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False-positive rates in screening for trisomies 18 and 13: a comparison between first-trimester combined screening and a cfDNA-based approach

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Abstract

Purpose To determine the false-positive rates (FPR) associated with screening for trisomy 18/13 using first-trimester combined screening (FTCS) and an ultrasound plus cfDNA-based approach (US-cfDNA), which includes a detailed ultrasound examination, a cfDNA analysis and a FTCS reflex backup test for cases with uninformative results.

Methods This is a sub-analysis of a randomized controlled trial, which was performed between 2015 and 2016. Pregnant women with a normal first-trimester ultrasound examination at 11–13 weeks' gestation (NT < 3.5 mm, no anomalies) were randomized into two groups: FTCS and US-cfDNA screening. The overall FPR in screening for trisomies 18/13 and 21 was compared with the FPR in screening for trisomy 21 alone. Pregnancies were considered screen positive if the risk for trisomy 21 was 1:100 and for trisomy 18 and 13, 1:20 each.

Results The study population consisted of 688 pregnancies in each study arm. In the FCTS group, median delta NT was 0.0 mm, free beta-hCG and PAPP-A 0.96 and 1.11 MoM. In the US-cfDNA group, median delta NT was 0.0 mm. In 10 pregnancies, the cfDNA analysis was uninformative. In the FTCS and in the US-cfDNA group, the FPR in screening for trisomy 21 was 2.5% and 0%. In both groups, the overall FPR was not increased by adding screening algorithms for trisomies 18 and 13.

Conclusion In conclusion, the addition of screening for trisomies 18 and 13 to screening for trisomy 21 does not significantly change FPR. This is true for both the FTCS and the US-cfDNA-based approach.

Keywords Aneuploidy · First trimester · Nuchal translucency · cfDNA · Trisomies 18 and 13

Introduction

Most antenatal screening programs for chromosomal abnormalities focus on trisomy 21 [1, 2]. So far, first-trimester combined screening (FTCS) based on maternal age, fetal nuchal translucency (NT) thickness, free beta-hCG and PAPP-A is considered as the gold standard. The detection rate is about 90% for a false-positive rate of 3–5% [3, 4]. Further improvement can be achieved if the additional ultrasound markers such as nasal bone (NB), tricuspid blood flow (TCF) and ductus venosus flow (DVF) are taken into account [1].

This approach is increasingly being challenged by cell-free DNA (cfDNA) screening [5, 6]. In a recently published randomized controlled trial, we compared the performance of FTCS to an approach, which includes a detailed ultrasound examination, a cfDNA analysis and a FTCS reflex backup test for cases with uninformative cfDNA test results [7]. In this study, a detailed ultrasound examination was performed prior to the randomization to either the cfDNA-based or the FTCS arms. In case of a significantly increased NT (≥ 3.5 mm) or in the presence of a fetal structural defect, the pregnancies were not randomized, and invasive testing was offered. The rationale for this management is the fact that

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chromosomal abnormalities other than those which cfDNA technology can detect are especially common in these two populations. Compared to FTCS, we observed a significantly lower false-positive rate in the cfDNA-based arm.

Both the FTCS and cfDNA-based screening tests use algorithms for trisomies 18 and 13 that are separate from screening for trisomy 21 [5, 8]. In affected cases, median fetal NT is about 5.5 and 4.0 mm, PAPP-A is 0.2 and 0.3 MoM and free beta-hCG is 0.2 and 0.5 MoM for trisomies 18 and 13, respectively [9]. In a study from the Fetal Medicine Foundation UK based on 56,376 normal cases, 122 cases with trisomy 18, and 61 with trisomy 13, the detection rate using each FTCS algorithm for trisomies 18 and 13 alone was about 91% and 87%, respectively. The false-positive rate was 0.2% for each trisomy. If used in combination with the trisomy 21 algorithm, the detection rate increased to 95% for both trisomies 18 and 13. This is due to the fact that some of the fetuses of the trisomies 18 and 13, which are missed by the trisomy 18 or 13 algorithm, are detected by the trisomy 21 algorithm. The false-positive rates of the three algorithms also overlap. Thus, the use of all three algorithms together increases the overall false-positive rate by only 0.1% instead of 0.4% [8]. Since the overall false-positive rate of screening for all three trisomies is less than simply adding their individual false-positive rates, all three algorithms need to be considered together to accurately evaluate the FTCS test performance for trisomies 18 and 13.

