Original Article

Application of ultrasound combined with noninvasive prenatal testing in prenatal testing

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Background: Both noninvasive prenatal testing (NIPT) and prenatal ultrasound are widely used in clinical settings due to their safety, noninvasiveness, and accuracy, showing high detection rates for fetal chromosomal aneuploidies and structural abnormalities. However, whether the combined application of these two techniques has higher clinical applicability remains to be demonstrated.

Methods: The clinical and laboratory data of 3,050 pregnant women who underwent NIPT were collected. The clinical feasibility and health economics of NIPT were investigated by analyzing the accuracy, postnatal follow-up results, and population applicability of NIPT. In addition, an analysis ultrasonography, NIPT, and karyotyping results were performed to evaluate the combined application of ultrasonography and NIPT in screening fetal chromosomal abnormalities.

Results: NIPT could accurately detect trisomies 21, 18, and 13, and was highly sensitive and specific in detecting other autosomal and sex chromosomal aneuploidies. The positive rates of chromosomal abnormalities in the presence of 1 or 2 or more ultrasound markers were 7.5% and 29.2%, respectively, indicating that ultrasonography combined with NIPT should be preferred for the detection of fetal chromosomal abnormalities.

Conclusions: Health economic analysis revealed NIPT to be superior to conventional serologic screening in terms of accuracy and socioeconomics. Ultrasound and NIPT are complementary to each other and the combined techniques can improve the screening ability of fetal chromosomal abnormalities and provide clinicians with more diagnostic information.

Keywords: Ultrasonography; noninvasive prenatal test; prenatal diagnosis; fetal chromosomal abnormalities

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Introduction

Chromosomal abnormalities are one of the primary causes of birth defects (1). Children with chromosomal abnormalities usually have structural malformations in various systems and mental deficiencies, with death occurring in severe cases. Screening and diagnosis in the prenatal period are particularly important for avoiding chromosomal abnormalities in newborns. Currently,

karyotyping immediately after amniocentesis or cord blood puncture is the gold standard for detecting fetal chromosomal aberrations; however, 0.5–1% of cases of this procedure are associated with miscarriage and intrauterine infection (2), and it is not indicated for some pregnant women with contraindications. Therefore, safe and effective screening methods for fetal chromosomal abnormalities are urgently needed for clinical settings.

Ultrasonography is effective in detecting structural abnormalities in all fetal systems (3) and has the advantages of being noninvasive, accurate, and rapid. Studies have shown that abnormalities in fetal ultrasound structures and/or the presence of soft markers are directly related to aneuploidies (4,5), with the aneuploidy risk increasing with the number of ultrasound structural abnormalities (6). Proper ultrasound performed during any stage of a pregnancy allows for the early detection of fetal structural abnormalities and the assessment of a patient's risk of carrying a fetus with a chromosomal disorder. However, for some cases without structural abnormalities or unclear structural abnormalities, or affected by ultrasound doctor technology and machine resolution, ultrasound cannot accurately diagnose.

The cell-free fetal DNA (cffDNA) in the peripheral blood of pregnant women carries the genetic information of a fetus. Noninvasive prenatal testing is a screening method to extract maternal blood to detect fetal chromosome abnormalities. It can avoid the birth of fetus with chromosome abnormalities, reduce the delivery of fetus with birth defects, and avoid fetal abortion or infection caused by invasive testing. As a noninvasive and accurate prenatal screening method, noninvasive prenatal testing (NIPT) can detect and analyze cffDNA using high-throughput sequencing technology and is thus able to assess the risks of fetal chromosomal disorders (7). It can effectively detect chromosomal aneuploidies such as trisomy 21 (T21), trisomy 18 (T18), or trisomy 13 (T13). Meanwhile, with the increase in read depth, NIPT has also shown certain capability in detecting the copy number variations (CNVs), which has been increasingly applied in clinical settings.

In recent years, most studies on NIPT have focused on its ability to screen for chromosomal disorders, but few follow-up studies on low-risk populations and studies on the applicability of NIPT in special populations (e.g., older pregnant women) have been conducted. Many studies involve a small number of cases and lack of analysis on the health economics of NIPT, while the clinical feasibility of NIPT combined with prenatal ultrasound for screening aneuploidy and other abnormalities remains uncertain. We thus investigated the use of NIPT for aneuploidy screening for postnatal follow-up in special populations; based on the results of prenatal ultrasound, we also explored the role of prenatal ultrasound combined with prenatal NIPT for the screening of aneuploidies.

