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Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test

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KEYWORDS: cell-free DNA; fetal trisomy; first-trimester combined test

ABSTRACT

Objectives *Cell-free DNA (cfDNA) analysis of maternal blood for detection of trisomies 21, 18 and 13 is superior to other methods of screening but is expensive. One strategy to maximize performance at reduced cost is to offer cfDNA testing contingent on the results of the first-trimester combined test that is used currently. The objectives of this study were to report the feasibility of implementing such screening, to examine the factors affecting patient decisions concerning their options for screening and decisions on the management of affected pregnancies and to report the prenatal diagnosis of fetal trisomies and outcome of affected pregnancies following the introduction of contingent screening.*

Methods *We examined routine clinical implementation of contingent screening in 11 692 singleton pregnancies in two National Health Service (NHS) hospitals in the UK. Women with a risk ≥ 1 in 100 (high-risk group) were offered options of invasive testing, cfDNA testing or no further testing, and those with a risk between 1 in 101 and 1 in 2500 (intermediate-risk group) were offered cfDNA testing or no further testing. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or by examination of the neonates.*

Results *In the study population of 11 692 pregnancies, there were 47 cases of trisomy 21 and 28 of trisomies 18 or 13. Screening with the combined test followed by invasive testing for all patients in the high-risk group potentially could have detected 87% of trisomy 21 and 93% of trisomies 18 or 13, at a false-positive rate of 3.4%; the respective values for cfDNA testing in the high- and intermediate-risk groups were 98%, 82% and 0.25%. However, in the high-risk group, 38% of women chose invasive testing and 60% chose cfDNA testing; in the intermediate-risk group 92% opted for cfDNA testing. A prenatal diagnosis was made in 43 (91.5%) pregnancies with trisomy 21 and all pregnancies with trisomies 18 or 13. In many affected pregnancies the parents chose to avoid testing or termination and 32% of pregnancies with trisomy 21 resulted in live births.*

Conclusions *Screening for fetal trisomies by cfDNA analysis of maternal blood, contingent on the results of the combined test, can be implemented easily in routine clinical practice. In the high-risk group from the combined test, most but not all women chose cfDNA testing rather than invasive testing. Performance of screening for trisomy 21 was superior by the cfDNA test than by the combined test. However, prenatal detection of trisomies and pregnancy outcome depend not only on performance of screening tests but also on parental choice.*

INTRODUCTION

Screening for trisomy 21 in all National Health Service (NHS) hospitals in England is performed using the first-trimester combined test which involves measurement of fetal nuchal translucency (NT) thickness and serum levels of β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), with potential prenatal detection of about 90% of fetuses with trisomy 21 and 95% of those with trisomies 18 or 13, at a false-positive rate (FPR) of 5%[1,2]. Recent evidence suggests that the performance of screening may be improved by analysis of cell-free DNA (cfDNA) in maternal blood. A meta-analysis of clinical validation studies reported detection rates (DRs) for trisomies 21, 18 and 13 of 99%, 96% and 91%, respectively, at an overall FPR of 0.35%[3]. Consequently, there has been

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widespread uptake of cfDNA testing in routine clinical practice and we have demonstrated that this is feasible during the first trimester of pregnancy[4–6]. For screening for the major trisomies in the general population, cfDNA testing can be used either as a first-line method of screening or contingent on the results of the combined test performed at 11–13 weeks' gestation. Contingent screening could potentially lead to a very high DR and very low invasive-testing rate, at a considerably lower cost than would be possible using cfDNA testing as a first-line method of screening, based on current cfDNA testing costs[7–9].

In this study we examine the clinical implementation of cfDNA testing, contingent on the results of the combined test, in routine screening for fetal trisomies. The objectives were, first, to report the feasibility of implementing such screening, second, to examine the factors affecting patient decisions concerning their options for screening and decisions on the management of affected pregnancies and, third, to report the prenatal diagnosis of fetal trisomies and outcome of affected pregnancies following the introduction of contingent screening.

METHODS

Study design and participants

This was a prospective study including women with a singleton pregnancy attending one of two NHS hospitals in England (King's College Hospital, London, and Medway Maritime Hospital, Gillingham, Kent) for routine care between October 2013 and February 2015. Implementation of contingent screening was approved by the National Research Ethics Committee (REC reference 13/LO/0885).

