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Original Article

Diagnostic differences between patients opting for non-invasive prenatal testing and patients having traditional prenatal diagnosis

Shufa Yang[1,2], Jianyi Lv[1], Yanmei Si[2], Xiaoyan Du[1], Zhenwen Chen[1]

[1]*School of Basic Medical Science, Capital Medical University, Beijing Key Laboratory of Cancer Invasion and Metastasis Research, Beijing 100069, China;* [2]*Laboratory of Genetics and Metabolism, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China*

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Abstract: Objective: To analyze possible missed chromosomal aberrations by utilization of cell-free fetal DNA (cffDNA) testing. Methods: A retrospective study of two cohorts who underwent amniocentesis or cffDNA testing was conducted. A total of 15,220 patients were included in amniocentesis group and 9,821 patients in cffDNA group. Part of the cffDNA group was followed up and informed of the results of invasive prenatal diagnosis. Chromosomal aberrations of amniocentesis group were classified according to the testing range of cffDNA testing and compared to the cffDNA group. Results: Chromosomal aberration rates in the two groups were 6.52% (992/15220) and 0.58% (57/9821), respectively. 3.75% (all patients), 3.33% (advanced maternal age), or 3.4% (positive results of serum screening) chromosome aberrations would have been missed since they exceeded the cffDNA range. Pathogenic chromosomal aberrations beyond the cffDNA testing range were estimated as 0.81%, 0.62% and 0.78% in the above three categories in amniocentesis group. Furthermore, unclear pathogenic chromosomal aberrations could be missed approximately by 1.01%, 0.92% and 0.97% in the corresponding categories in the amniocentesis group. Conclusion: With the availability of cffDNA testing, an increasing number of patients tend to refuse invasive prenatal diagnosis. This may lead to missed diagnosis of chromosomal aberrations during prenatal screening.

Keywords: Chromosomal aberrations, amniocentesis, cell-free fetal DNA testing, noninvasive prenatal testing

Introduction

Chromosomal aberrations account for a large portion of congenital malformations. Autosomal aneuploidy, sex chromosome aneuploidy (SCA), balanced structural rearrangement and unbalanced structural rearrangement are the common types of chromosomal aberrations in prenatal diagnosis. Hereinto, trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13) and SCA are the most common chromosomal aneuploidies, which account for 25-43%, 8.1-17.88%, 0.4-3.28% and 9.81-11.6% of total chromosomal aberrations, respectively [1-4]. Noninvasive prenatal testing using cell-free fetal DNA (cffDNA) has shown relatively high sensitivity, specificity, and positive predictive value for detection of common autosomal aneuploidies (T21, T18, T13), since its introduction into clinical practice [5-8]. An increasing number of patients tend to select cffDNA testing instead of invasive prenatal diagnosis [9, 10]. In addition, a growing number of obstetricians plan to offer cffDNA testing to pregnant women as a preferred option [11].

Traditional samples employed in prenatal diagnosis include amniotic fluid and chorionic villous tissues. The subsequent analyses in invasive prenatal diagnosis include karyotype analyses, quantitative fluorescent PCR assay, fluorescence *in situ* hybridization, and array-based comparative genomic hybridization [12, 13]. Karyotype analyses of amniotic fluid cells, which remains the predominant prenatal diagnostic method especially in China, is capable of detecting most chromosome aberrations. However, only common autosomal aneuploidies (T21, T18 and T21), sometimes including SCA, are assessed by cffDNA testing in most laboratories [14]. The consequence is that some chro-

mosome aberrations exceeding the cffDNA testing range would be missed for diagnosis in patients with the same indications for amniocentesis during prenatal testing.

To analyze chromosome aberrations missed by the cffDNA test, two cohorts, receiving amniocentesis for chromosome karyotype or cffDNA testing, were retrospectively summarized in this study.

Materials and methods

Subjects

A retrospective study of two cohorts was conducted. The first cohort included 15,220 women who underwent amniocentesis for chromosome karyotype analysis at the Laboratory of Genetics and Metabolism of Beijing Obstetrics and Gynecology Hospital from January 2010 to November 2016. Patient information was derived from the laboratory requisition forms. The following data were collected: ages of pregnant women receiving amniocentesis, indications for amniocentesis, and results of chromosome analyses.

