Thieme

First Trimester Contingent Screening for Aneuploidies with Cell-Free Fetal DNA in Singleton Pregnancies – a Swiss Single Centre Experience

Ersttrimesterscreening auf Aneuploidien mittels zellfreier fetaler DNA bei Einlingsschwangerschaften – Erfahrungen aus einem schweizerischen Level-3-Referenzzentrum

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ABSTRACT

Introduction
Switzerland was amongst the first countries to offer cell-free fetal DNA (cffDNA) testing covered by the health insurance to pregnant women with a risk ≥ 1 : 1000 for trisomies at first trimester combined screening (FTCS). The aim of this study is to evaluate the implementation of this contingent model in a single tertiary referral centre and its effect on gestational age at diagnosing trisomy 21.

Materials and Methods
Between July 2015 and December 2020 all singleton pregnancies at 11–14 weeks of gestation without major fetal malformation were included and stratified according to their risk at FTCS. Statistical analysis was performed by GraphPad Version 9.1 for Windows.

Results
4424 pregnancies were included. Of 166 (3.8 %) pregnancies with a NT ≥ 3.5 mm and/or a risk ≥ 1 : 10 at FCTS, 130 (78.3 %) opted for direct invasive testing. 803 (18.2 %) pregnancies had an intermediate risk, 692 (86.2 %) of them opted for cffDNA first. 3455 (78.1 %) pregnancies had a risk < 1 : 1000. 63 fetuses were diagnosed with trisomy 21, 47 (74.6 %) directly by invasive procedures after FTCS, 16 (25.4 %) by cffDNA first.

Conclusions
Most women choose cffDNA or invasive testing as second tier according to national guidelines. Despite the delay associated with cffDNA testing after FCTS, 75 % of all trisomy 21 are still diagnosed in the first trimester with this contingent screening model.

ZUSAMMENFASSUNG

Einleitung
Die Schweiz war eines der ersten Länder, das schwangeren Frauen mit einem ≥ 1 : 1000-Risiko für Trisomien von den Krankenkassen vergütete cffDNA-Tests (cffDNA: zellfreie fetal DNA) nach dem kombinierten Ersttrimesterscreening (FTCS) angeboten hat. Ziel dieser Studie war es, die Umsetzung dieses Screeningmodells in einem Level-3-Referenzzentrum und die Auswirkungen auf das Schwangerschaftsalter bei der Trisomie-21-Diagnose auszuwerten.

Material und Methoden
Zwischen Juli 2015 und Dezember 2020 wurden alle Einlingsschwangerschaften ohne fetale Fehlbildungen in den Schwangerschaftswochen 11–14 in die Studie aufgenommen und gemäß ihrem Risiko beim FTCS in Risikogruppen unterteilt. Die statistische Analyse wurde mit GraphPad Version 9.1 für Windows durchgeführt.

Ergebnisse
Insgesamt wurden 4424 Schwangerschaften in die Studie eingeschlossen. Von 166 (3,8 %) Schwangerschaften mit einer Nackentransparenz (NT) von ≥ 3,5 mm und/oder einem Risiko von ≥ 1 : 10 beim FTCS entschieden sich 130 (78,3 %) für direkte invasive Testmethoden. Bei 803 (18,2 %) Schwangerschaften wurde das Risiko als intermediär eingestuft, und 692 (86,2 %) der Schwangeren entschieden sich, erst einen cffDNA-Test durchzuführen. Bei 3455 (78,1 %) Schwangerschaften war das Risiko < 1 : 1000. 63 Feten wurden mit Trisomie 21 diagnostiziert, davon 47 (74,6 %) direkt durch invasive Eingriffe nach dem FTCS und 16 (25,4 %) erst mit cffDNA.

Schlussfolgerungen
Die meisten Frauen wählten cffDNA oder eine invasive Testmethode als Folgeuntersuchung gemäß den nationalen Richtlinien. Obwohl eine cffDNA-Analyse nach dem FCTS zu Verzögerungen führt, werden mit diesem Kontingent-Screening immer noch 75 % aller Fälle mit Trisomie 21 im 1. Trimester diagnostiziert.

