Seeing the unseen

The importance of prenatal screening

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CHAPTER 8

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ELIMINATING FIRST TRIMESTER COMBINED TESTING: CONSEQUENCES FOR EARLY DETECTION OF *significant fetal anomalies*

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Abstract

Objective: To determine whether implementation of cell-free DNA (cfDNA) testing for aneuploidy as first-tier test and subsequent abolition of first trimester combined testing (FCT) affected the first trimester detection (<14 weeks) of certain fetal anomalies.

Methods: We performed a geographical cohort study in two Fetal Medicine Units between 2011 and 2020 including 705 fetuses with prenatally detected severe brain, abdominal wall and congenital heart defects. Cases were divided into two groups: before (n=396) and after (n=309) cfDNA introduction. Primary outcome was the first trimester detection rate (<14 weeks) overall and for non-chromosomal anomalies solely.

Results: Overall, gastroschisis, AVSD and HLHS were detected more often in first trimester in the before compared to the after group, respectively 54.5% vs 18.5% (P =0.004), 45.9% vs 26.9% (P = 0.008) and 30% vs 3.4% (P =0.005). After exclusion of chromosomal anomalies identifiable through cfDNA testing, the detection of AVSD remained higher in the before group (43.3% vs 9.5%, P = 0.02), leading to a possible earlier gestation at termination (TOP). The TOP rate did not differ among the groups. In the after group, referrals for suspected anomalies following a dating scan between 11-14 weeks significantly increased from 17.4% to 29.1% (P <0.001).

Conclusion: This study underscores the value of a scan dedicated to fetal anatomy in the first trimester as we observed a decline in the early detection of certain fetal anomalies (detectable in the first trimester) subsequent to the abolition of FCT.

Introduction

Prenatal screening has changed tremendously in the recent years.[1, 2] Non-invasive-prenatal testing (NIPT), based on the analysis of cell free DNA (cfDNA), is replacing first trimester combined testing (FCT) as the first-tier screening test for common fetal aneuploidies (trisomy 21, 18 and 13) worldwide.[3-5] Moreover, routine ultrasound screening in the first trimester has become increasingly important and available. A first trimester anomaly scan (FTAS) carried out between 11-14 weeks according to a standardized protocol, has the potential to identify all cases with fetal structural anomalies as acrania or holoproscencephaly, abdominal wall defects and close to 90% of atrioventricular septal defects (AVSD) and hypoplastic left heart syndrome (HLHS).[6]

In the Netherlands, FCT was introduced in January 2007 as part of the national screening program and all pregnant individuals could choose for FCT on a voluntary basis. In April 2014 cfDNA screening was introduced as a second-tier test after an increased risk for aneuploidy based on FCT or increased risk based on preceding pregnancy with trisomy 21, 18 or 13.[7] From 2017 the implementation of the cfDNA was evaluated as a first-tier screening test.[8] As a result of the superior test characteristics of cfDNA screening, the uptake of FCT declined dramatically over the years and is no longer offered to future parents as of October 2021.[9] For both FCT and cfDNA screening, a financial contribution of €175 was required until April 2023, irrespective of the type of insurance.[8-10]
Besides prenatal screening for fetal aneuploidies, the Dutch government offers two reimbursed ultrasound scans in pregnancy. An early scan is offered around 10 weeks of gestation for pregnancy dating followed by a second trimester anomaly scan (STAS) to detect fetal structural anomalies.[10, 11] Since September 2021, a first trimester routine anomaly scan at 13 weeks is offered, but only in a research setting and not part of standard care.[12]

With the introduction of cfDNA screening as a first-tier test, the first trimester scan conducted between 11 and 14 weeks' pregnancy as part of FCT rapidly declined in our country.[6] We hypothesized that this decline could impede early detection of certain fetal anomalies. The aim of our study was to investigate the first trimester detection of fetal structural anomalies visible in the first trimester (chromosomal and non-chromosomal) over a 10-year period, evaluating the time periods before and after the introduction of cfDNA screening and prior to the introduction of the first trimester routine anomaly scan at 13 weeks in a research setting.

Methods

Design and participants

This is a retrospective cohort study of all prenatally detected cases with a certain fetal anomaly diagnosed in the two Fetal Medicine Units in the Amsterdam region, Amsterdam UMC location AMC and VUmc between January 2011 and December 2020. The Medical Ethics Com-

mittee of the Amsterdam UMC approved this study (W21_029). This manuscript is written in alignment with Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[13] We focused on fetal anomalies that are detectable in the first trimester of pregnancy[6] and we classified them according to one of the main anomaly groups: severe brain anomalies, abdominal wall defects or severe congenital heart defects (CHD). Severe brain anomalies included, acrania-exencephaly-anencephaly sequence, alobair holoproscencephaly and encephalocele. Abdominal wall defects included omphalocele, gastroschisis and other abnormalities associated with abdominal wall defects (body stalk anomaly, pentalogy of Cantrell, OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects). Severe CHDs included atrioventricular septal defects (AVSD) and hypoplastic left heart syndrome (HLHS).

