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First-trimester risk assessment based on ultrasound and cell-free DNA *vs* combined screening: a randomized controlled trial

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KEYWORDS: aneuploidy; cfDNA; first trimester; nuchal translucency; trisomy 21

ABSTRACT

Objective *This was a randomized controlled trial to compare risk assessment by first-trimester combined screening (FTCS) with an approach that combines a detailed ultrasound examination at 11–13 weeks' gestation and cell-free DNA (cfDNA) analysis.*

Methods *Pregnant women with a normal first-trimester ultrasound examination at 11–13 weeks' gestation (fetal nuchal translucency (NT) ≤ 3.5 mm and no fetal defects) were randomized into one of two groups. In the first group, risk of aneuploidy was assessed using FTCS based on the most recent UK Fetal Medicine Foundation algorithm. In the second group, risk assessment was based on ultrasound findings and cfDNA analysis. An additional tube of blood was collected for FTCS in case the cfDNA analysis was uninformative. Primary outcome was false-positive rate in screening for trisomy 21. A case was considered false positive if the karyotype was not trisomy 21 and if the risk for trisomy 21 was >1:100, irrespective of the method of risk calculation. Results were compared using 95% CIs using the Clopper–Pearson method.*

Results *Between October 2015 and December 2016, 1518 women with singleton pregnancy underwent first-trimester screening. Thirty-one (2.0%) pregnancies were not eligible for randomization due to increased NT (> 3.5 mm) and/or fetal defect. After exclusion of women who declined randomization (*n = *87) and cases of fetal death and loss to follow-up (*n = *24), 688 pregnancies were randomized into the FTCS arm and 688 into the ultrasound + cfDNA analysis arm. There were no differences in maternal and gestational age, maternal weight and BMI, ethnicity, use of assisted reproduction and cigarette smoking between the two arms. In the ultrasound + cfDNA analysis arm, median risk for trisomy 21 was 1 in 10 000. None of the cases had a risk above 1: 100 (95% CI, 0.0–0.5%). In the FTCS arm, the median risk for trisomy 21 was 1 in 3787 and in 17 cases, the risk was higher than 1:100, which corresponds to 2.5% (95% CI, 1.5–3.9%) of the FTCS study-arm population.*

Conclusion *Our study has shown that first-trimester risk assessment for trisomy 21 that includes a detailed ultrasound examination as well as NT measurement and is followed by cfDNA testing is associated with a significant reduction in the false-positive rate compared with FTCS. This approach obviates the need for maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A in screening for fetal aneuploidy. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

There is an ongoing debate regarding how cell-free DNA (cfDNA) screening can best be incorporated into current prenatal screening algorithms for chromosomal abnormalities. Although the test performance of cfDNA screening is better than that of first-trimester combined screening (FTCS), at present, the cost of cfDNA testing is generally deemed too high for it to be adopted as the primary method of screening[1,2]. In addition, FTCS includes a detailed ultrasound examination of the fetus that allows for an early detection of a significant proportion of fetal structural defects[3]. The combination of a detailed fetal ultrasound examination and nuchal translucency (NT) measurement also increases

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the detection rates of chromosomal abnormalities that are currently not detectable by cfDNA analysis[4–8]. Therefore, many see significant value in a contingent model in which FTCS serves as a triage examination for determining whether cfDNA analysis is required. Depending on the computed risk for trisomy 21 (T21) and the presence of major defects, the pregnancy is classified as at high, low or intermediate risk. The high-risk group is offered invasive testing and the low-risk group is managed expectantly. cfDNA testing is performed in the intermediate-risk group only. This approach reduces the false-positive rate (FPR) of FTCS and, depending on the applied cut-off, it can also increase the detection rate. Furthermore, it significantly decreases the number of pregnancies in which cfDNA analysis is performed. The number of cfDNA tests performed can be adjusted by changing the cut-offs used to define the various risk groups.

