Effect of Cell-Free DNA Screening vs Direct Invasive Diagnosis on Miscarriage Rates in Women With Pregnancies at High Risk of Trisomy 21
A Randomized Clinical Trial

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IMPORTANCE Cell-free DNA (cfDNA) tests are increasingly being offered to women in the first trimester of pregnancies at a high risk of trisomy 21 to decrease the number of required invasive fetal karyotyping procedures and their associated miscarriages. The effect of this strategy has not been evaluated.

OBJECTIVE To compare the rates of miscarriage following invasive procedures only in the case of positive cfDNA test results vs immediate invasive testing procedures (amniocentesis or chorionic villus sampling) in women with pregnancies at high risk of trisomy 21 as identified by first-trimester combined screening.

DESIGN, SETTING, AND PARTICIPANTS Randomized clinical trial conducted from April 8, 2014, to April 7, 2016, in 57 centers in France among 2111 women with pregnancies with a risk of trisomy 21 between 1 in 5 and 1 in 250 following combined first-trimester screening.

INTERVENTIONS Patients were randomized to receive either cfDNA testing followed by invasive testing procedures only when cfDNA test results were positive (n = 1034) or to receive immediate invasive testing procedures (n = 1017). The cfDNA testing was performed using an in-house validated method based on next-generation sequencing.

MAIN OUTCOMES AND MEASURES The primary outcome was number of miscarriages before 24 weeks’ gestation. Secondary outcomes included cfDNA testing detection rate for trisomy 21. The primary outcome underwent 1-sided testing; secondary outcomes underwent 2-sided testing.

RESULTS Among 2051 women who were randomized and analyzed (mean age, 36.3 [SD, 5.0] years), 1997 (97.4%) completed the trial. The miscarriage rate was not significantly different between groups at 8 (0.8%) vs 8 (0.8%), for a risk difference of −0.03% (1-sided 95% CI, −0.68% to 0.62%; P = .47). The cfDNA detection rate for trisomy 21 was 100% (95% CI, 87.2%-100%).

CONCLUSIONS AND RELEVANCE Among women with pregnancies at high risk of trisomy 21, offering cfDNA screening, followed by invasive testing if cfDNA test results were positive, compared with invasive testing procedures alone, did not result in a significant reduction in miscarriage before 24 weeks. The study may have been underpowered to detect clinically important differences in miscarriage rates.

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Prenatal screening for Down syndrome has evolved considerably over the last 2 decades. Combined first-trimester screening, which is based on maternal age, fetal nuchal translucency measurement, and maternal serum screening, has been adopted in most countries, yielding a 90% detection rate at a 5% false-positive rate. Fetal karyotyping by amniocentesis or chorionic villus sampling (CVS) is offered when risk of trisomy 21 surpasses a predefined cutoff value, thus defining a patient as at high risk.

Cell-free DNA (cfDNA)-based analyses of maternal plasma have a sensitivity of more than 99% for Down syndrome in pregnancies at high risk of this syndrome without the associated risks of an invasive procedure. Safety has been the most widely used argument for implementing cfDNA testing in high-risk patients without evidence of its superiority over immediate use of invasive testing. This theoretical advantage of cfDNA testing prior to any invasive testing over immediate use of amniocentesis or CVS for karyotyping has not been tested in a randomized trial and should be balanced against the lower sensitivity and specificity of cfDNA in the detection of trisomy 21. In addition, cfDNA testing may overlook other common aneuploidies and structural chromosomal anomalies that are detected by conventional karyotyping following invasive testing.

This randomized clinical trial compared rates of miscarriage after either cfDNA testing with invasive procedures only when cfDNA test results were positive vs immediate invasive testing procedures in pregnancies at high risk of trisomy 21 as identified by first-trimester combined screening.

Methods

Study Design

The study protocol and the statistical analysis plan are provided in Supplement 1 and Supplement 2. This study was a 2-year, nationwide, open-label randomized clinical trial conducted in 64 centers throughout France. It compared the miscarriage rates following either cfDNA testing (followed by invasive testing if cfDNA test results were positive) or direct fetal karyotyping by invasive testing procedures in women with pregnancies with a risk of trisomy 21 between 1 in 5 and 1 in 250 following combined first-trimester screening. Ethics committee approval was obtained and funding was received from the French Ministry of Health. Eligible women who provided consent to take part were registered in a secured database (https://www.bionuqual.org/echo.php).

