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Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10–11 weeks' gestation and the combined test at 11–13 weeks

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KEYWORDS: cell-free DNA; combined test; fetal fraction; first trimester; prenatal diagnosis; screening; trisomies

ABSTRACT

Objective *To examine in a general population the performance of cell-free DNA (cfDNA) testing for trisomies 21, 18 and 13 at 10–11 weeks' gestation and compare it to that of the combined test at 11–13 weeks.*

Methods *In 2905 singleton pregnancies, prospective screening for trisomies was performed by chromosome-selective sequencing of cfDNA in maternal blood at 10–11 weeks' gestation and by the combined test at 11–13 weeks' gestation.*

Results *Median maternal age of the study population was 36.9 (range, 20.4–51.9) years. Results from cfDNA analysis were provided for 2851 (98.1%) cases and these were available within 14 days from sampling in 2848 (98.0%) cases. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or clinical examination of the neonates. Of the 2785 pregnancies with a cfDNA result and known trisomic status, cfDNA testing correctly identified all 32 cases with trisomy 21, nine of 10 with trisomy 18 and two of five with trisomy 13, with false-positive rates of 0.04%, 0.19% and 0.07%, respectively. In cases with discordant results between cfDNA testing and fetal karyotype, the median fetal fraction was lower than in those with concordant results (6%* vs *11%). Using the combined test, the estimated risk for trisomy 21 was ≥ 1/100 in all trisomic cases and in 4.4% of the non-trisomic pregnancies.*

Conclusion *The performance of first-trimester cfDNA testing for trisomies 21 and 18 in the general population is similar to that in high-risk pregnancies. Most false-positive and false-negative results from cfDNA testing could be avoided if the* a priori *risk from the combined test is taken into account in the interpretation of individual risk.*

INTRODUCTION

Several studies have shown that cell-free DNA (cfDNA) analysis of maternal blood can detect about 99% of cases of trisomy 21, 97% of trisomy 18 and 92% of trisomy 13, with respective false-positive rates (FPR) of approximately 0.1%, 0.2% and 0.2%[1]. Most of these studies were retrospective, using stored samples from pregnancies with known outcome, or prospective, using samples from high-risk pregnancies undergoing invasive testing[1]. There are also some studies reporting on the clinical implementation of cfDNA testing in routine screening for trisomies in the general population, but most of these studies do not provide data on complete pregnancy outcome and they cannot be used for assessment of the screening performance.

Only three studies in the general population reported outcome data on nearly all cases examined[2–4]. The first study examined stored plasma samples from 2049 singleton pregnancies that underwent combined screening at 11–13 weeks' gestation. They obtained results from cfDNA testing in 95.1% of cases and correctly identified all eight cases of trisomy 21 and the two with trisomy 18, with an FPR of 0.1%[2]. The second study performed cfDNA testing prospectively at a median gestational age of 16 (range, 11–21) weeks in 1916 singleton pregnancies[3]. The test did not provide a result in 3.8% of cases and there was loss to follow-up in 5.8% of cases. Of the 1741 pregnancies with cfDNA results and outcome data, the test correctly identified all eight cases of trisomy 21, two with trisomy 18 and one with trisomy 13; there was only one false-positive result for trisomy 18, but in this case there was low-grade maternal mosaicism for trisomy 18. The third study performed cfDNA testing prospectively in 2042 singleton pregnancies at 17 (range, 8–39) weeks' gestation[4]. Outcome was based on prenatal or postnatal karyotyping or clinical examination of the neonate. The

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trisomic status of the fetus could not be ascertained in only 3.5% of cases, either because of loss to follow-up or because the patient miscarried and the products of conception were not karyotyped. Results from cfDNA testing were provided for 99.1% of cases, and of the 1952 with known outcome, the test correctly identified all five cases of trisomy 21 and the two with trisomy 18, with an FPR of 0.5%.