Health economics is a branch of economics, which is used to solve economic problems in the field of health. It is also closely related to medicine, hygiene, demography and sociology. Our study also evaluated the clinical feasibility and social benefits of NIPT through health economic analysis.

We present the following article in accordance with the STARD reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-21-617/rc).

Methods

Patients

The data from 3,050 pregnant women who underwent NIPT were collected. The inclusion criteria were as follows: (I) with a gestational age of ≥12 weeks; (II) singleton pregnancy; and (III) available for prenatal ultrasound and serological examinations as required by the study. The exclusion criteria were the following: (I) multiple pregnancies (multiple pregnancies were excluded from this study for more accurate analysis of the obtained NIPT results and not because NIPT is infeasible for multiple pregnancies); (II) a history of allogeneic blood transfusion within the last 6 months in the mother; (III) a mother with immune disease; and (IV) a mother with known chromosomal abnormalities or a history of malignancy, blood transfusion, bone marrow transplantation, or stem cell therapy that might interfere with the accuracy of NIPT testing. Detailed information including name, telephone number, age, date of birth, week of gestation, last menstrual period, mode of conception, times of pregnancies and deliveries, number of fetuses, and serological screening results were recorded for patients who met the enrollment criteria. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of the Second Hospital of Hebei Medical University (No. 2017-R208) and informed consent was taken from all the patients.

Methods and equipment

Ultrasonography was performed using a GE Voluson

E10/E8 diagnostic ultrasound machine (GE Healthcare, Chicago, IL, USA) with 2- to 5-MHz probes, with a mechanical index (MI) of <1. High-throughput gene sequencing for NIPT was performed with a BGISEQ-500 gene sequencer (MGI, Shenzhen, China). Karyotyping was performed using the trypsin-Giemsa banding technique on a Leica CW4000 karyotyping system (Leica, Wetzlar, Germany).

Ultrasound examination

For ultrasound examination, the pregnant woman was placed in a supine position on the examination bed, with her abdomen fully exposed. Level III ultrasound of the fetus was performed by a sonographer according to the British Fetal Medicine Foundation screening criteria. Patients were grouped according to the abnormalities of fetal structures or soft markers among the ultrasound findings.

NIPT procedure

After inclusion and exclusion criteria were applied, 8–10 mL of peripheral blood was collected from the enrolled mothers and placed in free DNA collection tubes (Xinle, China). Plasma was separated after pretreatment, and cffDNA was extracted from the plasma and purified. After end repair and modification, the DNA library was constructed for up sequencing. Multiple library samples were mixed in a certain ratio and then subjected to gene sequencing. The sequencing results were submitted for human database comparison, bioinformatic analysis, and logical calculations, so as to identify the risks for fetal chromosomal abnormalities. For those at high risk of NIPT failure, amniocentesis was applied, as this is recommended for karyotyping.

Amniocentesis

For amniocentesis, the pregnant woman was asked to empty her bladder and lie flat in a supine position on the operating table. Preoperative ultrasound was used to routinely assess the fetal heart, fetal position, and the basic condition of the uterine cavity and to determine the puncture site and direction. After the puncture site was disinfected, 15–20 mL of amniotic fluid was collected and immediately inoculated in amniotic fluid medium for amniotic fluid cell culture and karyotype analysis. Postoperative ultrasound was performed to check the basic conditions of the placenta, amniotic fluid, and fetal heart.

Research methods

Postnatal follow-up was performed for the mothers with low-risk NIPT results; for mothers with high-risk NIPT results, amniocentesis was performed to confirm the karyotype and assess the efficacy of NIPT screening.

Information on the age, serological screening, and ultrasound examination of the enrolled mothers was collected and recorded. These participants were grouped by advanced maternal age (AMA), serologic screening results, and ultrasound findings, and the feasibility of NIPT in each group was analyzed. The groupings were delineated as follows: (I) the age-based groups consisted of an AMA group (with a maternal age of ≥35 years) and a non-AMA group (with a maternal age of <35 years); (II) serological result-based groups consisted of a serological screening high-risk group (T21 risk value ≥1/270 and T18 risk value ≥1/350), a serological screening borderline-risk group (T21 risk value between 1/1,000 and 1/270; T18 risk value between 1/1,000 and 1/350), and a serological screening low-risk group (T21 risk value <1/1,000 and T18 risk value <1/1,000); (III) ultrasound soft markers included (i) nuchal translucency thickness (NT)/nuchal fold (NF) (NT ≥2.5 mm at 11–13^{+6} gestational weeks and/or NF ≥6 mm as measured in the cerebellar plane at 15–24 gestational weeks; (ii) single umbilical artery; (iii) mild dilatation of the lateral ventricles (unilateral or bilateral posterior horns of the lateral ventricles with an internal diameter of 10–15 mm); (iv) choroid plexus cysts in the lateral ventricles; (v) bright spots in the ventricles; (vi) Echo enhancement of the intestinal canal (limited intestinal canal echogenicity similar or stronger compared to that of bone tissue); (vii) renal pelvis dilation (unilateral or bilateral anterior-posterior diameter of the renal pelvis in cross-section of both kidneys >4 mm at 20 gestational weeks, >5 mm at 20–30 weeks, and >7 mm at 30 weeks); (viii) missing or dysplastic nasal bone; (ix) and short femur (femur length below 2 standard deviations for the normal gestational age).

