ISUOG VIRTUAL INTERNATIONAL SYMPOSIUM 2021

State-of-the-art Ultrasound Imaging in Obstetrics and Gynecology

17-18 April 2021

REGISTER NOW ▸

Using ultrasound together with other technologies to improve the lifelong health of women and babies

- Two streams of scientific content over two days, delivered through the ISUOG virtual platform which will exceed your expectations
- A mixture of lectures and practical, interactive training, including scan demonstrations, pattern recognition sessions and case report discussion
- Leading international and local experts in obstetrics, gynecology and imaging
- Live program delivered from 7:30 - 18:30 Calgary, Canada time (Mountain Daylight Time)
- Content available on Demand, at a time, pace and location to suit you until 17 May 2021
- All non-member registration fees include a 12-month ISUOG basic membership

Provisional program

Sessions will run simultaneously, providing two streams of content both days.

Highlights include:

- Obstetrics: the first trimester, beyond the routine mid-trimester fetal ultrasound scan, screening to improve pregnancy outcomes, fetal growth and health, ultrasound in labor, and more
- Gynecology: ectopic pregnancy, miscarriage, endometriosis, menopause, ovarian tumors, tubal and uterine pathology, and more
- Special sessions include advanced imaging/MRI, fetal therapy and COVID

The symposium will be co-chaired by:

Jo-Ann Johnson (Canada),
Denise Pugash (Canada)

Symposium Advisory Group

Shabnam Bobdiwala (UK)
George Condous (Australia)
Karen Fung-Kee-Fung (Canada)
Jon Hyett (Australia)
Simon Meagher (Australia)
Liona Poon (Hong Kong)
Angela Ranzini (USA)
Magdalena Sanz Cortes (USA)

Who should attend?

This interactive course is designed for Maternal Fetal Medicine (MFMs), OB-GYNs, Radiologists, Sonographers, Geneticists, Researchers, Trainees/ Residents and other maternity care providers. The program will appeal to a wide global audience, with a focus on North American educational needs.

See you ONLINE in 2021! For more information, please visit:
isuog.org/event/17th-isuog-international-symposium.html

isuog.org

Ultrasound Obstet Gynecol 2019; **53**: 208–213
Published online 7 December 2018 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.20160

Routine first-trimester screening for fetal trisomies in twin pregnancy: cell-free DNA test contingent on results from combined test

S. GALEVA[1,2], L. KONSTANTINIDOU[1], M. M. GIL[1,3], R. AKOLEKAR[2] and K. H. NICOLAIDES[1]

[1]*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK;* [2]*Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, UK;* [3]*Universidad Francisco de Vitoria, UFV, Pozuelo de Alarcón, Madrid, Spain*

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

ABSTRACT

Objective *To report on the routine clinical implementation of cell-free DNA (cfDNA) analysis of maternal blood for trisomies 21, 18 and 13, contingent on the results of the first-trimester combined test in twin pregnancy.*

Methods *Screening for trisomies 21, 18 and 13 was carried out in 959 twin pregnancies by assessment of a combination of maternal age, fetal nuchal translucency thickness, and serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A at 11–13 weeks' gestation in two UK NHS hospitals. Women in the high-risk group (risk ≥ 1 in 100) were offered the option of invasive testing, cfDNA testing or no further testing, and those in the intermediate-risk group (risk 1 in 101 to 1 in 2500 in the first phase of the study and 1 in 101 to 1 in 500 in the second phase) were offered cfDNA or no further testing. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or examination of the neonates.*

Results *In 42 (4.4%) of the 959 pregnancies, there was termination, miscarriage or stillbirth with no known karyotype or there was loss to follow-up. The 917 pregnancies with known trisomic status of both twins included six that were discordant for trisomy 21, four that were discordant for trisomy 18 and 907 with no trisomy 21, 18 or 13. Following combined screening, 47 (5.1%), 203 (22.1%) and 667 (72.7%) of the pregnancies were classified as high risk, intermediate risk and low risk, respectively. The high-risk group included five (83.3%) cases of trisomy 21 and three (75.0%) of trisomy 18. The cfDNA test was carried out in 224 pregnancies and results were provided in 214 (95.5%); this group included six pregnancies with trisomy 21, three with trisomy 18 and 206 with no trisomy 21, 18 or 13. The cfDNA test classified correctly as screen positive all six cases of trisomy 21 and two of the three with trisomy 18, and as screen negative for each of the trisomies all 206 unaffected pregnancies. Contingent screening led to prenatal detection of all cases of trisomy 21 and three of four with trisomy 18.*

