OBSTETRICS

Obstetrical, perinatal, and genetic outcomes associated with nonreportable prenatal cell-free DNA screening results

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BACKGROUND: The clinical implications of nonreportable cell-free DNA screening results are uncertain, but such results may indicate poor placental implantation in some cases and be associated with adverse obstetrical and perinatal outcomes.

OBJECTIVE: This study aimed to assess the outcomes of pregnancies with nonreportable cell-free DNA screening in a cohort of patients with complete genetic and obstetrical outcomes.

STUDY DESIGN: This was a prespecified secondary analysis of a multicenter prospective observational study of prenatal cell-free DNA screening for fetal aneuploidy and 22q11.2 deletion syndrome. Participants who underwent cell-free DNA screening from April 2015 through January 2019 were offered participation. Obstetrical outcomes and neonatal genetic testing results were collected from 21 primary-care and referral centers in the United States, Europe, and Australia. The primary outcome was risk for adverse obstetrical and perinatal outcomes (aneuploidy, preterm birth at <28, <34, and <37 weeks' gestation, preeclampsia, small for gestational age or birthweight <10th percentile for gestational week, and a composite outcome that included preterm birth at <37 weeks, preeclampsia, small for gestational age, and stillbirth at >20 weeks) after nonreportable cell-free DNA screening because of low fetal fraction or other causes. Multivariable analyses were performed, adjusting for variables known to be associated with obstetrical and perinatal outcomes, nonreportable results, or fetal fraction.

RESULTS: In total, 25,199 pregnant individuals were screened, and 20,194 were enrolled. Genetic confirmation was missing in 1165 (5.8%), 1085 (5.4%) were lost to follow-up, and 93 (0.5%) withdrew; the final study cohort included 17,851 (88.4%) participants who had cell-free DNA, fetal or newborn genetic confirmatory testing, and obstetrical and perinatal outcomes collected. Results were nonreportable in 602 (3.4%) participants. A sample was redrawn and testing attempted again in 427; in 112 (26.2%) participants, results were again nonreportable. Nonreportable results were associated with higher body mass index, chronic hypertension, later gestational age, lower fetal fraction, and Black race. Trisomy 13, 18, or 21 was confirmed in 1.6% with nonreportable tests vs 0.7% with reported results (P=.013). Rates of preterm birth at <28, 34, and 37 weeks, preeclampsia, and the composite outcome were higher among participants with nonreportable results, and further increased among those with a second nonreportable test, whereas the rate of small for gestational age infants was not increased. After adjustment for confounders, the adjusted odds ratios were 2.2 (95% confidence interval, 1.1—4.4) and 2.6 (95% confidence interval, 0.6—10.8) for aneuploidy, and 1.5 (95% confidence interval, 1.2—1.8) and 2.1 (95% confidence interval, 1.4—3.2) for the composite outcome after a first and second nonreportable test, respectively. Of the patients with nonreportable tests, 94.9% had a live birth, as opposed to 98.8% of those with reported test results (adjusted odds ratio for livebirth, 0.20 [95% confidence interval, 0.13—0.30]).

CONCLUSION: Patients with nonreportable cell-free DNA results are at increased risk for a number of adverse outcomes, including aneuploidy, preeclampsia, and preterm birth. They should be offered diagnostic genetic testing, and clinicians should be aware of the increased risk of pregnancy complications.

Key words: adverse perinatal outcomes, cell-free DNA screening, noninvasive prenatal screening, preeclampsia, preterm birth

Introduction

Noninvasive prenatal testing with cell-free DNA (cfDNA) is increasingly used for aneuploidy screening. Despite high sensitivity and specificity, some cfDNA tests do not yield a result.[1—4] The clinical significance and appropriate follow-up of nonreportable tests remain uncertain.

The most common reason for test failure is inadequate fetal cfDNA, although this can also occur when sequencing results are uninterpretable or implausible.[2,3] Fetal cfDNA arises from apoptosis of placental trophoblasts, and the quantity and quality reflect placental growth and function; a small or poorly functional placenta may be associated with aneuploidy and some adverse obstetrical and perinatal outcomes, such as hypertension, fetal growth restriction, and preterm birth.[5—8] It has been hypothesized that poor placental implantation can result in a lower quantity of fetal cfDNA early in gestation.[6,9] Although some previous studies have reported an association between fetal cfDNA and chromosomal abnormalities and other adverse obstetrical and perinatal outcomes, these have been limited by small sample sizes and incomplete follow-up of all outcomes.[2,4,5,9,10]

The objective of this study was to determine outcomes of pregnancies with

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AJOG at a Glance

Why was this study conducted?