This contingent screening approach to cfDNA testing can be challenged in several ways. Firstly, there are still some pregnancies affected by T21 that are present in the 'low-risk' group. Those would remain unidentified if cfDNA analysis is not performed in this group[2,9]. Since the exclusion of the low-risk group from cfDNA analysis is based on economic considerations, this approach may become difficult to justify as the cost of cfDNA testing decreases further[10]. The recommendation to proceed straight to invasive diagnosis in the high-risk group is somewhat harder to contest, especially in the presence of an increased NT or fetal structural defects. This group includes a significant number of fetuses that have a chromosomal abnormality that is detectable only through invasive testing and not by cfDNA analysis[4–8].

An approach in which every woman with a normal ultrasound examination at 11–13 weeks' gestation undergoes cfDNA testing for aneuploidy may be a reasonable, though less cost-effective, option. Under these circumstances, the need for biochemical markers, such as free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A), is questionable. However, first-trimester biochemical markers may be useful in cases in which cfDNA analysis is uninformative[11]. In these cases, the best risk assessment would likely be based on FTCS (reflex approach), especially when a redraw of maternal blood for repeat cfDNA testing is challenging for logistical reasons. Such an approach would require storage of a serum sample at the time of the cfDNA testing since it is often too late to draw blood for maternal serum marker analysis when it becomes apparent that cfDNA analysis will not yield a result.

In this randomized controlled trial, we set out to compare the screening performance of FTCS with an approach that uses the combination of a detailed ultrasound examination and cfDNA analysis. In the latter, a combined test was performed only if cfDNA testing was uninformative. FPR was used as primary outcome parameter as, in terms of absolute numbers, this is the most crucial parameter in screening that is of major importance to physicians, health authorities and patients alike.

METHODS

This was a randomized controlled study of women undergoing first-trimester screening, performed at the prenatal medicine department of the University of Tuebingen, Germany.

In our department, first-trimester risk assessment is performed routinely at 11–13 weeks' gestation. In addition to crown–rump length and NT measurements, it also includes a detailed ultrasound examination based on ISUOG guidelines[12,13]. All operators who perform this examination are certified by the UK Fetal Medicine Foundation (FMF). A specific risk for aneuploidy is not calculated if the NT measurement is >3.5 mm or if a fetal anomaly is identified. These cases are deemed to be at a very high risk for chromosomal abnormalities and are offered invasive testing.

For the purposes of this study, pregnant women with a normal first-trimester ultrasound examination were randomized into two groups. In the first group, the risk of aneuploidy was assessed using FTCS (maternal and gestational age, fetal NT thickness, and maternal levels of serum PAPP-A and free β-hCG). The combined risk for T21 was computed based on the most recent FMF algorithm[12,14–17]. In the second group, risk assessment was based on ultrasound findings and cfDNA screening (US+cfDNA group).

cfDNA analysis was performed by Cenata GmbH (Tuebingen, Germany) using the Harmony® Prenatal Test (Roche/Ariosa Diagnostics, Inc., San Jose, CA, USA) as described previously[18–20]. In brief, chromosome-selective cfDNA analysis was performed using DANSR™ (Digital ANalysis of Selected Regions) and included a simultaneous microarray-based assay of non-polymorphic (chromosomes 13, 18, 21, X and Y) and polymorphic loci to estimate chromosome proportion and fetal fraction. The FORTE™ (Fetal fraction Optimized Risk of Trisomy Evaluation) algorithm was used to provide patient-specific risk assessments for trisomy.

In cases in which cfDNA analysis was performed, an additional tube of blood was collected in anticipation of an uninformative cfDNA testing result in a certain proportion of cases. This serum sample was centrifuged at 4000 rpm for 5 min, aliquoted and stored at −80 °C. In cases in which no result was obtained by cfDNA analysis, the serum sample was thawed gently and free β-hCG and PAPP-A levels were measured. The risk for T21 was then computed using the FTCS method.

Irrespective of the method of risk calculation used (FTCS or US+cfDNA), an invasive test was offered to patients with risk >1 in 100. This is in concordance with the guidelines of the UK National Screening Committee and the risk cut-off that the Harmony Prenatal Test uses to define a 'high-risk' result. All pregnant women and healthcare providers were informed about the risk assessment as soon as the results were available.