The second cohort included all 9,821 pregnant women receiving cffDNA testing at the Laboratory of Genetics and Metabolism from August 2015 to October 2016. Data collection included ages of pregnant women receiving cffDNA testing, indications for cffDNA testing, and results of cffDNA testing.

Experimental design

In the amniocentesis group, chromosome karyotype analysis for amniotic fluid was carried out as previously described [4]. Clinical indications included: advanced maternal age (AMA), positive result of maternal serum screening (MSS), increased nuchal translucency (NT), history of chromosome abnormality in pregnancy, abnormal ultrasound findings, chromosomal abnormality carrier in parents, positive results of cffDNA testing, and history of abnormal pregnancy. AMA was defined as ≥35 years of age at the expected date of delivery. Increased NT was defined as ≥2.5 cm (95th percentile). Abnormal karyotype was classified into the following groups: autosomal aneuploidy, SCA, balanced structural rearrangement, unbalanced structural rearrangement, mosaic, polymorphism, and others. Frequently reported chromosome aberrations were classified as a subgroup. For fetuses carrying structural rearrangements, chromosome polymorphisms, or marker chromosomes, both parents were advised to take chromosome analyses of peripheral blood to confirm the pathogenic effect of the aberrations.

The cffDNA testing was performed as reported by Fuman Jiang *et al* [15]. Clinical indications included AMA, MSS, abnormal ultrasound findings, increased NT and history of abnormal pregnancy. Screening for T21, T18 and T13 were offered by cffDNA testing currently according to local policy [16]. For patients receiving cffDNA testing from August 2015 to December 2015, an active follow-up process (fax and phone) was undertaken to collect the information of the patients whether invasive prenatal diagnosis was done or not. For patients with high risk of aneuploidy with cffDNA testing, the outcome information confirmed by karyotype analysis was collected through follow-up or by referring to patient medical records.

Before taking amniocentesis or cffDNA testing, all the patients consulted with obstetricians or certified genetic counselors, and informed consent was obtained from every patient.

Data analysis

Statistical analyses were performed with the use of SPSS 15.0 software. Categorical variables were compared by Chi-square test. Continuous variables were compared with independent-samples by *t* test. A *p*-value <0.05 was considered significant.

Results

Demographics

The mean age for amniocentesis group vs. cffDNA group was 34.51 ± 4.46 years vs. 32.52 ± 4.15 years. There was no significant difference between ages of the two cohorts. In the amniocentesis group, the five most common indications were AMA (9,347/15,220, 61.41%), MSS (4,736, 31.12%), increased NT (270/15,220, 1.77%), positive results of cffDNA testing (253/15,220, 1.66%), history of chromosome abnormality pregnancy (231/15,220, 1.52%) and abnormal ultrasound findings (218/15,220, 1.43%). 149 patients (0.97%) received amniocentesis for parental chromo-

Table 1. General information on patients receiving amniocentesis and cell-free DNA testing

	Amniocentesis	CffDNA
Age (X ± S)[a]	34.51 ± 4.46	32.52 ± 4.15
Indications No. (%)		
AMA	9347 (61.41)	4370 (44.50)
MSS	4736 (31.12)	1037 (10.56)
Increased NT	270 (1.77)	161 (1.64)
Positive results of cell-free DNA testing	253 (1.66)	0
History of chromosome abnormality pregnancy	231 (1.52)	0
Abnormal ultrasound findings	218 (1.43)	117 (1.19)
Chromosomal abnormality carrier in parents	95 (0.62)	0
History of abnormal pregnancy	54 (0.35)	11 (0.11)
No identifiable indication	0	4125 (42.00)[b]
Others	16 (0.11)	0
Total	15220 (100)	9821 (100)

AMA: advanced maternal age; MSS: positive result of maternal serum screening; Increased NT: increased nuchal translucency (NT). a. $P>0.05$. b. most cases got negative results of maternal screening.

somal abnormality or history of abnormal pregnancy. In cffDNA group, AMA (4,370/9,821, 44.50%), MSS (1,037/9,821, 10.56%), increased NT (161/9,821, 1.64%), abnormal ultrasound findings (117/9,821, 1.19%), and history of abnormal pregnancy (11/9,821, 0.11%) were the most common reasons for cffDNA testing. 4,125 patients (42.00%) received cffDNA testing for no identifiable indications, and most of these patients displayed negative results of maternal screening.