Introduction

Screening for chromosomal aneuploidies was revolutionised by the detection of cell-free fetal DNA (cffDNA) and the development of non-invasive prenatal testing (NIPT) [1, 2]. While over decades, the detection rate (DR) of trisomy 21 could be improved from only 30% to 90% at a false positive rate (FPR) of 5% by first trimester combined screening (FTCS), cffDNA has a DR of Down syndrome of 99% at a very low FPR of 0.04% [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. The DRs for trisomy 13 and 18 are similar, as is the performance of cffDNA in twin pregnancies [9, 10, 11, 12]. Despite the excellent performance, cffDNA remains a screening test and confirmation of high risk cffDNA results through invasive testing is mandatory [13, 14]. Due to its high costs, most health care systems do not offer cffDNA screening to all pregnant women. Therefore, different models on direct cffDNA or contingent screening have been proposed [15, 16, 17].

In Switzerland all pregnant women are reimbursed for FTCS by their health insurance provider, including a detailed ultrasound exam by a certified sonographer with measurement of the fetal nuchal translucency (NT) as well as biochemical analysis. Invasive testing is covered by the health insurance in case of fetal anomalies seen in ultrasound, increased NT > 95th percentile or risk for any trisomy at FTCS ≥ 1 : 380.

Since July 2015, as one of the first countries worldwide, Switzerland implemented cffDNA into routine screening in a contingent manner. If at FTCS combining maternal age (MA) and NT with the biochemical serum markers β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) the risk for trisomy 21, 18 or 13 is ≥ 1 : 1000, cffDNA for these trisomies is offered as a second tier of screening to singleton and twin pregnancies [18, 19]. If the risk is < 1:1000 cffDNA can be performed at the patient's own cost. In singleton pregnancies, cffDNA for sex chromosomes is offered at no additional cost.

The aim of the study is to evaluate the implementation of this contingent screening for aneuploidies into routine screening in singleton pregnancies in a single tertiary referral centre in Switzerland and its effect on gestational age at diagnosing trisomy 21.

Material and Methods

Inclusion and exclusion criteria

We performed a retrospective analysis of all singleton pregnancies seen at our ultrasound department at the university hospital of Bern for first trimester screening between July 15th 2015 and December 31st 2020 who agreed to further use of their data. Inclusion criteria were singleton pregnancies, a fetal crown-rump length (CRL) of 45 to 84 mm, fetal viability and patient's acceptance of contingent first trimester screening. In addition, women referred for second opinion of a first trimester scan that showed an increased NT or increased risk at FTCS were included, if first trimester assessment for aneuploidies was not yet completed. Exclusion criteria were multiple pregnancies, pregnancies with major fetal malformations, refusal of general consent and referral after completed first trimester aneuploidy assessment for specific questions.

Data collection and risk stratification

The following risk factors were recorded: maternal age (MA), maternal BMI, maternal ethnicity, parity, mode of conception and nicotin abuse. Multiples of the medians for free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) were calculated by Viewpoint 5.6.25.284 (GE, Mountainview, CA, USA) based on the algorithm provided by the Fetal Medicine Foundation (FMF) London [20]. Screening was performed by expert sonographers according to the guidelines of the Swiss Society of Obstetrics and Gynecology (SGGG) [19]:

- In a first step, aneuploidy screening combining MA with NT, β-HCG and PAPP-A is offered to all women. The risk calculations were performed in Viewpoint 5.6.25.284 (GE, Mountainview, CA, USA) [20].
- In a second step, cffDNA is offered and covered by health insurance if the risk for any of the trisomies 13, 18 or 21 is ≥ 1 : 1000.

In case the NT is > 95th percentile the costs of an invasive procedure are covered by health insurance, in case the NT is ≥ 3.5 mm (99th percentile) or in case the risk at FTCS is ≥ 1 : 10 an invasive procedure is recommended [19, 21].

We stratified the patients according to their risk into four groups. Group "increased NT": NT ≥ 3.5 mm, group "high risk": risk for any trisomy 13, 18 or 21 ≥ 1 : 10, group "intermediate risk": risk for any trisomy < 1 : 10 to ≥ 1 : 1000 and group "low risk": risk for all trisomies < 1 : 1000. For historical reasons, in patients presenting a risk ≥ 1 : 380 for any trisomy, an invasive procedure is still covered by the insurance [19, 22]. Therefore the "intermediate risk" group is subdivided into subgroup 1 with a risk < 1:10 to ≥ 1:380 and subgroup 2 with a risk for any trisomy < 1:380 to ≥ 1:1000. Patient choices according to risk group and results of the different screening and testing options were analysed.

If a cffDNA was performed, we assessed the technique used and fetal fraction stated. cffDNA can be analysed by next generation sequencing (NGS) or more targeted sequencing using chromosome selective sequence analysis (CSS) or single nucleotide polymorphism (SNP)-based analysis [9, 23]. In case of a high risk cffDNA result, we analysed the results of subsequent karyotyping via invasive prenatal testing or postnatal diagnosis.

