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Screening for trisomies by cfDNA testing of maternal blood in twin pregnancy: update of The Fetal Medicine Foundation results and meta-analysis

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KEYWORDS: cell-free DNA; first-trimester screening; meta-analysis; non-invasive prenatal testing; trisomy 21; trisomy 18; trisomy 13; twin pregnancy

ABSTRACT

Objectives *To report on the routine clinical implementation of cell-free DNA (cfDNA) analysis of maternal blood for trisomies 21, 18 and 13 in twin pregnancy and to define the performance of the test by combining our results with those identified in a systematic review of the literature.*

Methods *The data for the prospective study were derived from screening for trisomies 21, 18 and 13 in twin pregnancies at 10 + 0 to 14 + 1 weeks' gestation. Two populations were included; first, self-referred women to the Fetal Medicine Centre in London or Brugmann University Hospital in Brussels and, second, women selected for the cfDNA test after routine first-trimester combined testing at one of two National Health Service hospitals in England. This dataset was used to determine the performance of screening for the three trisomies. Search of MEDLINE, EMBASE, CENTRAL (The Cochrane Library), ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform (ICTRP) was carried out to identify all peer-reviewed publications on clinical validation or implementation of maternal cfDNA testing for trisomies 21, 18 and 13 in twin pregnancy. A meta-analysis was then performed using our data and those in the studies identified by the literature search.*

Results *In our dataset of 997 twin pregnancies with a cfDNA result and known outcome, the test classified correctly 16 (94.1%) of the 17 cases of trisomy 21, nine (90.0%) of the 10 cases of trisomy 18, one (50.0%) of the two cases of trisomy 13 and 962 (99.4%) of the 968 cases without any of the three trisomies. The literature search identified seven relevant studies, excluding our previous papers because their data are included in the current study. In the combined populations of our study and the seven studies identified by the literature search, there were 56 trisomy-21 and 3718 non-trisomy-21 twin pregnancies; the pooled weighted detection rate (DR) and false-positive rate (FPR) were 98.2% (95% CI, 83.2–99.8%) and 0.05% (95% CI, 0.01–0.26%), respectively. In the combined total of 18 cases of trisomy 18 and 3143 non-trisomy-18 pregnancies, the pooled weighted DR and FPR were 88.9% (95% CI, 64.8–97.2%) and 0.03% (95% CI, 0.00–0.33%), respectively. For trisomy 13, there were only three affected cases and two (66.7%) of these were detected by the cfDNA test at a FPR of 0.19% (5/2569).*

Conclusions *The performance of cfDNA testing for trisomy 21 in twin pregnancy is similar to that reported in singleton pregnancy and is superior to that of the first-trimester combined test or second-trimester biochemical testing. The number of cases of trisomies 18 and 13 is too small for accurate assessment of the predictive performance of the cfDNA test.* Copyright © 2019 ISUOG. *Published by John Wiley & Sons Ltd.*

INTRODUCTION

In singleton pregnancies, cell-free DNA (cfDNA) analysis of maternal blood provides effective screening

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for trisomies 21, 18 and 13. A recent meta-analysis of clinical validation studies reported that, in the combined total of 1963 cases of trisomy 21 and 223 932 non-trisomy-21 singleton pregnancies, the pooled weighted detection rate (DR) was 99.7% (95% CI, 99.1–99.9%) and the false-positive rate (FPR) was 0.04% (95% CI, 0.02–0.07%); in a total of 563 cases of trisomy 18 and 222 013 unaffected pregnancies, the pooled weighted DR and FPR were 97.9% (95% CI, 94.9–99.1%) and 0.04% (95% CI, 0.03–0.07%), respectively, and, in a total of 119 cases of trisomy 13 and 212 883 unaffected singleton pregnancies, the pooled weighted DR and FPR were 99.0% (95% CI, 65.8–100%) and 0.04% (95% CI, 0.02–0.07%), respectively[1]. In contrast to singleton pregnancies, data on cfDNA testing in twins are very limited; the meta-analysis reported that only five studies had examined twin pregnancies prospectively and, in a total of 24 cases of trisomy 21 and 1111 non-trisomy-21 cases, the DR was 100% and FPR was 0%[1].

In previous studies, we reported our data on cfDNA testing for trisomies in twins. In the first study, which included stored and prospectively collected samples, the cfDNA test classified correctly 11 of the 12 cases of trisomy 21, the one case of trisomy 18, the one case of trisomy 13 and all 241 non-trisomic pregnancies[2]. In the second study, we reported the results of prospective screening in twin pregnancies; the cfDNA test classified correctly 11 of the 12 cases of trisomy 21, all five cases of trisomy 18 and all 334 non-trisomic pregnancies[3]. In the third study, we reported performance of cfDNA testing in 417 prospectively examined pregnancies; the cfDNA test identified correctly all eight cases of trisomy 21, three of the four cases of trisomy 18, not the single case of trisomy 13 and 403 (99.8%) of the 404 non-trisomic pregnancies[4]. In the fourth study, we performed cfDNA testing in pregnancies identified by the combined test as being at intermediate or high risk for trisomy; the cfDNA test identified correctly all six cases of trisomy 21, two of the three cases of trisomy 18 and all 206 non-trisomic pregnancies[5].

The objectives of this study are, first, to report our updated experience on prospective first-trimester screening for trisomies 21, 18 and 13 in twins by cfDNA testing and, second, to carry out a meta-analysis of all studies on cfDNA testing in twin pregnancies published up to 9 March 2019.

