 32.89 (25/76) | 16.10 |

a. Cases in which a woman's fetal fraction increased from the initial draw to the redraw are considered to have a positive change in fetal fraction. b. Cases in which a woman's fetal fraction decreased from the initial draw to the redraw are considered to have a negative change in fetal fraction.

## Discussion

*Limitations*

A significant limitation of this study is the small sample size, a reflection of the low prevalence rates of the conditions detected on NIPT. Moreover, many patient charts were incomplete, mostly due to loss of patients to follow up and missed appointments, which may contribute to an underestimation of prevalence rates in our present findings. Our sample population, as described in the Methods section, has higher than average rates of obesity, diabetes, and asthma. These are all confounding factors that may impact measures such as fetal fraction and accordingly, the number of no call reports seen.

*22q11.2 Deletion Syndrome Results*

This retrospective pilot study of NIPT patients in a general obstetrical population found that fully a third of high risk results received by Montefiore Medical Center were attributed to testing for 22q11.2DS. Given the low PPV of testing for 22q11.2DS, it is not surprising that all 5 women who elected confirmatory diagnostic

testing were found to have false positive NIPT results. This outcome emphasizes the importance of thorough pre- and post-test counseling regarding not only the features of 22q11.2DS, but also the meaning of a high risk result for 22q11.2DS in relation to a high risk result for any aneuploidies on the NIPT panel. As seen here, the low positive predictive values of 22q11.2DS mean that many women may undergo diagnostic testing unnecessarily even if rates of follow-up testing remain low.

Patients who receive high risk results for 22q11.2DS require extra care and attention from clinicians, since the decision to do confirmatory diagnostic testing is complicated by the low likelihood that the screen will be a true positive relative to other conditions on NIPT panel. In total, 37.5% of our patients who had a high risk result for 22q11.2DS elected diagnostic testing. This is in contrast to 56.67% of patients who elected diagnostic testing for all other high risk results or 62.96% of women if those who miscarried are excluded.

It is possible that, in their decision making process, some patients weigh a low PPV and a “milder phenotype” versus the risks of an invasive diagnostic procedure and decide against confirmatory testing. The idea that women may find a 22q11.2DS result less concerning is further supported by the decisions made by those who are found to have true positive results. Although none of the women in this study who elected diagnostic testing were found to have true positive results, a recent study published by Natera found that of 11 women found to be carrying a pregnancy affected by 22q11.2DS through microarray analysis, only 2 chose to terminate (Gross et. al, 2016). This may have implications regarding the clinical utility of adding 22q11.2DS and other microdeletion syndromes to NIPT panels.

*No Call Reports and Redraw Success Rates*

According to internal data published on no call reports, redraw success rates range from 41% for women with the lowest initial fetal fraction (<2.0%) and highest maternal weight (>220 lbs) to 87% for women with the highest initial fetal fraction that did not yield results (>3.4%) and lowest maternal weight (<165 lbs) for the Panorama Screen (Natera, 2015). Other studies also report redraw success rates in this range (Zhou et al, 2015; Kinnings et al., 2015).

Redraw success rates in this study for individuals with low fetal fraction and low fetal fraction with analytic factors on their initial NIPT report were significantly lower, at 28.75% for analysis of the full NIPT panel or 36.25% if partial results are considered as successful. One explanation for this discrepancy is that the study population is known to have high rates of obesity. However, if only women who weigh less than 220 lbs are considered from the data set, redraw success rates in this population still remain low at 37.5%, or 42.5% including partial results.

Although a direct explanation cannot be provided for the low redraw success rate in Montefiore's population, these results may suggest that fetal fraction and redraw success depend on factors other than maternal weight and gestational age. One study found that women of Afro-Caribbean descent had significantly lower fetal fractions than Caucasian women (Ashoor et al., 2013). It is possible that genetic or cultural factors associated with ethnicity are impacting NIPT results in this cohort of obstetrical patients, as nearly a third of the population that Montefiore Medical Center serves identifies as African-American. Future studies exploring conditions

specific to this population as opposed to other more frequently studied cohorts would need to be done to understand differences in fetal fraction more completely.

