High Positive Predictive Value (PPV) of Cell-Free DNA (cfDNA) Testing in a Clinical Study of 10,000 Consecutive Pregnancies

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**Abstract**

**Background:** Cell-free DNA (cfDNA) analysis in maternal blood for the detection of fetal Down syndrome is gradually replacing first trimester screening. We present here a large clinical series of 10,000 consecutive pregnancies.

**Objectives:** To study the reliability of cell-free DNA (cfDNA) analysis in maternal blood for the detection of fetal trisomy 21, 18 and 13 in a clinical setting in 10,000 consecutive pregnancies with variable risk. cfDNA testing has been evaluated in an increasing number of pregnancies mainly at high risk for fetal trisomy, and some studies have suggested that its positive predictive value (PPV) might be lower in low-risk populations.

**Study design:** CfDNA testing using the Harmony™ Prenatal Test was performed in 10,000 consecutive pregnancies with high or low a-priori risk for fetal trisomy 21, 18 and 13.

**Results:** In 147 (1.47%) of the 10,000 pregnancies a high-risk cfDNA testing result indicated trisomy 21 (n=121), trisomy 18 (n=15) or trisomy 13 (n=11). It failed to detect 5 trisomies (2 trisomies 21, 2 trisomies 18, and 1 trisomy 13). Five false-positive results were recorded (4 in the high and 1 in the low risk population). The overall positive predictive value (PPV) was 96%, with a PPV of 96% in the high-risk (>1/200) population and 97% in the low risk (<1/200) population.

**Conclusions:** In this large clinical series of 10,000 consecutive pregnancies, cfDNA testing proved very reliable in detecting fetal trisomy 21, 18 and 13, with a very high PPV both in high and low risk populations.

**Introduction**

First trimester screening using the combined test (CT), is still worldwide the most widely used test in the prenatal screening for fetal aneuploidies such as Down syndrome. It assesses the risk for fetal trisomy 21 and 18 (and usually also trisomy 13) based on maternal age, two maternal serum markers (Pregnancy-Associated Placental Protein (PAPP-A) and free-beta HCG (free β-hCG)), and the measurement of fetal nuchal translucency (NT) [1].

Patients with a CT risk above a certain cut-off (usually between 1/200 or 1/300) are offered follow-up investigations: until recently this was mainly genetic testing of material obtained by an invasive tests (i.e. amniocentesis-AC or chorionic villus sampling-CVS). As invasive procedures carry a risk of miscarriage of up to 1% [2].

AC and CVS are now progressively being replaced by non-invasive prenatal testing (NIPT) based upon analysis of cell-free DNA (cfDNA) in maternal blood [3-8]. NIPT has been introduced in 2011 in Hong Kong and the US, and has now become the most popular genetic test in many countries worldwide [9].

CIDNA testing cannot only be offered as a secondary test after the CT, but also as a primary screening test. Compared to NIPT, the CT has a high false-negative rate of up to 20% (the existence of Down syndrome although the CT did not show an increased risk), and a very high false-positive rate (5%) leading to unnecessary follow-up testing: 95% of women with increased CT risk carry a baby without trisomy, so the PPV of the CT is only 5%. However, some studies have suggested that the positive predictive value (PPV) of cfDNA testing is not high in low risk populations [4,10-12].

We evaluated the first 10,000 consecutive samples sent to our center for cfDNA analysis to investigate the PPV of NIPT in both the high and low risk pregnancy population.

**Materials and Methods**

Between March 2013 and January 2015 more than 10,000 samples were sent to GENDIA (Genetic Diagnostic Network, Antwerp, Belgium) for cfDNA testing. The results of the first 10,000 consecutive cfDNA tests are presented in this study. All patients received detailed information on all aspects of cfDNA testing before the test, and were referred to a specific NIPT website (www.DownsyndromeNIPT.info) developed by GENDIA for additional info.

All patients gave written informed consent and filled out an application form in which the following data were recorded:
identification data, maternal age, mode of conception, duration of pregnancy, single or multiple pregnancy, indication for cfDNA testing, results of any prior CT and history of genetic disease in the family.

Blood was collected from the 10th week of gestation onwards in Streck DNA BCT blood tubes, and sent out for testing with express mail to Ariosa Diagnostics Inc. (San Jose, California, USA), where the Harmony™ Prenatal Test was performed as previously published [3,8]. Samples that did not generate a result were classified as low (<4%) fraction of fetal cfDNA, inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, and failed analysis. In these cases a repeat sampling was offered (at no additional cost) to the patient. Ariosa Diagnostics reports individual risk scores for trisomies 21, 18 and 13 below 1% as "low risk", whereas risk scores above 99% were reported as "indicative of fetal trisomy".