METHODS

Update of The Fetal Medicine Foundation results

Study design and participants

The data for this study were derived from prospective screening for trisomies 21, 18 and 13 in twin pregnancies at 10 + 0 to 14 + 1 weeks' gestation. Two populations were included; first, self-referred women to the Fetal Medicine Centre in London, which is a private clinic[6], or the Brugmann University Hospital in Brussels, which is a public hospital, and, second, women selected for the cfDNA test after routine first-trimester combined testing in one of two National Health Service hospitals in England[5,7]. The patients were examined between October 2012 and January 2018. The study was approved by the appropriate ethics committees (NREC reference 13/LO/0885, NREC reference 19/HRA/0576, CE 2014/5).

We recorded maternal characteristics and medical history, including maternal age and racial origin (white, black, South Asian, East Asian or mixed), method of conception (natural or assisted conception requiring the use of ovulation drugs or by *in-vitro* fertilization), cigarette smoking during pregnancy (yes or no) and parity (parous or nulliparous if no previous pregnancy ≥ 24 weeks' gestation). An ultrasound scan was carried out to determine gestational age from the measurement of crown–rump length[8] of the larger fetus and chorionicity by examining the junction of the intertwin membrane with the placenta[9].

Women provided written informed consent and maternal blood (20 mL) was collected into either Cell-Free DNA BCT® tubes (Streck, Omaha, NE, USA) or Roche Cell-Free DNA Collection Tubes (Roche, Pleasanton, CA, USA). These were shipped via courier to Ariosa Diagnostics, Inc. (San Jose, CA, USA) where they were processed within 7 days after collection. Targeted cfDNA testing for fetal trisomy was performed using the Harmony™ prenatal test, as described previously[10–13]. Harmony uses Digital ANalysis of Selected Regions (DANSR) assays targeting sequences on chromosomes 13, 18 and 21 for chromosome quantitation and single-nucleotide polymorphisms on chromosomes 1–12 for fetal-fraction measurement. Products of the DANSR assays can be quantified using either next-generation sequencing or a custom microarray; both were used during the course of this study. The data were analyzed using the fetal fraction-optimized risk of trisomy evaluation (FORTE) algorithm, which calculates probability scores for fetal trisomy, with > 1% considered to be high probability. In cases in which the cfDNA test did not provide a result, the parents were offered repeat testing or to rely on the results of the combined test in deciding whether to have an invasive test. In cases with a high-risk result on the cfDNA test, the parents were advised to consider having invasive fetal karyotyping before deciding on the further management of their pregnancy.

Patient characteristics, results of the investigations and pregnancy outcome were recorded in a database. The outcomes were divided into, first, trisomy 21, 18 or 13 if the karyotype on analysis of chorionic villi, amniotic fluid or neonatal blood demonstrated the relevant trisomy in one or both fetuses, second, no trisomy 21, 18 or 13 if the karyotype was normal or both neonates were phenotypically normal, third, no known karyotype in both fetuses because the pregnancy resulted in termination, embryo reduction, miscarriage or stillbirth and no karyotyping of fetal tissue was carried out, and, fourth, outcome unknown because the pregnancy was lost to follow-up.

Statistical analysis

Descriptive data are presented as median (interquartile range (IQR)) for continuous variables and as *n* (%) for categorical variables.

Systematic review and meta-analysis

Literature search and study selection

Searches of MEDLINE via PubMed, EMBASE and CENTRAL (The Cochrane Library) were performed to identify clinical validation or implementation studies on maternal cfDNA testing in screening for aneuploidy in twin pregnancy; additionally, ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform (ICTRP) were searched for ongoing or recently completed trials. The study period was from January 2011, when the first such study was published, to 9 March 2019; the initial search was performed on 8 December 2018 and this was updated with autoalerts in MEDLINE. A list of relevant citations was generated from these databases using the search strategies detailed in Appendix S1. This review was registered in PROSPERO international database for systematic reviews (reference: CRD42019121506).

The abstracts of citations were examined by two reviewers (M.M.G., S.G.) to identify all potentially relevant articles, which were then examined in full-text form. Reference lists of relevant original and review articles were searched manually for additional reports. Agreement on potential relevance was reached by consensus and by consultation with a third reviewer (K.H.N.).

The inclusion criteria were peer-reviewed study reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidy in twin pregnancy, in which data on pregnancy outcome were provided for at least 85% of the study population. Proof-of-principle articles and studies in which the laboratory scientists carrying out the tests were aware of fetal karyotype or pregnancy outcome were excluded. We also excluded case–control studies because they tend to introduce an optimistic bias to the estimates of diagnostic performance.

Data extraction and meta-analysis

Data regarding sample size, gestational age at analysis, method used for cfDNA testing and DR or sensitivity and FPR or specificity for non-mosaic trisomies 21, 18 and 13 were obtained from each study included in the systematic review and documented in contingency tables. In the construction of these tables, we used the results from the cfDNA test and excluded cases of known mosaicism and those in which the test failed to give a result. In the calculation of FPR, we included all euploid cases and cases with aneuploidy other than the one under investigation. Authors were contacted when clarification was required in the interpretation of their data.

We extracted data from the primary studies to obtain the four cell values of a diagnostic 2×2 table to calculate test accuracy measures of DR and FPR. The analyses were stratified according to type of aneuploidy (trisomy 21 or trisomy 18). We calculated DR and FPR with corresponding 95% CI for individual studies and displayed them in forest plots to investigate heterogeneity. We pooled the DR and FPR estimates using bivariate random-effects regression models. The bivariate model assumes that logit transformations of DR and FPR are correlated negatively and follow a bivariate normal distribution[14]. We computed the positive and negative likelihood ratios from the pooled estimates of DR and FPR. Heterogeneity among studies was quantified with the variance of the logit of accuracy indices as estimated by the bivariate model.