Gil et al. recently reviewed the test performance of fDNA in screening for trisomies 18 and 13. Their meta-analysis was based on more than 210,000 normal pregnancies, 563 trisomy 18 and 119 trisomy 13 cases. They found that the detection and false-positive rates were 97.9% and 0.04% for trisomy 18 and 99.0% and 0.04% for trisomy 13, respectively [5].

In the present study, we set out to determine the false-positive rates associated with screening for trisomy 18 and 13 using FTCS and the ultrasound and cfDNA-based approach, which includes a detailed ultrasound examination, a cfDNA analysis and a FTCS reflex backup test for cases with uninformative results. For this purpose, we performed a secondary analysis of our previously published randomized controlled trial with the addition of a larger, retrospective dataset.

Methods

This study makes use of two datasets. The data in the first cohort were obtained prospectively during a randomized controlled study [7]. The second dataset was obtained retrospectively and consists of women who underwent first-trimester risk assessment between 2009 and 2017. Both cohorts were examined at the Prenatal Medicine Department of the University of Tuebingen, Germany.

We perform FTCS at 11–13 weeks' gestation based on the crown-rump length and NT measurement as well as the biochemical markers free beta hCG and PAPP-A [2, 10]. Furthermore, a detailed ultrasound examination is performed in all cases and includes early fetal echocardiography. The examinations are performed in accordance with recommendations and the guidelines published by the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG) and the German Society of Ultrasound in Medicine (DEGUM) society by operators who are certified by the Fetal Medicine Foundation (FMF), UK [11, 12]. If the NT measurement is equal to or greater than 3.5 mm or if a fetal anomaly is identified, the patient is considered to be at high risk for chromosomal defects and is offered invasive testing without a formal calculation of aneuploidy risk. All clinically relevant ultrasound and screening information is recorded in the Viewpoint database (GE Healthcare, Munich). Outcome data are added as soon as they become available.

Prospectively collected dataset

Between October 2015 and December 2016, we performed a randomized controlled trial where we compared false-positive rates associated with first-trimester risk screening for trisomy 21 using FTCS and an ultrasound plus cfDNA-based approach which includes a detailed ultrasound examination, a cfDNA analysis and a FTCS reflex backup test for cases with uninformative results. The results of the original RCT were published elsewhere [7].

Briefly, this population consisted of unselected patients who presented to our unit for routine first-trimester screening. The initial step included nuchal translucency measurement and fetal anatomic survey. Only singleton pregnancies with a crown-rump measurement of 45–84 mm, fetal NT thickness of less than 3.5 mm, and those without major fetal structural defects were included in the trial. After signing an informed consent, the subjects were randomized to undergo one of the two screening protocols: FTCS group (risk assessment was based on maternal and gestational age, fetal NT thickness, maternal serum PAPP-A and free beta hCG levels) and an ultrasound plus cfDNA-based screening group. In this group, an additional sample of serum was collected and stored in anticipation of the fact that in a certain proportion of the cases, the cfDNA testing would be uninformative. In cases where no result was obtained by cfDNA analysis, the serum sample was used to measure the maternal free beta-hCG and PAPP-A levels and a risk assessment using FTCS (reflex test) was completed [13].

In the current sub-analysis, we compare the same two groups. However, in contrast to our previous two

publications where we evaluated screening for trisomy 21, in this study, we focus on the risk for trisomies 18 and 13.