Conclusion *This study has demonstrated the feasibility of introducing cfDNA testing, contingent on the results of the first-trimester combined test for major trisomies, in a routine population of twin pregnancies.* Copyright © *2018 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

In singleton pregnancy, screening for the major trisomies using a combination of fetal nuchal translucency (NT) thickness, serum β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) can detect about 90% of cases of trisomy 21, 18 or 13, at a false-positive rate (FPR) of 5%[1,2]. In twin pregnancy, use of the combined test can achieve a similarly high detection rate (DR) for trisomy 21 as in singletons, but with a higher FPR of about 6%[3]. A more effective method of screening for trisomy 21 is provided by analysis of cell-free DNA (cfDNA) in maternal blood; a recent meta-analysis of clinical validation studies reported that, in the combined total of 1963 cases of trisomy 21 and 223 932 of non-trisomy-21 singleton pregnancies, the weighted pooled DR and FPR were 99.7% (95% CI, 99.1–99.9%) and 0.04% (95% CI, 0.02–0.07%), respectively[4]. In twin pregnancies, the performance of screening for trisomy 21 by cfDNA is encouraging, but the number of cases reported is small; in a total of 24

Correspondence to: Prof. K. H. Nicolaides, Fetal Medicine Research Institute, King's College Hospital, 16–20 Windsor Walk, Denmark Hill, London SE5 8BB, UK (e-mail: kypros@fetalmedicine.com)

Accepted: 16 October 2018

cases of trisomy 21 and 1111 non-trisomy-21 cases, the DR was 100% (95% CI, 95.2–100%) and FPR was 0.0% (95% CI, 0.0–0.003%), respectively[4].

In 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 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[5,6]. We have reported previously on the clinical implementation of such a policy in singleton pregnancies[7,8].

The objective of this study was to examine the clinical implementation of cfDNA testing, contingent on the results of the combined test, in routine first-trimester screening for fetal trisomies in twin pregnancies.

METHODS

Study design and participants

This was a prospective study in women with a twin pregnancy attending one of two National Health Service (NHS) hospitals in England (King's College Hospital, London, and Medway Maritime Hospital, Kent) for routine care between October 2013 and January 2018. 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, we recorded maternal demographic characteristics and medical history, measured maternal serum free β-hCG and PAPP-A (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA) and carried out an ultrasound scan to determine gestational age from the measurement of the fetal crown–rump length[9] of the larger fetus, and chorionicity by examining the junction of the intertwin membrane with the placenta[10], to diagnose any major fetal abnormalities and measure fetal NT thickness. The measured NT thickness was expressed as a difference from the expected normal mean for gestation (delta value)[11]. Similarly, the measured free β-hCG and PAPP-A were converted into multiples of the median values adjusted for maternal characteristics, gestational age and chorionicity[3,12].

The estimated risk for trisomy 21 and that for trisomy 18 or 13 were calculated. In the case of monochorionic twins, a risk was given for the whole pregnancy; in dichorionic twins a risk was given for each fetus and the highest of the two was used for stratification. Women in the high-risk group (risk ≥ 1 in 100) were offered the option of chorionic villus sampling (CVS), cfDNA testing or no further testing; this cut-off was selected because it is used by the NHS for offering invasive testing. Women in the intermediate-risk group (risk 1 in 101 to 1 in 2500 in the first phase of the study and 1 in 101 to 1 in 500 in the second phase) were offered cfDNA or no further testing. Women in the low-risk group (risk < 1 in 2500 in the first phase of the study and < 1 in 500 in the second phase) were reassured that fetal trisomy was unlikely and no further testing was necessary.