Exclusion criteria were maternal age < 18 years, CRL measurement > 84 mm or < 45 mm, and multiple

pregnancy, including vanishing twins. The clinically relevant ultrasound and screening information was recorded in the Viewpoint database (GE Healthcare, Munich, Germany). Information on maternal weight and height was extracted from patient records. Ethnicity, smoking status and mode of conception were assessed using a questionnaire and this information was also recorded in the Viewpoint database. Outcome data were added as soon as they became available. The primary-analysis population included participants for whom both the screening results and results of newborn examination or genetic testing (pre- or postnatal) were available.

Potential trial participants were given written information about the study and were counseled by a member of the perinatal medicine department staff. All women who agreed to participate in the trial provided written informed consent. Randomization was done by our local research institute in a 1:1 ratio (FTCS *vs* US+cfDNA), using computer-generated random numbers. The study was set up as an investigator-initiated trial. Approval for the study was obtained from the local ethics committee (No. 572/2015BO1). The study was registered in the International Standard Randomised Controlled Trial Number registry (ISRCTN No. 11174071).

Statistical analysis

Power calculation was based on the assumption that, in a general population, combined screening with a 1:100 cut-off is associated with a FPR of 2.0%[21]. This rate is based on the maternal age distribution of England and Wales in 2011. However, in previous prospective German first-trimester studies, we have observed a higher proportion of women with advanced maternal age ($\geq$ 35 years). Therefore, FPR was estimated to be 2.5%[22,23]. Since fetuses with increased NT of > 3.5 mm were not randomized in this study, FPR was assumed to be reduced to 1.5%.

For the US+cfDNA arm, we assumed a FPR of 0.1% and a cfDNA testing no-result rate of 3.0%. In the latter cases, we planned to estimate the risk for T21 using FTCS with a 1:100 cut-off. Thus, the final FPR in this arm was estimated to be 0.13%, an increase of 0.03%. To demonstrate a significant difference between the FPRs in the two study arms with a power of 80% and a significance level of 0.05, we estimated that we needed 674 women in each study arm.

The primary focus of the study, FPR in first-trimester screening for T21, was analyzed by comparing 95% CI

Table 1 Ultrasound findings in 31 pregnancies excluded from study due to increased nuchal translucency (NT) (> 3.5 mm) and/or presence of fetal defect

Karyotype	*CRL (mm)*	*NT (mm)*	*Fetal defect*
T21	55.6	4.5	—
T21	66.5	9.3	—
T21	61.8	3.7	—
T21	53.8	3.9	Cardiac defect
T21	72.5	3.7	Cardiac defect
T21	70.2	3.2	Agenesis of ductus venosus
T21	73.3	1.9	Cardiac defect
T18	60.0	1.4	Spinal defect, cardiac defect
T13	55.5	6.0	Cardiac defect, polydactyly
T13	79.5	3.5	Cardiac defect, polydactyly, facial cleft
Monosomy X	46.0	3.5	Cardiac defect
Triploidy	46.0	3.5	Cardiac defect, abnormal posterior fossa, bilateral hydrothorax, echogenic kidneys, abnormal hands
Normal	58.0	6.6	Noonan syndrome
Normal	68.7	4.2	Cornelia de Lange syndrome: radius aplasia, abnormal hand, cleft palate
Normal	67.5	1.5	Roberts syndrome, phocomelia
Normal	53.2	5.2	Body-stalk anomaly
Normal	48.3	6.9	Diaphragmatic hernia, facial cleft
Normal	72.2	3.1	Diaphragmatic hernia, retrognathia
Normal	66.0	4.0	Cardiac defect
Normal	65.2	2.8	Cardiac defect
Normal	84.0	4.8	Occipital encephalocele
Normal	57.2	6.5	Unexplained fetal hydrops
Normal	83.7	2.3	Obstructive uropathy
Normal	65.8	3.2	Obstructive uropathy
Normal	63.2	2.8	Thoracic cyst
Normal	61.1	1.8	Mesenteric cyst
Normal	66.5	9.3	—
Normal	65.4	3.6	—
Normal	62.6	3.6	—
Normal	53.1	4.3	—
Normal	71.6	3.6	—