Statistical analysis

Statistical analysis was performed on GraphPad version 9.1 for Windows (GraphPad Software, San Diego CA). Continuous variables were analysed using the Student t-test or Kruskal-Wallis test, while proportions were evaluated utilising the Fisher's exact test or chi^2 test where appropriate. Statistical significance was considered achieved when p was less than 0.05.

Results

Between July 15th 2015 and December 31st 2020, 5106 singleton pregnancies were screened in the first trimester. 682/5106 (13.4%) were excluded due to various reasons: in 152 (3.0%) pregnancies fetal anomalies were found, 423 (8.3%) declined screening, 66 (1.3%) opted directly for an invasive procedure mostly due to known genetic problems in the family or in a previous pregnancy, 34 (0.7%) opted directly for cffDNA, 2 (0.04%) had preimplantation diagnosis and in 5 (0.1%) no screening was performed without any apparent reason noted. The remaining 4424/5106 (86.6%) patients agreed to first trimester screening. In 122/4424 (2.8%) pregnancies the NT was ≥ 3.5 mm (group "increased NT"), in 44/4424 (1.0%) the risk for any trisomy was ≥ 1 : 10 ("high risk" group), in 803/4424 (18.2%) the risk was < 1 : 10 to ≥ 1 : 1000 ("intermediate risk" group) and in 3455/4424 (78.1%) the risk was < 1 : 1000 ("low risk" group). In the "intermediate risk" group in 395/4424 (8.9%) the risk was < 1 : 10 to ≥ 1 : 380 (subgroup 1), in 408/4424 (9.2%) the risk was < 1 : 380 to ≥ 1 : 1000 (subgroup 2). Patient characteristics stratified according to risk group are depicted in ▶ **Table 1**. The risk parameters of aneuploidy screening are distributed as expected, maternal age was highest in the "high risk" group and lowest in the "low risk" group, the NT decreases significantly from group "increased NT" to the "low risk" group, b-HCG and PAPP-A decrease and increase respectively the same way.

Patient choices for further testing stratified according to risk group as well as the results of testing are depicted in ▶ **Table 2**. A steady decrease in patients opting for invasive procedures was noted from group "increased NT" with 107/122 (87.7%) to "low risk" group with 6/3455 (0.2%) (p < 0.0001). Opposite to invasive testing, patients opting for cffDNA increased from the "high risk" group with 20/44 (45.5%) to the "intermediate risk" subgroup 1 and 2 with 330/395 (83.5%) and 362/408 (91.6%) respectively. The number of high risk cffDNA results did not differ significantly between the two "intermediate risk" subgroups 1 and 2 with 11/330 (3.3%) and 8/362 (2.2%) respectively (p = 0.49); there is a trend towards more pathological results from invasive procedures in subgroup 1 compared to subgroup 2 with 4/38 (10.5%) and 0/4 (0%) respectively, however the difference is not significant (p > 0.99).

Detailed results for the different groups

All results of invasive testing and cffDNA are depicted in ▶ **Table 2** and ▶ **Table 3**. A few other considerations are as follows:

- In the "high risk" group, in 14/23 (60.9%) patients opting for invasive procedure the NT was ≥ 95th percentile, in the remaining 9/23 (39.1%) the NT was < 95th percentile.
- In the "intermediate risk" subgroup 1, 13/38 (34.2%) women opted for invasive procedure due to a NT ≥ 95th percentile and 25/38 (65.8%) due to an increased risk at FTCS with a NT < 95th percentile.
- In the "intermediate risk" subgroup 2, 3/4 (75%) of patients opting for invasive procedures had a fetus with a NT ≥ 95th percentile, 1/4 (25%) had a NT < 95th percentile but still a risk ≥ 1 : 1000. One couple opted for confirmation of trisomy 21 postnatally. No genetic testing was performed for the trisomy 18 cffDNA result on parents' request, and an apparently healthy child was delivered.
- In the "low risk" group, 6/3455 (0.2%) opted for invasive diagnoses, 3 due to a NT ≥ 95th percentile but still a risk < 1 : 1000 at FTCS. The invasive procedure revealed a VOUS on array-CGH in one case, further 3 women had an invasive procedure on

▶**Table 1** Patient characteristics stratified according to their risk category in first trimester screening for trisomies.