All patients in this study were asked if they would receive information on prenatal screening and if so received information on the first trimester screening for fetal aneuploidies (either FCT or cfDNA screening according to the time period) and ultrasound screening for structural anomalies In the Netherlands, pre-test counseling is centrally organized and regulated, administered by certified and highly trained counselors. The quality of prenatal screening is consistently monitored and evaluated to ensure high standards of care.[12] FCT combines the analysis of maternal serum screening of free beta-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A), an ultrasound in first trimester between 11 and 13+6 weeks of gestation to measure NT together with maternal age. FCT was considered abnormal when the risk of fetal trisomy 21, 18 or 13 was increased to >1:200 for either one of the three trisomies.

Additionally, all pregnant individuals were all offered a reimbursed dating scan and a second trimester routine anomaly scan (STAS). First trimester screening (either FCT or cfDNA screening) was not offered in case of a NT measurement ≥3.5 mm (>99th percentile) or when fetal anomalies were observed on ultrasound, given the high a priory risk for chromosomal anomalies. In these cases, patients were referred to a Fetal Medicine Unit (FMU) for an advanced ultrasound scan and offered invasive prenatal genetic testing for chromosomal analysis. Moreover, in case of an increased risk for fetal aneuploidy after FCT (used cut-off risk in the Netherlands was 1:200) or abnormal cfDNA screening, patients were also referred for counselling on invasive diagnostics. In line with policies from other countries, chorionic villus sampling (CVS) was performed from 11 weeks' gestation and amniocentesis from 16 weeks' gestation onwards [14, 15]. Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) analysis was routinely used and from 2012 onwards, chromosome microarray analysis (CMA) was performed if QF-PCR was normal. If QF-PCR was abnormal for chromosome 13, 18, or 21 karyotyping was performed to determine heredity. Whole exome sequencing (WES), the most current high standard test, is offered prenatally from 2020 onwards in cases with multiple ultrasound anomalies with normal QF-PCR and CMA and for cases with severe CHD, preceding postnatal cardiac surgery.

Study Group and coding/definitions

We obtained all cases with certain fetal anomalies that are potentially detectable in first trimester,

irrespective of additional anomalies or chromosomal anomalies from our prenatal database. Secondly, cases were divided in two groups, *before* (01-01-2011 until 01-04-2017) and *after* (01-04-2017 until 31-12-2020), the evaluation of cfDNA as a first-tier screening test. Subsequently, cases were allocated to one of the groups based on the date of the ultrasound examination in our FMU. If there were multiple pregnancies with the same underlying genetic condition causing a fetal anomaly one family, only the first pregnancy was included. Cases with a physiological omphalocele (an umbilical herniation before 12 weeks of gestation of which the size is not larger than the abdomen and without the presence of extracorporeal liver[16, 17]) were excluded from our study. If multiple congenital anomalies were present, we classified cases according to their dominant lesion (for example, anencephaly combined with a small omphalocele was coded as anencephaly, omphalocele with polydactyly was coded as omphalocele, AVSD with talipes equinovarus was coded as AVSD). Both balanced and unbalanced AVSD were included. Hypoplastic left heart syndrome included cases with and without mitral valve stenosis or atresia. Gestational age at detection was based on the first ultrasound in our FMU. We defined a chromosomal anomaly as any aneuploidy or chromosomal aberration probably detectable by cfDNA screening (>10 size resolution Mb).

Data collection

We evaluated the local obstetric databases and electronic patients records to collect information on maternal and fetal characteristics; maternal age, obstetric history, positive family history for congenital anomalies, singleton or multiple gestation, type of ultrasound leading to referral, gestational age according to dating scan, participation on prenatal screening (FCT or cfDNA), NT measurement, gestational age at detection, structural anomalies on ultrasound. We retrieved information on additional ultrasound examinations, possible invasive testing (pre- or postnatally) and pregnancy outcome. Postpartum follow-up was obtained to confirm the diagnosis. In case of a live birth, final diagnosis of the fetal anomaly was based on the results of postnatal examination. In cases resulting in termination of pregnancy, miscarriage or stillbirth, final diagnosis of the fetal anomaly was based on post-mortem examination, and if not performed, based on the last ultrasound.