Women provided written informed consent and maternal blood (20 mL) was sent via courier to the USA for cfDNA testing (Harmony™ Prenatal Test, Ariosa Diagnostics, Inc., San Jose, CA, USA)[13,14]. Digital analysis of selected regions (DANSR) by chromosome-selective sequencing or microarray was used to quantify chromosomes 21, 18 and 13. Risk scores for trisomies 21, 18 and 13 were provided as a percentage with ranges capped at $> 99\%$ and $< 0.01\%$. In cases in which the cfDNA test did not provide results, the parents were offered repeat testing or to rely on the results of the combined test in deciding whether to have an invasive test or not. In cases with a high-risk result from 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 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 of chorionic villi, amniotic fluid or neonatal blood was normal or both neonates were phenotypically normal, third, no known karyotype in both fetuses because the pregnancy resulted in termination or 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 and interquartile range (IQR) for continuous variables and as number and percentage for categorical variables. Comparisons between outcome groups were by Mann–Whitney *U*-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables.

The statistical software package R version 3.3.3 (https://www.R-project.org/) was used for data analyses.

RESULTS

Study population

During the study period, 977 women with a twin pregnancy and two live fetuses at 11–13 weeks' gestation were offered combined screening for trisomies; 959 (98.2%) accepted, but 42 (4.4%) of these were excluded from further analysis either because the pregnancy ended in termination, miscarriage or stillbirth with no known karyotype ($n = 29$) or they were lost to follow-up ($n = 13$).

Maternal and pregnancy characteristics of the 917 pregnancies with known trisomic status of both twins are summarized in Table 1; these included 740 (80.7%) dichorionic and 177 (19.3%) monochorionic twins. In the

Table 1 Characteristics of study population of 917 twin pregnancies, according to risk for trisomy 21 or trisomy 18/13

Characteristic	*Risk*		
	High (n = *47*)	*Intermediate* (n = *203*)	*Low* (n = *667*)
Maternal age (years)	36.2 (32.5–40.0)*	36.6 (33.3–38.9)*	32.0 (28.3–35.5)
Maternal body mass index (kg/m^2)	26.9 (22.1–30.5)	25.0 (22.6–28.1)	25.4 (22.3–30.0)
Racial origin			
White	33 (70.2)	153 (75.4)	512 (76.8)
Black	12 (25.5)	32 (15.7)	105 (15.7)
South Asian	2 (4.3)	12 (5.9)	24 (3.6)
East Asian	—	3 (1.5)	5 (0.7)
Mixed	—	3 (1.5)	21 (3.1)
Cigarette smoker	1 (2.1)	3 (1.5)	34 (5.1)
Parity			
Nulliparous	25 (53.2)	87 (42.9)	272 (40.8)
Parous	22 (46.8)	116 (57.1)	395 (59.2)
Method of conception			
Spontaneous	31 (66.0)	129 (63.5)	479 (71.8)
Assisted	16 (34.0)	74 (36.5)	188 (28.2)
Estimated risk for trisomy 21 or 18/13 (1 in *x*)	24 (5–57)*	463 (276–1182)*	3833 (1952–7321)
Patient choice for further testing			
Cell-free DNA test	27 (57.4)	186 (91.6)	—
Chorionic villus sampling	17 (36.2)	—	—
None	3 (6.4)*	17 (8.4)*	667 (100)

Data are given as median (interquartile range) or *n* (%). Pregnancies stratified according to higher of the two trisomy risks. Comparisons of high- and intermediate-risk groups with low-risk group made using Mann–Whitney *U*-test for continuous variables and chi-square or Fisher's exact test for categorical variables, with *post-hoc* Bonferroni correction with adjusted *P*-value of < 0.025. *Significant on comparison with low-risk group.

monochorionic twin pregnancies, there were no trisomic fetuses. In the dichorionic twin pregnancies, there were 10 in which one fetus was normal and the cotwin was trisomic (six cases of trisomy 21 and four of trisomy 18).

On the basis of the maternal age distribution and the age-related risk for these trisomies at 12 weeks' gestation, the expected numbers of cases of trisomy 21 and trisomy 18 or 13 in our monochorionic twin pregnancies were 0.6 (95% CI, 0.07–4.87) and 0.3 (95% CI, 0.02–4.32), respectively[15,16]. In the dichorionic twin pregnancies, on the assumption that the trisomic risk for each of the 1480 fetuses was the same as that in singleton pregnancies, the expected numbers of cases of trisomy 21 and trisomy 18 or 13 in our study population were 6.4 (95% CI, 3.03–13.54) and 3.4 (95% CI, 1.26–9.32), respectively, which were similar to the observed numbers of six and four, respectively[15,16].