During a routine visit at 11–13 weeks' gestation, patients were asked to provide information on demographic characteristics, obstetric history and education, and subsequently underwent the combined test. The estimated risks for trisomy 21 and trisomies 18 or 13 were calculated and the highest of the two was considered in the stratification of the population. In one of the two hospitals, the ultrasound scan, biochemical analysis, estimation of risk and post-test counseling of the women were undertaken during the same hospital visit. In the second hospital, analysis of the blood sample collected at the time of the ultrasound scan and estimation of risk were carried out on the following day, and women with a risk of $\geq$ 1 in 2500 were contacted by telephone and scheduled for another hospital appointment for further counseling. Women with risk of $\geq$ 1 in 100 (high risk), were offered the options of chorionic villus sampling (CVS), cfDNA testing or no further testing. Women with a risk between 1 in 101 and 1 in 2500 (intermediate risk) were offered the options of cfDNA testing or no further testing and those with a risk of $<$ 1 in 2500 (low risk) were reassured that fetal trisomies were unlikely and no further testing was necessary.

Women opting for cfDNA testing provided written informed consent. A maternal blood sample (20 mL) was sent via courier to the USA for cfDNA testing (Harmony^{TM} Prenatal Test, Ariosa Diagnostics, Inc., San Jose, CA, USA)[10–12].

The risk cut-off of 1 in 100 was selected for defining the high-risk group because this is the cut-off used by the NHS for offering invasive testing. The risk cut-off of 1 in 2500 was selected for offering the cfDNA test because we estimated previously that, in a population with the maternal age distribution found in England in 2011, such a policy potentially could result in cfDNA testing in about 25% of the population and a contingent policy would lead to the detection of about 97% of cases of trisomy 21 and 95% of trisomies 18 and 13[8].

Patient characteristics, results of the investigations and pregnancy outcome were recorded in a database. The outcomes were divided into the following categories: trisomy 21, 18 or 13 if the karyotype of chorionic villi, amniotic fluid or neonatal blood demonstrated the relevant trisomy; 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; no known karyotype because the pregnancy resulted in miscarriage or stillbirth and no karyotyping of fetal tissue was carried out; and unknown outcome because the pregnancy was lost to follow-up.

In a previous study on the first 6651 pregnancies that were recruited to the study, we explored the factors affecting patient decisions concerning their options for screening[13]. In this study we explored further these decisions and examined the performance of contingent screening and the effects on pregnancy outcome.

Statistical analysis

Descriptive data were presented as median (interquartile range (IQR)) for continuous variables and as n (%) for categorical variables. Comparisons between outcome groups were performed using Mann–Whitney U-test for continuous variables and χ^2-test or Fisher's exact test for categorical variables. Logistic regression analysis was used to determine which factors were significant predictors of opting for CVS in the high-risk group and opting for cfDNA testing in the intermediate-risk group. The statistical software package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses.

RESULTS

Study population

During the study period, 12 134 women were offered combined screening for detection of trisomies; 11 921 (98.2%) accepted, of which 229 (1.9%) were excluded from further analysis either because the pregnancy ended in termination, miscarriage or stillbirth with no known karyotype (n = 169) or they were lost to follow-up (n = 60).

Maternal and pregnancy characteristics of the 11 692 pregnant women with known outcome are summarized in Table 1. There were 47 cases of trisomy 21, 24 of trisomy 18, four of trisomy 13 and 11 617 without these

Table 1 Maternal and fetal characteristics of 11 692 women with singleton pregnancy, attending National Health Service hospitals, who were offered cell-free DNA (cfDNA) testing for trisomies (T) 21, 18 and 13, according to the results of the combined test at 11–13 weeks' gestation