	Increased NT (N = 122)	**High risk** (N = 44)	**Intermediate risk** (N = 803)	**Low risk** (N = 3455)	**p**
Maternal age (years)	35 [30–37]	37 [34–40]	36 [33–39]	32 [28–35]	< 0.0001
Maternal BMI (kg/m^2)	24.9 [21.3–27.9]	22.9 [19.9–26]	23.3 [21.2–27.3]	23.3 [21.3–27.5]	ns
Ethnicity					
▪ Caucasian	65 (53.3)	37 (84.1)	642 (80.0)	2865 (82.9)	< 0.0001
▪ Black	3 (2.5)	2 (4.5)	61 (7.6)	244 (7.1)	ns
▪ South Asian	1 (0.8)	1 (2.3)	27 (3.4)	143 (4.1)	ns
▪ East Asian	0 (0)	1 (2.3)	35 (4.4)	86 (2.5)	ns
▪ Mixed	2 (1.6)	0 (0)	20 (2.5)	101 (2.9)	ns
▪ Unknown	51 (41.8)	3 (6.8)	18 (2.2)	16 (0.5)	< 0.0001
Parity					
▪ Nulliparous	45 (36.9)	20 (45.5)	362 (45.1)	1820 (52.7)	< 0.0001
▪ Multiparous	77 (63.1)	24 (54.5)	441 (54.9)	1635 (47.3)	< 0.0001
Smoking	6 (4.9)	5 (11.4)	46 (5.7)	270 (7.8)	ns
Mode of conception					
▪ Spontaneous	101 (82.8)	39 (88.6)	711 (88.5)	3163 (91.5)	0.0008
▪ OD	4 (3.3)	3 (6.8)	23 (2.9)	134 (3.9)	ns
▪ IVF	3 (2.5)	1 (2.3)	64 (8.0)	155 (4.5)	0.0003
▪ Unknown	14 (11.5)	1 (2.3)	5 (0.6)	3 (0.1)	< 0.0001
CRL (mm)	65.5 [60–72.2]	69.3 [62.1–72.8]	67.7 [62.1–73.5]	64.9 [59.7–70.2]	< 0.0001
NT (mm)	4.55 [3.8–6.4]	2.73 [2.2–3.1]	1.92 [1.67–2.3]	1.71 [1.5–2.0]	< 0.0001
b-HCG-MoM	na	1.8 [0.71–3.0]	1.2 [0.70–1.9]	0.94 [0.64–1.4]	< 0.0001
PAPP-A-MoM	na	0.45 [0.30–0.71]	0.69 [0.44–1.0]	1.2 [0.82–1.6]	< 0.0001

The results are depicted in medians [IQR] or absolute numbers (percentage). Continuous variables were analysed using Kruskal-Wallis test, proportions were evaluated utilising chi^2 test. CRL = crown rump length; IVF = in vitro fertilisation; NT = nuchal translucency; OD = ovulation drugs; na = not applicable; ns = not significant.
Group "increased NT": NT ≥ 3.5 mm. Group "high risk": FTCS-result ≥ 1 : 10 for trisomy 13, 18 and/or 21, but NT < 3.5 mm. Group "intermediate risk-subgroup 1": FTCS-result < 1 : 10 to ≥ 1 : 380 for trisomy 13, 18 and/or 21. Group "intermediate risk-subgroup 2": FTCS-result < 1 : 380 to ≥ 1 : 1000 for trisomy 13, 18 and/or 21. Group "low risk": FTCS-result < 1 : 1000 for any trisomy.

request, all of them with normal karyotypes. Of note, in this group 14/3455 (0.4 %) further patients opted for an invasive procedure in the second or third trimester of pregnancy, due to anomalies only diagnosed later in gestation: 4 due to intrauterine growth restriction; 3 due to cardiac anomalies (1 double outlet right ventricle, 1 Fallot tetralogy and 1 corrected transposition of the great arteries); 3 due to skeletal dysplasia; 2 due to esophageal atresia; 1 due to echogenic kidneys and 1 due to singular umbilical artery and lateral neck cysts. 4/14 (28.6 %) revealed a pathological result, none of them a trisomy. In 97 (2.8 %) cases cffDNA was performed at patient's own cost and no high risk result was found.

Details on cffDNA and invasive diagnosis

In total, 816 patients chose cffDNA testing. 781/816 (95.7 %) of the tests were performed with NGS, 6/816 (0.7 %) with targeted sequencing while in 29/816 (3.6 %) tests no further information was available. In 593 (72.7 %) cases, testing for sex chromosome aneuploidies (SCAs) was performed on parents' request.

