Original Article

Evaluating clinical challenges in screening for chromosomal diseases in pregnancy with Cell-free DNA

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ABSTRACT

Cell-free DNA(CFDNA) is one of the diagnostic methods used in the trimesters of pregnancy to screen for chromosomal abnormalities. This study investigates and compares CFDNA and amniocentesis results and several factors affecting CFDNA results in a specified period.The present study was conducted on all pregnant mothers who underwent CFDNA tests due to high-risk screenings in the first or second stage of pregnancy or for any other reason. Based on this, about 2300 pregnant mothers with CFDNA tests were selected and included in the study by census, and the abnormal answers obtained after performing the diagnostic test were analyzed, which included false negative, false positive, and abnormal results. The cases that were not reported, especially the cases where the diagnostic test is performed due to the low fetal fraction, were investigated. 90 high-risk cases were investigated.The results revealed a statistically significant relationship between patients' weight and CFDNA results ($p<0.05$). The results also revealed that the highest sensitivity and specificity were related to trisomy 21 (Down syndrome). Also, a significant relationship was found between the numerical decrease in fetal fraction (less than 5) and the CFDNA result. However, no correlation was found between the number of pregnancies and the mother's gravidity and the CFDNA response.Thus, it seems to be the best screening method in suspected cases of Down syndrome. However, unlike other studies, no significant relationship was observed between BMI and CFDNA results in our study.

Keywords: Chromosomal abnormalities, Screening, Cell-free DNA,Amniocentesis,Fetal Fraction

Introduction

Non-invasive prenatal screening that uses Cell-free DNA present in the plasma of pregnant women offers great potential as a screening method for fetal aneuploidy. Cell-free DNA analysis became clinically available in 2011 and the American College of Obstetrics and Gynecology and the American Society of Maternal and Fetal Medicine recommended it as a screening option for women at risk of fetal aneuploidy before the 21st week of pregnancy [1]. Women over 33 years of age, fetuses with ultrasound findings indicating an increased risk of aneuploidy, women with a history of children with trisomy, parents who have a balanced Robertsonian translocation with an increased risk of trisomy 13 or trisomy 21, and women whose results of the screening test of the first trimester or the second trimester are positive are at the risk. Many women begin to perform screening tests to understand the risks that affect the fetus since screening for chromosomal abnormalities using the Cell-free DNA technique is non-invasive. Some women use this information to determine if further diagnostic tests are appropriate for them or not since available prenatal diagnostic tests are associated with a low but undeniable risk of miscarriage [2]. Circulating cell-free DNA of fetal origin includes about 3-13% of total mother cell-free DNA after 10 weeks of gestation. It is also thought to be derived primarily from the placenta. Cell-free DNA appears in the mother's circulation from early pregnancy and is quickly cleared from the mother's blood after 2 hours after delivery [3, 4]. The Cell-free DNA test only examines common trisomies and sex chromosome composition if requested. It can be done from the 9th week of pregnancy until the time of delivery. Some laboratories have approved different techniques for using Cell-free DNA as a fetal aneuploidy screening test. All data rely on

NGS (next-generation sequencing) technologies and advanced bioinformatics analyses [5-7]. Regardless of the type of technology used, results become mostly available within 7 to 10 days of maternal sampling. Despite the effectiveness of this test in screening pregnant mothers, its use is associated with some limitations. Affordability is one of the primary principles of medical screening. The cost of CFDNA screening can be one of the biggest challenges to extensive clinical use of this test. Another limitation is the amount of fetal DNA content or the Cell-free DNA in the mother's blood, which is of fetal origin and is necessary for accurate test results. Some laboratories require a minimum of 4% of fetal DNA content to report a result. However, other laboratories do not report fetal DNA content. The amount of fetal DNA content normally increases with increasing gestational age. Generally, the probability of failure in the screening test ranges from 1 to 8% depending on the laboratory and the technique used [8, 9]. The results may not be obtained in patients with fetal aneuploidies or those who are obese due to the low fetal DNA content. In this regard, 10% or more of patients weighing more than 250 pounds have a fetal DNA content of less than 4% [10].