Thus, amniocentesis and cffDNA testing groups used the same indications including AMA, MSS, increased NT, abnormal ultrasound findings and history of abnormal pregnancy.

Positive results in two cohorts

The incidence of abnormal karyotype was 6.52% (992/15,220) for all specimens in amniocentesis group. Chromosomal polymorphism and inversion of chromosome 9 were also included in chromosome aberrations. Autosomal aneuploidies were the most frequent abnormal karyotype, accounting for 42.64% (423/992) of all abnormal specimens. T21 (310/423, 73.29%), T18 (104/423, 24.59%) and T13 (8/423, 1.89%) constituted a large majority of autosomal aneuploidy. Balanced structural rearrangement, polymorphism, SCA, mosaic, and unbalanced structural rearrangement accounted for 20.46% (203/992), 15.02% (149/992), 9.17% (91/992), 8.47% (84/992), and 2.92% (29/992) of all abnormal specimens, respectively. The rest of the chromosome aberrations included marker chromosomes and triploidy (**Table 2**).

In the cffDNA group, 57 specimens exhibited positive results (57/9821, 0.58%). A total of 46 fetuses of these 57 patients carried chromosomal aberrations confirmed by karyotype analysis of amniotic fluid cells. Indications of these 46 patients with chromosome aberrations confirmed by amniocentesis included 21 patients with AMA, 8 patients with increased NT, 6 patients with MSS, 6 patients with no identifiable indications, and 2 patients with abnormal ultrasound findings. T21, T18 and T13 accounted for 64.91% (37/57), 26.32% (15/57), and 8.77% (5/57) respectively (**Table 3**). Of these patients, totally 11 cases including 4 cases of T21, 5 cases of T18 and 2 cases of T13 were false positive verified by karyotype analysis of amniocentesis (**Table 3**).

Selection of invasive prenatal diagnosis for patients with negative cffDNA testing

Pregnant women who received cffDNA testing from August 2015 to November 2016 were followed up to know whether they accepted invasive prenatal diagnosis. A total of 1,975 patients were followed up. Among them, 1,955 subjects showed negative results and the rest had positive results. Among the 1,975 patients, 66.39% (1,298/1,955) patients with negative results accepted follow-up; others failed the follow-up due to wrong phone numbers recorded in laboratory requisition forms or refusal to answer the call. Of the 1298 patients with negative results, three patients received amniocentesis because of abnormal ultrasound findings in the face, right heart, and reduction in the growth rate, respectively. The first fetus with aberrations in the face had a normal karyotype. This patient chose to terminate pregnancy

Table 2. Abnormal cytogenetic findings in patients undergoing amniocentesis versus cffDNA

Abnormal karyotype	Amniocentesis n (%)	CffDNA n (%)
Autosomal aneuploid	423 (42.64)	57 (100)
Trisomy 21[a]	310 (31.25)	37 (64.91)
Trisomy18[b]	104 (10.48)	15 (26.32)
Trisomy13	8 (0.81)	5 (8.77)
Others	1 (0.1)	-
Sex chromosome aneuploid	91 (9.17)	-
X	10 (1.01)	-
XXX	23 (2.32)	-
XXY	46 (4.64)	-
XYY[c]	11 (1.11)	-
XXYY	1 (0.1)	-
Balanced structural rearrangement	203 (20.46)	-
Inversion	147 (14.82)	-
Reciprocal translocation	35 (3.53)	-
Robertsonian translocation	21 (2.12)	-
Unbalanced structural rearrangement	29 (2.92)	-
Additional material on the chromosome	16 (1.61)	-
Deletion	12 (1.21)	-
Tas	1 (0.1)	-
Polymorphism	149 (15.02)	-
Mosaic	84 (8.47)	-
Others	13 (1.31)	-
Marker chromosome	10 (1.01)	-
Triploid	3 (0.3)	-
Total	992 (100)	57 (100)

[a]Includes 9 cases of Robertsonian trisomy 21, 5 cases of inversion trisomy 21, 1 case of marker chromosome trisomy 21, and 1 case of polymorphism trisomy 21. [b]Includes 2 cases of inversion trisomy 18 and 1 case of polymorphism trisomy 18. [c]Includes 1 case of inversion 47, XYY.

in 24 weeks. The second patient with abnormal ultrasound findings in the right heart carried normal karyotype, and selected to go on with her pregnancy. For the last fetus, the result of amniocentesis was reported as 46, XN, inv(9) (p11q12), a pericentric inversion of chromosome 9 which was inherited from its phenotypically normal father. Since this mutation was reported for an absence of clinical manifestation [17], the reduction in growth rate was unlikely related to the chromosomal inversion.