Outcome measures

Our primary outcome was first trimester detection rate (<14 weeks of gestation) of fetal structural anomalies before and after the introduction of cfDNA. We analyzed the total group and the group with exclusion of chromosomal anomalies identifiable through cfDNA testing separately, to minimize the effect of abnormal prenatal screening as possible confounder. Secondly, we evaluated the type of scan initiating referral, gestational age at referral, pregnancy outcome, termination of pregnancy (TOP) rate and gestational age of TOP. We described the primary outcome as number with percentages. We described continuous data as a mean with standard deviation (SD) or median with interquartile rage (IQR) when appropriate. To test for associations between categorical

variables, Chi-square test or Fisher-exact test was used. For numerical variables, comparisons were made using one-way ANOVA or Kruskal-Wallis when appropriate. We considered P <0.05 to be statistically significant. Statistical analyses were done using IBM Corp SPSS Statistics version 25.0 (IBM, Armonk, NY, USA) and RStudio version 4.0.3.[18]

Results

Study population

A total of 705 fetuses with either severe brain-and skull anomalies, abdominal wall defects or severe congenital heart defects (CHDs) were included in this study, 396 fetuses in the before cfDNA group and 309 in the after cfDNA group. The proportion of brain-and skull defects, abdominal wall defects and severe CHDs did not differ between the two groups, neither did the specific anomalies within the groups (Table 1). Mean maternal age was 33 years (SD=5.9) and median BMI and percentage of singleton gestation were similar among the two groups. We found no significant differences in the number of chromosomal anomalies with 43.7% (173/396) in the before group versus 40.7% (126/309) the after group (Table 1).

Table 2 presents the prenatal detection of the included certain fetal anomalies categorized into five gestational age groups over a 10 years period. The prenatal detection rate before 11 weeks of gestation was highest among "other abdominal wall defects" which includes body stalk anomaly, pentalogy of Cantrell and OEIS complex, with a prenatal detection rate of 22.2% (4/18) followed by cases with anencephaly (19.4%, 21/108) (Table 2). Overall, 67.2% (121/180) of severe brain- and skull defects were detected before 14 weeks of gestation with the highest detection rate of anencephaly within this group (83.3%, 90/108). Of the abdominal wall defects, 67.2% were detected before 14 weeks of gestation and 33.2% of the severe CHD's were detected before 14 weeks (Table 2).

Indication for scan leading to referral

Table 3 presents the indication for the scan leading to referral, divided into five gestational age groups. Cases were mainly referred between 11-14 weeks of gestation (51.7%, 365/705) and mostly because of suspected anomalies at a dating scan (22.5%,159/705) with a mean gestational age of 11+3 days (SD=7). In the after group, referrals for suspected anomalies following a dating scan between 11-14 weeks significantly increased from 17.4% (69/396) to 29.1% (90/309) (difference: 11.7% 95 CI 0.05 – 0.18, P <0.001). The gestational age of the dating scan did not differ between the two groups, with a mean gestational age of 11+2 (SD=7) in the before versus 11+3 (SD=7) in the after group. Referral because of abnormal prenatal screening, an increased NT or anomalies at STAS were similar among the two groups. In the before group, FCT was performed in 28% (111/396) with 46.8% (52/111) referred because of an increased risk of aneuploidy and 53.1% (59/111) because of anomalies at the FCT scan. Mean gestational age of FCT scan performance was 12+3 (SD=4).

Table 1. Baseline characteristics of included cases separated per time period

	Total n = 705	Before cfDNA n = 396	After cfDNA n = 309	
Parameter				P-value
Maternal age (mean,sd)	32.9 (5.9)	33.0 (6.1)	32.8 (5.8)	0.55
BMI (mean, sd)	24.4 (4.5)	24.2 (4.5)	24.7 (4.5)	0.15
Singleton gestation n (%)	663 (93.9)	370 (93.4)	293 (94.8)	0.54
Anomaly groups n (%)				
Severe brain-and skull defects	180 (25.6)	103 (26.2)	77 (24.9)	0.81
Anencephaly	108 (15.3)	61 (15.4)	47 (15.2)	1.00
Holoprosencephaly	54 (7.7)	32 (8.1)	22 (7.1)	0.74
Encephalocele	18 (2.6)	10 (2.5)	8 (2.6)	1.00
Abdominal wall defects	269 (42.0)	144 (36.3)	125 (40.4)	0.30
Omphalocele	191 (27.1)	101 (25.6)	90 (29.1)	0.32
Gastroschisis	60 (8.5)	33 (8.3)	27 (8.7)	0.96
Other †	18 (2.6)	10 (2.5)	8 (2.6)	1.00
Severe CHDs	256 (36.3)	149 (37.5)	107 (34.6)	0.46
AVSD	187 (26.5)	109 (27.5)	78 (25.2)	0.55
HLHS	69 (9.8)	40 (10.1)	29 (9.4)	0.85
Chromosomal anomalies ‡ n (%)	299 (42.4)	173 (43.7)	126 (40.7)	0.48
Trisomy 21	95 (13.4)	55 (13.8)	40 (12.9)	0.80
Trisomy 18	110 (15.6)	63 (15.9)	47 (15.2)	0.88
Trisomy 13	37 (5.2)	19 (4.8)	18 (5.8)	0.66
Triploidy	20 (2.8)	9 (2.3)	11 (3.6)	0.49
Monosomy X	13 (1.8)	9 (2.3)	4 (1.3)	0.50
Other §	24 (3.4)	18 (4.5)	6 (1.9)	0.09
Outcome n(%)				
Live birth	115 (16.3)	56 (14.1)	59 (19.1)	0.10
TOP	500 (70.9) ¶	287 (72.5)	213 (68.9)	0.35
IUFD	62 (8.8)	37 (9.3)	25 (8.1)	0.65
NND	28 (4.0)	16 (4.1)	12 (3.9)	1.00