Aneuploidy rate of up to 23% (due to low fetal DNA content or other unknown factors) has been reported for women who do not receive an interpretable result from the Cell-free DNA test. Women whose results are unreported, unclear, or non-interpretable through Cell-free DNA screening (a test result of 'no result') should receive further genetic counseling, and due to the increased risk of aneuploidy, a comprehensive evaluation and ultrasound examination should be provided for them. Although it is possible to perform frequent screening, it may delay the diagnosis of aneuploidy and potentially limit fertility options, and only 50-60% of repeated screen tests present a presentable result [9, 11]. All types of Cell-free DNA tests have high sensitivity and specificity for trisomy 18 and trisomy 21 (sensitivity and specificity of 99.3% and 99.8%, respectively, for trisomy 21 and sensitivity and specificity of 97.4% and 99.8%, respectively, for trisomy 18), regardless of the type of molecular technique used [5]. However, the positive predictive value in this population is lower than this value due to the lower prevalence of aneuploidy in the general population of pregnant women. This means that a limited number of women with a positive test result will have an infected fetus and more false positive test results will be false [12]. Another limitation of Cell-free DNA screening in the general population of pregnant women is that trisomies 13, 18, and 21 include a smaller proportion of the chromosomal abnormalities found in the general population of women. Traditional serum analyte screening methods provide a higher diagnosis of these chromosomal abnormalities and the risk of other adverse pregnancy outcomes [2, 13]. For example, a positive integrated screening test result may indirectly identify a fetus with an unbalanced chromosomal rearrangement other than trisomies 13, 18, or 21. A study of women with abnormal traditional screening test results and diagnostic tests estimated that up to 17% of clinically significant chromosomal abnormalities might not be diagnosed by most current cell-free DNA techniques [14]. Other limitations of this test include malignancy in pregnant women. The first report of a conflicting CFDNA screening test result and subsequently diagnosed maternal malignancy was published in 2013. In this case, CFDNA screening of the patient was positive for trisomy 13 and monosomy 18. The patient was selected for amniocentesis and karyotype and microarray were selected. The patient was subsequently diagnosed with a metastatic neurocarcinoma in the postpartum period [15]. Although Cell-free DNA is thought to have a high positive predictive value, a low false negative rate, and a low false positive rate, these values vary depending on the condition and the age of the woman. Failure in test results may be caused by a failure in the measurement, a low amount of fetal DNA content (the ratio of Cell-free DNA from pregnancy in the mother's serum compared to the mother's Cell-free DNA), or failed quality control criteria [16]. Some studies have indicated that maternal blood pressure, in vitro fertilization, and the use of certain drugs can be associated with an increased risk of failure in the Cell-free DNA test [17]. Since Cell-free DNA is increasingly used in the general population, an increase in the number of unsuccessful test results may be observed.

Materials and Methods

This retrospective descriptive study was conducted based on hospital and clinic data. Its purpose was to determine the clinical challenges in screening for chromosomal diseases in pregnancy by examining Cell-free DNA in people referring to medical centers affiliated with Jundishapur University of Ahvaz from 2020 to 2022. It was conducted on 2300 pregnant mothers who underwent the Cell-free DNA test due to the high-risk screenings of the first or second stage of pregnancy or for any other reason. The device used in this study is the package of NGS, ION PROTON, and IONA kit of PREMAITHA Company, England, with IVD. Accordingly, all pregnant mothers with Cell-free DNA tests followed by amniocentesis were selected and included in the study. The abnormal answers obtained after the diagnostic test were examined. False negative cases, false positive cases, examining anomalies (such as trisomies, Down syndrome, sex chromosomes, etc.), and non-reported cases, especially cases where a diagnostic test is performed due to the low fetal fraction were also examined. Among all cases, the results of 90 high-risk cases were examined. Also, the mother's demographic information and its relationship with the results of the Cell-free DNA Amniocentesis test were analyzed. Data were analyzed after collecting and coding in SPSS software (version 13, SPSS Inc., Chicago, IL). A statistically significant level of less than 0.05 was considered.