Comparison of chromosome aberrations between two cohorts with same indications

Comparison between pregnancies from the two cohorts was performed to evaluate the missed chromosome aberrations. Patients received amniocentesis and cffDNA testing because of AMA and MSS were investigated. Chromosome aberrations detected by karyotype analysis were categorized into common autosomal aneuploidy, SCA, polymorphism and others. Common autosomal aneuploidy could be detected by cffDNA testing. SCA, polymorphism, and others were out of cffDNA testing range. Common autosomal aneuploidies (including T21, T18 and T21) in amniocentesis group accounted for 2.77% in total patients, 0.92% in patients with AMA, and 1.92% in patients with MSS, respectively (**Table 4**).

Abnormal chromosome aberrations (including SCA, polymorphism, and others) that exceed the cffDNA testing range and would be missed accounted for 3.75%, 3.33% and 3.40% in total patients, patients with AMA, and patients with MSS in amniocentesis group respectively. There were significant differences between the amniocentesis group and cffDNA group ($P< 0.05$) in common autosomal aneuploidy detection rates of the three groups.

Pathogenic effect of possibly missed chromosomal abnormalities

To further analyze the pathogenic effect of possibly missed chromosomal abnormalities by cffDNA testing, chromosome aberrations were divided into three categories: pathogenic chromosomal aberrations, non-pathogenic chromosomal aberrations and unclear pathogenic chromosomal aberrations. Pathogenic chromosomal aberrations included autosomal aneuploidy, SCA, unbalanced structural rearrangement, and triploid. Pathogenic chromosomal aberrations were classified into two groups, within or exceeding the cffDNA testing range. Non-pathogenic chromosomal aberrations included balanced structural rearrangement inherited from parents, polymorphism, and a marker chromosome inherited from par-

Table 3. Follow-up of patients with positive results of cffDNA testing

	CffDNA[a]	Karyotype		
		Positive	Negative	PPV[b]
T21	37	33	4	89.2%
T18	15	10	5	66.7%
T13	5	3	2	60.0%
Total	57	46	11	80.7%

[a]patients with positive cffDNA testing. [b]positive predictive value.

ents. Other chromosomal aberrations (mainly including *de novo* balanced structural rearrangement, mosaicism, *de novo* marker chromosome) were classified as unclear pathogenic chromosomal aberrations.

Non-pathogenic chromosomal aberrations and unclear pathogenic chromosomal aberrations were out of the cffDNA testing range. Patients with pathogenic chromosome aberrations exceeding the cffDNA testing range accounted for 0.81% in total patients, 0.62% with AMA, and 0.78% with MSS in the amniocentesis group. In addition, unclear pathogenic chromosome aberrations accounted for 1.01%, 0.92%, 0.97% in the three groups mentioned above (**Table 5**). Significant differences were seen in the detection rates of pathogenic chromosomal aberrations within the cffDNA testing range between the amniocentesis group and the cffDNA group in total patients, patients with advanced maternal age, and patients with MSS ($P<0.05$).

Discussion

In this study, we present a comparison of chromosomal aberrations between two cohorts underwent amniocentesis and cffDNA testing. In the amniocentesis group a total of 992 (992/15220, 6.52%) fetuses were found to carry chromosome aberrations (see **Table 2**). These data were similar to previous reports by other studies [1, 3, 4, 18]. T21, T18 and T13 totally accounted for 42.54% (422/992), and other chromosome aberrations accounted for 57.46% (570/992). In the cffDNA group, 0.58% (57/9821) patients had positive results. This positive rate was lower than that reported by Taneja *et al* in a general obstetric population [19]. Lower age of cffDNA group in our study may have led to this difference.

In amniocentesis group, the most frequent indication was AMA (64.41%), followed by MSS (31.12%) (**Table 1**). In the cffDNA group, the most frequent indication was also AMA (44.50%), followed by positive result of MSS (10.56%) (**Table 1**). AMA and MSS were the most common indications to receive amniocentesis and cffDNA testing in clinical practice. Other common indications included increased NT, abnormal ultrasound findings, and history of abnormal pregnancy. Generally, patients with these indications should receive amniocentesis instead of cffDNA testing. However, in the clinical setting, many patients may prefer cffDNA testing instead of invasive prenatal diagnosis [9].