In addition to the lower success rate, 32.89% of samples were found to have a negative change in fetal fraction between initial draw and redraw. Although many studies, including this one, have shown that fetal fraction tends to increase with gestational age, these studies do not look at factors affecting fetal fraction changes in individuals (Rava et al., 2013; Ashoor et al., 2013; Kinnings et al., 2015). At this point it is unclear what other factors influence changes in fetal fraction in individuals between blood draws. One study demonstrated that fetal fraction is lower when maternal blood samples are taken immediately after physical activity, but then return to normal at 30 minutes post-exercise (Schlütter et al., 2014). This may suggest that fetal fraction is more dynamic than previously thought and that many factors that have yet to be elucidated can impact the amount of circulating fetal DNA in maternal plasma. This concept is further supported by the observation that although fetal fraction is thought to increase by 0.1% each week of gestation (Wang et al., 2013), many women who receive no call reports on an initial blood draw are able to obtain results upon redraw within a few weeks even if their original fetal fraction was well below the threshold for analysis.

Further research examining characteristics of women who showed a decrease in fetal fraction between draws compared with those who showed an increase in fetal fraction is needed. With increased understanding of this topic, it may be possible to determine which factors influence whether fetal fraction will increase or decrease during a pregnancy and provide more accurate success rates

to patients who receive a no call report. In addition, since recent studies have reported that low fetal fraction is associated with an increased risk for aneuploidy (Ashoor et al, 2013; Palomaki et al., 2015), a better understanding of what other factors can cause low fetal fraction could help to elucidate women at a higher risk after a result indicating low fetal fraction.

*Post-test Counseling Challenges*

The observation that over 9% of patients who received a no call or partial result chose to proceed with diagnostic testing suggests that the uncertainty of NIPT results may increase maternal anxiety. Certain factors causing a no call report, such as a low fetal fraction, have been linked to an increased risk for aneuploidy (Pergament et al., 2014). A redraw is typically offered in such cases, but it raises the question of whether or not patients should be counseled on the probability of a successful redraw as well as the potential implications of a delayed diagnosis. For these cases, it will be of the utmost importance to emphasize the recommendations of the American Congress of Obstetricians and Gynecologists (ACOG) in offering comprehensive ultrasound evaluation and diagnostic testing.

The significant number of 22q11.2DS and no call cases in this population means that detailed post-test counseling is required for many patients. Given the current shortage of genetic counselors and other clinicians who are able to provide genetic counseling, offering NIPT to the general population and including microdeletion syndromes could have a large impact on the field. It is possible that this could result in an increased number of women electing termination without

confirmatory testing, which is especially concerning in the context of the low PPVs of microdeletion syndromes.

Current ACOG guidelines regarding cell-free DNA screening advise against routine screening for microdeletion syndromes. It will be important for the ordering clinician to distinguish between the standard NIPT panel for aneuploidies versus an expanded panel that includes microdeletion syndromes when determining the appropriate panel for their patient. The NSGC, ACMG, and ACOG all recommend thorough patient education which includes pre- and post-test counseling for all NIPT patients. This issue has the potential to be exacerbated in the coming years, as offering NIPT to the general population will greatly increase demands on current genetic counseling resources and the quality of pre- and post-test counseling may suffer.

## Conclusion

This pilot study sets the stage for future research into NIPT utility, especially with regards to 22q11.2DS. It would be beneficial to obtain follow up data on women who receive high risk results as well as educating women about 22q11.2DS and surveying them to determine how recieving these results would impact decision making regarding their pregnancies. Additionally, a data set from a larger sample size that includes ethnic background and socioeconomic information would provide insight into any differences between populations. Qualitative research such as focus groups or interviews may reveal important facts about a particular population and its attitudes towards NIPT. These findings will be important for clinicians serving similar populations throughout the US.

It would also be helpful to further examine differences in fetal fraction changes across women of various ethnic backgrounds and socioeconomic classes. To help elucidate what factors affect fetal fraction, women could be given surveys immediately preceding blood draws for NIPT to observe characteristics such as diet, sleep, exercise, time of day, and maternal conditions. It would also be important to determine if fetal fraction levels are variable throughout a pregnancy despite a net increase with gestational age. This information could aid in providing more accurate risk assessments and redraw success rates for women who receive no call reports.