CRL, crown–rump length; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21.

using the Clopper–Pearson method and by chi-square test. The FPR for FTCS was calculated as a crude rate but then also adjusted based on the maternal age distribution in Germany in 2015[24]. Median maternal age in this population is 31.0 (interquartile range 27.0–35.0) years and 25.9% of women are 35 years and older. An adjustment for maternal age was not necessary for the cfDNA results as the calculated risk was 1:10 000 or less in 99.0%. Maternal and pregnancy characteristics were compared by Mann–Whitney *U*-test for continuous variables and by chi-square test for categorical variables. *P*-value was assessed at the two-sided significance level of 5%.

RESULTS

Between October 2015 and December 2016, 1518 women with singleton pregnancy underwent first-trimester screening. Thirty-one (2.0%) pregnancies were not eligible for randomization due to increased NT (> 3.5 mm) and/or fetal defect (Table 1). During the study period, there were seven pregnancies with T21. This is concordant with the expected number of cases based on the maternal age distribution of the study population (expected number and 95% prediction interval 6.8 (1.6–11.9)). All seven of these cases were detected based on increased NT and/or fetal defects. Furthermore, the fetuses with trisomy 18 (T18) and trisomy 13 (T13) were also identified by the first-trimester ultrasound examination. Thus, our study population did not include fetuses with any of these three trisomies.

Eighty-seven (5.9%) women declined randomization. Out of the remaining 1400 women, 699 were randomized into the FTCS arm and 701 into the US+cfDNA arm. In 24 (1.7%) cases, it was not possible to obtain an outcome, due to either loss to follow-up or a subsequent miscarriage or intrauterine death. Thus, our study population consisted of 688 pregnancies in each group (Figure 1).

Maternal characteristics of the study population are summarized in Table 2. In both groups, median maternal age was 33.9 years and median gestational age at the time of randomization was 12.7 weeks. There were no differences with respect to maternal and gestational age, maternal weight and BMI, ethnicity, use of assisted reproduction, and cigarette smoking between the two arms.

In the US+cfDNA arm, median delta NT was 0.0 mm. The distribution of NT measurements is shown in Figure 2. Median fetal fraction was 12.5%. cfDNA testing was uninformative in 10 (1.5%) cases (Table 3). The risk in these cases was established by FTCS. Median risk for T21 in the US+cfDNA group was 1 in 10 000. None of the cases had a risk for T21, T18 or T13 above 1:100 (95% CI, 0.0–0.5%). The distribution of risk for T21 is shown in Figure 3 and Table 4.

In the FTCS arm, the median values for delta NT, free β-hCG and PAPP-A were 0.0 mm, 0.96 multiples