The aim of this study was to report the results of clinical implementation of cfDNA testing for trisomies 21, 18 and 13 at 10–11 weeks' gestation in the general population, and compare its performance to that of the first-trimester combined screening test.

METHODS

The data for this study were derived from the clinical implementation of cfDNA testing in screening for trisomies 21, 18 and 13 at 10–11 weeks' gestation in women with singleton pregnancies. The women attended the Fetal Medicine Centre in London, UK, between October 2012 and January 2014. In addition to cfDNA testing, all women underwent the combined test at 11–13 weeks' gestation. In the first visit to the center, we recorded maternal characteristics and medical history, carried out an ultrasound examination to determine if the pregnancy was singleton with a live fetus and to estimate gestational age from measurement of the fetal crown–rump length (CRL). Maternal serum pregnancy-associated plasma protein A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) levels were measured (Thermo Scientific, Berlin, Germany) and 20 mL of maternal blood was collected in Streck cell-free DNA BCT™ tubes and sent via courier to the USA for cfDNA testing (Harmony™ Prenatal Test, Ariosa Diagnostics, Inc., San Jose, CA, USA)[5–7]. At the second visit, we combined maternal age with the results of the ultrasound measurement of fetal CRL, nuchal translucency (NT) thickness and serum concentrations of PAPP-A and free β-hCG levels to estimate the patient-specific risk for trisomy 21[8]. Patients were classified as high risk if the estimated risk was ≥ 1/100, which is the cut-off recommended by the UK National Screening Committee for invasive testing.

The results from cfDNA testing were presented as risk scores for trisomy 21, 18 and 13, which in most cases were either > 99% or < 1/10 000. In cases in which the cfDNA test did not provide results, the parents were offered repeat testing or they had to rely on the results of the combined test. In cases with a high-risk result, the parents were advised to consider having invasive fetal karyotyping before deciding on further management of their pregnancy. During the first part of the study, women with a low-risk result from cfDNA testing were reassured that the fetus was unlikely to be affected by these trisomies, irrespective of the results of the combined test[9]. In the second half of the study, the results of the combined test were used to derive the *a priori* risk for each trisomy and this was reduced by a factor of 100 for trisomy 21, 31 for trisomy 18 and 13 for trisomy 13[10].

Patient characteristics and results of the investigations were recorded in a fetal database. Results from invasive testing (obtained from laboratories) and pregnancy outcome (obtained from obstetricians, general practitioners or the patient) were recorded in the same database. The outcomes were divided into (1) trisomy 21, 18 or 13, if the karyotype of chorionic villi, amniotic fluid or neonatal blood demonstrated the relevant trisomy; (2) no trisomy 21, 18 or 13 if the karyotype of chorionic villi, amniotic fluid or neonatal blood was normal or the neonate was phenotypically normal; (3) no known karyotype because the pregnancies resulted in miscarriage or stillbirth and no karyotyping of fetal tissue was carried out; and (4) outcome unknown because the cases were lost to follow-up.

RESULTS

Study population

During the study period, we examined 2905 women with singleton pregnancies and a live fetus at 10 + 0 to 11 + 6 (median, 10 + 4) weeks' gestation. The median maternal age was 36.9 (range, 20.4–51.9) years and 1958 (67.4%) women were aged 35 years or older. The median maternal weight was 62.8 (range, 40.5–137.7) kg. The racial origin of the women was Caucasian in 2570 (88.5%), South Asian in 173 (6.0%), East Asian in 96 (3.3%), Afro-Caribbean in 21 (0.7%) and mixed in 45 (1.5%) women. 1555 (53.5%) women were parous and 1350 (46.5%) were nulliparous. Conception was spontaneous in 2438 (83.9%) of the pregnancies and 467 (16.1%) were the result of assisted reproduction techniques.

On the basis of the results of fetal karyotyping or clinical examination of the neonates, there were 34 cases of trisomy 21, 10 of trisomy 18, five of trisomy 13, 2787 without trisomy 21, 18 or 13, 48 miscarriages or stillbirths with unknown karyotype, and 21 that were lost to follow-up (Table 1).