Among 1,298 patients with negative results in cffDNA testing, only three patients received amniocentesis because of abnormal ultrasound findings in later ultrasound testing. These results indicated that most patients with negative results would not receive amniocentesis unless ultrasound testing shows positive finding. Thus, it appears that most patients with indications for amniocentesis would still prefer cffDNA testing to amniocentesis. The screening range of non-invasive prenatal testing is limited to common chromosomal aneuploidy (T21, T18 and T13) currently in clinical application according to local policy [16]. Other chromosomal aberrations (sex chromosome aneuploid, structural rearrangement, polymorphism, mosaic) will be missed, which can be detected through amniocentesis. Considering patients undergoing cffDNA testing shared the same indications with patients receiving amniocentesis (**Table 1**), it is meaningful to evaluate the possible missing chromosome aberrations out of the cffDNA testing range.

Positive rates of SCA, polymorphism, and other chromosome aberrations were 0.6%, 0.98% and 2.17% in the amniocentesis group (**Table 4**). All of these chromosome aneuploidies could not be detected by cffDNA testing. In patients with AMA in amniocentesis group, positive rates of SCA, polymorphism and other chromosome aberrations were 0.52%, 1.04% and 1.17%, respectively. In patients with MSS in the amniocentesis group, the corresponding rates were 0.53%, 0.87% and 2.00%, respectively. These chromosome aberrations would have been missed in patients who received cffDNA

Table 4. Comparison of abnormal results between two groups of all specimens, and those with indications of AMA and MSS

	Amniocentesis group			CffDNA group		
	Whole	AMA	MSS	Whole	AMA	MSS
CAA	422 (2.77%)	86 (0.92%)	91 (1.92%)	46[a] (0.47%)	21[b] (0.48%)	6[c] (0.58%)
SCA	91 (0.60%)	49 (0.52%)	25 (0.53%)	-	-	-
Polymorphism	149 (0.98%)	97 (1.04%)	41 (0.87%)	-	-	-
Others	330 (2.17%)	165 (1.77%)	95 (2.00%)	-	-	-
Negative	14228 (93.48%)	8950 (95.75%)	4484 (94.68%)	9775 (99.53%)	4349 (99.52%)	1031 (99.42%)
Total	15220 (100%)	9347 (100%)	4736 (100%)	9821 (100%)	4370 (100%)	1037 (100%)

AMA, advanced maternal age; MSS, positive result of serum screening; CAA, Common autosomal aneuploidy; SCA, Sex chromosome aneuploidy; [a,b,c]False positive cases had been excluded.

Table 5. Comparison of pathogenic effects between two groups with indications of AMA and MSS

	Amniocentesis group			CffDNA group		
	Whole	AMA	MSS	Whole	AMA	MSS
PCA	545 (3.58%)	144 (1.54%)	128 (2.7%)	46[c] (0.47%)	21[d] (0.48%)	6[e] (0.58%)
Within cffDNA[a]	422 (2.77%)	86 (0.92%)	91 (1.92%)	46[c] (0.47%)	21[d] (0.48%)	6[e] (0.58%)
Exceeding cffDNA[b]	123 (0.81)	58 (0.62%)	37 (0.78%)	-	-	-
NPCA	294 (1.93%)	167 (1.79%)	78 (1.65%)	-	-	-
UPCA	153 (1.01%)	86 (0.92%)	46 (0.97%)	-	-	-
Negative	14228 (93.48%)	8950 (95.75%)	4484 (94.68%)	9775 (99.53%)	4349 (99.52%)	1031 (99.42%)
Total	15220 (100%)	9347 (100%)	4736 (100%)	9821 (100%)	4370 (100%)	1037 (100%)

PCA, pathogenic chromosomal abnormalities; NPCA, non-pathogenic chromosomal abnormalities; UPCA, unclear pathogenic chromosomal abnormalities; [a]Pathogenic chromosomal abnormalities within cffDNA testing range; [b]Pathogenic chromosomal abnormalities exceeding cffDNA testing range; [c,d,e]False positive cases had been excluded.