Although the implementation of NIPT as a screening tool in the general population has increased accuracy compared to traditional screening methods, this new technology has also presented challenges in practice. Adding additional conditions such as 22q11.2DS to NIPT panels will affect pre and post-test counseling and complicate patient decision making. Overall, our study suggests patients need to be aware that NIPT does not replace diagnostic testing, that PPVs differ significantly for microdeletion syndromes, and that redraw success is variable. Additionally, understanding the dynamics of NIPT including no call reports and how the reason for a no call report may influence the likelihood of a successful redraw, and the relationship between fetal fraction and outcome data on the pregnancy and the fetus, may improve how NIPT is used on an individual level. Differences in the characteristics of the population being offered NIPT, including prior risk for aneuploidy, maternal characteristics such as weight, and attitudes toward diagnostic testing can also have an impact on the clinical utility of this screening tool.

## References


Agatisa, P.K., Mercer, M.B., Leek, A.C., Smith, M.B., Philipson, E., & Farrell, R.M. (2015). A first look at women's perspectives on noninvasive prenatal testing to detect sex chromosome aneuploidies and microdeletion syndromes. *Prenatal Diagnosis*, 35(7), 692-698.

American Congress of Obstetricians and Gynecologists. (2015). Cell-free DNA screening for fetal aneuploidy. Committee Opinion No. 640. *Obstet Gynecol*,126,e31-7.

Ashoor, G., Syngelaki, A., Poon, L. C. Y., Rezende, J. C., & Nicolaides, K. H. (2013). Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound in Obstetrics & Gynecology*, 41(1), 26-32.

Benn, P., Borell, A., Chiu, R., Cuckle, H., Dugoff, L., Faas, B., Gross, S., Johnson, J., Maymon, R., Norton, M., Odibo, A., Schielen, P., Spencer, K., Huang, T., Wright, D. & Yaron, Y. (2013). Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. *Prenat Diagnosis*, Jul;33(7):622-9. doi: 10.1002/pd.4139. Epub 2013 May 21.

Bianchi, D. W., Platt, L. D., Goldberg, J. D., Abuhamad, A. Z., Sehnert, A. J., & Rava, R. P. (2012). Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstetrics & Gynecology*, 119(5), 890-901.

Bianchi, D.W. & Wilkins-Haug, L. (2013). Integration of Noninvasive DNA Testing for Aneuploidy into Prenatal Care: What Has Happened Since the Rubber Met the Road? *Clin Chem*, 60(1), 78-87.

Bianchi, D. W., Parker, R. L., Wentworth, J., Madankumar, R., Saffer, C., Das, A. F., Craig, J.A., Chudova, D.I., Devers, P.L., Jones, K.W., Oliver, K., Rava, R.P., & Sehnert, A. J. (2014). DNA sequencing versus standard prenatal aneuploidy screening. *NEJM*, 370(9), 799-808.

Bianchi, D.W., Chudova, D., Sehnert, A.J., Bhatt, S., Murray, K., Prosen, T.L., Garber, J.E., Wilkins-Haug, L., Vora, N.L., Warsof, S., Goldberg, J., Ziainia, T., & Halks-Miller, M. (2015). Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies. *JAMA*, 314(2), 162-169.

Botto, L.D., May, K., Fernhoff, P.M., Correa, A., Coleman, K., Rasmussen, S.A., Merritt, R.K., O'Leary, L.A., Wong, L.Y., Elixson, E.M., Mahle, W.T., & Campbell, R.M. (2003). A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics*, 112,101–107.

Chetty, S., Garabedian, M. J., & Norton, M. E. (2013). Uptake of noninvasive prenatal testing (NIPT) in women following positive aneuploidy screening. *Prenatal Diagnosis*, 33(6), 542-546.

Dan, S., Wang, W., Ren, J., Li, Y., Hu, H., Xu, Z., Lau, T.K., Zhao, W., Huang, H., Xie, J., Sun, L., Zhang, X., Wang, W., Liao, S., Qiang, R., Cao, J., Zhang, Q., Zhou, Y., Zhu, H., Zhong, M., Guo, Y., Lin, L., Gao, Z., Yao, H., Zhang, H., Zhao, L., Jiang, F., Chen, F., Jiang, H., Li, S., Li, Y., Wang, J., Wang, J., Duan, T., Su, Y., & Zhang, X. (2012). Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11 105 pregnancies with mixed risk factors. *Prenatal diagnosis*, 32(13), 1225-1232.