Characteristic	*High risk* (n = *460*)	*Intermediate risk* (n = *3552*)	*Low risk* (n = *7680*)
Maternal age at visit (years)	36.1 (32.1–39.5)*	34.8 (30.8–38.4)*	29.9 (25.8–33.2)
Racial origin			
Caucasian	315 (68.5)	2548 (71.7)*	5309 (69.1)
Afro-Caribbean	90 (19.6)	633 (17.8)*	1584 (20.6)
South Asian	22 (4.8)	182 (5.1)	346 (4.5)
East Asian	21 (4.6)*	99 (2.8)*	137 (1.8)
Mixed	12 (2.6)	90 (2.5)*	304 (4.0)
Cigarette smoker	34 (7.4)	236 (6.6)*	686 (8.9)
Parity			
Nulliparous	176 (38.3)*	1391 (39.2)*	3907 (50.9)
Parous	284 (61.7)*	2161 (60.8)*	3773 (49.1)
Method of conception			
Spontaneous	444 (96.5)	3417 (96.2)*	7508 (97.8)
Assisted	16 (3.5)	135 (3.8)*	172 (2.2)
Level of education			
None/primary school	6 (1.3)	76 (2.1)	168 (2.2)
Secondary school	74 (16.1)	525 (14.8)*	1320 (17.2)
College qualification	146 (31.7)	1044 (29.4)*	2729 (35.5)
University	234 (50.9)*	1907 (53.7)*	3463 (45.1)
Previous pregnancy with aneuploidy	6 (1.3)*	50 (1.4)*	12 (0.2)
Fetal nuchal translucency (mm)	2.1 (1.7–2.9)*	1.8 (1.6–2.1)*	1.7 (1.5–1.9)
Estimated risk for T21 or T18/T13 (1 in *n*)	39 (70–10)*	993 (1627–458)*	8320 (14 128–4772)
Patient choice for further testing			
cfDNA test	276 (60.0)	3249 (91.5)	—
Chorionic villus sampling	173 (37.6)	—	—
No test	11 (2.4)	303 (8.5)	7680 (100)

Data are given as median (interquartile range) or *n* (%). Comparisons of high- and intermediate-risk groups with low-risk group by Mann–Whitney *U*-test for continuous variables and by chi-square or Fisher's exact test for categorical variables, with *post-hoc* Bonferroni correction: *adjusted $P < 0.025$.

trisomies. The expected number of cases of trisomy 21 and trisomies 18 or 13 in our study population, on the basis of the maternal age distribution and the age-related risk for these trisomies at 12 weeks' gestation, were 42 (95% CI, 30–56) and 22 (95% CI, 15–34), respectively, which were similar to the observed numbers of 47 and 28, respectively[14,15]. The mean maternal age in our study population was higher than in all pregnancies in England and Wales in 2013[16] (31.0 *vs* 30.0 years; Figure 1).

Following combined screening, 460 (3.9%), 3552 (30.4%) and 7680 (65.7%) patients were classified as high risk, intermediate risk, and low risk, respectively (Tables 1 and 2).

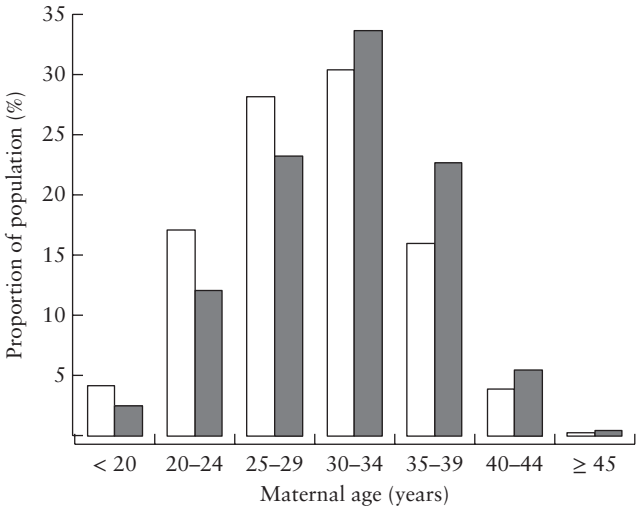

Figure 1 Comparison between age distribution in England and Wales in 2013[16] (□) and that in our study population of 11 692 women with singleton pregnancy attending National Health Service hospitals in the UK (■).

Potential performance of the combined test

The estimated risk from the combined test was ≥ 1 in 100 in 87% (41/47) of fetuses with trisomy 21, 92% (22/24) with trisomy 18, 100% (4/4) with trisomy 13 and 3.4% (393/11 617) of non-trisomic pregnancies (Table 2). Five cases of trisomy 21 and two of trisomy 18 were in the intermediate-risk group and one case of trisomy 21 was in the low-risk group. The distribution of risks for trisomic and unaffected pregnancies are given in Table 3.