Abbreviations: CHD, congenital heart defect; AVSD, atrioventricular septal defect; HLHS; hypoplastic left heart syndrome; TOP, termination of pregnancy; IUFD, intrauterine fetal death; NND, neonatal death.

† Other includes abnormalities usually accompanied by abdominal wall defects: body stalk anomaly, pentalogy of Cantrell, and OEIS complex

‡ Confirmed by invasive diagnostics or postnatal fetal blood or biopsy.

§ Other unbalanced translocations, deletions, duplications, inversions and mosaicisms and other chromosomal aberrations >10Mb that would have been detected with the additional findings by cfDNA.

¶ 4 of 500 were selective embryo reduction in twins.

Table 2. Prenatal detection and outcome of major fetal anomalies over a 10 years 'period

		Prenatal detection rate					Pregnancy outcome			
	Total	< 11 weeks	11-14 weeks	14-18 weeks	18-24 weeks	>24 weeks	TOP	IUFD	NND	Live birth
Defect	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Severe brain- and skull defects	180	22 (12.2)	99 (55.0)	29 (16.1)	30 (16.7)	0 (0)	160 (88.9)	7 (3.9)	10 (5.6)	3 (1.7)
Anencephaly	108	21 (19.4)	69 (63.9)	10 (9.3)	8 (7.4)	0 (0)	97 (89.8)	4 (3.7)	7 (6.5)	0 (0)
Holoprosencephaly	54	0 (0)	23 (42.6)	14 (25.9)	17 (31.5)	0 (0)	48 (88.8)	3 (5.6)	3 (5.6)	0 (0)
Encephalocele	18	1 (5.6)	7 (38.9)	5 (27.8)	5 (27.8)	0 (0)	15 (83.3)	0(0)	0 (0)	3 (1.7)
Abdominal wall defects	269	16 (5.9)	176 (65.4)	30 (11.2)	43 (16.0)	4 (1.5)	156 (60.0)	36 (13.4)	7 (2.6)	70 (26.0)
Omphalocele	191	11 (5.8)	144 (75.4)	15 (7.8)	18 (9.4)	3 (1.6)	131 (68.6)	27 (14.1)	4 (2.1)	29 (15.2)
Gastroschisis	60	1 (1.7)	22 (36.6)	12 (20.0)	24 (40.0)	1 (1.7)	12 (20.0)	5 (8.3)	2 (3.3)	41 (68.3)
Other †	18	4 (22.2)	10 (55.6)	3 (1.7)	1 (5.6)	0 (0)	13 (72.2)	4 (22.2)	1 (5.6)	0 (0)
Severe CHDs	256	0 (0)	85 (33.2)	40 (15.6)	124 (48.4)	7 (2.7)	184 (71.8)	19 (7.4)	11 (4.4)	42 (16.4)
AVSD	187	0 (0)	72 (38.5)	32 (17.1)	77 (41.1)	6 (3.2)	130 (69.5)	16 (8.6)	7 (3.7)	34 (18.2)
HLHS	69	0 (0)	13 (18.8)	8 (11.6)	47 (68.1)	1 (1.4)	54 (78.3)	3 (4.3)	4 (5.8)	8 (11.6)

Percentage are displayed as row %. Abbreviations: CHD, congenital heart defect; AVSD, atrioventricular septal defect; HLHS, hypoplastic left heart syndrome; TOP, Termination of pregnancy; IUFD, Intrauterine fetal death, NND, neonatal death

† Other includes abnormalities usually accompanied by abdominal wall defects: body stalk anomaly, pentalogy of Cantrell, and OEIS complex.