Publication bias was not analyzed given the limited power of available tests and the uncertainty about interpreting statistical evidence of funnel plot asymmetry as necessarily implying publication bias[15]. For trisomy 13, there was an insufficient number of cases for meaningful meta-analysis and we therefore computed average DR and FPR values.

We conducted statistical analyses using the metandi and midas commands in Stata software[16].

RESULTS

Update of The Fetal Medicine Foundation results

Study population

A total of 1122 twin pregnancies had cfDNA testing, but 125 (11.1%) of these were excluded from further analysis either because the cfDNA test did not provide a result ($n = 52$), the pregnancy ended in termination, miscarriage or stillbirth with no known karyotype ($n = 45$) or there was loss to follow-up ($n = 28$).

Of the 997 cases included in the study, 854 (85.7%) were dichorionic and 143 (14.3%) were monochorionic; the median maternal age was 38.0 (IQR, 34.5–41.0) years, the median maternal weight was 69.0 (IQR, 60.4–82.6) kg and the median gestational age at sampling was 12.1 (IQR, 10.7–12.9) weeks. Maternal racial origin was white in 772 (77.4%) pregnancies, South Asian in 65 (6.5%), East Asian in 32 (3.2%), black in 104 (10.4%) and mixed in 24 (2.4%). Conception was natural in 766 (76.8%) pregnancies and after use of assisted reproductive techniques in 231 (23.2%).

The study population of 997 pregnancies included 17 with trisomy 21, 10 with trisomy 18, two with trisomy 13 and 968 without trisomy 21, 18 or 13; one case of trisomy 18 was a monochorionic twin pregnancy in which both fetuses were affected, and all the other trisomic cases were a dichorionic pregnancy in which only one fetus was trisomic and the cotwin was non-trisomic.

Performance of screening

The cfDNA test classified correctly 16 (94.1%) of the 17 cases of trisomy 21, nine (90.0%) of the 10

cases of trisomy 18, one (50.0%) of the two cases of trisomy 13 and 962 (99.4%) of the 968 cases without any of the three trisomies. One case each of trisomy 21, trisomy 18 and trisomy 13 was classified as normal. In the non-trisomic group, four cases were classified as trisomy 13, one as trisomy 18 and one as trisomy 21 and, therefore, the combined FPR was 0.62% (6/968).

Systematic review and meta-analysis

Data sources

The search identified 329 potentially relevant citations, but 320 were excluded because they were not relevant, a conference abstract rather than a peer-reviewed paper, a review article or opinion, a study not on twins, a case–control study, a study on clinical implementation of cfDNA testing in screening for aneuploidy in which pregnancy outcome data were provided for < 85% of the study population or a proof-of-principle study reporting laboratory techniques rather than clinical validation of a predefined method of maternal blood cfDNA analysis (Figure 1). In total, nine relevant studies were identified[4,5,17–23] but two of these[4,5] were excluded from the meta-analysis because their data are included in the updated Fetal Medicine Foundation results presented above. The characteristics of the current study and the seven identified by the literature search are summarized in Table 1.

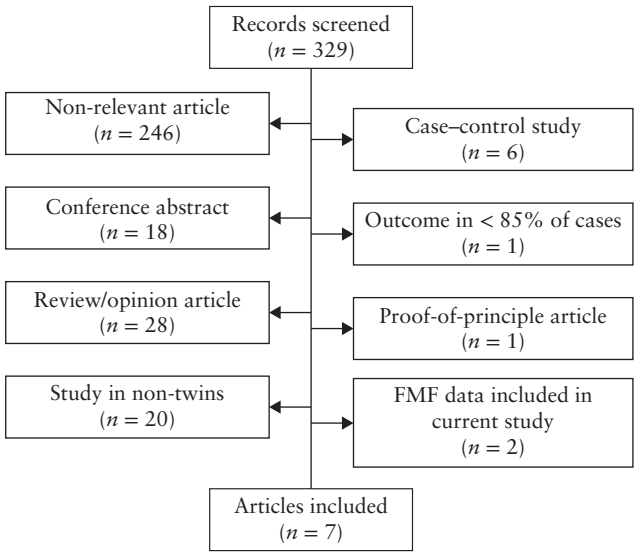

Figure 1 Flowchart summarizing selection from literature of studies for inclusion in systematic review. FMF, Fetal Medicine Foundation.

Methodological quality of selected studies

The methodological quality of the selected studies, assessed by the quality assessment tool for diagnostic accuracy studies (QUADAS-2)[24], is illustrated in Figure 2. This tool comprises four domains; each one is assessed in terms of risk of bias and the first three are also assessed in terms of concerns regarding applicability.

Risk of bias. The first domain relates to patient selection. A study was considered to be at low risk of bias if the cfDNA test was carried out in a consecutive or random sample of patients; all the studies were classified as being at high risk of bias because the samples were not explicitly stated to have been either consecutive or selected at random. The second domain relates to the index test; all included studies were considered to be at low risk of bias because the cfDNA test was carried out and the results given by the laboratory without prior knowledge of the fetal karyotype or pregnancy outcome. The third domain relates to the reference standard; all included studies were considered to be at low risk of bias because the method of diagnosing the chromosomal abnormality under investigation, including karyotyping or neonatal examination, was accepted to be true. The fourth domain relates to flow and timing. A study was considered to be at low risk of bias if, first, in the calculation of performance of screening, all patients in the study population had both a result from the cfDNA test and pregnancy outcome and, second, if the method of classifying the outcome result (invasive testing or clinical examination) was the same in all cases in the study population. All but one study[18] were classified as being at high risk of bias because cfDNA testing was not carried out or did not provide results in