Implementation and potential performance of the cfDNA test

The cfDNA test was carried out in 3698 pregnancies. These included 3525 from the high- and intermediate-risk

Table 2 Parental decision regarding further investigation and outcome in 11 692 women with singleton pregnancy attending National Health Service hospitals, according to estimated risk from combined test at 11–13 weeks' gestation

		High risk (n = 460)				*Intermediate risk (n = 3552)*			*Low risk (n = 7680)*
Outcome	n	*Total*	*CVS*	*cfDNA*	*No test*	*Total*	*cfDNA*	*No test*	*No test*
Trisomy 21	47	41	27	13	1	5	4	1	1
Trisomy 18	24	22	17	5	0	2	2	0	0
Trisomy 13	4	4	3	1	0	0	0	0	0
Non-trisomy	11 617	393	126	257	10	3545	3243	302	7679
Total	11 692	460	173	276	11	3552	3249	303	7680

Data are given as *n*. cfDNA, cell-free DNA testing; CVS, chorionic villus sampling.

Table 3 Distribution of risk from the combined test in 11 692 women with singleton pregnancy attending National Health Service hospitals, according to trisomic outcome

Risk cut-off	*Trisomy 21 (n = 47)*	*Trisomy 18 (n = 24)*	*Trisomy 13 (n = 4)*	*Unaffected (n = 11 617)*
≥ 1 in 10	30 (63.8)	19 (79.2)	4 (100)	62 (0.5)
≥ 1 in 20	36 (76.6)	20 (83.3)	4 (100)	101 (0.9)
≥ 1 in 50	38 (80.9)	21 (87.5)	4 (100)	208 (1.8)
≥ 1 in 100	41 (87.2)	22 (91.7)	4 (100)	393 (3.4)
≥ 1 in 500	46 (97.9)	23 (95.8)	4 (100)	1351 (11.6)
≥ 1 in 1000	46 (97.9)	24 (100)	4 (100)	2181 (18.8)
≥ 1 in 1500	46 (97.9)	24 (100)	4 (100)	2870 (24.7)
≥ 1 in 2000	46 (97.9)	24 (100)	4 (100)	3429 (29.5)
≥ 1 in 2500	46 (97.9)	24 (100)	4 (100)	3938 (33.9)
≥ 1 in 3000	46 (97.9)	24 (100)	4 (100)	4453 (38.3)
≥ 1 in 3500	47 (100)	24 (100)	4 (100)	4899 (42.2)

Data are given as *n* (%).

groups that opted for cfDNA testing and 173 from the high-risk group that opted for CVS but also had cfDNA testing for research purposes; in the latter group the blood test was collected before invasive testing.

cfDNA testing provided a result after first sampling in 97.3% (3599/3698) of cases. In 54 of the 99 cases with no result, a further blood sample was obtained and subsequently a result was provided in 34 (63.0%); consequently, cfDNA results were available for 98.2% (3633/3698) of cases. The median time interval between sending the blood to the laboratory for testing and receiving the results was 8 (range, 4–21) days, with 98.7% (3651/3698) of results (including failed result) being available within 14 days.

The 65 cases with no results from cfDNA testing included four for whom the test was done in addition to CVS and 61 for whom the parental choice was cfDNA testing. In 60 of the latter group no invasive tests were carried out and healthy babies were born; in one case detection of multiple fetal defects at 20 weeks led to a diagnosis of trisomy 18 by amniocentesis.

The cfDNA test was carried out in 44/47 cases of trisomy 21; for one case in the low-risk group and two in the high- or intermediate-risk group the parents opted against further investigations (Table 2). The cfDNA test classified correctly 97.7% (43/44) of affected cases as screen positive for trisomy 21; one case from the intermediate-risk group was a false negative. The cfDNA test was carried out in all 28 cases of trisomy 18 or 13. The cfDNA test classified correctly 87.5% (21/24) of affected cases as screen positive for trisomy 18; in three the test did not provide a result. The cfDNA test classified correctly 50% (2/4) of affected cases as screen positive for trisomy 13; in two there was a false-negative result.

In the 3633 cases with a cfDNA result there were 3564 with no trisomies 21, 18 or 13. The cfDNA test classified correctly 99.7% (3555/3564) of cases as screen negative for each of the trisomies; in nine (0.25%) there was a false-positive result, including one case of trisomy 21, four of trisomy 18 and four of trisomy 13. If cfDNA testing had been confined to screening for trisomy 21, the FPR would have been 0.03% (1/3564).