First trimester detection rate between groups

No differences were found in first trimester detection of anencephaly, holoprosencephaly and encephalocele between the two groups (Table 4). The first trimester detection rate for all abdominal wall defects was comparable between groups with 75.7% (109/144) detected in the before and 67.2% (84/125) in the after group (*P* =0.123). The slight decline in the after group was mainly due to the significantly lower detection of gastroschisis (Table 4). CHDs were detected more often in first trimester in the before group, with 41.6% (62/149) compared to 20.6% (22/107) in the after group (*P* <0.001). This applied to both AVSD (45.9% vs 26.9%; 95%CI 0.05-0.32, *P* =0.008) as HLHS (30% vs 3.4%: 95% CI 0.11 – 0.42 *P* =0.005). (Table 4).

In the before group, 37.5% (15/40) of HLHS cases were referred between 11-14 weeks of gestation, which is significantly more often compared to the after group 6.9% (2/29) (95% CI 0.12-0.48, *P* = 0.003). Of the 15 cases that were referred between 11-14 weeks in the before group, 40% (6/15) had an increased NT at the dating scan, 20% (3/15) suspected HLHS at FCT, and 40% (6/15) an increased risk at FCT. In the after group, the two cases were referred because of an increased NT at the dating scan.

First trimester detection rate between groups for non-chromosomal anomalies

First trimester detection rate of non-chromosomal severe brain-and skull anomalies was similar among groups (Table 5). As no chromosomal anomalies were found in cases with gastroschisis,

Table 3. Type of scan and indication leading to referral

Type of scan at referral	Total n = 705	Before cfDNA n = 396	After cfDNA n = 309
<11 weeks n (%)	95 (13.4)	51 (12.9)	44 (14.2)
Anomalies at dating scan	74 (10.5)	39 (9.8)	35 (11.3)
Early increased NT	21 (3.0)	12 (3.0)	9 (2.9)
11-14 weeks n (%)	365 (51.7)	216 (54.5)	149 (48.2)
Anomalies at dating scan	159 (22.5)	69 (17.4)	90 (29.1)
Increased NT	58 (8.2)	32 (8.1)	26 (8.4)
Anomalies at FCT scan	63 (8.9)	59 (14.9)	4 (1.3)
Increased risk FCT	52 (7.4)	52 (13.1)	0 (0)
Abnormal NIPT	32 (4.5)	3[†] (0.75)	29 (9.4)
Other indication for scan [§]	1 (0.14)	1 (0.25)	0 (0)
14-18 weeks n (%)	36 (5.1)	14 (3.5)	22 (7.1)
Anomalies at dating scan	7 (1.0)	4 (1.0)	3 (1.0)
Gender reveal scan [†]	22 (3.1)	6 (1.5)	16 (5.2)
Biometry scan [‡]	4 (0.6)	2 (0.5)	2 (0.6)
Other indication for scan [§]	3 (0.4)	2 (0.5)	1 (0.3)
18-24 weeks n (%)	204 (28.9)	112 (28.2)	92 (29.7)
Anomalies at STAS	204	112	92
≥24 weeks n (%)	5 (0.7)	3 (0.8)	2 (0.6)
Biometry scan [‡]	5	3	2

Abbreviations: NT, Nuchal Translucency; FCT, First trimester combined testing; STAS, second trimester anomaly scan.

[†]These women chose commercial NIPT in a foreign country and were referred to our FMU.

[†] These patients were referred after anomalies present at a gender scan.

[‡] Cases were referred because of anomalies after a biometry scan and were referred to our FMU for an advanced ultrasound in second or third trimester.

[§] 2 out of 4 had a scan due to performance of invasive diagnostics because of maternal age, 2 out of 4 had an extra ultrasound because of vaginal blood loss

detection in first trimester was similar to the overall group with inclusion of chromosomal anomalies (Table 4 and Table 5). The increased detection rate in the before group was mainly due to referrals following an abnormal FCT, since referrals after a dating scan or STAS were similar (Table 6).

The detection rate for non-chromosomal CHDs during the first trimester was higher in the before group compared to the after group. This applied only to non-chromosomal AVSD, with a detection rate of 42.3% (11/26) in the before group versus 9.5% (2/21) in the after group, difference: 32.8% 95% CI 0.10-0.55, *P* =0.02 (Table 5). In the before group, more cases were referred due to scans conducted between 11-14 weeks of gestation, accounting for 47.8% (11/26), compared to 14.2% (3/21) in the after group (*P* =0.03) (Table 6).