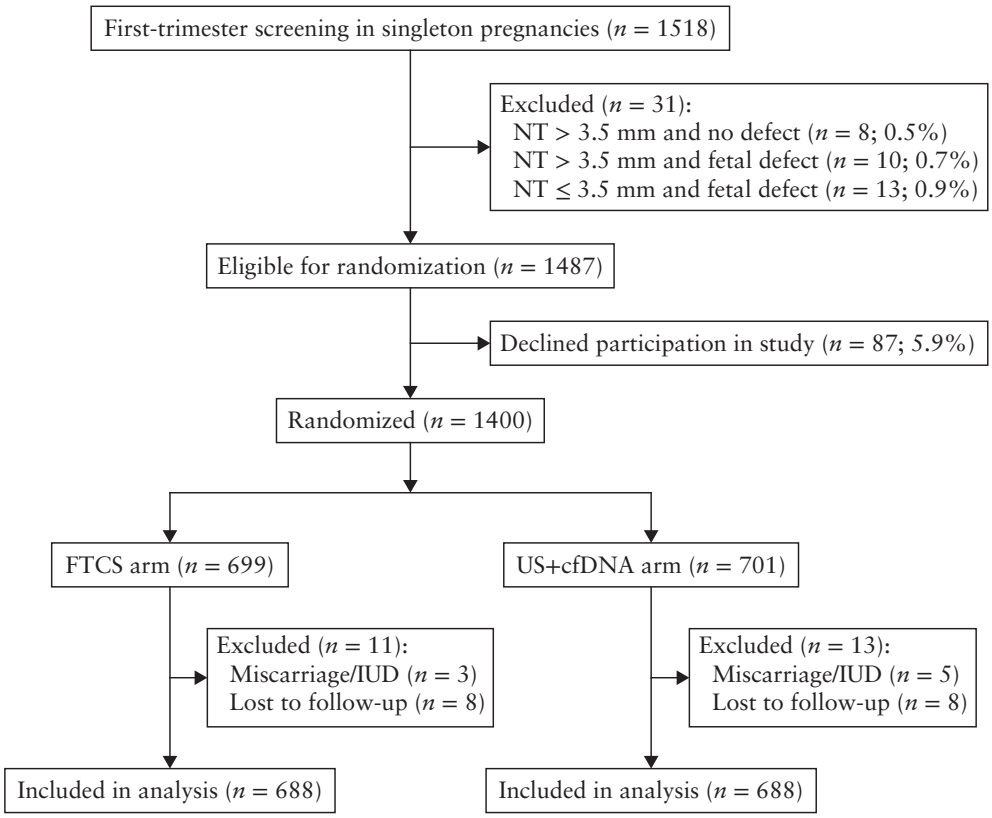

Figure 1 Flowchart showing inclusion of study population and randomization to first-trimester screening for trisomy 21 by first-trimester combined screening (FTCS) or combination of ultrasound examination and cell-free DNA testing (US+cfDNA). IUD, intrauterine death; NT, nuchal translucency thickness.

of the median (MoM) and 1.11 MoM, respectively (Table 3). The distribution of risk for T21 is shown in Figure 3. Median risk for T21 was 1 in 3787, and in 17 cases, the risk was higher than 1:100 (Table 4). This corresponds to 2.5% (95% CI, 1.5–3.9%) of the FTCS study-arm population, which is significantly greater than the risk in the US+cfDNA arm (chi-square test, $P < 0.0001$). After standardization for maternal age distribution in Germany, the risk would have been above 1:100 in 15 (2.2% (95% CI, 1.2–3.6%)) pregnancies ($P < 0.0001$).

In the FTCS group, 6 (35.3%) of the 17 women with high risk for T21 opted for invasive testing, and nine (52.9%) for additional cfDNA analysis. Two (11.8%) women decided against any further evaluation. There were six low-risk women in the FTCS group who also elected to have invasive testing, bringing the total number of women who underwent invasive testing to 12 (1.7%) in the FTCS arm. In contrast, in the US+cfDNA group, only two (0.3%) women underwent invasive testing. In all cases, the decision was based on the personal risk for trisomy or personal choice as there were no fetal defects in this group.

Table 2 Characteristics of 1376 women randomized to first-trimester screening for trisomy 21 by first-trimester combined screening (FTCS) or combination of ultrasound examination and cell-free DNA analysis (US+cfDNA)

Characteristic	*FTCS* (n = *688*)	*US+cfDNA* (n = *688*)	P
Maternal age (years)	33.9 (30.7–36.7)	33.9 (31.0–36.8)	0.498
Gestational age (weeks)	12.7 (12.3–13.1)	12.7 (12.4–13.1)	0.296
Maternal weight (kg)	66.0 (59.1–74.3)	65.4 (59.0–73.7)	0.260
Body mass index (kg/m^2)	23.4 (21.2–26.6)	23.4 (21.2–26.1)	0.596
Caucasian ethnicity	676 (98.3)	672 (97.7)	0.445
Cigarette smoking	23 (3.3)	19 (2.8)	0.531
Assisted reproduction	29 (4.2)	44 (6.4)	0.090

Data are given as *n* (%) or median (interquartile range).

DISCUSSION

Main findings

In this study, we have shown that, in screening for T21, a policy that is based on a detailed ultrasound examination at 11–13 weeks' gestation, followed by a cfDNA analysis and FTCS in cases with uninformative cfDNA testing, has a significantly lower FPR than has conventional FTCS. In the US+cfDNA group, there were no false-positive cases, while the age-adjusted FPR in the FTCS group was 2.5%. Our study was specifically designed to compare FPRs between two different screening approaches and not their detection rates. However, all T21 fetuses were identified by a detailed ultrasound examination.