Procedures

As part of routine practice in France, all women were informed about Down syndrome screening and were able to undergo first-trimester combined screening or sequential combined screening as part of their routine antenatal care. Women with a risk above 1 in 250 were considered screen positive. Practitioners performing nuchal translucency measurement as part of prenatal screening for trisomy 21 were licensed for nuchal translucency measurement and were registered in a perinatal network, which ensured continuous quality control monitoring. Biochemical assays and risk calculations were performed in 82 accredited laboratories that adhere to a nationwide quality policy.

All women with a pregnancy with a risk of Down syndrome between 1 in 5 and 1 in 250 were offered to enroll in this study at one of the participating prenatal diagnostic centers. Additional inclusion criteria were maternal age at least 18 years, singleton pregnancy, health care coverage by the national health care insurance system (Assurance Maladie), and gestational age between 11 and 18 weeks. Eligible women agreed a priori to fetal karyotyping by CVS or amniocentesis and provided written informed consent. Exclusion criteria were nuchal translucency greater than 3 mm and maternal pregnancy-associated plasma protein A or β human chorionic gonadotropin concentrations less than 0.3 or greater than 5 multiples of the median, as these characteristics have been associated with other chromosomal abnormalities. Cases with a fetal malformation found at the first ultrasound examination, a vanishing twin, or prior knowledge of any parental balanced translocation were also excluded.

Prior to patient inclusion in the study, face-to-face standardized information, counseling sessions, and printed information leaflets were provided to patients by health care professionals in each participating center. This content presented the benefits and limitations of both tests. This included the potential of the invasive test to find chromosomal abnormalities that can be overlooked by cfDNA testing, as well as the 1% additional risk of miscarriage associated with invasive procedures. Women’s preferences regarding cfDNA and invasive testing were recorded on eligibility (details will be described in a subsequent study).

Randomization to either cfDNA testing followed by invasive testing procedures only when cfDNA test results were positive or to direct use of invasive testing procedures was performed with a 1:1 ratio using the same centralized, secure web system. The randomization sequence was created by an independent data manager using a list of computer-generated random numbers (random block sizes of 2, 4, and 6) and was stratified according to each center. Patients, investigators, and all staff involved in the trial were aware of group allocation.
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to particals. For women in the cfDNA group, blood samples

drawn in each participating center, identified by a study
code, and shipped to the referral cytogenetic laboratory at

Necker-Enfants Malades Hospital in Paris. The cfDNA tech-
nique as well as the interpretation and reporting of the re-
sults are described in the eAppendix in Supplement 3. Posi-
tive cfDNA test results were always confirmed with

conventional invasive testing before management decisions
were undertaken. Women in the invasive prenatal diagnosis
group had karyotyping performed by one of the local accredit-
ed cytogenetics laboratories.13

**DNA Analysis**

Genomic DNA was extracted from 5 mL of plasma and used
for library preparation with end-repair, A-tailing, and adap-
tor ligation with a specific tag. Thereafter, sequencing of a pool
of 11 patients with 1 case of trisomy 21 (control sample) was
performed with a HiSeq 1500 (Illumina) using a rapid run for single
reads of 50 base lengths. After demultiplexing, read counts per
chromosome and z score computations were performed using the
R RAPIDR package, version 0.1.11.14 The z score is the dif-
erence in the number of reads of chromosome 21 in the test
and reference set divided by the standard deviation of the num-
ber of reads. A result was considered positive when the z score
was above +1.645.

The technique including sequencing and bioinformatics
tools as well as interpretation and rendering of cfDNA results
are detailed in the eAppendix in Supplement 3.

**Outcomes**

Cell-free DNA test results and cytogenetic results from inva-
sive testing were collected for participants according to ran-
domized group. Pregnancy outcomes were collected and cat-
egorized as follows: miscarriage before 24 weeks' gestation,
intrauterine fetal death (ie, spontaneous death of a fetus af-
after 24 weeks' gestation and before delivery), termination of

pregnancy, and live birth and perinatal death (restricted to in-
trapartum stillbirth and neonatal death before 6 days). The pri-
mary outcome was miscarriage before 24 weeks' gestation. This
is the outcome commonly used in articles on which the sample
size calculation was based, as detailed in the trial protocol15,16
and in recent review articles on this issue.17,18 In the initial pro-
tocol, a less well-defined term of “pregnancy losses” was used.
However, 24 weeks' gestation is the current definition for
viability.19 Thus, at this threshold, “extremely premature birth”
is more appropriate than “pregnancy loss.” Although the
French College of Gynecologists and Obstetricians recently at-
tempted to better define miscarriage and issued practical
guidelines,20 there is a lack of an internationally accepted set
of definitions for many terms used to describe pregnancy losses
and also a lack of a standardized French-English reciprocal ter-
minology or glossary.