Table 4. First trimester detection before and after introduction of cfDNA in all cases

	Before cfDNA		After cfDNA		
Defect	Total n	First trimester detection rate n (%)	Total n	First trimester detection rate n (%)	P-value
Severe brain- and skull defects	103	65 (63.1)	77	55 (71.4)	0.241
Anencephaly	61	49 (80.3)	47	40 (85.1)	0.518
Holoprosencephaly	32	12 (37.5)	22	11 (50.0)	0.361
Encephalocele	10	4 (40.0)	8	4 (50.0)	0.671
Abdominal wall defects	144	109 (75.7)	125	84 (67.2)	0.123
Omphalocele	101	84 (83.2)	90	72 (80.0)	0.572
Gastroschisis	33	18 (54.5)	27	5 (18.5)	**0.004**
Other †	10	7 (70)	8	7 (87.5)	0.588
Severe CHDs	149	62 (41.6)	107	22 (20.6)	**<0.001**
AVSD	109	50 (45.9)	78	21 (26.9)	**0.008**
HLHS	40	12 (30.0)	29	1 (3.4)	**0.005**

Abbreviations: CHD; congenital heart defect, AVSD; atrioventricular septal defect, HLHS; hypoplastic left heart syndrome

† Other includes abnormalities usually accompanied by abdominal wall defects: body stalk anomaly, pentalogy of Cantrell, and OEIS complex.

Table 5. First trimester detection of non-chromosomal anomalies before and after the introduction of cfDNA

	Before cfDNA		After cfDNA		
Defect	Total n	First trimester detection rate n (%)	Total n	First trimester detection rate n (%)	P-value
Severe brain- and skull defects	81	56 (69.1)	62	45 (72.5)	0.713
Anencephaly	60	48 (80.0)	45	38 (84.4)	0.558
Holoprosencephaly	11	4 (36.3)	9	3 (33.3)	0.888
Encephalocele	10	4 (40.0)	8	4 (50.0)	0.671
Abdominal wall defects	86	53 (61.6)	72	35 (48.6)	0.10
Omphalocele	40	27 (67.5)	38	24 (63.2)	0.687
Gastroschisis	33	18 (54.5)	27	5 (18.5)	**0.004**
Other †	10	7 (70)	7	6 (85.7)	0.603
Severe CHDs	56	16 (28.6)	49	3 (6.1)	**0.004**
AVSD	26	11 (42.3)	21	2 (9.5)	**0.020**
HLHS	30	5 (16.7)	28	1 (3.6)	0.195

Abbreviations: CHD; congenital heart defect, AVSD; atrioventricular septal defect, HLHS; hypoplastic left heart syndrome

†Other includes abnormalities usually accompanied by abdominal wall defects: body stalk anomaly, pentalogy of Cantrell, and OEIS complex.

Table 6. Type of scan and indication leading to referral of gastroschisis and non-chromosomal AVSD

Gastroschisis Type of scan at referral	Before cfDNA n = 33	After cfDNA n = 27
<11 weeks n,%	**1**	**0**
Dating scan	1	
11-14 weeks n,%	**17**	**9**
Dating scan	11	9
Anomalies at FCT scan	6	
14-18 weeks n,%	**1**	**6**
Gender reveal scan	1	6
18-24 weeks n,%	**14**	**12**
STAS	14	12
AVSD **Type of scan at referral**	**Before cfDNA** **n = 26**	**After cfDNA** **n = 21**
11-14 weeks n,%	**11**	**3**
Dating scan	2	2
Increased NT	2	1
Anomalies at FCT scan	3	
Increased risk FCT	4	
18-24 weeks n,%	**15**	**18**
STAS	15	18

Abbreviations: FCT; first trimester combined testing; STAS; second trimester anomaly scan.

Termination of pregnancy (TOP) between groups

TOP rate did not differ between the two groups, neither did the sub-analysis with exclusion of chromosomal anomalies (Table S1). The anomalies in the group with brain- and skull defects had the highest TOP rate, between 75%-91%. In the severe CHD group, cases with non-chromosomal HLHS were terminated predominantly and TOP rate did not differ between the two groups, respectively 79.1% (23/29) in the before group versus 78.6% (22/28) in the after group. TOP rate was low for gastroschisis compared to the other fetal anomalies, especially in the after group with a rate of 7.4% (2/27), but did not differ significantly between the two groups (Table S1).

The median gestational age of TOP did not differ among severe brain-and skull anomalies or in omphalocele or 'other' abdominal wall defects. If parents opted for TOP in case of a fetus with anencephaly, termination of pregnancy was usually in first trimester with a median gestational age of 12+4, this was similar among the two groups and similar for non-chromosomal anencephaly separately. Although only a small number of parents opted for TOP in case of a fetus with gastroschisis, gestational age of TOP differed among the before and after group (Table S2 and S3). In case of a fetus with an AVSD, parents opted for TOP earlier in pregnancy in the before group compared to the after group, respectively with a median gestational age of 15+3 (IQR, 13+5 - 21+3) versus 17+5 (IQR, 15+1 - 21+6) (P =0.030) (Table S2).

For non-chromosomal AVSD, gestational age of termination did not differ significantly between the two groups with respectively a median gestational age of TOP 16+5 (IQR, 13+1 – 22+3) versus 21+4 (20+0-22+1), *P* =0.15 (Table S3).