The ultrasound findings and NIPT results of patients with abnormal karyotypes were analyzed to comprehensively evaluate the value of ultrasound combined with NIPT in fetal chromosome detection.

Health economic analysis of NIPT

The selection of different screening strategies by 100,000 mothers was simulated, and a decision tree was

created. The following two strategies were included: (I) NIPT screening in which the mothers were first screened with NIPT, and those with high-risk NIPT screening results underwent genetic counseling and concurrent amniocentesis; (II) serological screening, in which the mothers were first screened serologically, and those at high risk for aneuploidy underwent genetic counseling and further amniocentesis + karyotyping. Pregnancies were terminated if karyotyping revealed the presence of aneuploidy in either strategy.

The health economic indicators included (I) incremental cost-effectiveness ratios (ICERs), which referred to the cost per birth of a child with aneuploidy prevented by a screening strategy, with a smaller ICER meaning better outcomes; and (II) safety index (SI), which referred to the number of normal fetal abortions per birth of a child with aneuploidy prevented by a screening strategy, with a lower SI indicating better results.

The total cost of the screening strategy was calculated as follows: total cost = number of cases receiving the screening method a child with aneuploidy prevented by a screening strategy, ling and further amniocentesis + karyotyping. Pregnancies were terminated if amniocentesis per case + number of positive cases of amniocentesis × average cost of termination of pregnancy + number of abortions caused by amniocentesis × cost of abortion per case + number of false-negative cases × social cost per patient. Meanwhile, the SI was calculated as follows: SI = number of abortions of normal fetuses caused by the screening strategy/number of births of infants with chromosomal aneuploidies prevented by the screening strategy.

Formula parameters including the incidence rates of T21, T18, and T13; the acceptance rate of either screening method; and the rate of abortions caused by amniocentesis were retrieved from the literature. The costs were obtained through surveys to inform the health economic analysis.

Statistical analysis

The numeration data are presented as n, and the composition ratios with percentages. Screening performance was evaluated using the methods used in screening tests, and the specificity [true negative/(true negative + false positive)], sensitivity [true positive/(true positive + false negative)], false-positive rate (FPR) [false positive/(true negative + false positive)], false-negative rate (FNR) [false negative/(true positive + false positive)], positive predictive value (PPV) [true positive/(true positive + false positive)], and negative predictive value (NPV) [true negative/(false negative + true negative)] were calculated for each aneuploidy disease.

Results

Chromosomal abnormalities detected by NIPT

After the exclusion and inclusion criteria were applied, a total of 3,050 pregnant women were deemed eligible for participation in this study. Among these women, were 2,954 pregnant women in the NIPT low-risk group and 96 pregnant women in the NIPT high-risk group including 25 cases with high risk for T21, 6 cases with high risk for T18, 4 cases with high risk for T13, 23 cases with high risk for other autosomal aneuploidy abnormalities, and 38 cases with high risk for sex chromosome aneuploidies (SCAs). All NIPT high-risk cases were diagnosed prenatally, and karyotypic abnormalities were detected prenatally in 35 cases, including 15 cases of T21, 2 cases of T18, and 9 cases of SCAs, while no T13 or other autosomal karyotypic abnormalities were detected. In addition, 346 out of 3,050 pregnant women indicated for amniocentesis underwent prenatal diagnosis. In the NIPT low-risk group, T21 was detected in 3 cases while SCA was detected in 6 cases (*Table 1*). It was found that the overall positive rate of fetal T13, T18, and T21 was 0.66%, which was higher than that in other reports (1–2 per 1,000). This might be explained by the inclusion or exclusion criteria in our study: all the pregnant women participating in this study were found to have fetuses with single or multiple ultrasound abnormalities on systematic ultrasound scans.