Stratification of risk and parental choice

Following combined screening, 47 (5.1%), 203 (22.1%) and 667 (72.7%) of the pregnancies were classified as high risk, intermediate risk and low risk, respectively. The high-risk group can be subdivided into a group with estimated risk of ≥ 1 in 30, which contained 27 (2.9%) cases, and another with a risk of 1 in 31 to 1 in 100, which contained 20 (2.2%) cases.

In the high-risk group, 36.2% (17/47) opted for CVS (including four cases of trisomy 21 and two of trisomy 18), 57.4% (27/47) for cfDNA testing (including one case of trisomy 21 and one of trisomy 18) and 6.4% (3/47) did not want any further investigations. In the subgroup with risk of ≥ 1 in 30, 55.6% (15/27) opted for CVS (including four cases of trisomy 21 and two of trisomy 18), 37.0% (10/27) for cfDNA testing (including one case of trisomy 21) and 7.4% (2/27) did not want any further investigations. In the intermediate-risk group, 91.6% (186/203) opted for cfDNA testing (including one case of trisomy 21) and 8.4% (17/203) did not want any further investigations.

Results of combined test

Combined screening with an estimated risk cut-off of 1 in 100 detected 83.3% (5/6) of cases of trisomy 21 and 75% (3/4) with trisomy 18. One case of trisomy 21 had a risk of 1 in 939 and this was identified by cfDNA testing. In three of the six cases of trisomy 21, the parents chose to continue with the pregnancy and, in the other three, they had embryo reduction. One case of trisomy 18 had a risk of 1 in 3450 and this case was identified by amniocentesis because, at the routine 20-week scan, the affected fetus had tetralogy of Fallot, clenched hands, strawberry-shaped head and growth restriction.

Implementation and performance of cfDNA test

In total, the cfDNA test was carried out in 224 pregnancies. These included 213 from the high- and intermediate-risk groups that opted for cfDNA testing and 11 from the high-risk group that opted for CVS but also had cfDNA testing for research; in the latter group, the blood test was collected before invasive testing. Results from testing were provided after first sampling for 95.5% (214/224) of cases and the median fetal fraction was 8.5%