In only 12/787 (1.5 %) cases cffDNA for rare autosomal trisomies (RATs) or segmental chromosomal aberrations was performed at the patient's own cost and request. Median fetal fraction [IQR] was 11.5 % [8.6–14.4 %]. In 9/787 (1.1 %) tests with a fetal fraction ≤ 4 % a result could be obtained; in 1/787 (0.1 %) case the fetal fraction was too low to obtain a result on multiple attempts. Overall, 29/816 (3.6 %) of the cffDNA revealed a high risk result (▶ **Table 2**). 16/19 (84.2 %) trisomy 21 cffDNA results were confirmed by karyotyping, 14 by invasive procedures, two postnatally using cord blood while in 3/19 (15.8 %) pregnancies a late

▶**Table 2** Patients choices stratified by risk group according to first trimester combined screening (FTCS) with the corresponding test results.

	Increased NT (N = 122)	**High risk** (N = 44)	**Intermediate risk-subgroup 1** (N = 395)	**Intermediate risk-subgroup 2** (N = 408)	**Low risk** (N = 3455)	**p**
Direct invasive procedure	107 (87.7)	23 (52.3)	38 (9.6)	4 (1.0)	6 (0.2)	< 0.0001
Pathological:	52 (48.6)	14 (60.9)	4 (10.5)	0 (0.0)	1 (16.7)	
▪ Trisomy 21	36 (33.6)	9 (39.1)	2 (5.3)	0 (0.0)	0 (0.0)	
▪ Trisomy 18	6 (5.6)	2 (8.7)	1 (2.6)	0 (0.0)	0 (0.0)	
▪ Trisomy 13	3 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
▪ Other	7 (6.5)	3 (13.0)	1 (2.6)	0 (0.0)	1 16.7)	
Normal	55 (51.4)	9 (39.1)	34 (89.5)	4 (100.0)	5 (83.3)	
cffDNA	7 (5.7)	20 (45.5)	330 (83.5)	362 (91.6)	97 (2.8)	< 0.0001
Pathological	2 (28.6)	8 (40.0)	11 (3.3)	8 (2.2)	0 (0.0)	
▪ Trisomy 21 confirmed	2 (28.6) yes	6 (30.0) 4/6 (2 mc)	8 (2.4) 7/8 (1 mc)	3 (0.8) 3/3	0 (0.0)	
▪ Trisomy 18 confirmed	0 (0.0)	2 (10.0) yes	0 (0.0)	1 (0.3) declined	0 (0.0)	
▪ Trisomy 13 comfirmed	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
▪ Other	0 (0.0)	0 (0.0)	3 (0.9)	4 (1.1)	0 (0.0)	
Normal	5 (71.4)	12 (60.0)	318 (96.4)	354 (97.8)	97 (100.0)	
No result	–	–	1 (0.3)	–	–	
No further testing	8 (6.6)	1 (2.3)	27 (6.8)	42 (10.3)	3352 (97.0)	< 0.0001

The results are depicted in absolute numbers (percentage). Proportions were evaluated utilising the chi^2 test.
"confirmed" = confirmed by invasive testing or postnatally using cord blood, "mc" = miscarriage.
Group "increased NT": NT ≥ 3.5 mm. Group "high risk": FTCS-result ≥ 1 : 10 for trisomy 13, 18 and/or 21. Group "intermediate risk- subgroup 1": FTCS-result < 1 : 10 to ≥ 1 : 380 for trisomy 13, 18 and/or 21. Group "intermediate risk-subgroup 2": FTCS-result < 1 : 380 to ≥ 1 : 1000 for trisomy 13, 18 and/or 21. Group "low risk": FTCS-result < 1 : 1000 for any trisomy.

miscarriage occurred before the invasive procedures could be carried out. Of the three cffDNA revealing a trisomy 18, two were confirmed by invasive diagnosis while in the third case no fetal anomalies were detected and an apparently healthy child was delivered without genetic testing. For 1/4 patients with a sex chromosome aneuploidy found on cffDNA a Klinefelter syndrome was confirmed by invasive diagnosis, the other three children were born apparently healthy without genetic testing. For 3/12 patients, expanded cffDNA testing revealed a high risk result, two were excluded by invasive testing, while in the third case an apparently healthy child was born.