Results and Discussion

The screening results of the first and second trimesters related to NT ultrasound (40 percent) and TR21 (35.6 percent) were formed. Most of the cases detected in the Cell-free test of the

study subjects were female (44.4%). Also, the majority of the participants in the Cell-free report were related to TR21 (43.3 percent), and 52.2% were related to 2021. The mean (standard deviation) age of the participants was 34.31 (5.51). The mean (standard deviation) weight of the participants was 26.77 (13.99). The mean (standard deviation) BMI of the participants was 17.62 (0.87). The mean (standard deviation) of the FF of them was 10.11 (3.35). The mean number of pregnancies of the participants was 30 (2.64).

Out of a total of 90 reported cases, 39 cases (43.3%) were related to trisomy 21, 9 cases (10%) were related to trisomy 18, 6 cases (6.7%) were related to trisomy 13, 4 cases (4.4%) were normal (Amniocentesis was performed due to the problem of echocardiography of the fetus, abnormal ultrasound and low z-score, or high clinical suspicion.). Also, 7 cases (7.8%) were not reported (generally due to the low fetal fraction), 11 cases (12.2%) included XO and 14 cases (15.6%) included XXY. A total of 54 cases (60%) included trisomies and 36 cases (40%) included sex chromosome disorders and other cases. It indicates the mean body mass index at different levels of Cell-free DNA. The comparison of the mean body mass indices of pregnant women based on the results of Cell-free DNA did not show a statistically significant difference (one-way analysis of variance test, P-value=0.058). No statistically significant difference was found in the comparison of the mean number of pregnancies based on the results of Cell-free DNA (P-value=0.062). It shows the mean body weight of pregnant women at different levels of Cell-free DNA. The comparison of the mean weight of pregnant women based on the results of Cell-free DNA showed a statistically significant difference (One-way analysis of variance test, P-value=0.045) **(Table 1)**. It shows the mean age of pregnant women at different levels of Cell-free DNA. The comparison of the mean age of pregnant women based on the results of Cell-free DNA revealed a statistically significant difference (one-way analysis of variance test, P-value=0.0495) **(Table 2)**. The mean number of fetal fraction in pregnant women shows different levels of Cell-free DNA. The comparison of the mean age of pregnant women based on the results of Cell-free DNA indicated a statistically significant difference (P-value=0.0345) **(Table 3**).

Out of 39 cases reported as trisomy 21 in CFDNA, 38 cases were also positive in amniocentesis (one XO case was reported). In other words, 100% sensitivity and 98.07% specificity were calculated. Out of the 9 reported cases of trisomy 18, 7 cases had trisomy 18 in amniocentesis and 2 cases were normal in amniocentesis. The sensitivity was 70% and the specificity was 97.56%. Out of the 6 reported cases of trisomy 13, 4 cases were positive for amniocentesis, 1 case had normal amniocentesis, and 1 case of trisomy 18 was reported. The sensitivity was 80% and the specificity was 97.6%. Out of all CFDNA cases, 4 cases were reported as normal, 3 of which underwent amniocentesis due to abnormal sonography, low z-score, and high clinical suspicion. Also, 1 case underwent amniocentesis due to abnormal echocardiography of the fetal heart and clinical suspicion. In his amniocentesis, 1 case of trisomy 18, 2 cases of XXY, and 1 case of XO were reported. Seven cases were not reported due to the numerical low fetal fraction (generally between 4 and 5) and their amniocentesis was reported as normal. Out of 11 reported cases of XO, 8 cases were also reported in amniocentesis. The sensitivity was 66.6% and the specificity was 96.2%. Out of 14 reported cases of XXY, 12 cases of XXY in amniocentesis were also reported. The sensitivity was 80% and the specificity was 97.4% **(Table 4)**. It shows the frequency of the screening results of the first and second trimesters based on the results of Cell-free DNA in the studied subjects. The results of the chi-score test indicated a statistically significant relationship based on the screening findings of the first and second trimesters in different groups (P-Value=0.005) **(Table 5)**. Gender matching in the comparison between amniocentesis and CFDNA was calculated with a sensitivity of 81.3% and a specificity of 88.8% **(Table 6)**.