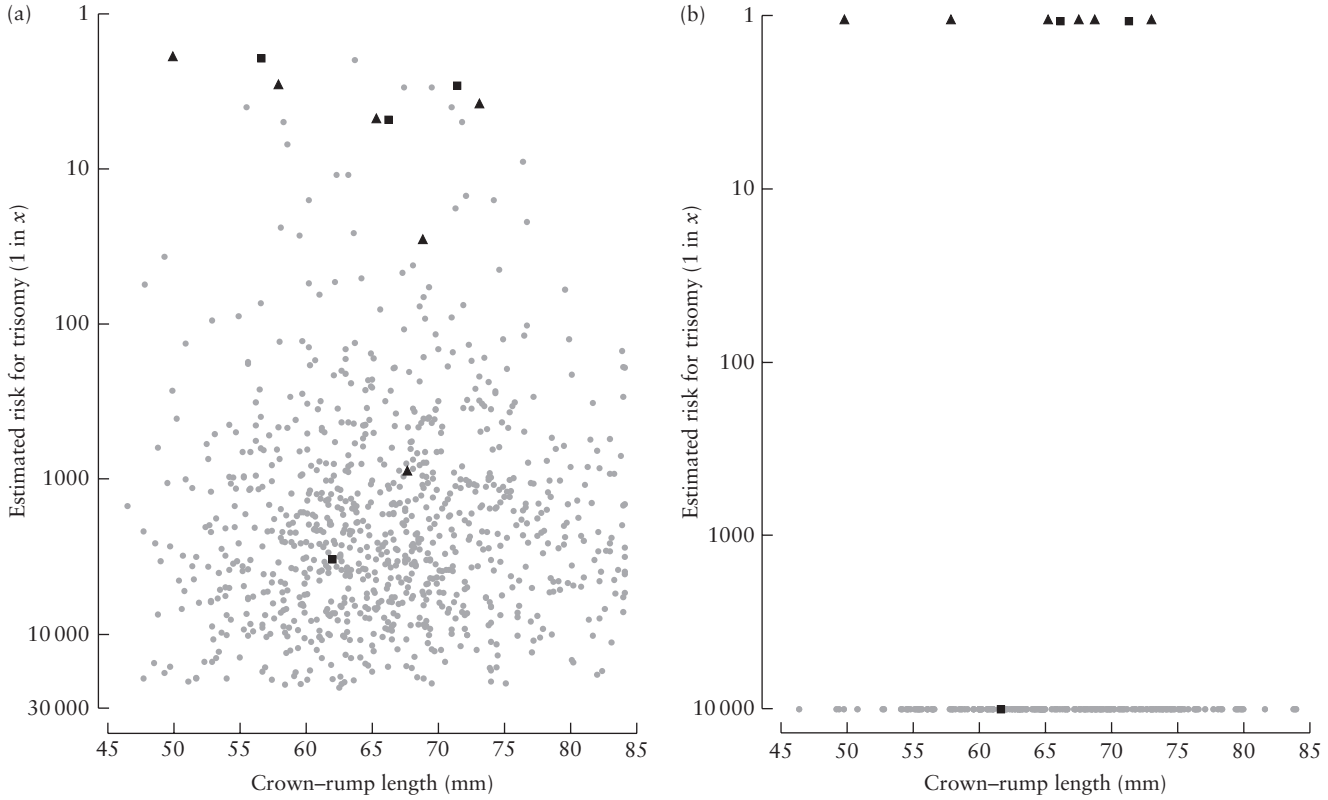

Figure 1 Distribution of estimated risk for trisomy in twin pregnancies, by combined test ($n = 917$) (a) and by cell-free DNA test ($n = 224$) (b), according to crown–rump length. ●, Cases without trisomy 21, 18 or 13; ▲, trisomy-21 cases; ■, trisomy-18 cases.

(range, 4–30%). The reasons for no result were insufficient fetal cfDNA for accurate evaluation in seven cases and that the sample did not meet thresholds for quality control in three. In seven of the 10 cases with no result, a further blood sample was obtained and a cfDNA result was provided in one. In eight of the nine cases with no result from the cfDNA test, the parents decided to avoid further testing, and in one the parents chose to have amniocentesis.

The group of 215 pregnancies with a cfDNA result (214 from first sampling and one from second sampling) included six with trisomy 21, three with trisomy 18 and 206 with no trisomy 21, 18 or 13. The cfDNA test classified correctly as screen positive all six cases of trisomy 21 and two of the three with trisomy 18, and as screen negative for each of the trisomies all 206 unaffected pregnancies.

The distribution of estimated risk for trisomies by the combined test and the cfDNA test is given in Figure 1.

Performance of contingent screening

The study population of 917 pregnancies with known trisomic status of both twins included six with trisomy 21, four with trisomy 18 and 907 with no trisomy 21, 18 or 13. Contingent screening led to prenatal detection of all cases of trisomy 21 and three of four with trisomy 18 (one case was classified as low risk by the combined test).

Invasive tests were carried out in 33 (3.6%) pregnancies in the study population. These included 17 (51.5%) for high-risk result from the combined test, two (6.1%) for positive result from the cfDNA test, one (3.0%) for failed cfDNA testing, five (15.2%) for fetal defects detected by ultrasound examination in the second trimester of pregnancy, seven (21.2%) for endoscopic laser separation of communicating placental vessels in association with severe twin–twin transfusion syndrome or selective fetal growth restriction, and one (3.0%) for prenatal diagnosis of sickle cell disease.

DISCUSSION

Principal findings

This study has demonstrated the feasibility of introducing cfDNA testing, contingent on the results of the first-trimester combined test for major trisomies, in a routine population of twin pregnancies. The observed number of trisomies was as expected on the basis of the maternal age distribution of the study population.

In our participating hospitals, about 98% of women attending for a routine ultrasound examination at 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, 36% of women opted for invasive testing, 57% for cfDNA testing and 6% for no further tests; in the subgroup with risk of ≥ 1 in 30, 56% opted for invasive testing. In the intermediate-risk group, 92% opted for cfDNA testing and 8% for no further tests. These results of patient choice

are very similar to those reported in our previous study on singleton pregnancies[8]. In the high-risk group, the choice between CVS and cfDNA testing was influenced by objective evidence derived from the patient-specific risk obtained from the combined test. In three of the six cases of trisomy 21, the parents chose to continue with the pregnancy and, in the other three, they had embryo reduction; there were no obvious differences between the two groups in terms of maternal age, race, parity or method of conception.