Results of cfDNA testing

Results of cfDNA testing were obtained after first sampling in 2782 (95.8%) of the 2905 cases. In 110 of the 123 cases with no result, a further blood sample was obtained and a result provided in 69 (62.7%) cases; consequently, cfDNA results were obtained for 2851 (98.1%) cases. The 54 cases with no result included one case for which the sample was not received by the laboratory, 38 cases with fetal fraction below the minimal requirement of 4% and 15 cases of assay failure. The median time interval between blood sampling and receiving results was 9 (range, 5–20) days, with 2848 (98.0%) results being available within 14 days of sampling. The median fetal fraction in the cases with a result was 11% (range, 4–40%).

Of the 54 pregnancies with no cfDNA result, there were 49 non-trisomic cases, two cases of trisomy 21 and three cases of miscarriage with no karyotype (Figure 1). Of the

Table 1 Results of cell-free DNA analysis of maternal blood and combined test in screening for trisomies 21, 18 and 13 in 2905 singleton pregnancies according to risk

		Cell-free DNA result			*Combined test*		
Trisomic status	n	*High-risk*	*Low-risk*	*No result*	*High-risk*	*Low-risk*	*No result*
Non-trisomic	2787	8	2730	49	124	2663	—
Trisomy 21	34	32	—	2	34	—	—
Trisomy 18	10	9	1	—	10	—	—
Trisomy 13	5	2	3	—	5	—	—
Not known	69	1	65	3	5	52	12

Data are presented as *n*.

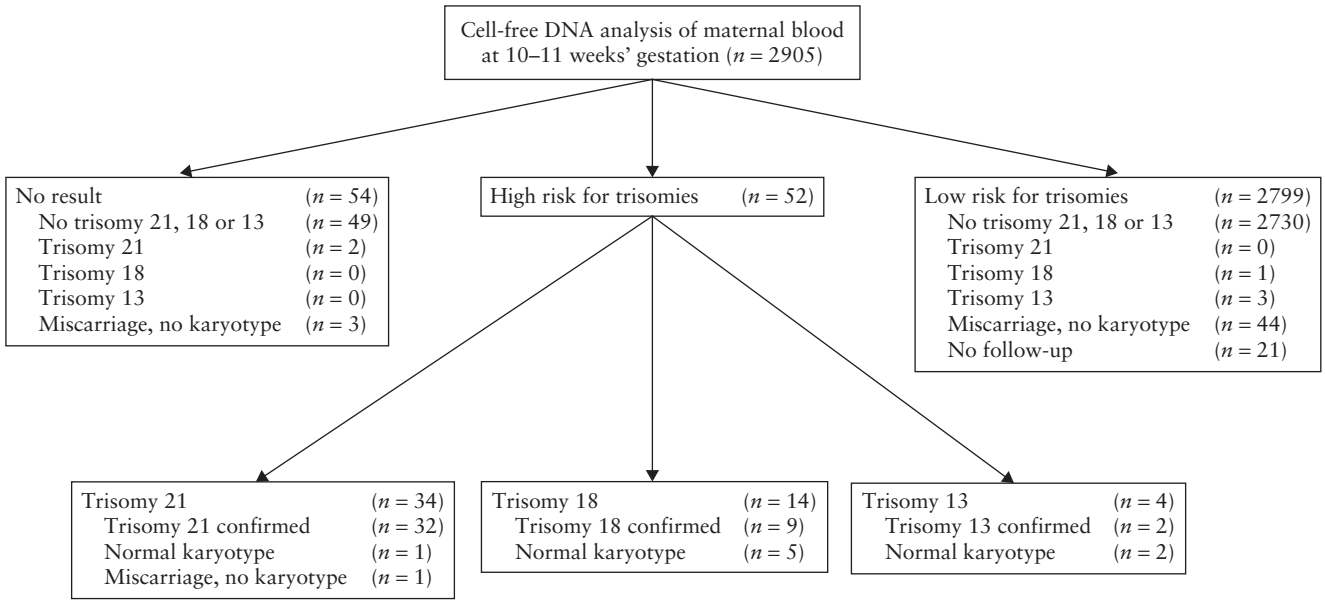

Figure 1 Flow-chart of pregnancy outcome according to results of cell-free DNA testing of maternal blood in 2905 singleton pregnancies.