Secondary outcomes included number and percentage of
invasive procedures performed in both treatment groups, per-
formance characteristics of cfDNA testing (including failed tests
and evaluation using sensitivity and specificity compared with
the reference standard karyotype or phenotype at birth), time

interval between blood sample receipt and result availability,
and diagnosis of chromosomal abnormalities in each group.
The false-positive rate of cfDNA testing was estimated post hoc
if a conventional cutoff z -score value of 3.0 had been used.2
Other secondary outcomes not reported in this article in-
clude the relationship between women's clinical characteris-
tics and cfDNA results, women's preferences regarding cfDNA
and invasive testing, and anxiety. The protocol also prespeci-
ed an economic analysis. At the time the protocol was writ-
ten in 2012, there was a scarcity of data on this topic and the

cfDNA test was much more expensive. However, we used an
in-house validated method based on next-generation sequenc-
ing to remain independent from private test providers. In a cost
assessment, this method would compare very unfavorably with
the more recent, lower-cost tests (with the introduction of au-
tomation technologies). More importantly, various strategies
for the implementation of cfDNA testing, including contin-
gent use, have now been evaluated and seem more appropri-
ate in terms of cost effectiveness,21,22 making the planned eco-

demic analysis less relevant.

Other pregnancy outcomes (intrauterine fetal death, ter-
mination of pregnancy, and live birth and perinatal death) were
analyzed as post hoc outcomes.

**Statistical Analysis**

The sample size was calculated hypothesizing a reduction in

the number of miscarriages using cfDNA testing. To observe a de-
crease in miscarriage from 1.5% to 0.5% between the invasive
and cfDNA testing groups, respectively, with a 1-sided \( \alpha = .05 \) and 80% power, 1250 patients per group were required, yielding a total

sample size of 2500 patients. These assumptions were derived
from the only existing randomized clinical trial, which found an
additional 1% miscarriage rate following invasive testing.16

All statistical analyses were performed using R software
version 2.11.1, with the statistical analysis plan prespecified be-
fore locking the database. The primary outcome underwent
1-sided testing; secondary outcomes underwent 2-sided test-
ing. For all tests used, \( P < .05 \) was considered statistically sig-
nificant. The baseline characteristics of the 2 treatment groups
were described as means and standard deviations or medians
and interquartile ranges (IQRs) for quantitative variables and
frequencies and percentages for qualitative variables.

For all analyses, patients without appropriate consent
forms were excluded. Regarding the analysis of the primary
outcome, the primary analysis set was defined as all patients
randomized excluding those with a missing outcome. The per-
protocol population was defined as patients from the pri-
mary analysis set analyzed according to the first procedure ac-
tually performed (cfDNA or invasive testing) and excluded
those who underwent neither cfDNA nor invasive testing. A post hoc analysis was performed analyzing patients accord-
ing to whether they underwent an invasive testing proce-
dure, whatever their allocation group (Figure).

Miscarriage rates (primary outcome) and intrauterine fetal
death rates (post hoc outcome) were compared between groups
using a 1-sided \( \chi^2 \) test on the primary analysis set.

Secondary end points were mainly descriptive; if not, they
were compared between groups using 2-sided tests: the \( \chi^2 \) test

\[ z \text{-score} = \frac{x - \mu}{\sigma} \]

\[ z = \frac{\text{test result} - \text{mean reference}}{\text{standard deviation}} \]

95% confidence interval:

\[ z \text{-score} \times 1.96 \]

Effect of Cell-Free DNA Screening on Miscarriage in Women With Pregnancies at High Risk of Trisomy 21
(the Fisher exact test as appropriate) was used for comparison of qualitative outcomes and the t test (or the Wilcoxon test for nonnormally distributed variables) was used for comparison of quantitative outcomes. Analyses of secondary end points were not adjusted for multiple comparisons and should be interpreted as exploratory.

A binomial generalized linear model with identity link was used to calculate the confidence intervals of the risk differences (cfDNA testing group minus invasive testing group). For the performance of cfDNA testing, 95% confidence intervals were calculated using binomial distribution.