Among the pregnant women who underwent amniocentesis, 56 were of advanced age and 288 were of non-advanced age. A total of 27 patients underwent serologic screening, including 10 cases in the low-risk group and 17 cases in the borderline-risk/high-risk groups. Ultrasound scans were performed in 336 patients, among whom 152 patients had ultrasound soft markers and 184 had no ultrasound soft markers. Analysis of the results of NIPT and amniocentesis in each group showed that the screening efficacy of NIPT was comparable between the advanced age group and the non-advanced age group (*Table 2*), indicating that NIPT is applicable to all age groups. Analysis of the characteristics of the serologic screening groups and the ultrasound soft marker groups with abnormal findings showed that NIPT had higher specificity and sensitivity and was therefore more practicable for assessing the risk of chromosomal disorders in fetuses conceived by pregnant

Table 1 Findings of NIPT and karyotyping (by amniocentesis)

Karyotyping	T21		T18		T13		Other autosomal abnormalities		SCAs	
	H	L	H	L	H	L	H	L	H	L
+ (n)	15	3	2	0	0	0	0	0	9	6
– (n)	10	318	4	340	4	342	23	323	29	302

H, NIPT high-risk group; L, NIPT low-risk group; +, abnormal karyotype; –, normal karyotype. NIPT, noninvasive prenatal testing; SCAs, sex chromosome aneuploidies.

Table 2 Detection of aneuploidies by NIPT in each group

Effectiveness index	Advanced maternal age (yes/no)		Results of serological screening		With ultrasound soft markers	
	Yes	No	Borderline/high risk	Low risk	Yes	No
Specificity	72.09% (31/43)	78.20% (208/266)	85.71% (12/14)	77.78% (7/9)	84.89% (118/139)	73.62% (120/163)
Sensitivity	76.92% (10/13)	72.73% (16/22)	66.67% (2/3)	100% (1/1)	84.62% (11/13)	71.43% (15/21)
PPV	45.45% (10/22)	21.62% (16/74)	50% (2/4)	33.33% (1/3)	34.38% (11/32)	25.86% (15/58)

NIPT, noninvasive prenatal testing; PPV, positive predictive value.

Table 3 Efficacy of NIPT in detecting chromosome aneuploidies

Effectiveness index	T21	T18	T13	Other autosomal abnormalities	SCAs
Specificity	96.95% (318/328)	98.84% (340/344)	98.84% (342/346)	93.35% (323/346)	91.24% (302/331)
Sensitivity	83.33% (15/18)	100% (2/2)	–	–	60% (9/15)
False-positive rate	3% (10/328)	1.16% (4/344)	1.16% (4/346)	6.65% (23/346)	8.76% (29/331)
False-negative rate	16.67% (3/18)	0 (0/2)	–	–	40% (6/15)
Positive predictive value	60% (15/25)	33.33% (2/6)	0 (0/4)	0 (0/23)	23.68% (9/38)
Negative predictive value	99.07% (318/321)	100% (340/340)	100% (342/342)	100% (323/323)	98.05% (302/308)

NIPT, noninvasive prenatal testing; SCAs, sex chromosome aneuploidies.

women with borderline/high risk on serologic screening and with ultrasound soft markers.

The detection efficacy was analyzed for NIPT, and the specificity, sensitivity, FPR, FNR, PPV, and NPV of NIPT for the detection of chromosome aneuploidies were obtained, as shown in *Table 3*. The results showed that NIPT had high specificities and sensitivities for the detection of T21, T18, and T13, which was consistent with the data in literature, demonstrating that NIPT is a safe and accurate prenatal screening technology that warrants wide application. Although NIPT has a high detection rate for chromosome aneuploidies, it still may yield false negatives, the frequency of which can be reduced by increasing the sequencing depth. Thus, NIPT can be used for a wider range of prenatal screening of chromosome aneuploidies; however, amniocentesis remains the gold standard for prenatal diagnosis of fetal chromosomal disorders, and there is currently no better alternative

Pregnancy outcomes in the NIPT low-risk group

A total of 1,813 pregnant women in the NIPT low-risk group were followed up, and the outcomes included 18 terminations (0.99%), 23 spontaneous abortions (1.27%), and 1,772 fetal births (97.74%). Among the fetuses born, 1,747 were in good developmental condition and 25 had