Overall, 198 (4.67 %) invasive procedures were performed due to increased risk at FTCS. 178 (89.9 %) opted for direct invasive procedure after FTCS and 20 (10.1 %) after cffDNA revealed a high risk result. 138/198 (69.7 %) of all invasive procedures were performed on patients from the "increased NT" and "high risk" group, of the 4258 pregnancies with a risk < 1 : 10 at FTCS only 60 (1.4 %) had an invasive procedure.

In this cohort, 63 (1.4 %) pregnancies were diagnosed with trisomy 21, while in three more a high suspicion for trisomy 21 due to a high risk cffDNA test could not be confirmed, as the pregnancy resulted in a late miscarriage. 47/63 (74.6 %) diagnoses were made by direct invasive procedures and 16/63 (25.4 %) by cffDNA first and then confirmed by karyotyping. 8/63 (12.7 %) women continued the pregnancy, while the remaining 55/63 (87.3 %) opted for termination of the pregnancy (TOP). 11/4424 (0.25 %) pregnancies were diagnosed with trisomy 18, nine by direct invasive procedure, the same applies for the 3/4424 (0.07 %) pregnancies diagnosed with trisomy 13. All of them opted for TOP. 6/7 (85.7 %) pregnancies with SCAs were diagnosed by direct invasive procedures. Details of distributions of trisomies stratified by risk group are depicted in ▶ **Table 4**.

Discussion

This study shows that if cffDNA is offered in a contingent model to all women with a risk ≥ 1 : 1000 at FTCS, 21.9 % of all women are eligible to cffDNA as second screening step. While 86.2 % of women at intermediate risk accept cffDNA as second tier, 5.2 % opt for direct invasive testing, often related to a NT > 95th percentile. On the other hand, nearly 80 % of women at a very high risk opt directly for invasive procedures. Therefore, with this contin-

▶**Table 3** Pathological results other than common trisomies by invasive testing or cffDNA.

	Increased NT (N = 122)	**High risk** (N = 44)	**Intermediate risk-subgroup 1** (N = 395)	**Intermediate risk-subgroup 2** (N = 408)	**Low risk** (N = 3455)
45,X0	4 (3.3)		1 (0.3)	1 (0.2)	
▪ Direct invasive testing	4 (100.0)		1 (100.0)	0 (0.0)	
▪ cffDNA	0 (0.0)		0 (0.0)	1 (100.0)	
▪ confirmed by invasive testing				declined	
47,XXX			1 (0.3)	1 (0.2)	
▪ Direct invasive testing			0 (0.0)	0 (0.0)	
▪ cffDNA			1 (100.0)	1 (100.0)	
▪ confirmed by invasive testing			declined	declined	
47,XXY			1 (0.3)		
▪ Direct invasive testing			0 (0.0)		
▪ cffDNA			1 (100.0)		
▪ confirmed by invasive testing			yes		
92,XXXX	1 (0.8)				
▪ Direct invasive testing	1 (100.0)				
▪ cffDNA	0 (0.0)				
Trisomy 22				1 (0.2)	
▪ Direct invasive testing				0 (0.0)	
▪ cffDNA				1 (100.0)	
▪ confirmed by invasive testing				no	
VOUS	2 (1.6)	2 (4.5)			
▪ Direct invasive testing	2 (100.0)	2 (100.0)			
▪ cffDNA	0 0.0)	0 (0.0)			
Multiple Triploidies				1 (0.2)	
▪ Direct invasive testing				0 (0.0)	
▪ cffDNA				1 (100.0)	
▪ confirmed by invasive testing				declined	
Deletion on Chromosome 7		1 (2.3)			
▪ Direct invasive testing		1 (100.0)			
▪ cffDNA		0 (0.0)			
Duplication of Chromosome 15			1 (0.3)		
▪ Direct invasive testing			0 (0.0)		
▪ cffDNA			1 (100.0)		
▪ confirmed by invasive testing			no		

The results are depicted in absolute numbers (percentage). Group “increased NT”: NT ≥ 3.5 mm. Group “high risk”: FTCS-result ≥ 1 : 10 for trisomy 13, 18 and/or 21. Group “intermediate risk- subgroup 1”: FTCS-result < 1 : 10 to ≥ 1 : 380 for trisomy 13, 18 and/or 21. Group “intermediate risk-subgroup 2”: FTCS-result < 1 : 380 to ≥ 1 : 1000 for trisomy 13, 18 and/or 21. Group “low risk”: FTCS-result < 1 : 1000 for any trisomy.

gent model, 75 % of all cases with trisomy 21 are still diagnosed by direct invasive procedure at 11–14 weeks of gestation and diagnosis is not necessarily postponed into the second trimester, an important fact to consider, as nearly 90 % of all pregnancies diagnosed with trisomy 21 are terminated.