Dar, P., Curnow, K.J., Gross, S.J., Hall, M.P., Stosic, M., Demko, Z., Zimmermann, B., Hill, M., Sjgurjonsson, S., Ryan, A., Banjevic, M., Kolacki, P.L., Koch, S.W., Strom, C.M., Rabinowitz, M., & Benn, P. (2014). Clinical experience and follow-up with large scale single-nucleotide polymorphism—based noninvasive prenatal aneuploidy testing. *American Journal of Obstetrics and Gynecology*, 211;527.e1-17.

Devers, P.L., Cronister, A., Ormond, K.E., Facio, F., Brasington, C.K., & Flodman, P. (2013). Noninvasive Prenatal Testing/Noninvasive Prenatal Diagnosis: the Position of the National Society of Genetic Counselors. *Journal of Genetic Counseling*, 22(3), 291-295.

Dobson, L., Reiff, E., Little, S., Wilkins-Haug, L., & Bromley, B. (2016). Patient Choice and Clinical Outcomes Following Positive Noninvasive Prenatal Screening for Aneuploidy with Cell-free DNA (cfDNA). *Prenatal Diagnosis*, Epub ahead of print.

Gil, M. M., Akolekar, R., Quezada, M. S., Bregant, B., & Nicolaides, K. H. (2014). Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal diagnosis and therapy*, 35(3), 156-173.

Grati, F. R., Malvestiti, F., Ferreira, J. C., Bajaj, K., Gaetani, E., Agrati, C., Grimi, B., Dulcetti, F., Ruggeri, A., De Toffol, S., Maggi, F., Wapner, R., Gross, S., & Simoni, G. (2014). Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. *Genetics in Medicine*, 16(8), 620-624.

Gregg, A.R., Gross, S.J., Best, R.G., Monaghan, K.G., Bajaj, K., Skotko, B.G., Thompson, B.H., & Watson, M.S. (2013). ACMG statement on noninvasive prenatal screening for fetal aneuploidy. *Genetics in Medicine*, 15, 395-398.

Gross, S. J., Stosic, M., McDonald-McGinn, D. M., Bassett, A. S., Norvez, A., Dhamankar, R., Kobara, K., Kirkizlar, E., Zimmermann, B., Wayham, N., Babiarz, J. E., Ryan, A., Jinnett, K. N., Demko, Z., & Benn, P. (2016). Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal

screening for 22q11. 2 deletion syndrome. *Ultrasound in Obstetrics & Gynecology*. 47(2), 177-183.

Hacıhamdioğlu, B., Hacıhamdioğlu, D. & Delil, K. (2015). 22q11 deletion syndrome: current perspective. *The Application of Clinical Genetics,* 8: 123-132.

Haghiac, M., Vora, N. L., Basu, S., Johnson, K. L., Presley, L., Bianchi, D. W., & Mouzon, S. H. (2012). Increased Death of Adipose Cells, a Path to Release Cell-Free DNA Into Systemic Circulation of Obese Women. *Obesity*, 20(11), 2213-2219.

Helgeson, J., Wardrop, J., Boomer, T., Almasri, E., Paxton, W. B., Saldivar, J. S., Dharajiya, N., Monroe, T.J., Farkas, D.H., Grosu, D.S., & McCullough, R. M. (2015). Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenatal Diagnosis*, 35(10), 999-1004.

Hochstenbach, R., Page-Christiaens, G.C.M.L, van Oppen, A.C.C., Lichtenbelt, K.D., van Harssel, J.J.T., Brouwer, T., Manten, G.T.R., van Zon, P., Elferink, M., Kusters, K., Akkermans, O., Ploos van Amstel, J.K., & Schuring-Blom, G.H. (2015). Unexplained False Negative Results in Noninvasive Prenatal Testing: Two Cases Involving Trisomies 13 and 18. *Case Reports in Genetics*, 2015, ePub.

Kinnings, S. L., Geis, J. A., Almasri, E., Wang, H., Guan, X., McCullough, R. M., Bombard, A. T., Saldivar, J.-S., Oeth, P., & Deciu, C. (2015). Factors affecting levels of circulating cell-free fetal DNA in maternal plasma and their implications for noninvasive prenatal testing. *Prenatal Diagnosis*, 35, 816–822.

Larion, S., Warsof, S.L., Romary, L., Mlynarczyk, M., Peleg, D., & Abuhamad, A.Z. (2015). Use of the Combined First-Trimester Screen in High- and Low-Risk Patient Populations After Introduction of Noninvasive Prenatal Testing. *J Ultrasound Med*, 34(8), 1423-1428.