Table 4 Follow-up results in the NIPT low-risk group

Pregnancy outcome	n (%)
Follow-up	1813
Pregnancy termination	18 (0.99)
Spontaneous abortions	23 (1.27)
Births	1,772 (97.74)
Well-developed	1,747 (96.36)
Congenital heart diseases	8 (0.44)
Isolated external ear malformation	2 (0.11)
Developmental retardation	2 (0.11)
Hearing abnormality combined with external ear deformity	1 (0.06)
Congenital esophageal atresia	1 (0.06)
Ichthyosis	1 (0.06)
Pigmentary incontinence	1 (0.06)
West syndrome	1 (0.06)
Hypospadias	1 (0.06)
Jaw-winking syndrome	1 (0.06)
Congenital ptosis	1 (0.06)
Intestinal torsion	1 (0.06)
Hip dysplasia	1 (0.06)
Congenital umbiliculitis	1 (0.06)
Anemia	1 (0.06)
Calf hemangioma	1 (0.06)

NIPT, noninvasive prenatal testing.

abnormal development. Congenital heart diseases (n=8, 0.44%) were among the most common diseases in the newborns, followed by isolated external ear malformation and postnatal growth retardation (n=2, 0.11%). Other diseases or syndromes included hearing abnormalities, congenital esophageal atresia, ichthyosis, pigmentary incontinence, West syndrome, hypospadias, jaw-winking syndrome, congenital ptosis, intestinal torsion, hip dysplasia, congenital umbiliculitis, anemia, and calf hemangioma, as detailed in *Table 4*. As no further examination was carried out in these children, it was unclear whether the developmental abnormalities were caused by genetic factors such as chromosome aneuploidy, CNVs, or mono/polygenic disorders. Thus, the possibility of fetal abnormalities could not be ruled out in the NIPT low-risk group. Meanwhile, the traditional karyotyping of cultured amniotic fluid cells has certain limitations and cannot accurately diagnose small-fragment CNVs and genetic disorders. Thus, genetic testing methods need to be carefully selected when providing genetic counseling and guidance to pregnant women. Particularly, when ultrasonography reveals the presence of structural malformations in the fetus, multiple genetic testing methods (e.g., karyotyping + CNV + whole exome testing) need to be considered to exclude the effects of genetic factors on fetal growth and development, so as to assist clinical diagnosis.

Relationships between ultrasound soft markers and fetal chromosomal abnormalities

Among the pregnant women whose fetal karyotype was clarified by amniocentesis, a total of 336 cases received ultrasound scans, with 152 of these cases possessing ultrasound soft markers and 184 having no ultrasound abnormality. Among these 152 mothers with ultrasound soft markers, 106 (69.74%) had a single soft marker, including bright ventricular spots (n=32, 21.05%), NT/NF thickening (n=19, 12.50%), and nasal bone hypoplasia or absence (n=15, 9.87%). Karyotyping confirmed abnormal karyotypes in 8 cases, and40 mothers (26.32%) had 2 ultrasound soft markers, with chromosome aneuploidy being confirmed in 5 of these cases. In addition, 6 mothers (3.95%) had 3 or more ultrasound soft markers, including 1 case of chromosomal abnormality, as detailed in *Table 5*. Therefore, the risk of fetal karyotype abnormalities increases with the increased number of ultrasound soft markers, and multiple genetic tests, including NIPT, are more feasible for this population.

Of the 18 fetuses with confirmed T21, 50% had no ultrasound abnormality (n=9), 27.8% had bright spots in the ventricles (n=5), 27.78% had nasal hypoplasia or absence (n=5), 16.67% had NT/NF thickening (n=3), 11.11% had short femurs (n=2), and 5.56% had renal pelvis dilation (n=1). Choroid plexus cysts in the lateral ventricles were noted in both fetuses with T18 (100%). Of the 15 fetuses with confirmed SCA, 73.33% had no ultrasound abnormality, 13.33% (n=2) had bright spots the ventricles, 6.67% (n=1) had NT/NF thickening, and 6.67% (n=1) had short femurs (*Table 6*). Analysis of the distribution of ultrasound soft markers in various chromosomal abnormalities suggests that the possibility of a fetal chromosomal abnormality should be considered if ultrasound scans reveal the presence of NT/NF thickening, nasal bone dysplasia, bright ventricular echogenic spots,

Table 5 Chromosomal abnormalities detected by ultrasound soft markers

Ultrasonographic abnormal marker	n	NIPT high risk	Abnormal karyotypes	Rate of abnormal karyotypes
Single soft marker				
NT/NF thickening	19	2	1	
Single umbilical artery	5	0	0	
Mild dilatation of the lateral ventricles	5	0	0	
Choroid plexus cysts in the lateral ventricles	4	2	2	
Bright spots in the ventricles	32	11	3	
Echo enhancement of the intestinal canal	5	1	0	
Renal pelvis dilation	10	1	0	
Nasal bone hypoplasia or absence	15	4	2	
Short femur	11	1	0	
Total	106	22	8	7.55% (8/106)
Two soft markers	40	8	5	12.50% (5/40)
Three or more soft markers	6	3	1	16.67% (1/6)

NT, nuchal translucency thickness; NF, nuchal fold; Notes: NIPT, noninvasive prenatal testing.