This study was conducted to understand whether pregnancies with nonreportable results of cell-free DNA (cfDNA) screening are at increased risk of adverse obstetrical and perinatal outcomes.

Key findings

Patients with nonreportable cfDNA results had twice the risk of aneuploidy when compared with those with reported results. Those with nonreportable cfDNA results and a euploid fetus had an increased risk of preeclampsia and preterm birth.

What does this add to what is known?

Patients with nonreportable cfDNA results may have a higher risk of adverse obstetrical and perinatal outcomes in addition to aneuploidy. They should be offered diagnostic testing, and their clinicians should be aware of increased risk of pregnancy complications.

nonreportable results of cfDNA screening in a large cohort of patients with complete genetic and obstetrical outcomes.

Materials and Methods

This was a prespecified secondary analysis of a multicenter prospective observational study of cfDNA screening for aneuploidy and 22q11.2 deletion syndrome.[11,12] All women screened for trisomy 13, 18, and 21 and the 22q11.2 deletion syndrome at participating centers were eligible. Enrolled patients consented to collection of pregnancy outcomes and newborn genetic testing, and all participants provided written consent. Chromosomal microarray, karyotype, or other confirmatory diagnostic testing was performed on all fetuses or newborns, and perinatal and obstetrical outcomes were collected. Participants were enrolled at 21 centers in 6 states or countries in the United States, Europe, and Australia. The study was approved by each site's institutional review board.

Participants

Eligible women underwent screening for aneuploidy and 22q11.2 deletion syndrome, were ≥18 years old and at ≥9 weeks' gestation, had a singleton pregnancy, and planned to deliver at a study site—affiliated hospital. Women were excluded if they received cfDNA results before enrollment, had had organ transplantation, ovum donation, or a vanishing twin, or were unwilling or unable to provide a newborn sample. Results of cfDNA screening were used by providers and patients as part of clinical care.

Variables collected included maternal and obstetrical characteristics, reason for nonreportable results, fetal fraction (proportion of cfDNA of fetal origin), and genetic, obstetrical, and perinatal outcomes, including preeclampsia, preterm birth, and small for gestational age (SGA).

Patients with nonreportable results for aneuploidy were compared with those with reported results. Those for whom only risk for 22q11.2 deletion syndrome was nonreportable were analyzed in the group with reported results.

Procedures

Analysis of cfDNA was performed as previously described (Natera Inc, San Carlos, CA).[11] In some cases, the laboratory algorithm indicates that repeated testing is not likely to be successful,[13] but decisions regarding repeated screening were made by patients and their local providers. We analyzed outcomes in patients with nonreportable results on cfDNA screening, including those with any nonreportable test, those with 2 nonreportable tests, and those with a first nonreportable test and subsequent successful test (Figure).

During enrollment, the cfDNA laboratory protocol was modified once, and results from both periods were combined for this analysis. After enrollment was completed, the laboratory developed an updated algorithm to improve detection and decrease nonreportable results. Results from that analysis are presented in the Supplemental Table.