Table 1. Comparison of the mean weight based on the Cell-free DNA results

Cell-free DNA	N	Mean	SD	P-Value
Tr21	39	05.78	03.14	
Tr18	9	78.78	17.7	
Tr13	6	67.70	85.16	045.0
Normal	4	50.81	03.13	
Not Reported	7	00.91	21.10	
XO	11	73.75	68.13	
XXY	14	26.77	33.14	

Table 2. Comparison of mean age based on the Cell-free DNA results

Cell-free DNA	N	Mean	SD	P-Value
Tr21	39	56.34	82.5	
Tr18	9	11.33	55.6	
Tr13	6	17.34	71.7	0.04
Normal	4	00.32	97.4	
Not Reported	7	00.34	83.4	
XO	11	36.34	61.4	
XXY	14	21.35	79.4	

Table 3. Comparison of the mean fetal fraction based on the Cell-free DNA results

Cell-free DNA	N	Mean	SD	P-Value
Tr21	39	11.22	3.25	
Tr18	9	11.1	2.45	
Tr13	6	10.26	4.5	0.0345
Normal	4	12.5	2.55	
Not Reported	7	4.33	4.75	
XO	11	10.45	2.42	
XXY	14	10.33	3.12	

Table 4. Comparison of the amniocentesis test results based on the Cell-free DNA results

Cell-free	Frequency	**amniocentesis**	sensitivity	specificity
Tr21	39	38	100%	98.7%

Tr18	9	10	70%	97.56%
Tr13	6	5	80%	97.6%
Normal	4	10	-	-
Not Reported	7	-	-	-
XO	11	12	66.6%	96.2%
XXY	14	15	80%	97.4%

Table 5. The frequency of the screening results of the first and second trimesters based on the results of Cell-free DNA in the studied subjects

Cell-free DNA	Screening results of the first and second trimesters					P-Value
	NT (n=36)	TR21 (n=32)	Age up to 35 n=16) (	Abnormal)Anomaly (n=32	patient Req. (n=1)	
Tr21	.7)15 (41	)19 (59.4	)4 (25.0	(20.0)1	(0.0)0	005.0
Tr18	(5.6)2	(9.4)3	)2 (12.5	(40.0)2	(0.0)0	
Tr13	(8.3)3	(0.0)0	)2 (12.5	(20.0)1	(0.0)0	
Normal	(8.3)3	(0.0)0	(0.0)0	(0.0)0	1 (100.0)	
Not Reported	(2.8)1	(6.3)2	)3 (18.8	(20.0)1	(0.0)0	
XO	(13.9)5	(12.5)4	)2 (12.5	(20.0)1	(0.0)0	
XXY	(19.4)7	(12.5)4	)3 (18.8	(0.0)0	(0.0)0	

Table 6. The level of fetal sex matching in Cell-free DNA and amniocentesis

		amniocentesis	
		male	female
Cell-free DNA	Male	35	4
	female	8	32

The results of the present study revealed that trisomy 21 was detected in 35.6% of the patients during the screening of the first and second trimesters of pregnancy. Also, based on CFDNA results, trisomy 21 was detected in 43.3% of patients, and trisomy 18 and trisomy 13, respectively, were detected in 10 and 6.7% of patients. Also, XO and XXY abnormalities were detected in 12.2 and 15.6% of patients, respectively. The study of Basaran *et al*. indicated that trisomy 21 was the most prevalent among patients based on CFDNA diagnosis. It also showed that the results of amniocentesis methods combined with CFDNA have a suitable diagnostic value for duplicate abnormalities in comparison with deletion abnormalities [18]. The study by Pescia *et al*. also showed that trisomy 21 was present in more than 60% of the fetuses examined by CFDNA [19]. It has been recently shown that the level of CFDNA is associated with some characteristics of pregnant mothers, such as BMI. Hence, it has been proven that CFDNA levels are higher in obese mothers with high BMI compared to thinner mothers [20]. Results have also shown that the increase in BMI level in obese mothers can be associated with necrosis of adipocyte cells. A previous study has shown that CFDNA levels were higher in obese women with preeclampsia compared to thin women [21]. Unexpectedly, there was no significant correlation between the patients' BMI level and CFDNA results in the present study ($p>0.05$). Unlike the present study, Agekili *et al*. showed a significant relationship between BMI and abnormalities detected by the CFDNA method [22]. The study by Brown *et al*. reported a significant relationship between BMI and CFDNA levels in pregnant women [23].