Retrospectively collected dataset

To increase the number of cases available for our analysis, we performed an additional search of the Viewpoint database (GE Healthcare, Munich). Retrospectively, we identified all pregnancies that were referred to our department for first-trimester risk assessment between 2009 and 2017. In this cohort, we included only singleton pregnancies with a CRL measurement of 45–84 mm. The NT thickness had to be less than 3.5 mm and free beta hCG and PAPP-A MoM levels had to be available. To remain consistent with the parameters of our prospective trial, and since our primary outcome was false-positive rate, we excluded those with a fetal structural or chromosomal abnormality. Of note is that this cohort included both women presenting for routine FTCS and those who were referred to us due to an abnormal screening result elsewhere, the latter being due to the tertiary referral nature of our department.

In the prospectively and retrospectively collected FTCS groups and in cases with an uninformative cfDNA result, we calculated the individual risk of trisomies 18 and 13 as well as for trisomy 21 based on maternal age, fetal NT and the serum markers free beta hCG and PAPP-A. The risk of trisomy was computed based on the most recent FMF UK algorithm. According to the guidelines of the UK National Screening Committee, we considered a FTCS result as "high risk", if the risk for trisomy 21, 18 or 13 exceeded 1:150 at term. At the time of screening, this corresponds to a risk cut-off of 1:107 for trisomy 21 and 1:20 for each, trisomies 18 and 13, respectively [14]. For the purpose of simplicity, we used a risk cut-off at the time of screening of 1:100 for trisomy 21 and 1:20 for trisomies 18 and 13 each to define a high-risk result.

In the cfDNA-based arm, the cfDNA analysis was performed by Cenata GmbH (Tuebingen, Germany) using the Harmony® Prenatal Test (Roche, Inc. (San Jose, CA, USA)) as previously described [15–17]. The Harmony® prenatal test uses a threshold of 1:100 for each of the trisomies to define a high-risk result.

Approval for the prospective study was obtained from the local ethics committee (no. 572/2015BO1). The original study was registered in the International Standard Randomized Controlled Trial Number registry (ISRCTN no. 11174071). Approval for the retrospective study was also obtained from the local ethics committee (no. 531/2018BO2).

Statistical analysis

In the two prospective and the retrospective dataset, we computed the false-positive rates for each of the trisomy algorithms alone and the overall false-positive rate for the combination of the three trisomy algorithms. For the latter, we considered a pregnancy to be "high risk" if either the trisomy 21, 18 or 13 risk was above the respective threshold. This overall false-positive rate was compared with the false-positive rate based on the trisomy 21 algorithm alone.

The false-positive rates were compared with Clopper and Pearson's 95% confidence intervals.

Results

Prospective screening study cohort

The prospectively collected cohort consisted of 1400 pregnancies that were randomized either in the FTCS group or in the cfDNA-based group. Prior to randomization, we excluded 31 cases due to an increased NT or a major fetal defect. This group contained all three cases of trisomies 18 and 13 and all other chromosomal abnormalities (seven cases with trisomy 21, one with monosomy X and one with triploidy). In 24 cases, it was not possible to obtain the outcome of the pregnancy; thus, there were 688 euploid pregnancies in each group that were eligible for a further analysis.

Table 1 demonstrates the characteristics of the two study groups. The fetal characteristics are summarized in Table 2. There was no significant difference between the two study groups [7].

Table 1 Characteristics of the two prospectively and the retrospectively collected study cohort

Study characteristics	Prospective study cohort		Retrospective study cohort $n=7510$
	FTCS $n=688$	US + cfDNA $n=688$	
Maternal age in years median (IQR)	33.9 (30.7–36.7)	33.9 (31.0–36.8)	33.6 (30.6–36.7)
Gestational age in weeks median (IQR)	12.7 (12.3–13.1)	12.7 (12.4–13.1)	13.0 (12.7–13.4)
Caucasian, *n* (%)	676 (98.3)	672 (97.7)	7,319 (97.5)
Smoking, *n* (%)	23 (3.3)	19 (2.8)	311 (4.1)
Assisted reproduction, *n* (%)	29 (4.2)	44 (6.4)	225 (3.0)