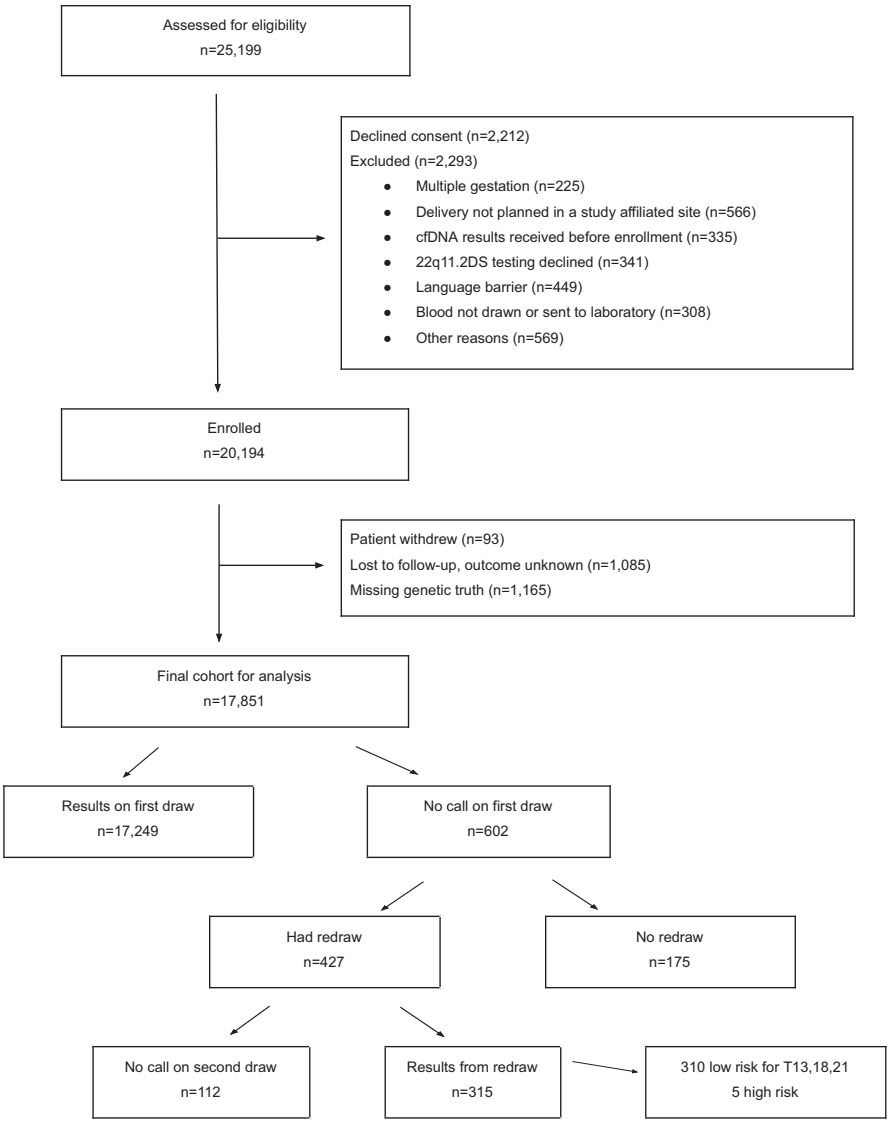
Genetic outcomes were assessed by analyzing fetal (chorionic villus sampling, amniocentesis, or products of conception) or infant (cord blood, buccal swab, or newborn blood spot) samples. A newborn sample was requested for chromosomal microarray analysis (CMA) in all cases; postnatal CMA was performed by an independent laboratory (Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA) blinded to clinical or other laboratory results. In cases without postnatal CMA confirmation, results from prenatal diagnostic testing, if available, were used for genetic confirmation.

For confirmatory CMA, DNA was prepared from neonates' cord blood, buccal smear, or dried blood spot. Copy number variants, including aneuploidies and 22q11.2 deletion syndrome, were identified using the Illumina single-nucleotide polymorphism (SNP)-based Infinium Global Screening Array platform (Illumina Inc, San Diego, CA). For quality assurance purposes, a concordance test was developed to confirm that cfDNA results and newborn samples were correctly paired using alignment between SNPs in the 2 samples; samples that could not be paired were excluded.

Outcomes

The primary outcome was the rate of adverse obstetrical and perinatal outcomes, including aneuploidy, preterm birth at <28, <34, and <37 weeks' gestation, preeclampsia, SGA birth, and a composite outcome that included preterm birth at <37 weeks, preeclampsia, SGA, and stillbirth at >20 weeks' gestation in patients who underwent cfDNA screening for aneuploidy (trisomy 13, 18, 21) and 22q11.2

FIGURE
Patient enrollment flowchart



cfDNA, cell-free DNA; *T*, trisomy.

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deletion syndrome. We compared outcomes between patients with 1 or 2 nonreportable tests and those with a successful second draw attempt, and also assessed the rate of adverse obstetrical and perinatal outcomes in the subset of patients with a euploid fetus who delivered at ≥20 weeks' gestation.