This inconsistency in the results may be due to the number of examined patients and the differences in the mean BMI of the examined subjects. In addition to BMI, previous studies have shown that maternal age can also be associated with CFDNA. The present study showed that the mean age of mothers who had chromosomal abnormalities, including trisomy 21, was higher compared to pregnant mothers who were normal in terms of CFDNA evaluation [24]. In the present study, a significant relationship was reported between the mean age of pregnant mothers and chromosomal disorders detected by the CFDNA method. BMI is affected by people's weight and height. Some studies have reported that weight gain in mothers can be associated with a decrease in CFDNA levels in them [20]. Some other studies have not shown a relationship between mother's weight and CFDNA level [25]. The present study revealed a significant relationship between patients' weight and CFDNA results. The study by Kal *et al*. showed that the use of the amniocentesis test was more sensitive in detecting monosomies compared to CFDNA [26]. The study by Basaran *et al*. showed that the predictive value of the CFDNA test for detecting duplicate abnormalities was higher compared to deletion abnormalities. It also showed that when using amniocentesis to diagnose genetic abnormalities, it is necessary to use other confirmation methods to confirm the abnormalities [18]. Norton et al showed that the percentage of detection of genetic abnormalities by cfDNA was lower compared to the gold standard method. In addition, this method is not able to detect abnormalities with high risk[27].The study by Scheffer *et al*. revealed that the low fetal fraction in cell-free tests is one of the significant reasons for test failure and no-cell result since a low fetal fraction can reflect an abnormal initial placenta and an adverse pregnancy outcome [28]. The present study also showed a significant relationship between the fetal fraction in the cell-free test, and the "unreported" results, indicating the necessity of performing a diagnostic test such as amniocentesis following these results. The results of the present showed that the sensitivity of the CFDNA method in determining trisomies 13, 18, and sex chromosome disorders was lower compared to trisomy 21. In other words, the detection sensitivity for trisomy 21 or Down syndrome was very high. In the present study, there was no association between the mother's gravidity or the number of pregnancies, and CFDNA test results.

Conclusion

Generally, it can be stated that the use of Cell-free DNA method, as it is a screening method, has a high sensitivity in cases of Down syndrome, but it is less sensitive in trisomy 13 and 18, and the diagnosis of problems related to the sex chromosome. Hence, it seems to be the best screening method in suspected cases of Down syndrome. Also, a relationship between CFDNA results, maternal age, pregnant mother's weight, and numerical low fetal fraction was found based on the data available in our study. However, in contrast to the assumptions and references, no association was observed between CFDNA results and BMI.