Patient decision in the high-risk group from combined test

In the high-risk group of 460 women, 173 (37.6%) opted for CVS, 276 (60.0%) for cfDNA testing and 11 (2.4%) did not want any further investigations. At the two participating hospitals, the method of screening for trisomies before the start of our study was the combined test. During a 1-year period before the onset of the study, screening was carried out in 10 271 pregnancies and the risk for trisomies was ≥ 1 in 100 in 407 cases; in 267 (65.6%) the women opted for invasive testing and 140 (34.4%) had no further investigations. Therefore, the introduction of cfDNA testing was associated with a

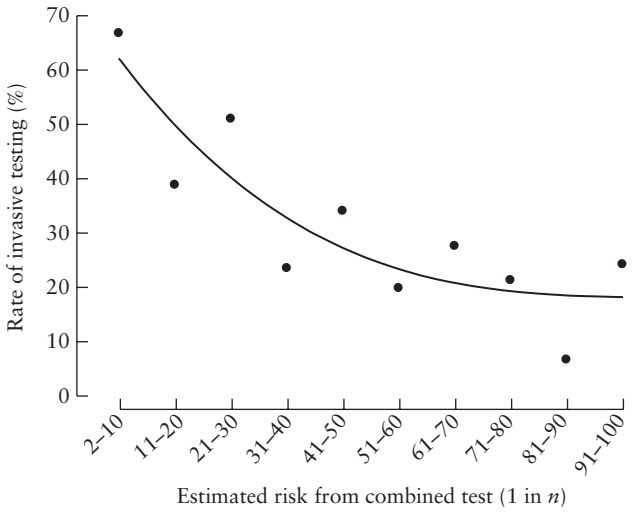

Figure 2 Relationship between estimated risk for trisomies from the combined test and maternal decision in favor of invasive testing in our study population of 11 692 women with singleton pregnancy.

43% reduction in the rate of invasive testing from 65.6% to 37.6%.

Regression analysis demonstrated that significant independent prediction of opting for CVS was provided by an increasing risk for trisomies (Figure 2) and increasing fetal NT, and opting against CVS was provided by being of Afro-Caribbean racial origin and attending the hospital in which the results from combined screening were not given on the same visit as the scan ($R = 0.315$; Table 4).

In the high-risk group, there were 41 cases of trisomy 21; in 27 the parents chose CVS, in 13 the cfDNA test and in one no further investigations. In the 13 cases with a positive cfDNA test, nine women had confirmatory CVS and four did not want further testing. In the total high-risk group, the rate of pregnancy termination for trisomy 21 was 92.6% (25/27) in those choosing invasive testing and 35.7% (5/14) in those choosing cfDNA testing or no further investigations ($P = 0.0002$).

Patient decision in the intermediate-risk group from combined test

In the intermediate-risk group, 91.5% (3249/3552) opted for cfDNA testing and 8.5% (303/3552) had no further investigations. The reason given by the women for their decision against further testing was that, first, they were happy with the risk from the combined test and did not want to endure the anxiety of awaiting for results of further tests ($n = 155$), second, they would never contemplate having termination of an affected pregnancy ($n = 73$), third, they received the results from the combined test after the visit for the scan and found it difficult or inconvenient to return to the hospital for further tests ($n = 42$), fourth, they considered the cfDNA test to be experimental and did not want to participate in research ($n = 29$) and, fifth, they did not want their blood to be sent for testing in another country ($n = 4$).

Regression analysis demonstrated that opting for cfDNA testing was associated with increasing maternal age and increasing risk for trisomies, and opting against testing was associated with being of Afro-Caribbean racial origin, cigarette smoking, being parous and attending the hospital in which the results from combined screening were not given at the same visit as the scan ($R = 0.128$; Table 4).

In the intermediate-risk group there were five cases of trisomy 21; in four the parents opted for cfDNA testing and in one no further investigations. In the group with cfDNA testing, the correct diagnosis was made in three, of which the parents opted for pregnancy termination in two and continued with the pregnancy in one.

Performance of contingent screening

Contingent screening led to the prenatal detection of 91.5% (43/47) of cases of trisomy 21 (Figure 3) and 100% (28/28) of trisomies 18 or 13. One case of trisomy 21 was in the low-risk group in which no further tests were offered, two cases were in the high- or intermediate-risk group but the mother chose to have no further investigations, and one was in the intermediate-risk group but the cfDNA test gave a false-negative result. In 74.4% (32/43) of the detected cases, parents opted for pregnancy termination and in 25.6% (11/43) they chose to continue with the pregnancy. Consequently, 31.9% (15/47) of trisomy 21 were live born.