The diagnosis of preeclampsia includes hypertension and proteinuria or the new onset of hypertension and other significant end-organ dysfunction with or without proteinuria after 20 weeks' gestation or postpartum in a previously normotensive woman[14]; local providers established the diagnosis of preeclampsia at each site. Preterm birth outcomes included delivery at <28, <34, and <37 weeks' gestation in women whose pregnancies continued past 20 weeks' gestation. SGA was defined as infant birthweight <10th percentile for gestational age.[15]

We compared baseline characteristics of those who received cfDNA results and those with nonreportable tests, including maternal age, nulliparity, gestational age at blood draw, body mass index (BMI), chronic hypertension, race, assisted reproduction, and smoking (none vs any smoking during pregnancy). We further compared use of diagnostic testing (amniocentesis or chorionic villus sampling), fetal fraction, and presence of a fetal anomaly.

Multivariable analyses were performed, adjusting for variables known to be associated with obstetrical and perinatal outcomes, nonreportable results, or fetal fraction.

Data collection

Research coordinators at each site recorded information using a secured computerized tracking system developed and managed by the Data Coordinating Center at the Biostatistics Center at George Washington University, Rockville, Maryland. Collected data included patient and obstetrical data, imaging reports, serum aneuploidy screening, and prenatal diagnosis results. After delivery, information on pregnancy complications, genetic testing or ultrasound findings, newborn features suggestive of genetic abnormality, major malformations, and other adverse outcomes was collected.

Study oversight

The study was funded by Natera (Natera Inc, Austin, TX), and was a collaboration between the clinical investigators and the sponsor. The first and last authors designed the protocol with the sponsor and had a majority vote in study design and data interpretation. There were no data confidentiality agreements between the authors, sites, or sponsor. All laboratory analyses were blinded to outcome data. Clinical and laboratory results were managed by the Data Coordinating Center, which independently matched the information and deidentified and analyzed the results.

Statistical analysis

The primary study had an initial planned sample of 10,000 participants based on birth prevalence of 22q11.2 deletion syndrome.[16] During the trial, concerns arose regarding prevalence of the 22q11.2 deletions, and the sample size was increased to 20,000.[17] All participants who had cfDNA testing and fetal or newborn

genetic confirmatory testing were eligible for this analysis. Continuous variables were compared using the Wilcoxon test and categorical variables using chi-square or Fisher exact test; logistic regression was used for multivariable analyses controlling for confounders.

Results

Study participants

From April 2015 through January 2019, 25,199 women were screened, and 20,194 were enrolled from 21 centers. Overall, 56.6% were enrolled in the United States and 43.4% in Europe or Australia. Of the enrolled participants, 1165 (5.8%) were missing genetic confirmation, 1085 (5.4%) were lost to follow-up, and 93 (0.5%) withdrew; the final study cohort included 17,851 (88.4%) participants who had cfDNA screening, fetal or newborn genetic confirmatory testing, and obstetrical and perinatal outcomes (Figure).

Mean maternal and gestational ages at enrollment were 33.5 years and 13.3 weeks, respectively; 44.1% of participants were nulliparous (Table 1). Overall, 100 (0.6%) had cfDNA after sonographic detection of a fetal anomaly, 109 (0.6%) after diagnosis of a cystic hygroma or nuchal translucency ≥3 mm, and 616 (3.5%) following high-risk results on serum analyte screening for aneuploidy.

Primary and secondary outcomes

There were 602 (3.4%) cases of patients with nonreportable results after the first cfDNA draw. Of these, 194 (32.2%) were because of low fetal fraction (≤2.8%). A similar number had DNA sequencing patterns in which the aneuploidy risk could not be interpreted and that were reported to be uninformative with fetal fraction >2.8% (n=197; 32.7%) or not measurable (n=211; 35.0%). These categories were developed by the laboratory statistics team on the basis of internal metrics, but the clinical significance of these different nonreportable categories has not been validated against external measures. Of the 602 patients with nonreportable results, 427 had a redrawn second attempt, and 112 (26.2%) again had a nonreportable test.