Table 6 Composition and distribution of ultrasound soft markers in abnormal chromosomal karyotypes

Ultrasound soft markers	n	Composition ratio (%)
T21 (n=18)		
NT thickening	3	16.67% (3/18)
Bright spots in the ventricles	5	27.78% (5/18)
Renal pelvis dilation	1	5.56% (1/18)
Nasal bone hypoplasia or absence	5	27.78% (5/18)
Short humerus or femur	2	11.11% (2/18)
Without any ultrasound soft marker	9	50% (9/18)
T18 (n=2)		
Choroid plexus cysts in the lateral ventricles	2	100% (2/2)
Sex chromosome abnormalities (n=15)		
NT thickening	1	6.67% (1/15)
Bright spots in the ventricles	2	13.33% (2/15)
Short femur	1	6.67% (1/15)
Without any ultrasound soft marker	11	73.33% (11/15)

NT, nuchal translucency thickness.

and/or choroid plexus cysts in a fetus. Other structural deformities should also be carefully screened.

Karyotypic abnormalities were confirmed by amniocentesis in 28 fetuses, including 18 cases of T21, 2 cases of T18, 8 cases of 47,XXY (Klinefelter syndrome), 4 cases of 47,XYY, and 3 cases of 45,X/46,XN mosaicism. The combined analysis of the results of karyotyping, NIPT, and ultrasound revealed that when an autosomal aneuploidy

Table 7 NIPT and ultrasound results of abnormal chromosome karyotypes

Karyotype	NIPT	Ultrasound findings	n
47, XN, +21	High risk for trisomy 21	NF thickening, short nasal bone, bright spots in left ventricle, and bilateral renal pelvis dilatation	1
		Bright spots in left ventricle	1
		Short femur; bright spots in left ventricle	1
		Nasal bone is not clearly seen; bright spots in left ventricle	1
		Nasal bone is not shown; excessive amniotic fluid; short femur; abnormal posture of right foot	1
		Excessive amniotic fluid	1
		Nasal bone is not clearly shown; abnormal development of right hand	1
		Ventricular septal defect; persistent left superior vena cava	1
		Nasal bone is not shown; abnormal bipedal posture; pericardial effusion	1
		NT thickening	1
		No obvious abnormality is seen	5
	Low risk	Excessive amniotic fluid	1
		Persistent left superior vena cava	1
		NF thickening; bright spots in left ventricle	1
47,XN, +18	High risk for trisomy 18	Ventricular septal defect; bilateral ventricular choroid plexus cysts; omphalocele; umbilical cord cysts	1
		Overlapping fetal fingers; bilateral ventricular choroid plexus cysts; omphalocele; umbilical cord cysts	1
47,XXY	Sex chromosome abnormalities	Bright spots in left ventricle	1
		Ventricular septal defect; bright spots in left ventricle	1
		No obvious abnormality is seen	4
	Low risk	Thickened skin of the neck; short femur; excessive amniotic fluid	1
		No obvious SCA	1
47,XYY	SCA	No obvious SCA	2
	Low risk	No obvious SCA	1
		–	1
45,X/46,XX	SCA	No obvious SCA	1
45,X/46,XY	High risk for trisomy 21	Cervical hygroma	1
	Low risk	No obvious abnormality	1

–, no ultrasound was performed. NIPT, noninvasive prenatal testing; NT, nuchal translucency thickness; NF, nuchal fold; SCA, sex chromosome aneuploidies.

occurred, there were high-risk NIPT results or abnormal ultrasound findings, or both, which were highly effective in detecting fetal chromosomal abnormalities (*Table* 7). NT/NF thickening, facial abnormalities, and neurological abnormalities are the more common ultrasound features of chromosomal variations. A sex chromosome aneuploidy (SCA) may be associated with low-risk NIPT results and no obvious abnormalities on ultrasound, which can result