The excellent performance of cffDNA screening for trisomy 21 has been demonstrated in many trials [9, 10, 11, 12] and indeed in our study all of the 16 high risk cffDNA results not leading to late miscarriage were confirmed by invasive testing.

▶**Table 4** Diagnosis of fetal trisomies amongst the different groups stratified according to the initial test.

	Increased NT (N = 122)	**High risk** (N = 44)	**Intermediate risk-subgroup 1** (N = 395)	**Intermediate risk-subgroup 2** (N = 408)	**Low risk** (N = 3455)
Trisomy 21	38 (31.1)	13 (29.5)	9 (2.3)	3 (0.7)	0 (0.0)
▪ Invasive testing	36 (94.7)	9 (69.2)	2 (22.2)	0 (0.0)	0 (–)
▪ cffDNA first	2 (5.3)	4 (30.8)	7 (77.8)	3 (100.0)	0 (–)
Trisomy 18	6 (4.9)	4 (9.1)	1 (0.3)	0 (0.0)	0 (0.0)
▪ Invasive testing	6 (100.0)	2 (50.0)	1 (100.0)	0 (–)	0 (–)
▪ cffDNA first	0 (0.0)	2 (50.0)	0 (0.0)	0 (–)	0 (–)
Trisomy 13	3 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
▪ Invasive testing	3 (100.0)	0 (–)	0 (–)	0 (–)	0 (–)
▪ cffDNA first	0 (0.0)	0 (–)	0 (–)	0 (–)	0 (–)
Sex chromosome anomalies	5 (4.1)	0 (0.0)	2 (0.5)	0 (0.0)	0 (0.0)
▪ Invasive testing	5 (100.0)	0 (–)	1 (50.0)	0 (–)	0 (–)
▪ cffDNA first	0 (0.0)	0 (–)	1 (50.0)	0 (–)	0 (–)

The results are depicted in absolute numbers (percentage).
Group "increased NT": NT ≥ 3.5 mm. Group "high risk": FTCS-result ≥ 1:10 for trisomy 13, 18 and/or 21. Group "intermediate risk- subgroup 1": FTCS-result < 1:10 to ≥ 1:380 for trisomy 13, 18 and/or 21. Group "intermediate risk-subgroup 2": FTCS-result < 1:380 to ≥ 1:1000 for trisomy 13, 18 and/or 21. Group "low risk": FTCS-result < 1:1000 for any trisomy.

The introduction of cffDNA into routine screening depends on national health care systems, economic resources, costs of FTCS and cffDNA as well as training and regular certification of NT assessment and cffDNA testing and finally also social acceptance [24, 25].

In Switzerland first trimester ultrasound screening as part of routine pregnancy care has a high uptake and acceptance [26]. As recently published, about 90% of women presenting for first trimester ultrasound decide to screen for fetal trisomies [26]. Switzerland was one of the first countries worldwide to implement cffDNA into routine screening in 2015 [18]. Our results demonstrate high adherence to the proposed model. 78.3% of all women with a very high risk for aneuploidies, either ≥ 1:10 at FTCS or a NT ≥ 3.5 mm, opt for direct invasive procedures, while 86.2% of women at intermediate risk FTCS between < 1:10 to ≥ 1:1000 chose cffDNA as second tier. Most women at low risk at FTCS continue the pregnancy without any further testing, we are only aware of 2.8% of women from the "low risk" group to opt for further testing, however some might have chosen to do so elsewhere without our knowledge.

Different results are published from other European countries. Denmark was the first country worldwide to offer FTCS to all pregnant women; since 2017 cffDNA is offered as an alternative to invasive testing for pregnancies at high risk, such as, amongst other criteria, the risk for trisomy 21 > 1:300 [27]. Only 20% of the women choose cffDNA over invasive testing in this high risk cohort [27]. The Netherlands was the last European country to implement prenatal screening [28]. In a nationwide trial, the TRIDENT 1 trial (Trial by Dutch Laboratories for Evaluation of Non-invasive prenatal Testing), cffDNA was offered to women with a risk > 1:200 in FTCS; 85% chose cffDNA over invasive procedures [29]. Of note, as the FTCS in the Netherlands is not free of charge, the uptake in 2016 was only 34% [28]. The Danish argue that women prefer to gain additional information from chromosomal microarray testing and therefore opt for invasive testing. In our population this additional information seems of less interest or the fear of complications of invasive procedures outweighs the need for more information. This is also interesting in the context of the possibility to expand cffDNA to RATs or segmental chromosomal aberrations or even whole genome screening. The Dutch TRIDENT-1 study showed that in high risk populations, many additional findings were indeed relevant. However, so far the low positive predictive value (PPV) known from screening for microdeletions and SCAs leads to a restrictive attitude, additionally there are ethical concerns towards whole genome cffDNA [30]. Our results do not answer the question of whether such expanded cffDNA screening is of interest to our population, however the rather restrictive attitude towards invasive testing even considering SCAs found on cffDNA or very high risk ≥ 1:10 at FTCS seems to justify the assumption that expanded diagnosis is not generally desired. The low uptake of expanded cffDNA screening at own costs also veers towards the same conclusion.