The combined test, at risk cut-off of 1 in 100, could have potentially identified five of six cases of trisomy 21 and three of four of trisomy 18, at a FPR of 4.3%. The number of affected cases is too small for accurate assessment of the performance of screening, but the results are consistent with the modelled performance of about 90% detection of the major trisomies at a FPR of 6%[3].

In the group undergoing cfDNA testing, results were provided for 96% of pregnancies; the failure rate in twin pregnancies was twice as high as that in our previous study on singleton pregnancies[8]. The cfDNA test detected all cases of trisomy 21 and two of three with trisomy 18 in the population having this test, at a FPR of 0%. As in the case of the combined test, the number of affected cases is too small for accurate assessment of the performance of cfDNA screening, but the results are consistent with those of previous reports[17–24].

Limitations

The main limitation of the study relates to the small number of trisomic pregnancies and the small number of cases that had cfDNA testing, preventing definitive conclusions being drawn in terms of performance of screening by these two methods.

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.

Previous studies of cfDNA testing in twin pregnancies

There are only seven prospective studies with complete follow-up reporting on the performance of cfDNA testing in twin pregnancies[18–24]. Two studies examined a routine population[23,24], three examined pregnancies at high-risk of aneuploidy[20–22], and two were in a mixed population of high- and low-risk pregnancies[18,19]. In the combined total of 31 cases of trisomy 21 and 2008 non-trisomic pregnancies, the detection rate was 100% and FPR was 0.05%. Although the number of twin pregnancies examined by cfDNA testing is considerably lower than that for singleton pregnancies[4], the results suggest the test is equally effective in identifying trisomy 21.

Conclusions

Clinical implementation of cfDNA testing contingent on the results of a previously performed first-trimester combined test is feasible and it 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 the 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 on reducing the rate of invasive testing and a small effect on the rate of live births with trisomy 21.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116).

Disclosure

The cost of collection and analysis of some of the samples was covered by Ariosa Diagnostics, Inc., San Jose, CA, USA. These organizations had no role in study design, data collection, data analysis, data interpretation or writing of the report.

REFERENCES

1. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011; **31**: 7–15.
2. Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. *Fetal Diagn Ther* 2014; **35**: 118–126.
3. Madsen HN, Ball S, Wright D, Tørring N, Petersen OB, Nicolaides KH, Spencer K. A reassessment of biochemical marker distributions in trisomy 21-affected and unaffected twin pregnancies in the first trimester. *Ultrasound Obstet Gynecol* 2011; **37**: 38-47.
4. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2017; **50**: 302–314.
5. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM. First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. *Ultrasound Obstet Gynecol* 2013; **42**: 41–50.
6. Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. *Fetal Diagn Ther* 2014; **35**: 185–192.
7. Gil MM, Giunta G, Macalli EA, Poon LC, Nicolaides KH. UK NHS pilot study on cell-free DNA testing in screening for fetal trisomies: factors affecting uptake. *Ultrasound Obstet Gynecol* 2015; **45**: 67–73.
8. Gil MM, Revello R, Poon LC, Akolekar R, Nicolaides KH. Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on the results from first-trimester combined test. *Ultrasound Obstet Gynecol* 2016; **47**: 45–52.
9. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
10. Sepulveda W, Sebire NJ, Hughes K, Odibo A, Nicolaides KH. The lambda sign at 10–14 weeks of gestation as a predictor of chorionicity in twin pregnancies. *Ultrasound Obstet Gynecol* 1996; **7**: 421–423.
11. Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2008; **31**: 376–383.
12. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2008; **31**: 493–502.
13. Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; **206**: 319.e1–9.