Table 8 A summary of the parameters required for the decision tree model

Parameters	Values
Incidence rate of T21	0.15% (8)
Incidence rate of T18	0.025% (9)
Incidence rate of T13	0.05% (9)
Acceptance rate of NIPT	70% (10)
Acceptance rate of serological screening	67% (10)
Sensitivity of NIPT	85%
False positive rate of NIPT	5.5%
Sensitivity of serological screening	54.5% (11)
False positive rate of serological screening	10% (11)
Rate of miscarriage following amniocentesis	0.8% (12)
Cost of NIPT (RMB)	2,500
Cost of serological screening (RMB)	270
Cost of genetic counseling (RMB)	20
Cost of amniocentesis and karyotyping (RMB)	2,000
Cost of pregnancy termination (RMB)	1,800
Cost of abortion (RMB)	1,500

NIPT, noninvasive prenatal testing.

in the delivery of a chromosomally abnormal fetus. Greater clinical attention needs to be paid to this type of SCA, and more accurate methods are needed to assist in the diagnosis of SCAs.

Health economic analysis

Parameters required for the health economic analysis were obtained after a literature review or based on our current study (*Table 8*). Parameters including the incidence rates of T21, T18, and T13; the acceptance rate of serological screening; the acceptance rate of NIPT; the sensitivity and the FPR of serological screening for chromosome aneuploidy (including T21 and T18); and the rate of miscarriage due to invasive prenatal diagnosis were obtained from literature. The sensitivity and FPR of NIPT screening for chromosome aneuploidies (including T21, T18, and T13) was obtained from the results of the present study. The costs of NIPT, serologic screening, amniocentesis, karyotyping, genetic counseling, termination of pregnancy, and abortion were obtained from field surveys.

Simulation of the application of NIPT or serological screening in 100,000 pregnant women for fetal chromosome aneuploidies showed that NIPT prevented 133.88 births at a total cost of 186,669,451 RMB, and the ICER of NIPT was 1,394,304 RMB; serological screening prevented the delivery of 63.90 fetuses with chromosome aneuploidies at a total cost of 38,631,043.5 RMB. According to a report released in 2003, the average lifetime cost of a new case of Down syndrome from the family and societal perspectives was 390,600 RMB and 450,000 RMB, respectively, and there is no clinical cure for the disease. Therefore, the additional social cost of serological screening was calculated to be 58,825,188 RMB, and the cost-effectiveness ratio of serological screening was 1,525,137 RMB. The number of abortions of chromosomally normal fetuses due to amniocentesis was 80.42 and 44.99 in the serological screening group and the NIPT group, respectively, with an SI of 1.26 and 0.34, respectively (*Table 9*). Therefore, NIPT can be considered a safe, accurate, economical, and clinically practicable prenatal screening technique compared to traditional serological screening.

Discussion

NIPT assesses the risk of T13, T18, and T21 in fetuses by detecting cffDNA fragments in the peripheral blood of pregnant women using high-throughput gene sequencing technology. In our current study, the specificity of NIPT for T21, T18, and T13 was 96.95%, 98.84%, and 98.84%, respectively, and the sensitivity for T21 and T18 reached 83.33% and 100%, respectively; both the false-positive and FNRs were below 16.67%, and the sensitivity and specificity were also generally consistent with those in other reports (13-15). However, NIPT also has the ability to detect other autosomal and sex chromosome abnormalities, but its specificity in our study was only 93.35% and 91.24%, respectively. When used for screening SCAs, its sensitivity was only 60%, and the FNR reached 40%, suggesting an unacceptably high rate of misdiagnosis. Deeper sequencing depth may be considered to obtain richer data and thus increase the accuracy of analysis when applied to other chromosomes.

In terms of predictive value, NIPT is a screening test that has the potential for false positives or false negatives and is not a complete substitute for prenatal diagnosis. We found the predictive value was relatively high in the NIPT low-risk group, with NPVs of more than 98% for various chromosomal aneuploidies; however, the predictive value

Table 9 Health economics analysis of 100,000 simulated pregnant women undergoing different screening strategies

Health economics data	Serological screening	NIPT
Total cost	38,631,043.5	186,669,451
Number of babies born with chromosome aneuploidies avoided	63.90	133.88
Social costs	58,825,188	–
Incremental cost-effectiveness ratio (ICER)	1,525,137	1,394,304
Number of abortions of chromosomally normal fetuses due to screening	80.42	44.99
Safety index	1.26	0.34

NIPT, noninvasive prenatal testing.