34 cases with a high-risk result for trisomy 21, there were 32 cases in which invasive testing confirmed trisomy 21, one case of miscarriage before planned chorionic villus sampling (CVS) and one case with a normal karyotype. Of the 14 cases with a high-risk result for trisomy 18, there were nine cases in which invasive testing confirmed trisomy 18 and five cases with a normal karyotype. Of the four cases with a high-risk result for trisomy 13, there were two cases in which invasive testing confirmed trisomy 13 and two cases with a normal karyotype. Of the 2799 cases with a low-risk result for all three trisomies, there were 2730 pregnancies with no trisomy 21, 18 or 13, one case of trisomy 18, three of trisomy 13, 44 of miscarriage and no karyotype and 21 cases that were lost to follow-up.

Of the 2836 pregnancies with known trisomic status, cfDNA testing correctly identified 32 of the 34 cases of trisomy 21 but did not provide results in two, nine of the 10 cases of trisomy 18 and two of the five cases of trisomy 13 (Table 1). Of the 2787 non-trisomic pregnancies, cfDNA testing correctly provided a low-risk result for each of the three trisomies in 2730 cases, did not provide a result in 49 and gave a false-positive result for trisomy 21 in one (0.04%) case, for trisomy 18 in five cases (0.19%) and for trisomy 13 in two cases (0.07%).

Results of combined screening

In the 2836 pregnancies with known trisomic status, the estimated risk for trisomy 21 at 11–13 weeks' gestation was ≥ 1/100 in all 49 trisomic pregnancies and in 124 (4.4%) of the 2787 non-trisomic pregnancies (Table 1).

Discordant results between cfDNA testing and fetal karyotype

The discordant results between cfDNA testing and fetal karyotype are summarized in Table 2. The median fetal fraction in the 12 cases with discordant results (6.0% (range, 4.2–8.7%)) was significantly lower than in the 2730 cases with concordant normal results (11.1% (range, 4.1–40.2%)) and the 43 cases with concordant abnormal results (9.6% (range, 4.7–20.4%)) (Mann–Whitney *U*-test, $P < 0.0001$).

In the 32 cases of trisomy 21 with concordant results, the median fetal fraction was 10.1% (range, 5.4–20.4%) and the median estimated risk from the combined test was 1/2 (range, 1/2 to 1/81). In the case with discordant results, the fetal fraction was 4.7% and the risk from the combined test was 1/6966.

In the nine cases of trisomy 18 with concordant results, the median fetal fraction was 9.6% (range, 4.7–14.7%)

Table 2 Summary of the 12 cases with discordant results between cell-free DNA analysis of maternal blood and fetal karyotype among 2905 singleton pregnancies

Cell-free DNA analysis of maternal blood		*Combined test result*	*Fetal karyotype*	*Outcome of pregnancy*
Result	*Fetal fraction (%)*			
Risk for trisomy 21				
High	4.7	1:6966	CVS: normal	Healthy live birth
Risk for trisomy 18				
High	4.2	1:906	—	Healthy live birth
High	4.3	1:496	PM: normal	Miscarriage (20 weeks)
High	5.1	1:1120	CVS: normal	Healthy live birth
High	5.6	1:34 483	CVS: normal	Healthy live birth
High	6.3	1:210	Amnio: del 18(p11.1)	Termination
Low	8.7	1:6	Amnio: trisomy 18	Termination
Risk for trisomy 13				
High	5.9	1:1645	CVS: normal	Healthy live birth
High	7.2	1:6152	CVS: normal	Healthy live birth
Low	6.2	1:2	CVS: trisomy 13	Termination
Low	6.0	1:2	CVS: trisomy 13	Termination
Low	8.6	1:2	CVS: trisomy 13	Termination