There were 28 cases of trisomies 18 or 13, of which the parents chose pregnancy termination in 23 (82.1%) and to continue with the pregnancy in five (17.9%). In three of the latter pregnancies there was miscarriage or fetal death and in two neonatal death.

Invasive tests were carried out in 2.7% (312/11 692) of the study population. These included 193 (1.7%) for high-risk result from the combined test ($n = 173$) or a positive result from the cfDNA test ($n = 20$) and 119 (1.0%) for other indications, including genetic testing for conditions such as sickle cell disease and karyotyping for fetal defects detected by ultrasound examination in the first and/or second trimester of pregnancy.

DISCUSSION

Main findings of the study

This study has demonstrated the feasibility of introducing cfDNA testing, contingent on the results of the first-trimester combined test for major trisomies, in routine clinical practice. The incidence of trisomies and rates with risk from the combined test of ≥ 1 in 100 and ≥ 1 in 2500 were higher than reported in the UK in 2013, because the age distribution of our population was higher[16].

In our participating hospitals, about 98% of women attending for a routine ultrasound examination at

Table 4 Prediction of opting for chorionic villus sampling (CVS) in the high-risk group and for cell-free DNA testing (cfDNA) in the intermediate-risk group, based on results of the combined test

	High risk: favors CVS		*Intermediate risk: favors cfDNA*	
Independent variable	*Univariate OR (95% CI)*	*Multivariate OR (95% CI)*	*Univariate OR (95% CI)*	*Multivariate OR (95% CI)*
Maternal age in years	1.016 (0.983 to 1.051)	—	1.093 (1.072–1.115)*	1.072 (1.049–1.096)*
Racial origin				
Caucasian	1	1	1	1
Afro-Caribbean	0.457 (0.267–0.782)*	0.258 (0.136–0.491)*	0.549 (0.417–0.724)*	0.359 (0.263–0.490)*
South Asian	1.800 (0.755–4.292)	—	0.653 (0.401–1.063)	—
East Asian	0.923 (0.372–2.291)	—	3.908 (0.956–15.973)	—
Mixed	1.500 (0.473–4.755)	—	0.645 (0.329–1.265)	—
Cigarette smoking	1.029 (0.501–2.112)	—	0.307 (0.219–0.431)*	0.513 (0.355–0.742)*
Parity				
Nulliparous	1	—	1	1
Parous	0.607 (0.413–0.894)*	—	0.550 (0.423–0.715)*	0.540 (0.407–0.717)*
Method of conception				
Spontaneous	1	—	1	—
Assisted	1.691 (0.323–4.590)	—	3.141 (1.153–8.553)*	—
Level of education				
None/primary school	1	—	1	—
Secondary school	2.708 (0.300–24.429)	—	1.627 (0.861–3.073)	—
College qualification	2.300 (0.261–20.250)	—	1.660 (0.903–3.053)	—
University	3.731 (0.429–32.439)	—	4.080 (2.208–7.539)*	—
Previous pregnancy with aneuploidy	3.373 (0.611–18.611)	—	0.837 (0.330–2.125)	—
Fetal NT in mm	1.859 (1.540–2.245)*	1.620 (1.307–2.008)*	1.004 (0.758–1.330)	—
Prior risk for T21 or T18/T13	1075.9 (0.00–1.19×10^{11})	—	2.108×10^{37} (1.98×10^{24}–2.25×10^{50})*	—
Adjusted risk for T21 or T18/T13	225.6 (51.0–998.7)*	44.2 (7.5–261.2)*	1.2×10^{57} (6.0×10^{23}–2.4×10^{90})*	1.0×10^{63} (1.5×10^{29}–6.7×10^{96})*
Hospital A	1	1	1	1
Hospital B	0.405 (0.250–0.654)*	0.307 (0.177–0.533)*	0.343 (0.270–0.435)*	0.371 (0.278–0.495)*

Significant predictors identified by logistic regression analysis: *$P < 0.0001$. Hospital A, patient informed of risk at time of visit; Hospital B, patient informed of risk after visit; NT, nuchal translucency; T, trisomy.