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References

1. Obstetricians A. Gynecologists. Screening for fetal aneuploidy. ACOG Practice bulletin no. 163. Obstetrics and Gynecology. 2016;127:e123-e37.
2. Gregg AR, Gross S, Best R, Monaghan K, Bajaj K, Skotko B, et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genetics in medicine. 2013;15(5):395-8.
3. Illanes S, Denbow M, Kailasam C, Finning K, Soothill P. Early detection of cell-free fetal DNA in maternal plasma. Early human development. 2007;83(9):563-6.
4. Bryant L. Non-invasive prenatal testing for Down's syndrome: psychologically speaking, what else do we need to know? : Taylor & Francis; 2014. p. 1-4.
5. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstetrics & Gynecology. 2012;119(5):890-901.
6. Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. Prenatal diagnosis. 2012;32(13):1233-41.
7. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genetics in medicine. 2011;13(11):913-20.
8. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, et al. Single-nucleotide polymorphism–based noninvasive prenatal screening in a high-risk and low-risk cohort. Obstetrics and gynecology. 2014;124(2 0 1):210.
9. Ashoor G, Syngelaki A, Poon L, Rezende J, Nicolaides K. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound in Obstetrics & Gynecology. 2013;41(1):26-32.
10. Willems P, Dierickx H, Vandenakker E, Bekedam D, Segers N, Deboulle K, et al. The first 3,000 non-invasive prenatal tests (NIPT) with the harmony test in Belgium and the Netherlands. Facts, views & vision in ObGyn. 2014;6(1):7.
11. Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, Zimmermann B, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism–based noninvasive prenatal aneuploidy testing. American journal of obstetrics and gynecology. 2014;211(5):527. e1-. e17.
12. Alamillo CM, Krantz D, Evans M, Fiddler M, Pergament E. Nearly a third of abnormalities found after first-trimester screening are different than expected: 10-year experience from a single center. Prenatal diagnosis. 2013;33(3):251-6.
13. Norton ME, Jelliffe-Pawlowski LL, Currier RJ. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. Obstetrics & Gynecology. 2014;124(5):979-86.
14. Wang Y, Chen Y, Tian F, Zhang J, Song Z, Wu Y, et al. Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. Clinical chemistry. 2014;60(1):251-9.
15. Grace MR, Hardisty E, Dotters-Katz SK, Vora NL, Kuller JA. Cell-free DNA screening: complexities and challenges of clinical implementation. Obstetrical & gynecological survey. 2016;71(8):477.
16. Burns W, Koelper N, Barberio A, Deagostino-Kelly M, Mennuti M, Sammel MD, et al. The association between anticoagulation therapy, maternal characteristics, and a failed CFDNA test due to a low fetal fraction. Prenatal Diagnosis. 2017;37(11):1125-9.
17. Bevilacqua E, Gil MM, Nicolaides KH, Ordoñez E, Cirigliano V, Dierickx H, et al. Performance of screening for aneuploidies by cell-free DNA analysis of maternal blood in twin pregnancies. Ultrasound in Obstetrics & Gynecology. 2015;45(1):61-6.
18. Basaran S, Has R, Kalelioglu IH, Sarac Sivrikoz T, Karaman B, Kirgiz M, et al. Clinical, Cytogenetic and Molecular Cytogenetic Outcomes of Cell-Free DNA Testing for Rare Chromosomal Anomalies. Genes [Internet]. 2022; 13(12).
19. Pescia G, Guex N, Iseli C, Brennan L, Osteras M, Xenarios I, et al. Cell-free DNA testing of an extended range of chromosomal anomalies: clinical experience with 6,388 consecutive cases. Genetics in Medicine. 2017;19(2):169-75.
20. Stupak A, Kwaśniewski W, Goździcka-Józefiak A, Kwaśniewska A. The Influence of Maternal Obesity on Cell-

Free Fetal DNA and Blood Pressure Regulation in Pregnancies with Hypertensive Disorders. Medicina (Kaunas). 2021;57(9).

21. Vora NL, Johnson KL, Basu S, Catalano PM, Hauguel-De Mouzon S, Bianchi DW. A multifactorial relationship exists between total circulating cell-free DNA levels and maternal BMI. Prenat Diagn. 2012;32(9):912-4.

24. Gerovassili A, Garner C, Nicolaides KH, Thein SL, Rees DC. Free fetal DNA in maternal circulation: a potential prognostic marker for chromosomal abnormalities? Prenatal Diagnosis. 2007;27(2):104-10.

23. Brown I, Fernando S, Menezes M, da Silva Costa F, Ramkrishna J, Meagher S, et al. The importance of ultrasound preceding cell-free DNA screening for fetal chromosomal abnormalities. Prenatal Diagnosis. 2020;40(11):1439-46.

25. Lüthgens K, Häbig K, Sonek J, Kagan KO. Screen-positive rate in Cell-free DNA screening for trisomy 21. Prenatal Diagnosis. 2023;n/a(n/a).

26. Lüthgens K, Grati FR, Sinzel M, Häbig K, Kagan KO. Confirmation rate of Cell-free DNA screening for sex chromosomal abnormalities according to the method of confirmatory testing. Prenatal Diagnosis. 2021;41(10):1258-63.

27. Norton ME, Baer RJ, Wapner RJ, Kuppermann M, Jelliffe-Pawlowski LL, Currier RJ. Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities. American Journal of Obstetrics and Gynecology. 2016;214(6):727.e1-.e6.

28. Scheffer PG, Wirjosoekarto SAM, Becking EC, Weiss MM, Bax CJ, Oepkes D, Sistermans EA, Henneman L, Bekker MN. Association between low fetal fraction in cell-free DNA testing and adverse pregnancy outcome: A systematic review. Prenat Diagn. 2021 Sep;41(10):1287-1295. doi: 10.1002/pd.6028. Epub 2021 Aug 18. PMID: 34350596; PMCID: PMC9292009.