Post hoc sensitivity analyses were performed to investigate the robustness of the results for the primary end point. A generalized linear model with random center effect was computed to handle the multiple-sites design. Multiple imputations by chained equations based on 2 different methods (random sample from observed values and logistic regression including randomization group, age, body mass index, and risk of trisomy 21 as covariates) were tested to handle missing data. In addition, a tipping-point analysis was carried out to evaluate the robustness of the results for the primary outcome by examining how it would be modified by different scenarios of missing data replacement.

Results

Patient Population
A total of 2592 women were assessed for eligibility at 57 centers between April 2014 and April 2016 (7 centers

Figure. Participant Flow in the Study of Cell-Free DNA Screening and Miscarriage in High-Risk Pregnancies

| 2592 Eligible patients invited to participate |
| 2111 Randomized |
| 1049 Randomized to cfDNA testing |
| 1062 Randomized to invasive testing |
| 481 Excluded |
| 33 Refused participation |
| 448 Participated only in preferences survey part of study |
| 15 Excluded |
| 13 Incorrect or missing consent form |
| 2 Randomized twice |
| 1034 Included in study |
| 1028 Underwent cfDNA testing as first screen as randomized |
| 6 Did not undergo cfDNA testing as first screen |
| 3 Refused group allocation and had invasive testing as first screen |
| 3 Refused group allocation and had neither cfDNA nor invasive testing recorded |
| 1017 Included in study |
| 745 Underwent invasive testing as first screen as randomized |
| 272 Did not undergo invasive testing as first screen |
| 148 Refused group allocation and had neither cfDNA nor invasive testing recorded |
| 96 Refused group allocation and had cfDNA testing as first screen |
| 26 Did not refuse group allocation but had neither cfDNA nor invasive testing recorded |
| 2 Did not refuse group allocation but had cfDNA testing as first screen |
| 1015 Included in primary analysis |
| 19 Excluded (unknown pregnancy outcome) |
| 1103 Included in per-protocol analysis a |
| 23 Excluded (unknown pregnancy outcome) |
| 1178 Included in post hoc analysis b |
| 38 Excluded (unknown pregnancy outcome) |
| 982 Included in primary analysis |
| 35 Excluded (unknown pregnancy outcome) |
| 731 Included in per-protocol analysis a |
| 15 Excluded (unknown pregnancy outcome) |
| 819 Included in post hoc analysis b |
| 16 Excluded (unknown pregnancy outcome) |

A total of 2592 women were assessed for eligibility at 57 centers between April 2014 and April 2016 (7 centers

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recruited no participants for the study). Among these women, 481 (18.6%) declined to participate prior to randomization, of whom 448 stated a preference for either cfDNA or invasive testing. A total of 2111 women were therefore randomized, 1049 to the cfDNA testing group and 1062 to the direct invasive testing group. After exclusion of patients without properly completed consent forms, 1034 patients were eligible for cfDNA testing and 1017 for invasive testing. Fifty-four women (2.6%) were lost to follow-up (19 [1.8%] in the cfDNA testing group and 35 [3.4%] in the invasive testing group). Participant flow through the trial is shown in the Figure. Both groups had similar demographics and screening results for trisomy 21 (Table 1).

Primary Outcome

There was no significant difference in miscarriage rates between the cfDNA and invasive testing groups (8 [0.8%] vs 8 [0.8%] miscarriages; risk difference, −0.23%; 1-sided 95% CI, −0.95% to 0.47; P = .47) (Table 2). Following cfDNA and invasive testing, miscarriages occurred at a median of 19.9 (IQR, 16.9-21.1) weeks’ gestation and 19.9 (IQR, 18.8-22) weeks’ gestation, respectively.

One woman randomized to the invasive testing group who experienced a miscarriage had refused to undergo invasive testing. When analyzing the data per protocol, there was no significant difference in miscarriage rates between cfDNA testing (n = 1103) and invasive testing (n = 733) (8 [0.7%] vs 7 [1%]; P = .30). No patient had a miscarriage following invasive testing performed after a positive cfDNA test result.

Secondary Outcomes

There were 84 (8.3%) and 751 (76.5%) invasive procedures performed in the cfDNA and invasive testing groups, respectively. Cell-free DNA testing was successful in 984 of the 1028 women (95.7%), who were randomized to the cfDNA testing group and received cfDNA testing as randomized. Forty-four samples (4.3%) failed quality checks (hemolysis of plasma samples; specimens received after 5 days). Three women with failed first tests declined to provide a second blood sample and 38 of 41 repeat samples were satisfactory, leading to successful cfDNA testing, including repeat testing, in 1022 (99.4%) of 1028 women.