When compared with patients who received results, those with nonreportable tests had similar maternal ages (33.7 vs 33.5 years; P=.37) and were equally likely to be nulliparous (47.1% vs 44.1%; P=.15), whereas the mean gestational age at the initial sample collection was greater for those with nonreportable tests (14.4 vs 13.3 weeks; P<.001). Those with nonreportable tests had higher BMI, particularly with 2 such tests (26.2 vs 31.3 vs 34.3 kg/m^2), and they were more likely to have chronic hypertension (4.0% vs 9.7% vs 17.3%, respectively). Fetal fraction was lower with nonreportable results, particularly in those with 2 nonreportable tests in whom the median fetal fraction was 2.6% vs 9.3% in the entire cohort (P<.001). The

TABLE 1
Characteristics of pregnancies with nonreportable results

Variable	Results with first draw N=17,249	No results with first draw N=602 (3.4%)	No results with second draw N=112 (0.6%)	Results vs no results with first draw
Mean maternal age (SD), y	33.5 (5.4)	33.7 (5.6)	34.9 (5.2)	P=.37
Nulliparity	7595 (44.1%)	281 (47.1%)	53 (49.1%)	P=.15
Mean gestational age at screening (SD), wk	13.3 (3.2)	14.4 (3.0)	14.3 (2.6)	P<.001
Mean BMI (SD), kg/m^2	26.2 (5.7)	31.3 (9.0)	34.3 (9.8)	P<.001
Median fetal fraction (IQR), %	9.4 (7.0–12.3)	4.5 (2.9–6.8)	2.6 (2.3–2.9)	P<.001
Race[a]				P<.001
Asian	1495 (8.7%)	37 (6.2%)	2 (1.8%)	
Black	1484 (8.6%)	85 (14.1%)	19 (17.0%)	
White	10,467 (60.7%)	344 (57.1%)	64 (57.1%)	
Latina	3215 (18.6%)	116 (19.3%)	23 (20.5%)	
Other/unknown	588 (3.4%)	20 (3.3%)	4 (3.6%)	
Pregnancy through assisted reproductive technology	876 (5.1%)	29 (4.8%)	5 (4.5%)	P=.77
Chronic hypertension	687 (4.0%)	58 (9.7%)	19 (17.3%)	P<.001
Never smoked in this pregnancy	16,507 (96.1%)	565 (94.7%)	102 (93.6%)	P=.16
Diagnostic testing	475 (2.8%)	69 (11.5%)	23 (20.5%)	P<.001
Fetal anomaly before testing	99 (0.6%)	1 (0.2%)	0	P=.27

BMI, body mass index; *IQR*, interquartile range.

[a] Race and ethnicity as reported by participants. If the participant did not report the information, the information from the medical record was used.

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TABLE 2
Outcomes of pregnancies with nonreportable results

Variable	Results with first draw N=17,249	No results with first draw N=602 (3.4%)	No results with second draw N=112 (0.6%)	Results with second draw[a] N=315 (1.8%)	Results vs no results with first draw
Pregnancy outcome					*P*<.001
Live birth	17,032 (98.8%)	568 (94.8%)	101 (90.2%)	311 (99.4%)	
IUFD/stillbirth	24 (0.1%)	6 (1.0%)	1 (0.9%)	1 (0.3%)	
Spontaneous loss	42 (0.2%)	7 (1.2%)	3 (2.7%)	0	
Elective termination	141 (0.8%)	18 (3.0%)	7 (6.3%)	1 (0.3%)	
Excluding aneuploidies, spontaneous loss at <20 wk, and elective termination	N=17,027	N=569	N=101	N=310	
PTB at 20.0–<37 wk	1166 (6.9%)	74 (13.0%)	21 (20.8%)	25 (8.1%)	*P*<.001
PTB at 20.0–<34 wk	260 (1.5%)	26 (4.6%)	7 (6.9%)	9 (2.9%)	*P*<.001
PTB at 20.0–<28 wk	59 (0.4%)	8 (1.4%)	2 (2.0%)	2 (0.7%)	*P*=.001
Preeclampsia	657 (3.9%)	53 (9.4%)	17/101 (16.8%)	27 (8.8%)	*P*<.001
SGA	1478 (8.8%)	55 (9.8%)	8 (7.9%)	33 (10.8%)	*P*=.40
Composite outcome (preeclampsia, SGA, PTB at 20–<37 wk, stillbirth)	2821 (16.6%)	147 (25.8%)	36 (35.6%)	71 (22.9%)	*P*<.001

IUFD, intrauterine fetal demise; *PTB*, preterm birth; *SGA*, small for gestational age.