14. Ashoor G, Syngelaki A, Wang E, Struble C, Oliphant A, Song K, Nicolaides KH. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 21–25.
15. Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. *Fetal Diagn Ther* 1995; **10**: 356–367.
16. Wright DE, Bray I. Estimating birth prevalence of Down's syndrome. *Journal Epidemiol Biostat* 2000; **5**: 89–97.
17. del Mar Gil M, Quezada MS, Bregant B, Syngelaki A, Nicolaides KH. Cell-free DNA analysis for trisomy risk assessment in first-trimester twin pregnancies. *Fetal Diagn Ther* 2014; **35**: 204–211.
18. Sarno L, Revello R, Hanson E, Akolekar R, Nicolaides KH. Prospective first-trimester screening for trisomies by cell-free DNA testing of maternal blood in twin pregnancy. *Ultrasound Obstet Gynecol* 2016; **47**: 705–711.
19. Le Conte G, Letourneau A, Jani J, Kleinfinger P, Lohmann L, Costa Pharm JM, Benachi A. Cell-free fetal DNA analysis in maternal plasma as screening test for trisomies 21, 18 and 13 in twin pregnancy. *Ultrasound Obstet Gynecol* 2018; **52**: 318–324.
20. Lau TK, Jiang F, Chan MK, Zhang H, Lo PSS, Wang W. Non-invasive prenatal screening of fetal Down syndrome by maternal plasma DNA sequencing in twin pregnancies. *J Matern-Fetal Neonatal Med* 2013; **26**: 434–437.
21. Huang X, Zheng J, Chen M, Zhao Y, Zhang C, Liu L, Xie W, Shi S, Wei Y, Lei D, Xu C, Wu Q, Guo X, Shi X, Zhou Y, Liu Q, Gao Y, Jiang F, Zhang H, Su F, Ge H, Li X, Pan X, Chen S, Chen F, Fang Q, Jiang H, Lau TK, Wang W. Noninvasive prenatal testing of trisomies 21 and 18 by massively parallel sequencing of maternal plasma DNA in twin pregnancies: Sequencing-based prenatal noninvasive fetal trisomy test in twin pregnancies. *Prenat Diagn* 2014; **34**: 335–340.
22. Du E, Feng C, Cao Y, Yao Y, Lu J, Zhang Y. Massively Parallel Sequencing (MPS) of cell-free fetal DNA (cffDNA) for Trisomies 21, 18, and 13 in twins pregnancies. *Twin Res Hum Genet* 2017; **20**: 242–249.
23. Tan Y, Gao Y, Lin G, Fu M, Li X, Yin X, Du J, Li J, LI W, Peng H, Yuan Y, Chen F, Jiang F, Zhang H, Lu G, Gong F, Wang W. Noninvasive prenatal testing (NIPT) in twin pregnancies with treatment of assisted reproductive techniques (ART) in a single center. *Prenat Diagn* 2016; **36**: 1–8.
24. Yang J, Qi Y, Hou Y, Guo F, Peng H, Wang D, Haoxin OY, Wang Y, Huang H, Yin A. Performance of non-invasive prenatal testing for trisomies 21 and 18 in twin pregnancies. *Molecular Cytogenetics* 2018; **11**: 47.

Ultrasound Obstet Gynecol 2019; **53**: 208–213
Published online 7 December 2018 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.20160

Cribado rutinario en el primer trimestre para trisomías fetales en el embarazo de gemelos: prueba de ADN fetal en función de los resultados de una prueba combinada

RESUMEN

Objetivo Informar sobre la aplicación clínica rutinaria del análisis de ADN fetal (cfDNA, por sus siglas en inglés) en la sangre materna para las trisomías 21, 18 y 13, en función de los resultados de la prueba combinada del primer trimestre en el embarazo gemelar.

Métodos Se llevó a cabo un cribado para las trisomías 21, 18 y 13 en 959 embarazos gemelares mediante la evaluación de una combinación de edad materna, grosor de la translucencia nucal del feto, y la ausencia en suero de la hormona gonadotrópica coriónica humana (subunidad B) y la proteína plasmática A asociada al embarazo a las 11–13 semanas de gestación en dos hospitales del NHS del Reino Unido. A las mujeres del grupo de alto riesgo (riesgo ≥1 en 100) se les ofreció la opción de realizar pruebas invasivas, la prueba de cfDNA o no realizar más pruebas, y a las del grupo de riesgo intermedio (riesgo 1 en 101 a 1 en 2500 en la primera fase del estudio y 1 en 101 a 1 en 500 en la segunda fase) se les ofreció la prueba de cfADN o no realizar más pruebas. El estado trisómico de los embarazos se determinó mediante el cariotipo prenatal o postnatal o el examen de los recién nacidos.