Table 1 Summary of characteristics of studies reporting on cell-free DNA (cfDNA) analysis of maternal blood in screening for trisomies (T) 21, 18 and 13 in twin pregnancy

Study	*Aneuploidy studied*	n	*Monochorionic* (n (%))	*T21* (n)	*T18* (n)	*T13* (n)	*Outcome known (%)*	*cfDNA method*	*GA (weeks)*	*Population*
Lau (2013)[17]	T21	12	2 (16.7)	1	—	—	100	MPSS	13 (11–20)	High risk
Huang (2014)[18]	T21,T18	189	33 (17.5)	9	2	—	100	MPSS	19 (11–36)	High risk
Tan (2016)[19]	T21	510	— (3.2)*	4	—	—	90	MPSS	12 (11–28)	Mixture
Du (2017)[20]	T21	92	39 (42.4)	2	—	—	100	MPSS	18 (14–23)	High risk
Le Conte (2018)[21]	T21,T18,T13	418	86 (20.6)	3	1	0	85	MPSS	16 (10–35)	Mixture
Yang (2018)[22]	T21,T18	432	95 (22.0)	4	1	—	91	MPSS	> 9	Mixture
Yu (2019)[23]	T21,T18,T13	1157	308 (26.6)	16	4	1	99	MPSS	18 (8–30)	Mixture
Current study	T21,T18,T13	997	143 (14.3)	17	10	2	94	Targeted	11 (10–14)	Mixture

Only first author is given for each study. Numbers reported are those after exclusion of cases without cfDNA test result or pregnancy outcome. Gestational age (GA) is given as mean (range) or actual value. *Value in original sample before exclusion for failed results and no follow-up. MPSS, massively parallel shotgun sequencing.

all cases and/or there was no complete follow-up and/or the method of determining outcome was not the same in all cases.

Concerns regarding applicability. In the context of screening for fetal aneuploidy by cfDNA analysis of maternal blood in the general population, the first domain relates to patient selection and all the studies were classified as being at high risk of concerns regarding applicability because, in these studies, the test was not carried out in the general population but in a mixture of low- and high-risk pregnancies, in which some of the patients had had another screening test before opting for cfDNA testing. In terms of the second and third domains (index test and reference standard, respectively), all studies were considered as being at low risk of concerns regarding applicability.

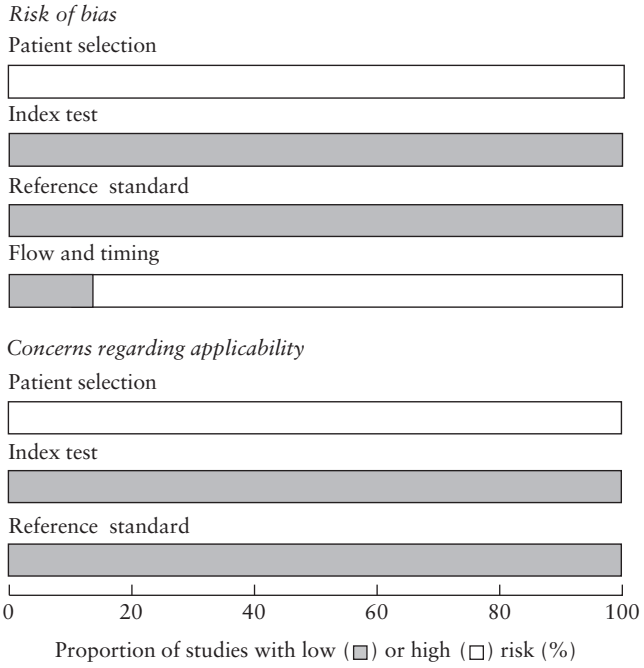

Figure 2 Summary of quality of included studies reporting on cell-free DNA testing for trisomies 21, 18 and 13, assessed using Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) checklist.

Method of analyzing samples

The studies included in the meta-analysis used one of two methods for analysis of cfDNA in maternal blood: massively parallel shotgun sequencing or targeted analysis (either by next-generation sequencing or by a custom microarray) (Table 1).

Nature of studies

All studies included in this meta-analysis were prospective; three studies were in high-risk pregnancies and five examined a mixture of high-risk and routine populations (Table 1). The proportion of monochorionic twin pregnancies ranged from 3.2% to 42.4%.

Meta-analysis and performance of screening for aneuploidy

The DR and FPR for each study, pooled weighted data and heterogeneity between studies (variance of the logit sensitivity and specificity) are provided in Tables 2 and 3; sensitivity and specificity are illustrated in Figures 3 and 4. Heterogeneity between studies was very low.

Trisomy 21. A total of eight studies reported on the performance of screening by cfDNA analysis for trisomy 21 in a combined total of 56 cases of trisomy 21 and 3718 non-trisomy-21 twin pregnancies (Table 2 and Figure 3). Among individual studies, the DR varied between 94.1% and 100% and the FPR varied between 0% and 0.24%. The pooled weighted DR and FPR were 98.2% (95% CI, 83.2–99.8%) and 0.05% (95% CI, 0.01–0.26%), respectively.