Performance of the cfDNA test was estimated for the 984 samples with satisfactory quality on first check. The detection rate (sensitivity) of cfDNA testing for trisomy 21 was 100% (95% CI, 97.2%-100%) (27/27) with a false-negative rate of 5.6% (95% CI, 4.2%-7.2%) (55/984) for the chosen z-score threshold (z = 1.645). The mean z score was 12.9 (SD, 5.6) in true-positive cases. In false-positives cases, all z scores were below 3 except for 2 cases with z scores of 3.5 and 14. In the latter case, this high z-score value was confirmed with another sample taken 3 months later, while amniocentesis ruled out trisomy 21. This indicated a possible confined placental mosaicism, although this could not be confirmed at birth. Had a z-score cutoff value of 3.0 been used, the false-positive rate would have been 0.2% (95% CI, 0.03%-0.7%).

The mean time interval between blood sample receipt and result availability was 13.0 (SD, 5.0) days and was less than 3 weeks for 903 women (88%) who underwent cfDNA testing.

### Table 1. Baseline Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cell-Free DNA Testing Group (n = 1034)</th>
<th>Invasive Testing Group (n = 1017)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>36.2 (5.2) 36.3 (4.9)</td>
<td>36.5 (5.0) 36.5 (4.9)</td>
</tr>
<tr>
<td>±35, No. (%)</td>
<td>966 (93.7) 968 (95.0)</td>
<td>951 (93.8) 953 (94.2)</td>
</tr>
<tr>
<td>±38, No. (%)</td>
<td>478 (46.1) 468 (46.0)</td>
<td>461 (45.4) 461 (45.3)</td>
</tr>
<tr>
<td>Gestational age on day of randomization, median (SD), wk</td>
<td>15.0 (1.3) 15.1 (1.3)</td>
<td>15.5 (1.3) 15.6 (1.3)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>67.4 (14.8) 68.0 (14.6)</td>
<td>67.3 (14.8) 68.0 (14.6)</td>
</tr>
<tr>
<td>Height, mean (SD), cm</td>
<td>164.4 (6.2) 164.7 (6.5)</td>
<td>164.5 (6.4) 164.8 (6.5)</td>
</tr>
<tr>
<td>Body mass index, mean (SD)*</td>
<td>24.9 (5.2) 25.1 (5.2)</td>
<td>24.9 (5.2) 25.1 (5.2)</td>
</tr>
<tr>
<td>±30, No. (%)</td>
<td>145 (14) 169 (16.6)</td>
<td>144 (14) 169 (16.6)</td>
</tr>
<tr>
<td>Parity, median (IQR)</td>
<td>1 (0-2) 1 (0-2)</td>
<td>1 (0-2) 1 (0-2)</td>
</tr>
<tr>
<td>First-trimester serum markers, No. (%) with data</td>
<td>961 (92.9) 927 (91.2)</td>
<td>950 (93.5) 916 (90.5)</td>
</tr>
<tr>
<td>Crown-rump length, mean (SD), mm</td>
<td>63.7 (8.3) 63.7 (7.9)</td>
<td>63.7 (8.3) 63.7 (7.9)</td>
</tr>
<tr>
<td>Nuchal translucency, mean (SD), mm a</td>
<td>1.85 (0.56) 1.81 (0.54)</td>
<td>1.85 (0.56) 1.81 (0.54)</td>
</tr>
<tr>
<td>Nuchal translucency multiple of the median, mean (SD)b</td>
<td>1.18 (0.37) 1.15 (0.35)</td>
<td>1.18 (0.37) 1.15 (0.35)</td>
</tr>
<tr>
<td>Human chorionic gonadotropin β fraction multiple of the median, mean (SD)</td>
<td>2.36 (1.09) 2.39 (1.13)</td>
<td>2.36 (1.09) 2.39 (1.13)</td>
</tr>
<tr>
<td>Pregnancy-associated plasma protein A multiple of the median, median (IQR)</td>
<td>0.73 (0.49-1.03) 0.66 (0.48-1.02)</td>
<td>0.73 (0.49-1.03) 0.66 (0.48-1.02)</td>
</tr>
</tbody>
</table>

**Abbreviation:** IQR, interquartile range.

a Calculated as weight in kilograms divided by height in meters squared.

b Nuchal translucency is sonographic measurement of the size of the translucent space behind the neck of the fetus and is correlated with risk of trisomy 21.

c Multiple of the median is a measure of how far an individual test result deviates from the median (multiple of the median value = patient value/median population value).

d First-trimester combined screening or sequential combined screening of trisomy 21.