Table 3 Risk parameters in first-trimester screening for trisomy 21 based on first-trimester combined screening (FTCS) or combination of ultrasound examination and cell-free DNA analysis (US+cfDNA)

Risk parameter	*FTCS* (n = *688*)	*US+cfDNA* (n = *688*)
Maternal age (years)	33.9 (30.7 to 36.7)	33.9 (31.0 to 36.8)
CRL (mm)	67.6 (62.6 to 72.9)	67.7 (62.7 to 72.8)
NT (mm)	1.9 (1.6 to 2.1)	1.8 (1.6 to 2.1)
Delta NT (mm)	0.0 (−0.2 to 0.2)	0.0 (−0.2 to 0.2)
Free β-hCG (MoM)	0.96 (0.64 to 1.44)	—
PAPP-A (MoM)	1.11 (0.76 to 1.51)	—
Fetal fraction	—	12.5 (9.8 to 16.0)
Failed cfDNA tests*	—	10 (1.5)
Free β-hCG in uninformative cfDNA tests (MoM)	—	0.66 (0.37 to 0.90)
PAPP-A in uninformative cfDNA tests (MoM)	—	0.73 (0.67 to 1.23)

Data are given as *n* (%) or median (interquartile range). *Risk in these cases was established by subsequent FTCS. β-hCG, β-human chorionic gonadotropin; CRL, crown–rump length; MoM, multiples of the median; NT, nuchal translucency thickness; PAPP-A, pregnancy-associated plasma protein A.

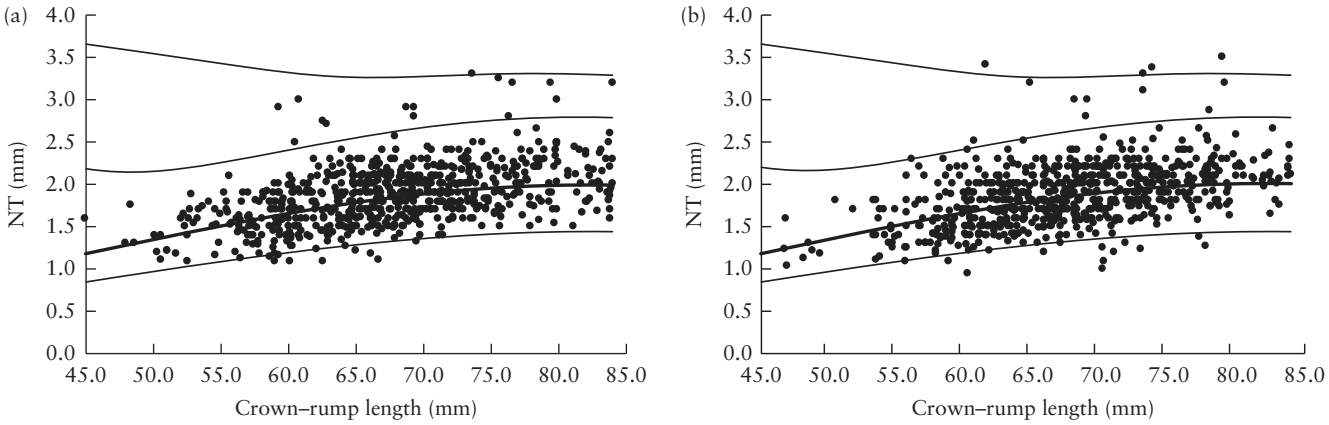

Figure 2 Distribution of nuchal translucency thickness (NT) measurements in women randomized to first-trimester screening for trisomy 21 by first-trimester combined screening (a) and those randomized to screening by combination of ultrasound examination and cell-free DNA testing (b). Lines represent 5th, 50th, 95th and 99th centiles.