Trisomy 18. A total of five studies reported on the performance of screening by cfDNA analysis for trisomy 18 in a combined total of 18 cases of trisomy 18 and 3143 non-trisomy-18 twin pregnancies (Table 3 and Figure 4). Among individual studies, the DR varied between 50.0%

Table 2 Studies reporting on application of cell-free DNA analysis of maternal blood in screening for trisomy 21 in twin pregnancy

	Trisomy 21		*Non-trisomy 21*	
Study	*Total* (n)	*Detection rate* (n (%, 95% CI))	*Total* (n)	*False-positive rate* (n (%, 95% CI))
Lau (2013)[17]	1	1 (100, 2.50–100)	11	0 (0, 0–28.49)
Huang (2014)[18]	9	9 (100, 66.4–100)	180	0 (0, 0–2.03)
Tan (2016)[19]	4	4 (100, 39.8–100)	506	0 (0, 0–0.73)
Du (2017)[20]	2	2 (100, 15.8–100)	89	0 (0, 0–4.06)
Le Conte (2018)[21]	3	3 (100, 29.2–100)	415	1 (0.24, 0.01–1.34)
Yang (2018)[22]	4	4 (100, 39.8–100)	396	0 (0, 0–0.93)
Yu (2019)[23]	16	16 (100, 79.4–100)	1141	0 (0, 0–0.32)
Current study	17	16 (94.1, 71.3–100)	980	1 (0.10, 0–0.57)
Pooled analysis (% (95% CI))*		98.2 (83.2–99.8)		0.05 (0.01–0.26)
Heterogeneity assessment		0.020		0.011
Positive likelihood ratio (95% CI)		1837 (369–9149)		
Negative likelihood ratio (95% CI)		0.018 (0.002–0.190)		

Only first author is given for each study. Cases with mosaicism were excluded from calculations. *Bivariate random-effects model.

Table 3 Studies reporting on application of cell-free DNA analysis of maternal blood in screening for trisomy 18 in twin pregnancy

	Trisomy 18		*Non-trisomy 18*	
Study	*Total* (n)	*Detection rate* (n (%, 95% CI))	*Total* (n)	*False-positive rate* (n (%, 95% CI))
Huang (2014)[18]	2	1 (50.0, 1.3–98.7)	187	0 (0, 0–1.95)
Le Conte (2018)[21]	1	1 (100, 2.5–100)	417	0 (0, 0–0.88)
Yang (2018)[22]	1	1 (100, 2.5–100)	399	0 (0, 0–0.92)
Yu (2019)[23]	4	4 (100, 39.8–100)	1153	0 (0, 0–0.32)
Current study	10	9 (90.0, 55.5–99.8)	987	1 (0.10, 0–0.56)
Pooled analysis (% (95% CI))*		88.9 (64.8–97.2)		0.03 (0.00–0.33)
Heterogeneity assessment		0		0
Positive likelihood ratio (95% CI)		2774 (388–19 823)		
Negative likelihood ratio (95% CI)		0.111 (0.030–0.411)		

Only first author is given for each study. Cases with mosaicism were excluded from calculations. *Bivariate random-effects model.

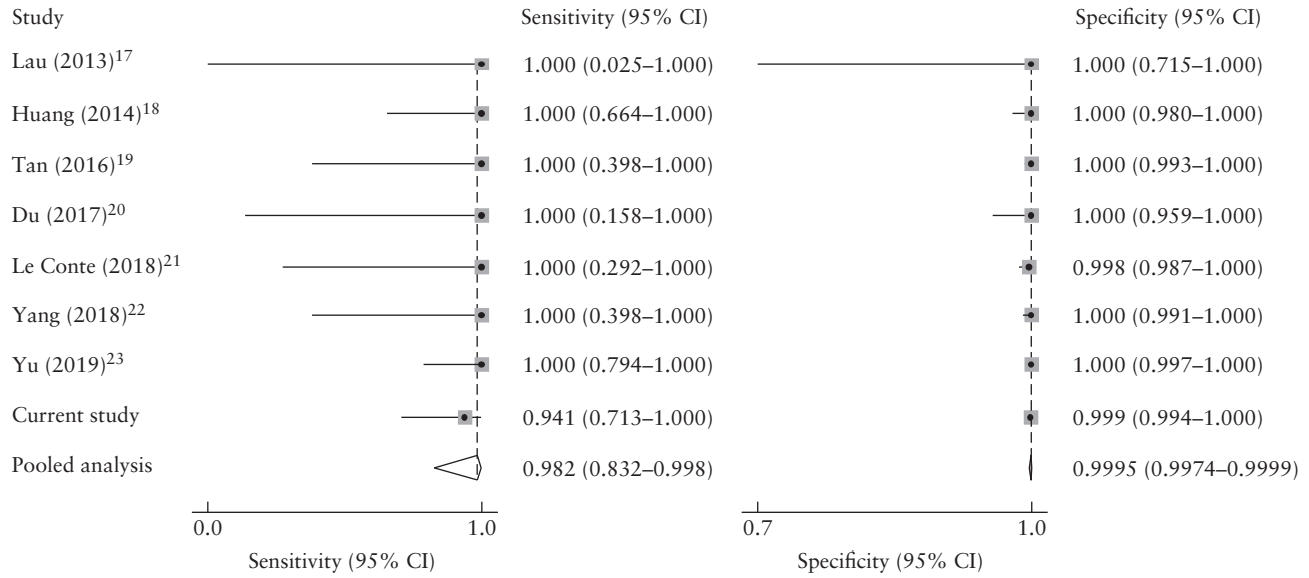

Figure 3 Forest plots of sensitivity and specificity with 95% CI and pooled weighted summary statistics using bivariate random-effects model in assessing performance of cell-free DNA analysis in screening for trisomy 21 in twin pregnancy. Only first author is given for each study.