Amnio, amniocentesis; CVS, chorionic villus sampling; del, deletion; PM, postmortem examination.

and the median estimated risk from the combined test was 1/4 (range, 1/2 to 1/14). In five cases with a positive result from cfDNA testing but disomy 18 on fetal karyotyping, the median fetal fraction was 5.1% (range, 4.2–6.3%) and the median estimated risk from the combined test was 1/906 (range, 1/210 to 1/34 483). In one of these five cases the first cfDNA test failed because of low fetal fraction, but after repeat testing 2 weeks later the result was > 99% for trisomy 18; amniocentesis at 16 weeks' gestation demonstrated a deletion in the short arm of chromosome 18. In one case cfDNA testing gave a low risk, but the risk from the combined test was 1/6; CVS was carried out and the quantitative fluorescence polymerase chain reaction (QF-PCR) result was reported as normal but the culture failed. Subsequently, amniocentesis was performed because ultrasound examination at 20 weeks' gestation demonstrated fetal growth restriction, choroid plexus cysts and flexion deformity of the hands, and both the QF-PCR and culture results indicated trisomy 18.

In two cases with concordant results for trisomy 13, the respective fetal fractions were 5.6% and 6.1% and the estimated risks from the combined test were 1/21 and 1/43, respectively. In two cases with a positive cfDNA result but normal fetal karyotype, the respective fetal fractions were 5.9% and 7.2% and the risks from the combined test were 1/1645 and 1/6152, respectively. In three cases at low risk for trisomy 13 from cfDNA testing and an abnormal result from fetal karyotyping, the risk from the combined test was 1/2.

DISCUSSION

Main findings of the study

This prospective study in women undergoing routine first-trimester screening for the major trisomies, by cfDNA analysis of maternal blood and by the combined test, examined 2905 cases and provided outcome data for nearly 98% of cases, which makes it possible to assess accurately the performance of screening.

The combined test, at a risk cut-off of 1/100, identified all cases of trisomy 21, 18 and 13, with an FPR of 4.4%. Screening by cfDNA analysis of maternal blood provided results in 98% of pregnancies and these were available within 2 weeks of sampling in 98% of cases. In the pregnancies with a cfDNA result, all cases of fetal trisomy 21 were detected, with an FPR of 0.04%. The test also detected nine of the 10 cases of fetal trisomy 18, with an FPR of 0.19%; in the one false-negative case, QF-PCR of chorionic villi reported disomy 18. The performance of screening for trisomy 13 was poorer, with only two of five affected cases being detected.

In cases of discordant results between cfDNA testing and fetal karyotype, the fetal fraction was lower than in those with concordant normal or abnormal results.

Limitations of the study

The median maternal age of the study population was 36.9 years, which is higher than the median age of 31.7 years in our NHS hospital in London[11]. The patients were self-selected and, inevitably, a high proportion of women were of advanced age and had conceived by assisted reproduction techniques. Nevertheless, the women did not have prior screening for trisomies by other methods and their results are representative of the general population.

Another limitation of the study relates to the high performance of the combined test. The results of cfDNA analysis were commonly available at the time of the ultrasound examination for measurement of fetal NT, and could have potentially biased the measurements.

Comparison with results of previous studies

Our findings on the performance of maternal blood cfDNA analysis in screening for trisomies 21 and 18

in a general population are compatible with the results of previous studies in high-risk pregnancies[1], but also with those that examined general populations[2–4]. Our detection rate of trisomy 13 was lower, however the number of cases we examined was too small for valid conclusions to be drawn. A meta-analysis of clinical validation or implementation studies of cfDNA testing reported that the weighted pooled detection rates in 809 cases of trisomy 21, 301 of trisomy 18 and 85 of trisomy 13 were 99.0% (95% CI, 98.2–99.6%), 96.8% (95% CI, 94.5–98.4%) and 92.1% (95% CI, 85.9–96.7%), respectively, with FPRs of 0.08% (95% CI, 0.03–0.14%), 0.15% (95% CI, 0.08–0.25%) and 0.20% (95% CI, 0.04–0.46%), respectively[1].