Risk scores <0.1% were reported as "normal" to the couples and referring doctor or midwife in written form. Results of fetal gender were revealed upon specific request from the couples. All risk scores >0.1% were discussed with gynecologists and/or midwives in order to determine follow up testing. The couples were counseled by a clinical geneticist, gynecologist or midwife, and confirmation of the NIPT result after chorionic biopsy (CVS) or amniocentesis (AC) was suggested in all cases with risk scores >1%. A questionnaire was sent to all couples that received a result after the date the pregnancy was due.

Results

Patients

During a 2-year period from March 2013 until January 2015 GENDIA received more than 10,000 blood samples from pregnant women for cfDNA testing. The first 10,000 consecutive tests are reported here. Results of the first 3,000 of these tests have already been reported separately [13]. The pregnant couples originated from the Netherlands (n=6,545), Belgium (n=2,293), Serbia (n=206), Romania (n=158), Luxembourg (n=149), Latvia (n=148), Slovenia (n=127), Lithuania (n=71), Finland (n=39) and other countries (n=264). The study included 155 twin pregnancies and 884 in-vitro fertilization (IVF) pregnancies (45 twin-IVF pregnancies).

At the time of cfDNA testing, the mean gestational age was 12 weeks and 4 days ± 2 days (range: 10-34 weeks), whereas the mean maternal age was 36 ± 4 yrs (range: 18-52 yrs).

Failures and fetal fraction

In 143 (1.43%) of the 10,000 pregnancies no result could be generated because of a low (<4%) fraction of fetal cfDNA, inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, or failed analysis. In these cases repeat sampling was offered: the NIPT failed also in the second sample in 57 pregnancies, whereas in 15 pregnancies no second attempt was made.

In total, a result was obtained in 9,928 of the 10,000 pregnancies, with an overall failure rate of 0.72% (Table 1). The mean fetal fraction (N=9,928) was 12.2 ± 4.4% (range: 4.0-32.5%), not taking into account the samples that were reported as failures (Figure 2). The cfDNA fraction in pregnancies with confirmed trisomy 21 (n=100) was 11.8 ± 4.0% (range: 4.7-23.3%), in the pregnancies with proven trisomy 18 (n=12) cfDNA fraction was 9.6 ± 5.0% (range: 4.6-21.7%), and in the pregnancies with proven trisomy 13 (n=9) cfDNA fraction was 9.4 ± 2.3% (range: 5.4-13.0%). The cfDNA fraction in twins (n=155) was 10.5 ± 4.1% (range: 4.5-24.2%), versus 12.3 ± 4.4% (range: 4.0-32.5%) in singletons (N=9773).

In IVF pregnancies (N=884) the cfDNA fraction was 11.5 ± 4.4% (range: 4.0-30.6%) versus 12.3 ± 4.4% (range: 4.0-32.5%) in non-IVF pregnancies (N=9044). The failure rate after the first NIPT was 3/155 (1.94%) in twins and 3/884 (3.51%) in IVF pregnancies, versus 109/9006 (1.21%) for singletons without IVF. The failure rate of early NIPT in week 10-13 (117/5830-2.00%) was significantly higher (P<0.05) than the failure rate of later NIPT in week 14-20 (26/4170-0.62%).

The turnaround time

The turnaround time (TAT) between the receipt of the sample at the GENDIA laboratory and the issuing of the cfDNA result to the patient/physician/midwife was 10 ± 2 days (range: 5-19 days), not taking into account the samples that were reported as failures (Figure 3), with 96.3% of the tests reported within 2 weeks after arriving of the sample in our lab.
Total pregnancies | Amount 10,000
---|---
Total tests with results | 9,928 (99.28%)
Failure rate during first NIPT | 143 (1.43%)
Total tests without results | 72 (0.72%)
T21 | 121
T18 | 15
T13 | 11
Total trisomies | 147
False- T21 | 2 (NIPT risk scores < 0.01% and 0.6%)
False- T18 | 2 (NIPT risk score < 0.01%)
False- T13 | 1 (NIPT risk score < 0.01%)
False+ T21 | 1 (NIPT risk score 86%, proven CPM)
False+ T18 | 3 (NIPT risk scores 2, 20 and 39%)
False+ T13 | 1 (NIPT risk score > 99%)
Confirmation of NIPT by invasive or postnatal testing | 121
Mosaicism | 1 true fetal mosaicism (correct NIPT diagnosis); 1 CPM for trisomy 21 (false + NIPT)

CPM=Confined placental mosaicism

Table 1: Results of 10,000 cfDNA testing samples.