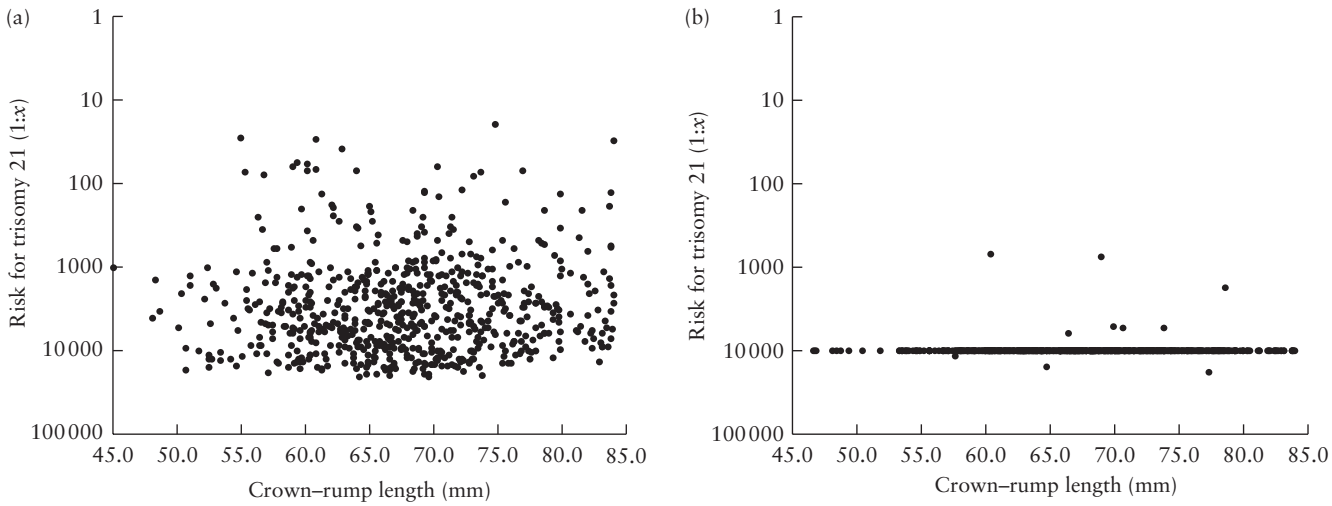

Figure 3 Distribution of risk for trisomy 21 in women randomized to first-trimester screening for trisomy 21 by first-trimester combined screening (a) or combination of ultrasound and cell-free DNA testing (b).

Table 4 Risk distribution in first-trimester screening for trisomy 21 (T21) based on first-trimester combined screening (FTCS) or combination of ultrasound examination and cell-free DNA analysis (US+cfDNA)

	FTCS (n = *688*)	*US+cfDNA* (n = *688*)
Median risk for T21	1 in 3787 (1605–8280)	1 in 10 000 (10 000–10 000)
T21 risk >1:100	17 (2.5)	0 (0)
T21 risk 1:100 to 1:999	79 (11.5)	2 (0.3)
T21 risk 1:1000 to 1:4999	302 (43.9)	1 (0.1)
T21 risk 1:5000 to 1:9999	163 (23.7)	4 (0.6)
T21 risk ≤1:10 000	127 (18.5)	681 (99.0)

Data are given as median (interquartile range) or *n* (%).

Comparison with previous studies

There is an ongoing discussion regarding whether cfDNA testing should be offered as first-line screening or as part of a contingent model in which cfDNA analysis is restricted to a group of patients selected based on their previous screening results[25,26].

The advantage of the first approach lies in its screening performance for T21, which is better than that of any other screening method[1,10]. However, disadvantages of a cfDNA-only approach include that no anatomical assessment of the fetus takes place and that in about 2% of cases the test fails quality metrics and no risk can be computed after the first blood draw[11]. Additionally, current costs of cfDNA analysis have been considered prohibitive in implementing it as a first-line screening test.