11–13 weeks' gestation accepted the offer of screening for fetal trisomies by the combined test and this was carried out successfully in all cases. In the high-risk group, 38% of women opted for invasive testing, 60% for cfDNA testing and 2% for no further tests. In the intermediate-risk group, 91.5% opted for cfDNA testing and 8.5% for no further tests. In the group undergoing cfDNA testing, results were provided for 98% of pregnancies.

The combined test had a potential detection rate of 87% for trisomy 21 and 93% for trisomies 18 or 13 at a FPR of 3.4%; the respective values for the cfDNA test were 98%, 82% and 0.25%. Contingent screening could have potentially identified most trisomic pregnancies at a very low invasive-testing rate if all women in the high-risk group who chose CVS or no further investigations would have chosen cfDNA testing. However, the theoretical performance of contingent screening for fetal trisomies is not synonymous with the rate of prenatal diagnosis and termination of affected pregnancies. In practice, many women identified by the combined test as being at high-risk chose invasive testing rather than the cfDNA test, some women in the screen-positive group did not want confirmatory diagnostic testing and many women with an affected fetus chose to continue with the pregnancy. In total, 32% of the pregnancies with fetal trisomy 21 resulted in live births. Consequently, health economic analyses which assume that, first, cfDNA testing in the high-risk group will replace the more expensive invasive tests and that such cost saving could be utilized for offering the cfDNA test to the intermediate-risk group and, second, improved prenatal detection of trisomic fetuses by the cfDNA test would result in a lower rate of affected live births and therefore in cost saving from postnatal care, may not be entirely valid[17–19].

In patients identified by the combined test as being at high risk for trisomies, the uptake of cfDNA testing was partly at the expense of invasive testing, but mainly as a new option in women who would have chosen previously to have no further investigations. We estimated that the introduction of cfDNA testing was associated with a 43% reduction in the rate of invasive testing. The choice between CVS and cfDNA testing was influenced by objective evidence derived from the patient-specific risk obtained from the combined test and the appearance of the fetus reflected in the measurement of NT, but also by parental attitudes in favor or against termination of a potentially affected pregnancy; termination was chosen by 93% of trisomy-21 cases in the CVS group, compared to 36% in those opting for the cfDNA test or no further investigations. An additional finding is that women of

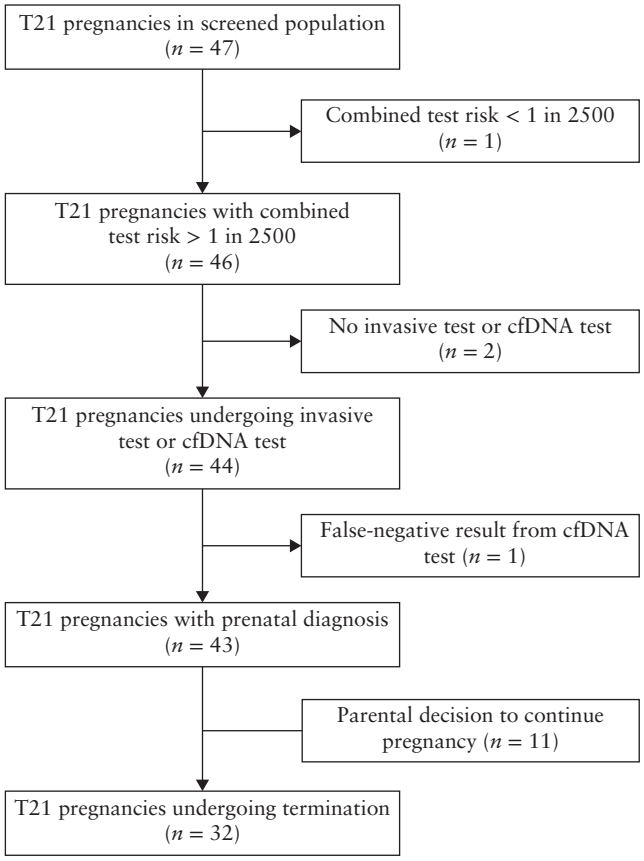

Figure 3 Flowchart summarizing prenatal diagnosis and management of pregnancies with fetal trisomy 21 (T21) in our study population of 11 692 women with singleton pregnancy.

Afro-Caribbean racial origin are more averse to invasive testing than Caucasian women, which presumably reflects cultural differences between the two groups in attitudes toward pregnancy termination and/or raising a child with a disability[20].