In the two previous prospective screening studies in a general population, the median gestational age at cfDNA testing was 16 and 17 weeks, respectively[3,4]. Our study focused on the application of cfDNA testing at 10–11 weeks because first-trimester screening and diagnosis of aneuploidies leads to early reassurance, for the majority of parents, that their fetus is unlikely to be trisomic, and for the few with an affected fetus, the parents have the option of an earlier and safer termination of pregnancy. The two-stage strategy of cfDNA testing at 10–11 weeks followed by the combined test retains the benefits of early detection of many major fetal defects and the prediction and potential prevention of a wide range of pregnancy complications[12].

Implications for clinical practice

There are essentially two options in the clinical implementation of cfDNA testing in screening for the major trisomies: firstly, routine screening of the whole population and secondly, contingent screening based on the results of first-line screening by another method, preferably the first-trimester combined test[1]. In the first option, it would be best to undertake screening in the first trimester, and our results establish the feasibility of such an approach.

cfDNA testing is not a diagnostic test and in the interpretation of individual patient results it is necessary to know the *a priori* risk for the given trisomy. On the basis of the results of our meta-analysis of cfDNA testing, the positive likelihood ratios for trisomies 21, 18 and 13 are 1238, 645 and 461, respectively[1]. It is therefore not surprising that in most of our cases with false-positive results, the estimated risk from the combined test was very low. The negative likelihood ratios for trisomies 21, 18 and 13 are 0.01, 0.03 and 0.08, respectively[1], and therefore, with a negative cfDNA result for these trisomies, there is a 100-fold, 31-fold and 13-fold reduction in the *a priori* risk[10]. In our three cases with a false-negative result for trisomy 13 the estimated risk from the combined test was 1/2, consequently their individual risk was not $< 1/10\,000$ as reported by the cfDNA test, but approximately 1/25 when the results of both the cfDNA test and the combined test were taken into account.

The use of the *a priori* risk in the interpretation of results from cfDNA testing is particularly important in cases with a low fetal fraction. The ability to detect the small increase in the amount of a given chromosome in maternal plasma in a trisomic compared to a disomic pregnancy is directly related to the relative proportion of the fetal to maternal origin of the cfDNA in maternal plasma[5,13–15]. In our cases with false-positive and false-negative results the median fetal fraction was lower than in those with concordant results between the cfDNA test and fetal karyotype.

The most accurate method for defining the *a priori* risk for each patient is the combined test[8]. However, it is unrealistic to expect that universal screening for trisomies by cfDNA testing would necessitate that all women should also have the combined test. Nevertheless, it is desirable that all women should have a high-quality first-trimester ultrasound scan, including measurement of fetal NT, which is a marker not only for trisomies but also for other aneuploidies, cardiac defects and many genetic syndromes. The *a priori* risk for trisomies derived from a combination of maternal age and fetal NT is certainly more accurate than that obtained from maternal age alone. As for the additional measurement of serum biochemical markers, this will essentially depend on the extent to which there is widespread uptake of first-trimester screening for pregnancy complications, such as pre-eclampsia.

Conclusions

This study has shown that routine screening for trisomies by cfDNA testing at 10–11 weeks' gestation is feasible, allowing diagnosis of aneuploidies and the option of first-trimester termination of pregnancy. The study has highlighted that firstly, cfDNA testing has a substantially lower FPR than the combined test, secondly, in cases of discordant results between the cfDNA test and fetal karyotype, the fetal fraction is lower than in those with concordant results and thirdly, in the interpretation of cfDNA results, particularly in cases with a low fetal fraction, the *a priori* risk should be considered.

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