Discussion

Main findings

This study evaluated the first trimester detection of certain fetal anomalies visible in the first trimester before and after the introduction of cfDNA as first-tier test. When comparing the before and after group, we observed a discrepancy of approximately 30% in the first trimester detection rate of gastroschisis (n=13), AVSD (n=28) and HLHS (n=11), favoring the period prior to the abolition of FCT. This discrepancy persisted after excluding chromosomal anomalies identifiable by cfDNA testing (n=299). When excluding these cases in the AVSD group (n=83), the detection of AVSD remained higher in the before group, with a first trimester detection rate of 39.1% compared to 9.5% in the after group. Since there were no underlying chromosomal anomalies in fetuses with gastroschisis, the first trimester detection rate was comparable to the overall group, showing a significant difference between the before and after cfDNA, respectively 54.5% versus 18.5%. A greater number of these patients underwent an ultrasound between 11-14 weeks as part of FCT in the before group, and were subsequently referred for suspected anomalies. This implies that there is anticipated benefit to conduct a standardized first trimester scan. Despite observing an elevated first trimester detection rate in the before group, we did not observe an increase in pregnancy terminations within this time frame.

Interpretation

We hypothesized that the decline of FCT and therefore the decline of a first trimester scan could impede first trimester detection of certain fetal anomalies. However, we were unable to validate our hypothesis regarding severe brain- and skull defects, omphalocele and other abdominal wall defects. We did not observe a reduction in the first trimester detection of anencephaly, holoprosencephaly and encephalocele. Given the severity of these anomalies, the majority of these cases will be detected at the dating scan around 11 weeks of gestation.[6] The detection of anencephaly before 11 weeks and as early as 9 weeks has also been reported elsewhere[19] and we know that the prenatal detection rate is positively influenced by appropriate training and application of a standardized protocol. Since these are life-limiting fetal conditions, early diagnosis is imperative for future parents to make reproductive autonomous choices. Similar to our results, without a fixed protocol the prenatal detection rate of anencephaly between 11 and 14 weeks by trained sonographers reached 86%.[20]

Despite a similar gestational age of dating scan performance between the before and after group, we found a higher number of referrals because of anomalies at the dating scan in the group after the introduction of cfDNA. Nevertheless, the impact of a dating scan on the first trimester detection rate appears to have its limits, as it did not yield an augmented first trimester detection rate after the

implementation of cfDNA. We observed that the impact on first trimester detection was not limited solely to cases with a chromosomal anomaly. More than 50% of parents that opted for FCT were referred due to anomalies discovered during the first trimester scan as part of FCT. As this scan was conducted at a more advanced gestation than the dating scan, the likelihood of detecting an anomaly in first trimester increased. This applied in particular to non-chromosomal AVSD and gastroschisis. Without a first trimester scan as part of FCT, these cases would have remain undetected until the STAS, as highlighted in a recent study.[21] Above emphasizes the significance of a first-trimester anomaly scan and affirms that dating scans conducted around 11 weeks of gestation cannot attain the same diagnostic effectiveness as an anomaly scan at 12-13 weeks of gestation for certain anomalies.

The first trimester detection of gastroschisis in our cohort was notably low with only 38.3% detected before 14 weeks of gestation in contrast to the 95-100% in existing literature.[22, 6, 23] In a prospective study with 44.859 pregnancies, all 104 cases of omphalocele, gastroschisis, and body stalk anomaly were identified during a first trimester scan conducted at 11+0 – 13.6 weeks of gestation.[6] The explanation for this discrepancy lies in the fact that within our cohort, there was no standardized protocol for a FTAS conducted independently, instead, it was exclusively performed as part of FCT. This also elucidates the noticeable discrepancy in first-trimester gastroschisis detection rate between the before and after group. Considering that gastroschisis is usually an isolated finding and chromosomal anomalies are rare[24, 25], gastroschisis will not be detected following an abnormal cfDNA screening in the current timeframe. Thus, conducting an additional first trimester scan can improve to identify these cases at an earlier gestation. Before the introduction of cfDNA, we observed a lower gestational age at termination of pregnancy (TOP). However, because of the limited sample size with only three cases terminated in the after group, it is not possible to draw any definitive conclusions on this matter.

If a FTAS is performed routinely with a structured protocol, also more than 90% of non-chromosomal AVSD and HLHS are detected in first trimester.[6, 23] In our retrospective study, the overall detection of non-chromosomal severe CHD's in the first trimester was below 30% as patients received only a standard dating scan and STAS. Although some chose for FCT in the before period, in the after group no FTAS was performed routinely. In line with a recent publication the earlier TOP we found in fetuses with AVSD (total group) might be attributed to the earlier detection[26], alternatively, it could be attributed to the high number of chromosomal anomalies (65/77, 84.4%). The lack of a significant difference in the gestational age of termination for non-chromosomal AVSD could be attributed to the small sample size. Though, based on the wide interquartile range we can only conclude that that before the abolition of the FCT, some non-chromosomal AVSD were terminated during the first trimester, whereas no cases were terminated in the first trimester during the period afterwards.