The comparison with other German speaking countries shows that in Germany FTCS is not covered by health insurance, however cffDNA testing is since 2022 [31]. In our cohort 3% of all pregnancies were diagnosed with a fetal anomaly at first trimester screening and were excluded from this analysis, most women opted for a direct invasive procedure in that case. We have no data on perfor-

mance of cffDNA screening without prior FTCS, however we assume that diagnosis of trisomies and possibly other pathologies is more often postponed into the second trimester with cffDNA screening only. Austria momentarily does not offer FTCS or cffDNA screening on a national basis but only on indication [32]. We strongly believe that FTCS and cffDNA in a contingent matter is superior to screening by cffDNA only or screening on indication only in order to detect trisomies.

Our results do not allow to draw any conclusions on PPV of SCAs found by cffDNA and three of four women declined further testing during or after pregnancy. Accordingly, cffDNA for SCAs should not be offered routinely. However, as most parents opt to find out the gender of the child, such results will remain incidental findings. This issue should be addressed very carefully in counseling as the current literature emphasises the lack of data on cffDNA test performance for SCAs in average risk pregnancies and the great variability of PPV across different aneuploidies due to their varying prevalence, and therefore testing for fetal gender could lead to results difficult to manage [33].

The chosen cut-off of ≥ 1 : 1000 in Switzerland was based on published models [34]. While at any given cut-off some cases of trisomy 21 will go undiagnosed, our results show a very low incidence of Down syndrome in the risk group < 1:380 to ≥ 1:1000, suggesting that only very few cases are missed in the group at risk < 1:1000, however we do not have the outcomes of all pregnancies to confirm that. Therefore, it seems reasonable to promote such a model of contingent screening for the general population until the costs of cffDNA come close to the costs of the biochemical markers βHCG and PAPP-A. The historically defined cut-off of ≥ 1:380 to reimburse invasive testing however according to our results could be abandoned as all women in whom a trisomy was diagnosed at a risk < 1:100 at FTCS opted for cffDNA anyway.

The only problem with contingent screening is still the time delay in obtaining cffDNA results. This shifts the diagnosis of trisomies away from the first and back into the second trimester, an achievement from the past 30 years of screening that is lost. Even though 75 % of all our diagnoses of trisomy 21 are based on direct invasive testing, it remains of utmost importance to accelerate cffDNA analysis for trisomy 21. Otherwise, and if cffDNA at some stage will be offered to all pregnant women in Switzerland due to drastic lowering of the costs or health care decisions, even offering cffDNA before a detailed first trimester scan will need to be discussed. Our results of contingent screening however are based on a detailed first trimester ultrasound excluding fetal anomalies. While pregnancies with fetal anomalies are at much higher risk of aneuploidies, only after their exclusion, 78 % of women can safely be considered at low risk without any need for further testing.

Conclusions

Offering cffDNA testing to all women at a risk > 1 : 1000 in FTCS for trisomy 13, 18, or 21 while recommending direct invasive procedures to those at a very high risk allows timely diagnosis of trisomy 21 within the first trimester scan in 75 % of patients, at an overall invasive procedure rate of 4.5 %.

Contributors' Statement

B.M. conceived and designed the study. A.P., L.R. and B.M. gathered the data and conducted the statistical analysis. F.B. and L.R. provided missing data. A.P. and B.M. wrote the first draft. F.T., S.S., S. A., L.R. and D.S. contributed essentially with intellectual input into the study design, result analysis and in the revision of the manuscript. All authors have read and approved the manuscript.

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Conflict of Interest

Florent Badiqué and Lorenz Risch work in one of the laboratories providing the cffDNA results, however they were not involved in the analysis of the results.

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