Table 2 Pregnancy characteristics of the two prospectively and the retrospectively collected study cohort

Pregnancy characteristics	Prospective study cohort		Retrospective study cohort $n=7510$
	FTCS $n=688$	US + cfDNA $n=688$	
Crown-rump length in mm median (IQR)	67.6 (62.6 to 72.9)	67.7 (62.7 to 72.8)	66.8 (61.9 to 72.3)
Fetal NT in mm median (IQR)	1.9 (1.6 to 2.1)	1.8 (1.6 to 2.1)	1.8 (1.6 to 2.1)
DeltaNT in mm median (IQR)	0.0 (−0.2 to 0.2)	0.0 (−0.2 to 0.2)	0.0 (−0.2 to 0.2)
Free beta hCG in MoM median (IQR)	0.96 (0.64 to 1.44)		1.02 (0.70 to 1.55)
PAPP-A in MoM median (IQR)	1.11 (0.76 to 1.51)		1.01 (0.69 to 1.41)
Uninformative test results[a], *n* (%)		10 (1.5)	

[a]In the 10 cases with an uninformative cfDNA result, fetal NT, free beta hCG and PAPP-A were 1.7 (1.4–2.1) mm, 0.66 (IQR 0.37–0.90) MoM, and 0.73 (IQR 0.67–1.23) MoM

FTCS-based risk distributions for trisomies 18 and 13 as well as the risk distribution for trisomy 21 are shown in Table 3. The median risks for trisomies 18, 13, and 21 are 1:15,109 (IQR 1:7277–1:28,015), 1:43,360 (IQR 1:19,991–1:82,842), and 1:3787 (IQR 1:1606–1:8267), respectively. Based on their individual algorithms, none of the risk assessments indicated an increased risk for trisomy 18 and one (0.1%) showed an increased risk for trisomy 13. Using the trisomy 21 algorithm only, the risk of trisomy 21 was found to be increased in 17 [2.5% (95% CI 1.4–3.9%)] cases. If all three algorithms were used together, the total false-positive rate remained the same [17 cases, 2.5% (95% CI 1.4–3.9%)]. Thus, the overall false-positive rate did not change by the addition of the trisomy 18 and 13 algorithms to the trisomy 21 algorithm.

The median risk of the ultrasound and cfDNA-based approach, which includes a reflex backup test for cases with uninformative cfDNA test results, was 1:10,000 (IQR 1:10,000–1:10,000) for each of the three risk algorithms. Table 4 shows the risk distributions for trisomies 18, 13 and 21. The screen positive rate is 0% as none of the subjects were identified as at an increased risk by any of the algorithms used for the three trisomies. With the ultrasound plus cfDNA-based approach, the overall test positive rate was significantly lower than with the FTCS approach alone.

Retrospective study cohort

The effect of the combined use of all three FTCS risk algorithms on the overall false-positive rate was further investigated in a large retrospective cohort including 7510 euploid pregnancies. The study and pregnancy characteristics are summarized in Tables 1 and 2. The findings are similar to those in the prospective study cohort.

Table 5 demonstrates the risk distributions in screening for trisomies 18, 13 and 21 in this population. An increased risk for trisomy 21 was noted in 459 [6.11% (95% CI 5.58–6.68%)] cases. There were 6 (0.1%) and 9 (0.1%) cases where the risk was increased for trisomies 18 and 13, respectively. Only 3 screen positive cases were added if the combination of all three algorithms was used. Thus, the screen positive rate remained at 6.15% (95% CI 5.62–6.72%).