Figure 2: Distribution of samples according to cfDNA fraction.

Trisomies

In a total of 10,000 NIPT pregnancies 147 (1.47%) cfDNA testing results indicated a high risk (>1%) for trisomy 21 (n=121), trisomy 18 (n=15) or trisomy 13 (n=11) (Table 1 and Figure 4).

All these women were counseled to confirm the cfDNA testing result by genetic testing using an invasive diagnostic procedure (CVS and/or AC).

If the cfDNA risk score was 0.1-1%, the results were discussed with the responsible obstetrician-gynecologist and/or midwife: depending upon a-priori risk (determined by maternal age, NT, or CT) the decision for further clinical management was taken.

The mean age of the women with a proven trisomic fetus (n=121) was 37 ± 4 yrs (range: 27-46 yrs), which is only slightly but significantly (P<0.0001) older than the 36 ± 4 yrs (range: 18 - 53 yrs) for the women with a normal NIPT result (N=9,781).
karyotype. One high-risk NIPT score for trisomy 13 (>99%) could also reveal cells with a normal risk score for trisomy 21, whereas AC only revealed cells with a normal karyotype. Among the 17 IVF pregnancies with abnormal NIPT results there were no false positives, which is in contrast to the high reported false-positive rate of CT due to low levels of pregnancy-associated plasma protein-A (PAPP-A) associated with IVF pregnancies (14). The overall positive PPV of cfDNA testing (calculated excluding the no-calls and excluding the 21 cases who did not have confirmatory testing) was 96% (121/126). For trisomy 21 the PPV was 99% (100/101), for trisomy 18 it was 80% (12/15) and for trisomy 13 it was 90% (9/10). In the high-risk population (trisomy risk >1/200) the PPV for all trisomies combined was 96% (91/95), whereas in the low risk population the PPV was 97% (30/31).

Testing

False-negatives

Overall, 5 false-negatives have been reported in the present study. Two cases of trisomy 18 and one case of trisomy 13 with a low risk NIPT result were found after AC testing, but two of these cases were found to have a mosaic karyotype with lower fetal fraction in trisomy 13 was 96% (91/95), whereas in the low risk population the PPV was 97% (30/31).

Detection of fetal sex

The fetal sex could be determined in all pregnancies with the exception of NIPT failures (N=9,928), twins where fetal sex is not determined (N=155) and 3 additional samples. In this study cfDNA analysis indicated a male fetus in 4,959 (50.0%) pregnancies and a female fetus in 4,811 (48.5%) pregnancies. Fetal sex was reported to the parents in 66% of the pregnancies. In only one case there was discordancy with postnatal phenotype. For this female neonate with confirmed mosaicism 46, XX (>90% cells) - 47, XXY (<10% cells) NIPT reported a male gender.

Main findings of the study

We evaluated cfDNA testing using the Harmony™ Prenatal test in 10,000 consecutive pregnancies in a clinical setting. The main indication for cfDNA testing (Figure 1) was increased maternal age (38.7%), followed by increased first trimester screening (CT) risk (12.3%), but in a large proportion of pregnancies (45.3%) there was no specific indication for cfDNA testing. Local policies restricting reimbursement of cfDNA testing to cases with increased CT risk, might thus fail to cover many women who desire such testing. The failure rate was low in our series as in only 0.72% of the pregnancies the cffDNA fraction was below the required threshold after repeated sampling and no cfDNA testing result could be obtained (Table 1 and Figure 4). The fetal fraction in pregnancies with confirmed trisomy 18 or 21 was not significantly different from that of euploid pregnancies, but the cffDNA fraction in trisomy 13 was significantly (P<0.01) lower. Also twin (P<0.0001) and IFV (P<0.0001) pregnancies were associated with lower cffDNA levels and a higher incidence of test failures. This is likely to be due to the smaller size of the placenta in IFV pregnancies leading to reduced cffDNA in maternal circulation [15-16].