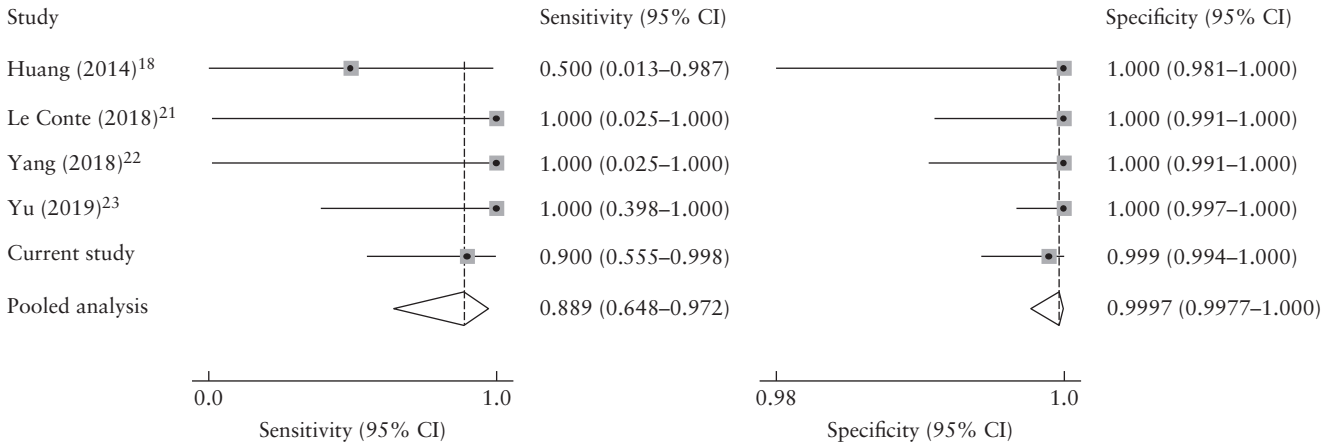

Figure 4 Forest plots of sensitivity and specificity with 95% CI and pooled weighted summary statistics using bivariate random-effects model in assessing performance of cell-free DNA analysis in screening for trisomy 18 in twin pregnancy. Only first author is given for each study.

and 100% and the FPR varied between 0% and 0.10%. The pooled weighted DR and FPR were 88.9% (95% CI, 64.8–97.2%) and 0.03% (95% CI, 0.00–0.33%), respectively.

Trisomy 13. A total of three studies reported on the performance of screening by cfDNA analysis for trisomy 13 in a combined total of three cases of trisomy 13 and 2569 non-trisomy-13 twin pregnancies. In our study, one of the two affected cases was detected by cfDNA testing at a FPR of 0.4% (4/995). In the second study[23], the one affected case was detected at a FPR of 0% (0/1156). In the third study[21], there were no cases of trisomy 13 but one false-positive result. In the combined results, the DR was 66.7% (2/3) and FPR was 0.19 (5/2569).

DISCUSSION

Principal findings

The results of our study and the meta-analysis of cfDNA testing of maternal blood in twin pregnancies suggest that the performance of the test for trisomy 21 may be similar to that in singleton pregnancies. In the combined total of 56 trisomy-21 and 3718 non-trisomy-21 twin pregnancies, the pooled weighted DR and FPR were 98.2% (95% CI, 83.2–99.8%) and 0.05% (95% CI 0.01–0.26%), respectively; in our meta-analysis of studies in singleton pregnancies, the pooled weighted DR in 1963 cases of trisomy 21 was 99.7% (95% CI, 99.1–99.9%) and the FPR in 223 932 non-trisomy-21 pregnancies was 0.04% (95% CI, 0.02–0.07%)[1]. In the combined total of 18 cases of trisomy 18 and 3143 non-trisomy-18 pregnancies, the pooled weighted DR and FPR were 88.9% (95% CI, 64.8–97.2%) and 0.03% (95% CI, 0.00–0.33%), respectively; in our meta-analysis of studies in singleton pregnancies, the pooled weighted DR in 563 cases of trisomy 18 was 97.9% (95% CI, 94.9–99.1%) and the FPR in 222 013 non-trisomy-18 pregnancies was 0.04% (95% CI, 0.03–0.07%)[1]. The number of twin pregnancies with trisomy 13 ($n = 3$) was too small for accurate assessment of DR. The average FPR for trisomy 13 of 0.19% (5/2569) seems slightly higher than the values reported in singleton pregnancy (0.04%; 95% CI, 0.02–0.07%)[1].

In our study, the method of cfDNA testing was targeted but, in all other studies, massively parallel shotgun sequencing was used. Similarly, our study was confined to pregnancies in the first trimester, whereas the other studies included pregnancies in the second, and some in the third, trimesters. Although there was no obvious difference in performance of screening between our study and the other studies, the small number of cases prevented meaningful subgroup analyses, including chorionicity, cfDNA method for analysis, background risk or gestational age at testing.

This study has not addressed the issue of cfDNA test failure because we have reported recently our experience in a larger cohort including both singleton and twin pregnancies[25]. In that study, we found that important contributors to cfDNA test failure are increased maternal weight, conception by *in-vitro* fertilization, black or South Asian racial origin, dichorionicity, nulliparity, low gestational age and low serum pregnancy-associated plasma protein-A and free beta human chorionic gonadotropin. Test failure after first sampling in dichorionic twins was 3.3-times higher than in singletons, but to a great extent this excess failure rate could be attributed to the fact that a considerably higher proportion of twins were conceived by *in-vitro* fertilization and more women were nulliparous.

Comparison with previous meta-analyses in twin pregnancies

Our previous meta-analysis on the performance of cfDNA testing for fetal aneuploidy in clinical validation or implementation studies included five studies in twin pregnancies; in a total of 24 cases of trisomy 21 and 1111 unaffected cases, the DR was 100% and FPR was 0%[1]. Another meta-analysis examined four studies that included both singletons and twins and three studies that included only twins; it was not possible to extract the number of twin pregnancies that were evaluated but the authors reported that the DR of trisomy 21 in twins was 89.4% (95% CI, 75–96%) and the FPR was 0.4%[26].