Of note is that the proportion of pregnancies with a positive screening result for trisomy 21 was higher in this group compared to the prospective study cohort. This is due to the fact that the subjects in the retrospective cohort included not only low-risk women but also those who were referred

Table 3 Risk distribution after first-trimester combined screening for trisomies 18, 13 and 21 in the prospective study cohort

Risk for trisomy in euploid fetuses	Trisomy 18 *n* (%)	Trisomy 13 *n* (%)	Trisomy 21 *n* (%)
> 1:10	0 (0)	1 (0.1)	0 (0)
1:10–1:99	3 (0.4)	0 (0)	17 (2.5)
1:100–1:999	14 (2.0)	6 (0.9)	79 (11.5)
1:1000–1:9999	222 (32.3)	80 (11.6)	465 (67.6)
≤ 1:10,000	449 (65.3)	601 (87.4)	127 (18.5)
False-positive rate[a]	0	1 (0.1)	17 (2.5)
Overall false-positive rate in screening for trisomies 18, 13 and 21[a]	17 (2.5)		

The risks were computed based on maternal age, fetal NT, free beta-hCG and PAPP-A

[a]Risk cut-off for trisomy 21 algorithm 1:100, trisomy 18 and 13 algorithms 1:20 each

Table 4 Risk distribution after ultrasound + cfDNA screening for trisomies 18, 13 and 21 in the prospective study cohort

Risk for trisomy in euploid fetuses	Trisomy 18 *n* (%)	Trisomy 13 *n* (%)	Trisomy 21 *n* (%)
> 1:10	0 (0)	0 (0)	0 (0)
1:10–1:99	0 (0)	0 (0)	0 (0)
1:100–1:999	1 (0.1)	0 (0)	2 (0.3)
1:1000–1:9999	3 (0.4)	2 (0.3)	5 (0.7)
≤ 1:10,000	684 (99.4)	686 (99.7)	681 (99.0)
False-positive rate[a]	0 (0)	0 (0)	0 (0)
Overall false-positive rate in screening for trisomies 18, 13 and 21[a]	0 (0)		

The risks were computed based on cfDNA testing including a reflex backup test for cases with uninformative cfDNA test results

[a]Risk cut-off for trisomy 21, 18 and 13 algorithms 1:100

Table 5 Risk distribution after first-trimester combined screening for trisomies 18, 13 and 21 in the retrospective study cohort

Risk for trisomy in euploid fetuses	Trisomy 18 *n* (%)	Trisomy 13 *n* (%)	Trisomy 21 *n* (%)
> 1:10	2 (0.0)	2 (0.0)	72 (1.0)
1:10–1:99	51 (0.7)	56 (0.7)	387 (5.2)
1:100–1:999	291 (3.9)	320 (4.3)	1,335 (17.8)
1:1000–1:9999	2353 (31.3)	1177 (15.7)	4440 (59.1)
≤ 1:10,000	4813 (64.1)	5954 (79.3)	1276 (17.0)
False-positive rate[a]	6 (0.1)	9 (0.1)	459 (6.1)
Overall false-positive rate in screening for trisomies 18, 13 and 21[a]	462 (6.1)		

The risks were computed based on maternal age, fetal NT, free beta-hCG and PAPP-A

[a]Risk cut-off for trisomy 21 algorithm 1:100, trisomy 18 and 13 algorithms 1:20 each

to our center due to abnormal screening results elsewhere. The women who were included in the prospective study, on the other hand, were only those who presented for routine screening.

Discussion

In this study, we have examined the false-positive rate in screening for trisomies 18 and 13 either by FTCS or by an ultrasound plus cfDNA-based approach. In practice, screening algorithms for these two trisomies are always used in conjunction with screening for trisomy 21. Therefore, one cannot look at false-positive rates in screening for trisomies 18 and 13 in isolation. The best estimate of how screening for these two trisomies alters the overall screen positive rate is to compare the false-positive rate of screening for trisomy 21 alone to the false-positive rate that is associated with screening for all three trisomies together. We have shown that the false-positive rates do not change significantly if one screens for all three trisomies or just trisomy 21. This holds true for both screening approaches used in our study. We have also shown that that the overall false-positive rate of FTCS was significantly higher than for the cfDNA-based approach.