In total cfDNA testing detected 147 trisomies, including 121 cases of trisomy 21, 15 cases of trisomy 18 and 11 cases of trisomy 13 (Table 1 and Figure 4). It failed to detect 5 trisomies. This included 2 cases of trisomy 18 and one case of trisomy 13, which were found after invasive testing for ultrasound abnormalities. One baby that was identified to have a translocation trisomy 21 received a low risk NIPT score of 0.01% and one with a free trisomy 21 had a NIPT risk score of 0.6%. Detection rates were 96% for trisomy 21, 82% for trisomy 18 and 86% for trisomy 13. This is somewhat lower than the sensitivity previously reported by others [8,13-17]. Of the total of 147 high-risk NIPT results, 121 were confirmed by follow-up genetic testing of fetal material obtained by CVS or AC. Only five false-positive results (3 cases of trisomy 18, one case of trisomy 13 and one case of trisomy 21) were reported, with four of these having intermediate NIPT risk scores (2, 20, 39 and 86%). Consequently, the overall PPV in our study was found to be very high (96%), reaching 99% for trisomy 21, and somewhat lower for trisomy 18 (80%) and trisomy 13 (82%). Also in the low risk population the overall PPV was demonstrated to be very high at 97%. These results contradict earlier smaller studies that suggested that the PPV is low in low-risk populations [4,10-12]. In a recent study in a population with average-risk (4) the PPV of NIPT was only 45.5% for trisomy 21 and 40.0% for trisomy 18. However, this study only included 1914 samples with few positives (5 true-positives versus 6 false-positives for trisomy 21; 2 true positives versus 3 false-positives
for trisomy 18; 1 true-positives versus 0 false-positives for trisomy 13). In another study, the PPV for trisomy 21 was 93% (38/41 true positives), but only 64% for trisomy 18 (16/25 true positives) and 38% for trisomy 13 (6/16 true positives). However, as cfDNA tests from various companies (including Natera, Ariosa, Verinata-Illumina and Sequenom) were used in this study (12), the PPV of the individual cfDNA test remains unclear. Theoretically, the PPV would be expected to be lower in low-risk populations compared to high-risk populations, due to the lower prevalence of aneuploidies in lower-risk women, whereas the amount of false-positives is not dependent on the a priori risk of a certain population. Recent, several large studies have suggested a high PPV in both high- and low-risk populations. In a commercial study of 17,885 samples with 356 aneuploidies, PPVs for both high (women >35 yrs) and low (women <35 yrs) risk women were at about 83 percent across all aneuploidies and 91% for trisomy 21 (5). A large study from China with 146,958 samples with 1,578 aneuploidies, showed a PPV for trisomy 21 of 92% (720/781 true positives), 77% for trisomy 18 (167/218 true positives), but only 33% for trisomy 13 (22/67 true positives) (7). Using the same test as in this study, Norton et al. [17] recently found the overall PPV to be 81 percent in almost 16,000 women from the general screening population. The high PPV reported in this recent large prospective series is in accordance with the high PPV reported in our study. As we and others have shown that the PPV is high in both high- and low-risk pregnancies. Thus cfDNA testing is likely replace the traditional CT as a primary screening method in the near future.

**Strengths and limitations of the study**

This is one of the largest studies to date reporting on the performance of cfDNA testing in a real life clinical context. Its main limitations relate to the fact that this is a clinical and not a research study and thus only has incomplete follow-up, making it unreasonable to estimate detection rates in a scientifically meaningful way. Despite its large sample size it is also not powered to do so. Furthermore, the lack of CT data in many pregnancies makes it difficult to compare NIPT to CT directly.

**Comparison with previous studies**

Our findings are compatible with the results of previous studies. The PPV is very high, both in high- and low-risk pregnancies, which contradicts earlier smaller4 or more heterogeneous [12] studies, but is in line with more recent large-scale studies in the general screening population [5,7,17].

**Conclusions**

CfDNA testing is a new non-invasive method of prenatal testing that proves to be reliable also in a clinical setting, with low failure rates, and low percentages of discordant results. Both in the high-risk and low-risk population the PPV was found to be much higher than for traditional CT. Consequently, cfDNA testing is likely to replace traditional screening methods for fetal aneuploidy, and reduce the number of invasive procedures in the coming years. The relatively high cost of cfDNA testing versus CT and the absence of (full) reimbursement of cfDNA testing in many countries are the main challenge for pregnant women who chose cfDNA testing at the moment [18].

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**References**