Liao *et al.* conducted a meta-analysis of studies reporting on cfDNA testing in twin pregnancy[27]. They included 10 studies, of which one was retrospective, three were a mixture of retrospective and prospective and six were prospective; five were cohort studies, two were case–control studies and three were a mixture of cohort and case–control studies. The authors did not set any criteria on the degree of follow-up. In a combined total of 69 cases of trisomy 21, the DR was 99% (95% CI, 92–100%), in 13 cases of trisomy 18 the DR was 85% (95% CI, 55–98%), in three cases of trisomy 13 the DR was 100% and in 2008 euploid pregnancies the FPR was 0.05%. Our meta-analysis included only prospective cohort studies with follow-up in at least 85% of cases to avoid reporting bias. Case–control studies were excluded because they tend to introduce an optimistic bias to the estimates of diagnostic performance.

Implications for clinical practice

This meta-analysis provides good evidence that the performance of cfDNA testing for trisomy 21 in twin pregnancies may be similar to that in singletons. In this respect, the performance of the cfDNA test is superior, both in terms of higher DR and substantially lower FPR, to that of the first-trimester combined test or second-trimester biochemical testing[28]. This is particularly important in the case of dichorionic twins in which both the incidence of aneuploidy and the invasive procedure-related risk of pregnancy loss are increased compared to in singletons. If the pregnancies are discordant for aneuploidy and the parents choose selective feticide, the subsequent risk of miscarriage or early preterm birth increases with gestational age at feticide[29]; in this respect it would be

preferable to offer screening leading to prenatal diagnosis in the first than in the second trimester.

A positive or high-risk cfDNA result should be confirmed by invasive testing. In the case of high risk for trisomy 21 on first-trimester combined screening and positive cfDNA result for trisomy 21, the diagnostic test can be chorionic villus sampling. In the case of trisomy 18 or 13, a positive result should be followed by a detailed ultrasound examination and, if the characteristic defects associated with the trisomy are detected, chorionic villus sampling can be carried out; if no defects are detected on the scan, the preferred diagnostic test is amniocentesis to avoid an erroneous result due to placenta-confined mosaicism.

On the other hand, a negative or low-risk cfDNA result reassures that the fetus is unlikely to be affected by the trisomy under investigation. The posterior risk for a given patient can be obtained by multiplying the prior risk by the negative likelihood ratios calculated in this meta-analysis; the risk for trisomies 21 and 18 is reduced by a factor of 56 and 9, respectively. For example, if prior screening by the combined test had shown that the risk for trisomy 21 was 1 in 10 and cfDNA testing gives a low-risk result, the chance that the fetus is affected is 1 in 560; in contrast, if the risk for trisomy 18 from the combined test was one in two and cfDNA testing gives a low-risk result, the chance that the fetus is affected is one in 18.

Limitations

Contrary to our previous meta-analysis in singleton pregnancies, the number of published studies analyzing the performance of cfDNA testing in twin pregnancies is limited and, consequently, the number of affected cases included in this meta-analysis is considerably smaller. However, the results reported in the literature for trisomies 21 and 18 present low heterogeneity and are therefore likely to represent the true performance. There was an insufficient number of trisomy-13 cases to assess accurately performance.

On assessment of the quality of the included studies, all were considered to be at high risk of selection bias and at high risk of concerns regarding applicability in relation to patient selection because they were not performed as part of routine primary screening but were carried out in preselected populations. However, the ability to detect aneuploidy with cfDNA analysis is dependent upon assay precision and fetal DNA percentage in the sample rather than the prevalence of the disease in the study population. Most studies were also classified as being at high risk of bias in relation to flow and timing. This is essentially because cfDNA testing did not provide results in all cases, follow-up was incomplete or the method of determining outcome was not the same in all cases. However, such criticisms could be applied to any clinical study; all methods of traditional screening occasionally fail to give a result and no screening study in pregnancy can have complete follow-up, especially because some women miscarry and karyotyping is not carried out.

Conclusions

Performance of cfDNA testing for trisomy 21 in twin pregnancies is similar to that reported in singleton pregnancies and is superior to that of the first-trimester combined test or second-trimester biochemical testing. The number of cases of trisomies 18 and 13 was too small for accurate assessment of predictive performance of the cfDNA test.

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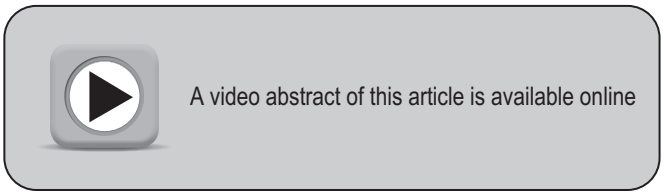
SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Appendix S1 Search strategies



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Detección de trisomías mediante la prueba del ADN fetal de la sangre materna en el embarazo de gemelos: actualización de los resultados de *The Fetal Medicine Foundation* y metaanálisis

RESUMEN

Objetivos Informar sobre la implementación clínica rutinaria del análisis de ADN fetal (cfDNA, por sus siglas en inglés) de la sangre materna para las trisomías 21, 18 y 13 en embarazos de gemelos y definir el rendimiento de la prueba mediante la combinación de los resultados de este estudio con los identificados en una revisión sistemática de la literatura.