Chromosomal anomalies identified in both groups are summarized in Table 2 and Table 3. Overall, there were 28 (2.8%) and 49 (5%) chromosomal abnormalities diagnosed in the cfDNA and invasive testing groups, respectively (risk difference, −2.23%; 95% CI, −3.93% to −0.54%; P = .01). These included 1 (0.1%) and 11 (1.1%) anomalies other than trisomy 21, respectively (risk difference, −1.02%; 95% CI, −1.71% to −0.34%; P = .003) (Table 2). In the cfDNA group, the anomaly was a tetrasomy 12p found on amniocentesis after ultrasound revealed multiple malformations at mid trimester. The anomalies in the invasive testing group are summarized in Table 3 and described in detail in the eTable in Supplement 3.
Table 2. Primary, Secondary, and Post Hoc Outcomes in the Cell-Free DNA and Invasive Testing Groups (Primary Analysis Set)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>No. (%)</th>
<th>Cell-Free DNA Testing Group (n=1015)</th>
<th>Invasive Testing Group (n=982)</th>
<th>Absolute Risk Difference, % (95% CI)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscarriage &lt;24 weeks’ gestation</td>
<td>8 (0.8)</td>
<td>8 (0.8)</td>
<td>−0.03 (−0.68 to ∞)</td>
<td>.47*</td>
<td></td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosomal anomalies</td>
<td>28 (2.8)</td>
<td>49 (5)</td>
<td>−2.23 (−3.93 to −0.54)</td>
<td>.01*</td>
<td></td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>27 (2.7)</td>
<td>38 (3.9)</td>
<td>−1.21 (−2.77 to 0.35)</td>
<td>.13*</td>
<td></td>
</tr>
<tr>
<td>Other anomalies</td>
<td>1 (0.1)</td>
<td>11 (1.1)</td>
<td>−1.02 (−1.71 to −0.24)</td>
<td>.003*</td>
<td></td>
</tr>
<tr>
<td>Post hoc outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrauterine fetal death</td>
<td>9 (0.9)</td>
<td>9 (0.9)</td>
<td>−0.03 (−0.72 to ∞)</td>
<td>.47*</td>
<td></td>
</tr>
<tr>
<td>Miscarriage + intrauterine fetal death</td>
<td>17 (1.7)</td>
<td>17 (1.7)</td>
<td>−0.06 (−1.01 to ∞)</td>
<td>.46*</td>
<td></td>
</tr>
<tr>
<td>Termination of pregnancy</td>
<td>30 (3)</td>
<td>39 (4)</td>
<td>−1.02 (−2.62 to 0.59)</td>
<td>.21*</td>
<td></td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>23</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Othera</td>
<td>7</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live birth</td>
<td>965 (95.1)</td>
<td>924 (94.1)</td>
<td>0.98 (−1.01 to 2.97)</td>
<td>.33*</td>
<td></td>
</tr>
<tr>
<td>Perinatal death</td>
<td>3 (0.3)</td>
<td>2 (0.2)</td>
<td>0.09 (−0.35 to 0.53)</td>
<td>&gt;.99</td>
<td></td>
</tr>
</tbody>
</table>

* Absolute risks differences were calculated as the cell-free DNA group minus the invasive testing group, with 1-sided 95% CIs for 1-sided tests and 2-sided 95% CIs for 2-sided tests, respectively.

a One-sided test.

b Two-sided test.

c Pregnancy outcomes were collected and categorized as follows: miscarriage before 24 weeks’ gestation, intrauterine fetal death (ie, spontaneous death of a fetus after 24 weeks’ gestation and before delivery), termination of pregnancy, and live birth and perinatal death (restricted to intrapartum stillbirth and neonatal death before 6 days).

Post Hoc Analysis of the Primary Outcome

The post hoc analysis among patients analyzed according to whether they underwent an invasive procedure also showed no significant difference in miscarriage rates (9/1178 [0.8%] vs 7/819 [0.9%]; risk difference, −0.09%; 1-sided 95% CI, −0.76% to ∞; P = .41).