Resultados En 42 (4,4%) de los 959 embarazos, hubo interrupción, aborto o éxitus fetal sin cariotipo conocido o no se hizo seguimiento. Los 917 embarazos con estado trisómico conocido de ambos gemelos incluyeron seis que eran discordantes con la trisomía 21, cuatro que eran discordantes con la trisomía 18 y 907 sin trisomía 21, 18 o 13. Tras el cribado combinado, 47 (5,1%), 203 (22,1%) y 667 (72,7%) de los embarazos se clasificaron como de alto riesgo, riesgo intermedio y bajo riesgo, respectivamente. El grupo de alto riesgo incluyó cinco (83,3%) casos de trisomía 21 y tres (75,0%) de trisomía 18. La prueba de cfDNA se realizó en 224 embarazos y se obtuvieron resultados de 214 (95,5%) de ellos; este grupo incluyó seis embarazos con trisomía 21, tres con trisomía 18 y 206 sin trisomía 21, 18 ó 13. La prueba de cfDNA clasificó correctamente como positivo en los seis casos de trisomía 21 y dos de los tres con trisomía 18, y como negativo en cada una de las trisomías en los 206 embarazos no afectados. El cribado contingente dio lugar a la detección prenatal de todos los casos de trisomía 21 y tres de los cuatro con trisomía 18.

Conclusión Este estudio ha demostrado la factibilidad de introducir la prueba de cfDNA, contingente con los resultados de la prueba combinada del primer trimestre para trisomías principales, en una población rutinaria de embarazos gemelares.

双胎妊娠胎儿三体常规孕早期筛查：无细胞 DNA 检测取决于综合检测结果

摘要

目的：报告第 21、18 与 13 对染色体三体变异母体血液无细胞 DNA (cfDNA)分析的常规临床实施，取决于双胎妊娠早期妊娠综合检测结果。

方法：在英国的两家 NHS 医院进行 11-13 周孕期产妇年龄、胎儿颈部半透明厚度、以及无血清β-人绒毛膜促性腺激素与妊娠相关血浆蛋白-A 综合评估，针对 959 个双胎孕妇进行第 21、18 与 13 对染色体三体变异筛查。高危组（风险机率≥1%）孕妇可以选择有创检测、cfDNA 检测或无后续检测，中等风险组（研究第一阶段的风险机率在 1:2500 至 1:101 之间，第二阶段的风险机率在 1:500 至 1:101 之间）孕妇可以选择 cfDNA 检测或无后续检测。通过产前或产后核型分析或新生儿检查确定孕妇的三体状态。

结果：在 959 个孕妇中，42 个(4.4%)孕妇出现无已知核型的终止妊娠、流产或死产，或者随访中发现新生儿死亡。在 917 个已知双胎三体状态的孕妇中，6 个出现第 21 对染色体三体变异不一致，4 个出现第 18 对染色体三体变异不一致，907 个并未出现第 21、18 或 13 对染色体三体变异。综合筛查之后，47 (5.1%)、203 (22.1%)与 667 (72.7%)个孕妇分别归入高危组、中危组与轻度危险组。高危组包括 5 (83.3%)个第 21 对染色体三体变异案例，3 (75.0%)个第 18 对染色体三体变异案例。对 224 个孕妇进行了 cfDNA 检测，214 (95.5%)个孕妇有检测结果。该组包括 6 个第 21 对染色体三体变异孕妇，3 个第 18 对染色体三体变异孕妇和 206 个并无第 21、18 或 13 对染色体三体变异的孕妇。cfDNA 检测结果将全部 6 个第 21 对染色体三体变异孕妇正确归为筛查呈阳性，3 个孕妇中有 2 个发现第 18 对染色体三体变异，全部 206 个未受影响的三体孕妇都被归为筛查呈阴性。产前检测酌情筛查发现全部孕妇都有第 21 对染色体三体变异，4 个孕妇中有 3 个发现第 18 对染色体三体变异。

结论：此次研究证实了在双胎妊娠常规人群中引入 cfDNA 检测的可行性，具体取决于针对大多数三体变异患者的孕早期综合检测结果。