Métodos Los datos para el estudio prospectivo se derivaron del cribado de las trisomías 21, 18 y 13 en embarazos de gemelos entre 10+0 a 14+1 semanas de gestación. Se incluyeron dos poblaciones: la primera, las mujeres que acudieron sin recomendación de especialista al Centro de Medicina Fetal de Londres o al Hospital Universitario Brugmann de Bruselas y, la segunda, las mujeres seleccionadas para la prueba de cfDNA después de la prueba combinada rutinaria del primer trimestre en uno de los dos hospitales del Servicio Nacional de Salud de Inglaterra. Este conjunto de datos se utilizó para determinar el rendimiento de la detección de las tres trisomías. Se realizó una búsqueda en MEDLINE, EMBASE, CENTRAL (The Cochrane Library), ClinicalTrials.gov y en la Plataforma Internacional del Registro de Ensayos Clínicos (ICTRP, por sus siglas en inglés) de la Organización Mundial de la Salud para identificar todas las publicaciones revisadas por pares sobre la validación clínica o la implementación de pruebas de cfDNA materno para las trisomías 21, 18 y 13 en el embarazo de gemelos. A continuación, se realizó un metaanálisis de los datos propios y de los de los estudios identificados por la búsqueda bibliográfica.

Resultados En el conjunto de datos propios de 997 embarazos de gemelos con un resultado de cfDNA conocido, la prueba clasificó correctamente 16 (94,1%) de los 17 casos de trisomía 21, nueve (90,0%) de los 10 casos de trisomía 18, uno (50,0%) de los dos casos de trisomía 13 y 962 (99,4%) de los 968 casos sin ninguna de las tres trisomías. La búsqueda bibliográfica identificó siete estudios relevantes, que excluyeron los artículos de los autores de este estudio ya que sus datos están incluidos en este estudio. En las poblaciones combinadas de este estudio y los siete estudios identificados por la búsqueda bibliográfica, hubo 56 embarazos gemelares con trisomía-21 y 3718 sin trisomía-21; la tasa de detección (TD) combinada ponderada y la tasa de falsos positivos (TFP) fueron del 98,2% (IC 95%: 83,2–99,8%) y del 0,05% (IC 95%: 0,01–0,26%), respectivamente. En el total combinado de los 18 casos de trisomía-18 y los 3143 embarazos sin trisomía-18, la TD combinada ponderada y la TFP fueron del 88,9% (IC 95%, 64,8–97,2%) y del 0,03% (IC 95%, 0,00–0,33%), respectivamente. Para la trisomía 13, sólo hubo tres casos afectados y dos (66,7%) de ellos fueron detectados por la prueba de cfDNA con una TFP del 0,19% (5/2569).

Conclusiones La bondad del desempeño de la prueba de cfDNA para la trisomía 21 en el embarazo de gemelos es similar a la reportada en embarazos con feto único y es superior a la de la prueba combinada del primer trimestre o a la de la prueba bioquímica del segundo trimestre. El número de casos de trisomías 18 y 13 es demasiado pequeño para una evaluación precisa de la bondad de predicción de la prueba de cfDNA.

双胞胎妊娠母体血液 cfDNA 检测筛查三体性：胎儿医学基金会（Fetal Medicine Foundation）最新研究成果与元分析

摘要

目标：报告双胞胎妊娠母体血液无细胞 DNA（cfDNA）分析的常规临床实施情况，以及综合考虑我们的检测结果与文献系统检索中发现的结果，进而确定测试性能。

方法：前瞻性研究的数据来自 10+0 至 14+1 周孕龄双胞胎孕妇的 21、18 和 13 三体性筛查。研究人群分为两组：第一组是向位于伦敦的胎儿医学中心或位于布鲁塞尔的布鲁格曼大学附属医院自荐参加研究的孕妇，第二组是在英格兰的两家 NHS 医院中的一家接受常规孕早期综合检测后遴选出来参加 cfDNA 测试的孕妇。根据这些数据确定三阶段三体性筛查的性能。研究中检索了 MEDLINE、EMBASE、CENTRAL（实证医学资料库）、ClinicalTrials.gov 及世界卫生组织国际临床试验注册平台（ICTRP），以甄选针对双胞胎妊娠母体 cfDNA 检测 21、18 和 13 三体性筛查临床验证或实施的全部经同行评审的出版物。随后根据我们的检测结果，以及文献检索所得的研究数据进行了元分析。

结果：根据 997 个双胞胎妊娠母体 cfDNA 检测数据及已知结果，对 17 个三体 21 案例中 16(94.1%)个、10 个三体 18 案例中 9(90.0%)个、2 个三体 13 案例中 1(50.0%)个、968 个无上述任何三体性的案例中 962(99.4%)个的检测结果进行了正确分类。文献检索中发现 7 个相关的研究项目，不包括我们之前发表的论文（因为当前的研究项目包括了这些数据）。在我们的研究项目的几组人群及文献检索中发现的 7 个研究项目中，有 56 个三体 21 与 3718 个非三体 21 双胞胎妊娠孕妇，合并加权检测率（DR）与假阳性率（FPR）分别为 98.2%(95% CI, 83.2-99.8%)和 0.05%(95% CI, 0.01-0.26%)。在合计总共 18 个三体 18 与 3143 个非三体 18 妊娠案例中，合并加权 DR 与 FPR 分别为 88.9%(95% CI, 64.8-97.2%)和 0.03%(95% CI, 0.00-0.33%)。至于三体 13，只有 3 个受影响案例，其中 2 个(66.7%)是通过 cfDNA 检测发现的，FPR 为 0.19%(5/2569)。

结论：双胞胎妊娠孕妇三体 21 cfDNA 检测的性能与单生儿妊娠孕妇类似，优于孕早期综合测试或孕中期生化检测。三体 18 与三体 13 的案例数太少，无法准确评估 cfDNA 检测的预测性能。© ISUOG 2019 版权所有。John Wiley & Sons Ltd.出版