[a] No call with first draw, successful second attempt.

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rates of conception with assisted reproduction and of smoking did not differ (Table 1).

There were 133 genetically confirmed trisomies in the cohort, as confirmed by pre- or postnatal diagnostic testing. This included 100 cases of trisomy 21, 18 cases of trisomy 18, and 15 cases of trisomy 13. The rate of nonreportable results with the initial draw varied by trisomy, and was 3% (3/100) in trisomy 21, 11% (2/18) in trisomy 18, and 33% (5/15) in trisomy 13 (*P*<.001). The median fetal fraction in trisomy 21 pregnancies was similar to that of the entire cohort at 9.7% (2.5–32.1), whereas it was 6.8% (3.7–13.1) in trisomy 18 and 5.9% (1.8–13.9) in trisomy 13 pregnancies. Overall, in 10 (7.5%) pregnancies affected with trisomy, the cfDNA screen was nonreportable with the initial draw. Four patients submitted a second test; 1 case of trisomy 21 resulted as high-risk, 1 of trisomy 18 resulted as low-risk, and the other 2 were again nonreportable. The rate of aneuploidy in reported cases was 0.7% (123/17,890) vs 1.7% (10/602) in patients with nonreportable results (*P*=.013).

After excluding cases with aneuploidy, elective terminations, and spontaneous losses at <20 weeks, the rates of preterm birth at <28, <34, and <37 weeks and preeclampsia were increased in patients with a nonreportable test (Table 2). The rate of preterm birth at <34 weeks was 1.5% in patients with a cfDNA result, and increased to 4.6% with a first and 6.9% with a second nonreportable test. Preeclampsia also increased with nonreportable tests, from 3.9% to 9.4% and 16.8% with 0, 1, and 2 nonreportable tests, respectively. The rate of the composite outcome was 16.6% in cases with reported results, as opposed to 25.8% in cases with 1 and 35.6% with 2 nonreportable tests. The rate of live birth, when evaluating the outcome of all pregnancies and including elective terminations, was significantly higher in patients with reportable results than in those with no results after the first and second draw (98.8% vs 94.8% vs 90.2%). In patients with low-risk results on a second draw, the rate of live birth was 99.4%, similar to the rate found in patients with an initial low-risk result.

After adjusting for maternal and gestational age, the adjusted odds ratio (aOR) for aneuploidy was 2.1 (95% confidence interval [CI], 1.1–4.0) after the first nonreportable test and 1.8 (95% CI, 0.4–7.3) after the second. The aORs for the composite outcome, adjusted for gestational age, BMI, and chronic hypertension, were 1.5 (95% CI, 1.2–1.8) and 2.1 (95% CI, 1.4–3.2) after the first and second nonreportable result. Regarding individual obstetrical and perinatal outcomes, the aORs were 2.2 (95% CI, 1.4–3.4) for preterm birth at <34 weeks' gestation, 1.4 (95% CI, 1.0–1.9) for preeclampsia, and 1.3 (95% CI, 0.9–1.7) for SGA. The aORs after a second nonreportable result were further increased for preterm birth at <34 weeks (2.7; 95% CI, 1.2–6.0) and for preeclampsia (2.0; 95% CI, 1.1–3.7), but not for SGA (1.1; 95% CI, 0.5–2.3). The chance of live birth was lower, with aOR of 0.20 (95% CI, 0.13–0.30) after 1

TABLE 3
Unadjusted and adjusted risk

Variable	No results with first draw N=602		No results with second draw N=112	
	OR	aOR	OR	aOR
Aneuploidy[a] (T13, 18, 21)	2.4 (1.2–4.5)	2.1 (1.1–4.0)	2.4 (0.6–10.0)	1.8 (0.4–7.3)
Live birth[b]	0.22 (0.15–0.33)	0.20 (0.13–0.30)	0.12 (0.06–0.23)	0.11 (0.06–0.23)
PTB at <34 wk[c]	3.1 (2.0–4.6)	2.2 (1.4–3.4)	4.6 (2.1–10.0)	2.7 (1.2–6.0)
Preeclampsia[c]	2.6 (1.9–3.4)	1.4 (1.02–1.95)	4.9 (2.9–8.2)	2.0 (1.1–3.7)
SGA[c]	1.1 (0.9–1.5)	1.3 (0.94–1.68)	0.9 (0.4–1.8)	1.1 (0.5–2.3)
Composite outcome (preeclampsia, SGA, PTB at <37 wk, stillbirth)[c]	1.8 (1.4–2.1)	1.5 (1.2–1.8)	2.8 (1.8–4.1)	2.1 (1.4–3.2)

aOR, adjusted odds ratio; *OR*, odds ratio; *PTB*, preterm birth; *SGA*, small for gestational age; *T*, trisomy.