A binomial generalized linear model with identity link including site as a random effect did not fit. When modeling logit link, the standard error of the β coefficient including center as a random effect was very close to the one estimated by the model without center as a random effect (SE[β] = 0.50205 vs 0.50199 without site effect). Multiple imputations of missing data based on random sampling from observed values and multiple logistic regression led to comparable results (risk differences, −0.02% [1-sided 95% CI, −0.68% to ∞; P = .47] and −0.03% [1-sided 95% CI, −0.67% to ∞; P = .48], respectively). Results from a tipping-point analysis are presented in the eFigure in Supplement 3.

Post Hoc Analysis of Other Pregnancy Outcomes

Following cfDNA and invasive testing, respectively, there were 9 (0.9%) and 9 (0.9%) intrauterine fetal deaths (risk difference, −0.03%; 1-sided 95% CI, −0.72% to ∞; P = .47) and 3 (0.3%) and 2 (0.2%) perinatal deaths (risk difference, 0.09%; 95% CI, −0.35% to 0.53%; P = .99), and 30 (3%) and 39 (4%) of women underwent termination of pregnancy (risk difference, −1.02%; 95% CI, −2.62% to 0.59%; P = .21) (Table 2).

There was no significant difference in overall pregnancy loss rates between the cfDNA and invasive testing groups (17 [1.7%] vs 17 [1.7%]; risk difference, −0.06%; 1-sided 95% CI, −1.01% to ∞; P = .46) (Table 2). Pregnancy losses occurred at a median gestational age of 21.3 (IQR, 18-23.1) weeks and 20 (IQR, 17.1-22) weeks, respectively.

When analyzing the data per protocol, there was no significant difference in pregnancy loss rates between cfDNA testing (n = 1103) and invasive testing (n = 733) (17 [1.5%] vs 14 [1.9%], respectively; risk difference, −0.37%; 1-sided 95% CI, −1.40% to ∞; P = .27). This remained true in the post hoc analysis between the cfDNA testing (n = 1178) and invasive testing (n = 819) (20 [1.7%] vs 14 [1.7%], respectively; risk difference, −0.01%; 1-sided 95% CI, −0.98% to ∞; P = .49).

Discussion

In this randomized clinical trial, there was no significant decrease in the risk of miscarriage before 24 weeks in high-risk pregnancies after cfDNA testing followed by invasive testing only in women with a positive cfDNA test result vs immediate invasive testing. The multicenter design of this study included invasive procedures performed in 57 different centers; this makes the result more generally applicable. To our knowledge, this is the first randomized clinical trial comparing miscarriage rates following cfDNA and invasive testing in women with pregnancies at high risk of trisomy 21 by combined screening in the first trimester of pregnancy.

The total miscarriage rate in the invasive procedure group was 0.8%, significantly lower than the commonly expected...
Effect of Cell-Free DNA Screening on Miscarriage in Women With Pregnancies at High Risk of Trisomy 21

Original Investigation Research

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This study did not show a reduction of miscarriage or intrauterine fetal death associated with use of cfDNA in high-risk cases. However, there were far fewer invasive procedures in the cfDNA group (81 vs 751 in the immediate invasive testing group), and invasive procedures have been associated with extremely rare but severe outcomes, such as maternal septicemia and death.28-30 None of these occurred in this randomized clinical trial. If safety is no longer an argument for cfDNA testing over karyotyping, then the risk of overlooking rare chromosomal anomalies in high-risk pregnancies should be carefully evaluated. In this study, standard karyotyping identified other chromosomal abnormalities in 1.5% of high-risk pregnancies. The use of chromosomal microarray analysis, which has now become the first-line genetic test on amniotic fluid or chorionic villi in many laboratories, could have increased this rate of discovery of abnormalities even further.31-33 Indeed, the additional value of chromosomal microarray analysis compared with karyotyping was reported to be as high as 6% when there are fetal anomalies on ultrasound and in 0.5% to 2.5% of cases in pregnancies with advanced maternal age or at high risk following first-trimester combined screening.32,31,33,34