Leach, M. W. (2015). Unjustified: The imbalance of information and funding with noninvasive prenatal screening. *AJOB Empirical Bioethics*, 6(1), 21-30.

Levy, B., & Norwitz, E. (2013). Non-invasive prenatal aneuploidy testing: technologies and clinical implication. *MLO: Medical Laboratory Observer*, *45*(6), 8-10.

McNamara, C.J., Limone, L.A., Westover, T., & Miller, R.C. (2015). Maternal source of false-positive fetal sex chromosome aneuploidy in noninvasive prenatal testing. *Obstet Gynecol*, 125(2), 390-2.

Montefiore Hospital. (2013). Montefiore Medical Center Community Health Needs Assessment and Implementation Strategy 2013. Retrieved March 17, 2016

from http://www.montefiore.org/documents/communityservices/2013-CHNA1-with-CSS-Inventory.pdf

Montefiore Hospital. (2014). Montefiore 2014-2017 Community Services Plan. Retrieved March 17, 2016 from http://www.montefiore.org/documents/communityservices/Montefiore-2014-2017-Community-Services-Plan.pdf

Natera, Inc. (2015). *Report Supplement for No Result* [Brochure]

Neufeld-Kaiser, W. A., Cheng, E. Y., & Liu, Y. J. (2015). Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. *BMC Medicine*, 13(1), 129.

Nicolaides, K. H., Syngelaki, A., Gil, M., Atanasova, V., & Markova, D. (2013). Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenatal diagnosis*, 33(6), 575-579.

Palomaki, G. E., Kloza, E. M., Lambert-Messerlian, G. M., Haddow, J. E., Neveux, L. M., Ehrich, M., van den Boom, D., Bombard, A. T., Deciu, C., Grody, W. W., Nelson, S. F., & Canick, J. A. (2011). DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genetics in Medicine*, 13(11), 913-920.

Palomaki, G. E., Kloza, E. M., Lambert-Messerlian, G. M., Boom, D., Ehrich, M., Deciu, C., Bombard, A. T., & Haddow, J. E. (2015). Circulating cell free DNA testing: are some test failures informative?. *Prenatal diagnosis*, *35*(3), 289-293.

Pettit, K. E., Hull, A. D., Korty, L., Jones, M. C., & Pretorius, D. H. (2014). The utilization of circulating cell-free fetal DNA testing and decrease in invasive diagnostic procedures: an institutional experience. *Journal of Perinatology*. 34, 750-753.

Pergament, E., Cuckle, H., Zimmermann, B., Banjevic, M., Sigurjonsson, S., Ryan, A., Hall, M.P., Dodd, M., Lacroute, P., Stosic, M., Chopra, N., Hunkapiller, N., Prosen, D. E., McAdoo, S., Demko, Z., Siddiqui, A., Hill, M., & Rabinowitz, M. (2014). Single-nucleotide polymorphism–based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstetrics & Gynecology*, 124(2, PART 1), 210-218.

Rava, R. P., Srinivasan, A., Sehnert, A. J., & Bianchi, D. W. (2014). Circulating fetal cell-free DNA fractions differ in autosomal aneuploidies and monosomy X. *Clinical Chemistry*, 60(1), 243-250.

Roman, A., Desai, N., Krantz, D., Liu, H.P., Rosner, J., Vohra, N., Rochelson, B. (2014). Maternal serum analytes as predictors of IUGR with different degrees of placental vascular dysfunction. *Prenatal Diagnosis*, 34(7), 692-698.

Samango-Sprouse, C., Banjevic, M., Ryan, A., Sigurjonsson, S., Zimmermann, B., Hill, M., Hall, M. P., Westemeyer, M., Saucier, J., Demko, Z., & Rabinowitz, M. (2013). SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. *Prenatal diagnosis*, 33(7), 643-649.

Schlütter, J.M., Hatt, L., Bach, C., Kirkegaard, I., Kolvraa, S., & Uldbjerg, N. (2014). The cell-free fetal DNA fraction in maternal blood decreases after physical activity. *Prenatal Diagnosis*, 34(4), 341-344.