[a] aORs controlled for maternal age and gestational age at first draw; [b] Adjusted for gestational age at first draw, maternal body mass index, and chronic hypertension; [c] Aneuploidies and cases with termination or less at <20 weeks' excluded, adjusted for gestational age at first draw, maternal body mass index, and chronic hypertension.

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nonreportable test and 0.11 (95% CI, 0.06–0.23) after 2 (Table 3). Finally, we compared outcomes on the basis of reason for nonreportable results, and found a higher rate of the composite outcome with fetal fraction ≤2.8% compared with >2.8% (31.3% vs 26.6%; *P*=.03). Although the rate of aneuploidy did not differ by fetal fraction, the numbers were small (Table 4).

With the updated algorithm, the no-call rate decreased to 1.4% (N=250). Of these, 182 had a redraw, and 28 had a second no-call result. The rate of preterm birth at <34 weeks was 1.6% in cases with results, and 5.6% and 14.3% in cases with a first and second nonreportable test, respectively (*P*<.001 in comparison of reported vs nonreportable results after first draw). The rate of preeclampsia increased from 4.0% to 7.3% and 19.1%, and the composite outcome was 16.8%, 25.0%, and 42.9% with 0, 1, and 2 nonreportable results, respectively (Supplemental Table).

Comment

Principal findings

These findings demonstrate that patients with nonreportable results on this SNP-based cfDNA screening test are at increased risk for aneuploidy, preterm birth, and preeclampsia. Overall, 7.5% of pregnancies with aneuploidy had a nonreportable result, and nonreportable cfDNA results doubled the risk of aneuploidy. The risk of adverse obstetrical and perinatal outcomes increased further when a redraw was again nonreportable, although the risk of aneuploidy was no longer significant, likely because of a small number of cases. In patients with a successful second draw, obstetrical and perinatal risks were lessened, although the number of cases was small. The risk of adverse outcomes was not explained by the increased rate of aneuploidy given that the risk was elevated in euploid pregnancies.

Results in the context of what is known

Many adverse obstetrical and perinatal outcomes are associated with abnormal placental development.[18] Investigators have hypothesized that cfDNA levels may be altered in patients with obstetrical complications mediated by impaired placentation, although studies have yielded conflicting results, and few have prospectively evaluated outcomes of nonreportable cfDNA screening; most have focused on fetal fraction or maternal characteristics predicting nonreportable results, and have not assessed obstetrical and perinatal outcomes in large cohorts.[3,9,19] Although most nonreportable results in most series are because of inadequate fetal DNA, this can also occur because of inability to interpret the sequencing results and inconclusive data.[20–22] The latter can result from changes in the maternal genome (eg, malignancy), a vanishing twin, high sequencing variation, or the presence of complex or multiple fetal or placental genomic variants. Several studies have now reported on the significance of low fetal fraction, but there are far fewer studies investigating nonreportable cfDNA screening based on all causes in large cohorts. Furthermore, there are few studies investigating the underlying biology of nonreportable results that are not specifically because of low fetal fraction.

A subanalysis of the TRIDENT-2 study from the Netherlands found that preeclampsia and aneuploidy were increased in the 295 women with test failure because of low fetal fraction, although the results did not adjust for maternal age or BMI.[23] A retrospective cohort study from Australia reported that first-trimester fetal fraction was inversely correlated with preeclampsia risk,[24] whereas another found that although first-trimester fetal fraction was lower in women who developed hypertensive disorders of pregnancy, this was no longer significant after adjusting for maternal age, race, BMI, and chronic hypertension.[25] Similar results have been

TABLE 4
Outcomes according to reason for nonreportable results and fetal fraction