Limitations
This study has several limitations. First, although the invasive testing rate was considerably less following cfDNA testing, the observed 5.6% false-positive rate was higher than the average 0.1% reported.3,35 This higher false-positive rate was deemed acceptable to improve cfDNA sensitivity since this was the first randomized trial evaluating this test. Although this induced more secondary invasive procedures and could therefore have increased the miscarriage rate in this group, no patient undergoing invasive testing following a positive cfDNA test result had a miscarriage. The exploratory post hoc analysis also suggested no additional miscarriage or pregnancy loss risks in women who underwent invasive procedures. Second, in this study, 17% of women declined randomization and up to 24% of women initially randomized to invasive testing refused their allocated test. This is a potential limitation because this participant attrition occurred after counseling about a 0.5% to 1% risk of procedure-related pregnancy loss. However, higher dropout rates in women assigned to invasive testing may have reflected an attempt to benefit from cfDNA testing free of charge through the study. Indeed, cfDNA has become widely available through private laboratories at a substantial cost. This prompted national health insurances to consider various risk cutoffs for offering second-line cfDNA testing following combined first-trimester screening.32,33,34 This dropout rate could also reflect a better acceptance of cfDNA testing vs invasive procedures. Third, cfDNA testing was only performed for trisomy 21, while the potential for cfDNA to rule out other chromosomal anomalies is likely to improve in the near future. Fourth, the assessment of clinical value of some of the chromosomal anomalies detected in the invasive group could be controversial. Nevertheless, it is difficult to discuss what

Table 3. Summary of Chromosomal Anomalies Other Than Trisomy 21 Detected by Karyotyping in 751 Women in the Invasive Testing Group*

<table>
<thead>
<tr>
<th>Case</th>
<th>Chromosomal Anomaly</th>
<th>Typical Clinical Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apparently balanced reciprocal translocation between chromosomes 5 and 17 inherited from the father</td>
<td>No obvious clinical manifestation</td>
</tr>
<tr>
<td>2</td>
<td>Balanced robertsonian translocation inherited from the father</td>
<td>No obvious clinical manifestation</td>
</tr>
<tr>
<td>3</td>
<td>Type II confined placental mosaicism (trisomy 12)</td>
<td>No obvious clinical manifestation</td>
</tr>
<tr>
<td>4</td>
<td>Apparently balanced reciprocal translocation between chromosomes 2 and 13 occurring de novo</td>
<td>No obvious clinical manifestation</td>
</tr>
<tr>
<td>5</td>
<td>Mosaic trisomy 13</td>
<td>Syndromic developmental delay/intellectual disability</td>
</tr>
<tr>
<td>6</td>
<td>3q11.2q13.11 mosaic trisomy: 1 clone with an interstitial deletion of the long arm of chromosome 3 and a marker derived from chromosome 3 and 1 clone with normal chromosome 3 and the same marker</td>
<td>Syndromic developmental delay/intellectual disability</td>
</tr>
<tr>
<td>7</td>
<td>Two distinct clones: 1 with a small-ring X (containing XIST locus) and 1 with a small-ring X and a large-ring X (which does not contain XIST locus)</td>
<td>Syndromic developmental delay/intellectual disability</td>
</tr>
<tr>
<td>8</td>
<td>Mosaic trisomy 13 resulting from a homologous robertsonian translocation</td>
<td>Syndromic developmental delay/intellectual disability</td>
</tr>
<tr>
<td>9</td>
<td>Monosomy X (45,X)</td>
<td>Turner syndrome: short stature, gonadal dysgenesis, and multisystemic complications (eg, congenital heart defects, renal and skeletal anomalies)</td>
</tr>
<tr>
<td>10</td>
<td>Mosaic 45,X and 46,XY</td>
<td>Turner syndrome</td>
</tr>
<tr>
<td>11</td>
<td>47,XXY</td>
<td>Klinefelter syndrome: tall stature, hypogonadism, infertility, variable cognitive impairments</td>
</tr>
</tbody>
</table>

*Anomaly descriptions according to the 2016 International System for Human Cytogenomic Nomenclature are available in the eAppendix in Supplement 3.
chromosomal anomaly may modify pregnancy management, as this may also vary according to prenatal counseling, ultrasound findings, parental preferences, and local laws. Fifth, this randomized clinical trial showed no statistical difference in the risk of miscarriage before 24 weeks with more than 2000 women with pregnancies at high risk of Down syndrome randomized. However, the study may have been underpowered to identify potentially clinically important reductions in miscarriage with cfDNA testing. Sixth, it was not possible to conduct the planned economic analysis. Because of this, the cost implications of the study findings could not be estimated. Seventh, because of the potential for type I error due to multiple comparisons, the analyses of the secondary end points should be considered exploratory.

Conclusions

Among women with pregnancies at high risk of trisomy 21, offering cfDNA screening followed by invasive testing if cfDNA test results were positive, compared with direct invasive testing, did not result in a significant reduction in miscarriage before 24 weeks. The study may have been underpowered to detect clinically important differences in miscarriage rates.