Limitations of the study

The study was not designed for comparison of the combined and cfDNA tests for performance of screening and the number of trisomic pregnancies was too small for valid conclusions to be drawn. The results on the uptake of various options of screening and management of affected pregnancies, depending on risk categories defined by the combined test, highlight some general principles concerning the factors that influence patient decisions. However, the exact rates of uptake of a specific option may not be generalizable to all populations from different racial and socioeconomic backgrounds in different countries and healthcare systems.

Comparison with findings from previous studies

Our study examined the implementation of contingent screening and the results of both the combined and cfDNA tests were used in clinical management. Three previous routine prenatal screening population studies compared the performance of traditional first- and/or second-trimester tests with that of the cfDNA test, but the results of the latter were not used in the clinical management of the pregnancies[4,21,22]. The first study examined stored plasma samples from singleton pregnancies that underwent combined screening at 11–13 weeks' gestation; in the 1949 cases with both cfDNA and combined test results, all 10 trisomic pregnancies were detected by both tests, at a FPR of 0.1% for the cfDNA test and 4.5% for the combined test[4]. The second study performed cfDNA testing in women undergoing a variety of traditional first- and/or second-trimester tests at 17 (range, 8–39) weeks; in the 1914 pregnancies with outcome data, both tests identified correctly all eight trisomic pregnancies, at a FPR of 0.5% for the cfDNA test and 4.2% for the traditional tests[21]. The third study performed cfDNA testing in women undergoing the first-trimester combined test at 10–14 weeks; in the 15 841 pregnancies with outcome data, the cfDNA test detected all 38 cases of trisomy 21, at a FPR of 0.06%, whereas the combined test detected 79% of affected pregnancies, at a FPR of 5.4%[22]. There are also two clinical implementation studies in which cfDNA testing was used for clinical management and the results were compared retrospectively to those of traditional testing[6,23]. The first study performed cfDNA testing and second-trimester triple serum screening at a median gestational age of 16 (range, 11–21) weeks; in the 1741 pregnancies with cfDNA results and outcome data, the test identified correctly all 11 trisomic pregnancies, at a FPR of 0.06%, whereas the triple test identified only six (54.5%) of the trisomies, at a FPR of 14.1%[23]. The second study performed cfDNA testing at 10–11 weeks' gestation and the combined test at 11–13 weeks; in the 2785 pregnancies with cfDNA results and outcome data, the test identified correctly all 32 cases with trisomy 21, 9/10 with trisomy 18 and 2/5 with trisomy 13, at a total FPR of 0.3%, whereas the combined test identified correctly all trisomic pregnancies at a FPR of 4.4%[6].

Our study has highlighted that, in the period prior to the introduction of cfDNA testing, the uptake of invasive testing in the high-risk group was only about 66% and the offer of cfDNA testing to high-risk pregnancies was associated with only a modest decrease in the rate of invasive testing and the majority of those who accept the new test are those who would have opted previously for no further investigations. Previous studies utilizing traditional methods of screening reported that the uptake of invasive testing in women identified as being at increased risk for trisomies varied between 46% and 78%[24–29]. Similarly, a previous study in high-risk pregnancies undergoing traditional screening reported that after the introduction of cfDNA testing the rate of patient uptake of invasive testing decreased by 17%, from 47% to 39%[30].

Regarding termination of pregnancy following prenatal detection of trisomy 21, this was chosen in 74% of our patients. This finding is compatible with that of a recent systematic review of termination rates in the USA (1995–2011); the weighted mean termination rate

was 67% (range, 61–93%) among population-based studies and 85% (range, 60–90%) among hospital-based studies[31].

Conclusions

In healthcare systems offering routine screening for trisomy 21 by the first-trimester combined test, incorporating the option of cfDNA testing for some patients is feasible. Such contingent screening could potentially lead to the prenatal detection of a higher proportion of affected pregnancies and a lower invasive-testing rate than in screening by the combined test alone. However, in clinical practice, prenatal detection of trisomies and pregnancy outcome depend not only on performance of screening tests but also on parental choice. Consequently, clinical implementation of cfDNA testing, contingent on the results of the combined test, may have only a modest impact in reducing the rate of invasive testing and a small effect on the rate of live births with trisomy 21.

DISCLOSURES

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