We have concentrated on the false-positive rate as primary outcome parameter because in terms of absolute numbers, this is the most crucial screening parameter that is of major importance to physicians, health authorities, and patients alike.

The primary focus of this study was to perform a secondary analysis of a published prospective first-trimester screening study that compared the false-positive rates in screening for trisomy 21 using FTCS and an ultrasound and cfDNA-based approach, both implemented after excluding fetuses with NT ≥ 3.5 mm and/or those with structural anomalies. In contrast to the original study, we set out to examine the false-positive rate associated with screening for trisomies 18 and 13 alone and in combination with trisomy 21 algorithm. Unexpectedly, we noted that in the case of FTCS, the false-positive rate in screening for all three trisomies was essentially the same as in screening for trisomy 21 alone. To investigate this phenomenon further, we evaluated the false-positive rate in a larger dataset, which was collected retrospectively. In contrast to the randomized controlled study population, the retrospective dataset included not only low-risk women but also those who were referred to our unit

due to increased risk. Consequently, the false-positive rate in screening for trisomy 21 was higher in this cohort than in the RCT population. However, we again found that the overall false-positive rates in screening for all three trisomies and in screening for trisomy 21 alone were almost identical. This is due to the fact that there is a significant overlap between the three FTCS algorithms. The risk for each of the three trisomies increases with increasing NT measurement and decreasing PAPP-A level [1]. Therefore, most cases that had an increased risk for trisomies 18 and 13 will have been already identified as being at risk for aneuploidy based on the trisomy 21 screen. The fact that in retrospective dataset which consisted of low- and high-risk cases, the additional false-positive cases were de facto the same as in the prospective screening dataset highlights that this observation is consistent and independent from the adjusted risk for trisomy 21.

The very low false-positive rate, which we found in the ultrasound and cfDNA cohort, is consistent with previous studies. In a meta-analysis from the "German institute for Quality and Efficiency in Health Care (IQWIG)", the false-positive rates in screening for trisomies 18 and 13 by cfDNA were 0.06% and 0.04% with the corresponding detection rates being 93% and 84% [18]. In a meta-analysis by Gil et al., the false-positive rates were similar but the detection rates were higher: 98% and 99% for trisomies 18 and 13, respectively [5]. Petersen et al. published positive predictive values based on 450 abnormal cfDNA test results. The positive predictive values for trisomies 13, 18, and 21 were 45%, 76%, and 84%, respectively [19]. However, since positive predictive values are dependent on the prevalence of the aneuploidy in the population, these vary greatly according to maternal age, history, and ultrasound findings.

The false-positive rate patterns, which we found in the FTCS cohort, are also consistent with previous studies. In a large study based on more than 50,000 euploid pregnancies and 578 fetuses with common trisomies, the overall false-positive rate was 3.1%. It was 3.0% in screening for trisomy 21 only and, as a separate screen, 0.4% for trisomies 18 and 13 [8]. When the three algorithms were used jointly, the detection rates for trisomies 21, 18 and 13 were 91%, 97% and 94%, respectively. In contrast, the detections were only 90%, 91% and 87% if the three algorithms were applied separately.

In conclusion, the addition of screening for trisomies 18 and 13 to screening for trisomy 21 does not significantly change the false-positive rate. This is true for both the FTCS and the ultrasound and cfDNA-based approach.

Author contributions KOK: conceptualization, project development, formal analysis, funding acquisition, project administration, manuscript writing and editing; JS: manuscript writing and editing; AS: formal analysis; HA: formal analysis; PW: data collection and analysis; NP: data analysis; MH: manuscript writing and editing.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This is a secondary analysis of a randomized controlled study at the University of Tübingen. Approval for the prospective study was obtained from the local ethics committee (no. 572/2015BO1). The original study was registered in the International Standard Randomized Controlled Trial Number registry (ISRCTN no. 11174071). Approval for the retrospective study was also obtained from the local ethics committee (no. 531/2018BO2).

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