Nicolaides *et al.* estimated that using FTCS as primary screening for T21 and performing cfDNA testing for pregnant women with a risk of > 1:3000, the detection and screen-positive rates would be 97% and 0.4%, respectively[27]. Similarly, in the RAPID study, all patients with a risk for T21 ≥ 1 in 1000 based on either FTCS or a quadruple screening test were offered cfDNA analysis. In those with risk ≥ 1:150, invasive testing was also offered. The results were in line with those predicted by mathematical modeling[28].

Miltoft *et al.*[29] reported on 6449 women who underwent combined screening for T21. In this study, women with a risk of ≥ 1:1000 underwent cfDNA testing. The authors then compared conventional combined screening with referral for invasive testing at a risk ≥ 1 in 300 with a contingent screening model in which cfDNA screening was offered to those with a risk between 1 in 100 and 1 in 1000. While the detection rate was 100% in both groups, the FPR was reduced significantly from 3% with conventional combined screening to 1.2% with the contingent policy[29].

The decision to offer cfDNA testing to women in the intermediate-risk group or to all except those in the high-risk group is usually primarily based on financial rather than clinical considerations[30]. However, this is likely to be a temporary limitation as the price of cfDNA testing has been decreasing since its introduction and is expected to continue to do so. Even though limiting cfDNA analysis to the intermediate-risk group offers a high detection rate, approximately 1.5–5% of T21 pregnancies remain in the low-risk group[2,27,31–33]. Therefore, the only way that a 99% detection rate for T21 would be realized, as is possible with a primary cfDNA testing approach, would be not to restrict cfDNA analysis to a small subpopulation. However, our data suggest that such a primary cfDNA testing approach may come with a significant disadvantage if ultrasound is omitted from first-trimester screening.

One could argue that, if first-trimester aneuploidy screening does not involve maternal serum markers, there would be a significant loss in the detection of other chromosomal abnormalities. Abnormal serum marker levels may be useful in identification of rare chromosomal abnormalities but much less so than for the common trisomies[34]. However, our results suggest that a detailed first-trimester ultrasound examination followed by

cfDNA testing in the majority of the population leads to a low FPR and allows for an excellent detection rate of common and rare chromosomal abnormalities as well as fetal structural anomalies. This approach obviates the need for maternal serum free β-hCG and PAPP-A measurements.

Apart from screening for fetal aneuploidy, one can argue that screening for pre-eclampsia is an essential part of first-trimester pregnancy assessment and therefore maternal serum markers cannot be omitted from any protocol[35,36]. O'Gorman *et al.* demonstrated, however, that at a 10% FPR, first-trimester screening for early and late pre-eclampsia (< 37 and > 37 weeks' gestation) without the serum markers PAPP-A or placental growth factor (PlGF) results in only a slightly lower detection rate than is obtained with inclusion of these serum markers[37]. If screening for pre-eclampsia without biomarkers were to reach the same detection rate, the cut-off that defines the high-risk group could be lowered.

Limitations of the study

We acknowledge that our study is confined to a single specialized center. The identification of defects in this study was facilitated by expertise in ultrasound examination that may not be available everywhere. However, our first-trimester ultrasound protocol follows ISUOG recommendations[13] and thus should be generally reproducible.

Thirty-one (2.0%) pregnancies were excluded before randomization due to an increased NT measurement of > 3.5 and/or due to fetal anomaly. This group contained all seven cases with T21. Although other fetal problems were identified in the majority of the remaining 24 cases, in screening for T21 specifically, these belonged to the 'false-positive group'. If all non-T21 cases contained within this group were included in the calculations, the FPR in screening for T21 would increase by about 1.5%, irrespective of whether biochemical markers or cfDNA analysis was used.

Conclusion

Our study has shown that first-trimester screening for T21 that includes a detailed ultrasound examination as well as NT measurement and is followed by cfDNA testing is associated with a significant reduction in the FPR compared with conventional FTCS. This approach obviates the need for maternal serum free β-hCG and PAPP-A measurements in screening for fetal aneuploidy.

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Cenata GmbH (Tübingen, Germany) performed the cfDNA analysis. Roche/Ariosa Diagnostics, Inc. (San Jose, CA, USA) provided the kits for the Harmony® Prenatal Test.

Disclosure

M.S. is an employee of Roche Sequencing Solutions Inc.

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