Clinical implications

A scan dedicated to fetal anatomy in the first trimester not only enables earlier detection but also grants time for further delineation of the anomaly and to do genetic testing, especially important in CHD.[27] This creates the opportunity for future parents to prepare for the neonatal period or overthink termination of pregnancy without time pressure in case abortion is restricted in the second trimester of pregnancy. For instance, in most European nations, the prevailing gestational limit for TOP is set at 12 weeks.[28] Besides early TOP correlates with reduced reported grief and lower maternal mortality rates[29], highlighting the importance of supportive healthcare environments in these sensitive situations.[30] Earlier diagnosis of severe CHD was linked to decision to choose for TOP, irrespective of extra-cardiac or genetic anomalies in a recent study with 158 cases.[26] On the contrary, early detection ensures tailored prenatal care, assisting medical teams in preparing for potential birth complications or postnatal interventions, This potentially reduce neonatal morbidity, leading to lower healthcare costs. However, the diagnosis of CHD in the first trimester is challenged by the heart's size, unfavorable fetal position and frequent involuntary movements. Several studies have highlighted its increased false positive rate, as anomalies identified in the first trimester may evolve or undergo reclassification later in pregnancy.[31]

The success of a first trimester prenatal screening program is not only determined by cfDNA testing, but depends on offering a FTAS as well. Future research should focus on the right moment of FTAS, before or after cfDNA testing and evaluate its additional healthcare costs.[32] However, a recent study showed that FTAS before cfDNA testing is a more cost-effective strategy for non-invasive prenatal aneuploidy screening compared to cfDNA alone.[33]

Strengths and limitations

The past decade we gathered a substantial volume of cases with certain fetal anomalies. Given that Amsterdam UMC serves as the central referral facility for peripheral hospitals and midwifery practices in cases of suspected fetal anomalies, we have likely encompassed all prenatal cases involving severe brain and skull defects, abdominal wall defects, and severe CHDs in the Amsterdam region. Consequently, our cohort is likely representative of other regions across the Netherlands.

Caution should be taken with interpreting our results due to the retrospective character of the study. Abnormal prenatal screening could have been a confounder for the detection of HLHS in the first trimester. In the total group we found a higher first trimester detection rate before introduction of cfDNA, but in the subgroup analysis with exclusion of chromosomal anomalies we did not find a difference in first trimester detection between the two groups. This was due to the fact that a greater number of cases involving HLHS were referred because of an increased NT measurement at the dating scan and abnormal FCT, and with that mothers received a FTAS in a Fetal Medicine Unit leading to in earlier detection of HLHS. This did not apply for cases with

AVSD, as the first trimester detection rate was higher in the group before cfDNA, this was similar for both the total group and solely the non-chromosomal anomalies. Moreover, within the ten-year study period, gender scans, mostly conducted at 15 weeks of gestation, have been introduced. Therefore, a large amount of gastroschisis cases (6/27, 22%) in the after period were identified during these scans subsequent leading to referral to our FMU. However, gender scans will not lead to an increase the first trimester detection of this anomaly, and do not have the capability to identify AVSD and HLHS, in contrast to a first-trimester scan designed for assessing fetal anatomy.[6]

Thirdly, our study exclusively encompassed cases identified prenatally, consequently omitting postnatally diagnosed cases which has its influence on the denominator. Given that we did not include these missed prenatal cases, the actual prenatal detection rate will be lower. Besides, postmortem confirmation of the prenatally detected fetal anomalies was not performed in all cases, leaving us unable to provide any details on false positive diagnosis in particular in AVSD cases.

Conclusion

This study presents evidence of a decline in prenatal detection of gastroschisis, HLHS and AVSD during the first trimester in the period after the implementation of cfDNA. In the absence of chromosomal anomalies, this decline remained for gastroschisis and AVSD. This implies that there exists an added benefit to conduct a first trimester scan, as there was before the abolition of FCT. Although we observed a noticeable increase in referrals for anomalies at the dating scan in the period after the introduction of cfDNA, there was not a corresponding increase in the first trimester detection of fetal anomalies. Therefore, it is likely that incorporating an additional first trimester scan around 13 weeks of gestation could enhance the overall first trimester detection of certain fetal anomalies, especially for non-chromosomal AVSD and gastroschisis.

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Supplemental material

Table S1. *Rate of TOP for non-chromosomal anomalies*
Table S2. *Gestational age of TOP all cases*
Table S3. *Gestational age of TOP non-chromosomal anomalies*