Sifakis, S., Koukou, Z., & Spandidos, D. (2015). Cell-free fetal DNA and pregnancy-related complications (Review). *Mol Med Reports*, 11, 2367-2372.

Snyder, M. W., Simmons, L. E., Kitzman, J. O., Coe, B. P., Henson, J. M., Daza, R. M., Eichler, E.E., Shendure, J., & Gammill, H. S. (2015). Copy-number variation and false positive prenatal aneuploidy screening results. *NEJM*, 372(17), 1639-1645.

Tiller, G.E., Kershberg, H.B., Goff, J., Coffeen, C., Liao, W., Sehnert, A.J. (2015). Women's views and the impact of noninvasive prenatal testing on procedures in a managed care setting. *Prenatal Diagnosis*, 35(5), 428-433.

Taglauer, E.S., Wilkins-Haug, L., & Bianchi, D.W. (2014). Review: Cell-free fetal DNA in the maternal circulation as an indication of placental health and disease. *Placenta*, 35, Supp A, Trophoblast Research Vol. 28, S64-S68.

Taylor-Phillips, S., Freeman, K., Geppert, J., Agbebiyi, A., Uthman, O., Madan, J., Clarke, A., Quenby, S. & Clarke, A. (2016). Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis. *BMJ Open*, Jan 18;6(1):e010002. doi: 10.1136/bmjopen-2015-010002.

U.S. Census Bureau. (2014). State & county quickfacts: New York (city), New York. Retrieved March 17, 2016 from http://quickfacts.census.gov/qfd/states/36/3651000.html

Wang, E., Batey, A., Struble, C., Musci, T., Song, K., & Oliphant, A. (2013). Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenatal diagnosis*, *33*(7), 662-666.

Wapner, R. J., Martin, C. L., Levy, B., Ballif, B. C., Eng, C. M., Zachary, J. M., ... & Jackson, L. (2012). Chromosomal microarray versus karyotyping for prenatal diagnosis. *NEJM*, 367(23), 2175-2184.

Wapner, R. J., Babiarz, J. E., Levy, B., Stosic, M., Zimmermann, B., Sigurjonsson, S., Wayham, N., Ryan, A., Banjevic, M., Lacroute, P., Hu, J., Hall, M.P.,

Demko, Z., Siddiqui, A., Rabinowitz, M., Gross, S.J., Hill, M., & Benn, P. (2015). Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol*, 212(3), 332-e1.

Wax, J.R., Cartin, A., Chard, R., Lucas, F.L., & Pinette, M.G. (2015). Noninvasive prenatal testing: impact on genetic counseling, invasive prenatal diagnosis, and trisomy 21 detection. *J Clin Ultrasound*, 43(1), 1-6.

Weaver, C. (2013). Tough calls on prenatal tests. *Wall Street Journal*, B1, April 3, 2013.

Yao, H., Jiang F., Hu, H., Gao, Y., Zhu, Z., Zhang, H., Wang, Y., Guo, Y., Liu, L., Yuan, Y., Zhou, L., Wang, J., Du, B., Qu, N., Zhang, R., Dong, Y., Xu, H., Chen, F., Jiang, H., Liu, Y., Zhang, L., Tian, Z., Liu, Q., Zhang, C., Pan, X., Yang, S., Zhao, L., Wang, W., Liang, Z. (2014). Detection of fetal sex chromosome aneuploidy by massively parallel sequencing of maternal plasma DNA: initial experience in a Chinese hospital. *Ultrasound Obstet Gynecol*, 44, 17–24.

Zhang, H., Gao, Y., Jiang, F., Fu, M., Yuan, Y., Guo, Y., Zhu, Z., Lin, M., Liu, Q., Tian, Z., Zhang, H., Chen, F., Lau, T.K., Zhao, L., Yi, X., Yin, Y., & Wang, W. (2015). Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. *Ultrasound Obstet Gynecol*, 45(4), 530-8.

Zhou, Y., Zhu, Z., Gao, Y., Yuan, Y., Guo, Y., Zhou, L., Liao, K., Wang, J., Du, B., Hou, Y., Chen, Z., Chen, F., Zhang, H., Yu, C., Zhao, L., Lau, T.K., Jiang, F., & Wang, W. (2015). Effects of Maternal and Fetal Characteristics on Cell-Free Fetal DNA Fraction in Maternal Plasma. *Reproductive Sciences*, 22, 1429-1435.