Variable	Results with first draw N=17,249	No results with first draw FF >2.8% N=197	No results with first draw FF ≤2.8% N=194	No results with first draw FF not reported N=211	Comparison of FF ≤2.8% vs FF>2.8%
Diagnostic testing	475 (2.8%)	19 (9.6%)	29 (15.0%)	21 (10.0%)	*P*=.11
Fetal anomaly before testing	99 (0.6%)	1 (0.5%)	0	0	*P*=1.00
Aneuploidy (T13, 18, 21)	123 (0.7%)	2 (1.0%)	4 (2.1%)	4 (1.9%)	*P*=.45
Live birth	17,032 (98.8%)	188 (95.9%)	179 (92.8%)	201 (95.7%)	*P*=.18
Excluding aneuploidies, spontaneous loss at <20 wk, and elective termination	N=17,027	N=188	N=182	N=199	
PTB at <34 wk[a,b]	260 (1.5%)	11 (5.9)	8 (4.4)	7 (3.5%)	*P*=.53
Preeclampsia[a,b]	657 (3.9%)	24 (12.8%)	20 (11.2%)	9 (4.6%)	*P*=.64
SGA[a,b]	1478 (8.8%)	15 (8.0%)	21 (11.8%)	19 (9.7%)	*P*=.22
Composite outcome (preeclampsia, SGA, PTB at <37 wk, stillbirth)[a,b]	2821 (16.6%)	50 (26.6%)	57 (31.3%)	40 (20.1%)	*P*=.32

FF, fetal fraction; *PTB*, preterm birth; *SGA*, small for gestational age; *T*, trisomy.

[a] Aneuploidies excluded; [b] Excluding cases with termination or loss at <20 weeks.

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reported by some,[26–28] whereas others have found elevated first-trimester cfDNA levels to be associated with subsequent preeclampsia.[29–31] Most of these previous studies have been single-center, retrospective reports limited by small numbers and focused on fetal fraction rather than nonreportable tests.

Clinical and research implications

The increased risk of trisomies 13 and 18 in patients with nonreportable cfDNA screening tests has been previously recognized.[2,10,32,33] Importantly, although the nonreportable rate for trisomy 21 in our cohort was not increased above euploid cases, there were 3 cases of trisomy 21 associated with nonreportable tests. These findings support the recommendations of professional societies such as the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, and Society for Maternal-Fetal Medicine that patients with nonreportable cfDNA tests be offered genetic counseling and the option of further evaluation, including diagnostic testing.[34,35]

These results have important implications for prenatal care providers given that this test is increasingly used. Some cases of aneuploidy will fail to be detected because of no-call results. Patients with no-call results should be offered genetic counseling and discussion of options for further evaluation for aneuploidy, including fetal diagnostic testing. Those with normal genetic testing are at increased risk for adverse obstetrical and perinatal outcomes and should undergo appropriate further evaluation and surveillance.

Strengths and limitations

Although our study provides an important contribution to the understanding of nonreportable cfDNA screening results, there were several limitations. The results may not be generalizable to all laboratories and cfDNA analysis techniques because methods for measurement of fetal fraction and criteria for nonreportable tests differ. Many variables affect fetal fraction, which may represent an increase in maternal cfDNA, a decrease in placental cfDNA,

or both; we were not able to quantify these differences. The factors leading to nonreportable results in cases with adequate or unmeasurable fetal fraction were not clearly delineated in the clinical reports. Some diagnoses, such as preeclampsia, were made by local providers, and use of low-dose aspirin to decrease preeclampsia risk was not reported. Finally, analysis of some outcomes was limited by small numbers.

Conclusions

This large, multicenter study with comprehensive prospective data on pregnancy outcomes demonstrates that patients with nonreportable cfDNA results are at increased risk for adverse obstetrical and perinatal outcomes. Patients with no-call results should be offered genetic counseling and discussion of options for further evaluation for aneuploidy, including diagnostic fetal testing.

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Data sharing requests should be submitted to the corresponding author (M.E.N.) for consideration. Requests will be considered by the study publication committee, and access may be limited by patient consent considerations. Study protocol and statistical analysis plan will be available upon request. Individual patient data will not be available. Access to deidentified data may be granted following submission of a written proposal and a signed data sharing agreement. Files will be shared using a secure file transfer protocol. Data will be available immediately following publication and ending 1 year after article publication.

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