Table 6. Comparison of age between two cohorts with indications of AMA and MSS

	Whole[a]		AMA[b]		MSS[c]	
	Amniocentesis	CffDNA	Amniocentesis	CffDNA	Amniocentesis	CffDNA
Mean	34.51	32.52	37.24	36.11	29.96	29.36
Std	4.46	4.15	2.26	1.88	3.28	3.08

[a]P>0.05. [b]P<0.05. [c]P<0.05.

testing, if they refused further invasive diagnostic procedures.

Detection of chromosome aberrations has important clinical significance in pregnancy and early childhood management. However, many chromosome aberrations are not associated with structural anomalies on ultrasound assessment and may have relatively mild phenotypes. If patients with indications for amniocentesis actually receive cffDNA testing and have negative results, chromosome aberrations exceeding the cffDNA testing limitation would have been missed. In the amniocentesis group, pathogenic chromosome aberrations undetected by cffDNA testing, and unclear pathogenic aberrations accounted for 0.81% and 1.01%, respectively. In patients with AMA, the corresponding rates were 0.62% and 0.92%, and in patients with MSS were 1.92% and 0.97%, respectively. All of them would have been left out, and babies with chromosomal disease would have been born, if they refused further invasive diagnostic procedures when getting negative cffDNA testing results.

The clinical application of cffDNA testing resulted in a significant decrease in invasive diagnosis [9, 10]. Marketing efforts of laboratories performing cffDNA testing and patients' increasing concerns about adverse pregnancy outcomes by invasive diagnostic testing played key roles in this transition. Some surveys have shown that more obstetricians would offer cffDNA testing to women at high and average risk [11]. Patients preferred cffDNA testing mainly

in fear of procedure-induced pregnancy loss following amniocentesis and chorionic villus sampling (CVS) [20]. Although common aneuploidies are most prevalent, other chromosomal aberrations exceeding cffDNA testing may be associated with severe intellectual disabilities. CffDNA testing is more expensive than traditional karyotype analysis at present (2300¥ vs. 1000¥) in China. In addition, positive results of cffDNA testing should be confirmed by karyotype analysis. However, pregnant women are given all the information and they have opportunity to make a choice. Although cffDNA testing is less economical and accurate than karyotype analysis at this time, many patients select cffDNA testing. Nevertheless, cffDNA testing cannot replace karyotype analysis except for patients with a contraindication for amniocentesis. The current shift away from diagnostic testing may cause unexpected consequences as Evans stressed [21]. However, cffDNA testing with high sensitivity can help to detect more common aneuploidies; for example,in the cffDNA group, 6 patients with no identifiable indication were identified as having trisomy. It is meaningful to discuss the trade-off between limited and complete evaluation. A similar discussion arose after clinical application of five-chromosomes FISH (13, 18, 21, X, Y) nearly 20 years ago, which concerned the trade-off between five chromosome FISH and complete karyotype [22, 23]. After appropriate education and counseling for pregnant patients, an appropriate set of indications [21] for cffDNA testing may help to balance the higher procedural risk and lower risk but less information in the choice of diagnostic assays.

In this study, data of the amniocentesis group and the cffDNA group were collected in different periods. Thus, these were not perfectly controlled groups, which may have caused heterogeneity in patients with the same indications. As shown in **Table 6**, a significant difference ($P<0.05$) was seen in age subgroups between the two cohorts with AMA or positive result of MSS. It is noteworthy that such heterogeneity may contribute to the significant difference in detecting rates of common autosomal aneuploidy and pathogenic chromosome aberrations between the two cohorts.

In conclusion, with the clinical application of cffDNA testing, an increasing number of patients tend to refuse invasive prenatal diagnosis. This will lead to more chromosomal aberrations missed during the prenatal screening process. The occurrence rate of chromosome aneuploidies, pathogenic chromosomal abnormalities, and unclear pathogenic chromosomal abnormalities out of the cffDNA testing range were analyzed in our research. These data will help the doctor to evaluate the cffDNA test and inform patients regarding the advantages and disadvantages of the noninvasive assay.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhenwen Chen, School of Basic Medical Science, Capital Medical University, Beijing Key Laboratory of Cancer Invasion and Metastasis Research, 10 You An Men Wai Xi Tou Tiao Road, Fengtai District, Beijing 100069, China. Tel: (86) 10-83911839; Fax: (86) 10-83911839; E-mail: czwen@ccmu.edu.cn

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