of NIPT was relatively low for positive results, with the PPVs being 60%, 33.33%, and 23.68% for T21, T18, and SCA, respectively. T13 and other autosomal aneuploidies in all the NIPT high-risk cases were confirmed to be chromosomally normal by amniocentesis. Therefore, the use of a high-risk NIPT result alone is not feasible for the diagnosis of karyotype abnormalities. Research has shown that confined placental chimerism is the most common cause of false negatives and false positives during NIPT (16). Furthermore, maternal CNVs also increase the risk of false positives during NIPT (17) because NIPT cannot fully distinguish cffDNA from maternal DNA, and abnormal maternal chromosomal alterations may be mistaken for fetal chromosomal abnormalities. False positives are also associated with maternal malignancy (18) or the stillbirth of one twin (19). False negatives in NIPT are associated with placental chimerism or with low levels of fetal DNA in peripheral blood that are not sufficiently high to be detected and analyzed. For instance, obesity and other pregnancy-related factors may cause low levels of cffDNA, leading to false negatives (20). Therefore, the decision to terminate a pregnancy in a pregnant woman with high-risk NIPT results should not be immediately made; rather, detailed genetic counseling should be performed, and the pregnant woman should be advised to undergo prenatal diagnosis to clarify the karyotype.

Pregnant women with low-risk NIPT results and without other indications for amniocentesis usually do not opt for invasive prenatal diagnosis, and the follow-up and prognostic analysis of this low-risk population is particularly important. We followed up 1,813 pregnant women with low-risk NIPT results, among whom 18 (0.99%) terminated the pregnancy and 23 (1.27%) experienced spontaneous abortions, mostly due to accidents or poor cervical function. There were 1,747 fetuses born with normal development, accounting for 96.36% of the total number of births, and the majority of low-risk pregnant women had good pregnancy outcomes. However, 25 newborns (3.64%) were found to have neonatal abnormalities after birth. Thus, the possibility of fetal abnormalities could not be fully ruled out in the NIPT low-risk group.

Our study also found that congenital heart diseases (n=8, 0.44%) were among the most common diseases in the newborns born by low-risk mothers, followed by isolated external ear malformation and postnatal growth retardation. Other diseases or syndromes included hearing abnormalities, congenital esophageal atresia, ichthyosis, pigmentary incontinence, West syndrome, hypospadias, jaw-winking syndrome, congenital ptosis, intestinal torsion, hip dysplasia, congenital umbiliculitis, anemia, and calf hemangioma. Follow-up color Doppler ultrasonography plays a crucial role in screening for fetal structural malformations during the genetic counseling and guidance for pregnant women with high-risk NIPT results (21). In our study, prenatal ultrasound accurately diagnosed fetal cardiac anomalies, external ear malformations, ichthyosis, hypospadias, congenital esophageal atresia, intestinal torsion, and other structural malformations in the pregnant women with a low-risk NIPT result; in addition, fetal chromosomal variations were identified after prenatal diagnosis. Thus, ultrasonography in combination with or as a complement to NIPT is particularly important for the multifaceted assessment of fetal growth and development. In addition, hearing abnormalities, ichthyosis, pigmentary incontinence, familial ptosis, and jaw-winking syndrome are mostly monogenic disorders (22-26). Detailed family history-taking during prenatal genetic counseling and enhanced screening for poly/monogenic disorders are particularly important for preventing birth defects in newborns.

The risk of abnormal fetal chromosomal variations increases with maternal age and is thus particularly pronounced among women of AMA. In this study, the screening efficacy of NIPT was comparable between the AMA group and non-AMA group, indicating that NIPT is applicable to all age groups. However, invasive prenatal diagnosis should be recommended first for AMA women during genetic counseling, and NIPT should be applied with caution. Adequate informed consent is required for prenatal testing or genetic counseling.

Traditional serologic screening, when used in combination in the first and second trimesters, can also be used to assess the risk of fetal chromosomal abnormalities; however, it can also be influenced by many factors, such as maternal age, weight, and gestational week. At present, it is considered that those with low-risk serological screening results should not undergo other chromosomal aneuploidy screening, so as to avoid false positives (27). NIPT is feasible in pregnant women with borderline-risk serological screening results to identify fetal chromosomal variations. For those with high-risk serological screening results, NIPT should be only applied with caution. If NIPT is applied in this population, the gestational weeks must be accurately calculated, and important information such as age and weight must be correctly collected. Nevertheless, prenatal diagnosis is still preferred (28). Therefore, in clinical practice, tailored genetic counseling and rational recommendations should be given according to the specific situation of the pregnant woman.

Although prenatal serological screening is less expensive, our study yielded a sensitivity of only 54.5% in screening for fetal chromosomal aneuploidies (including T21 and T18) (11). NIPT is more effective in screening chromosomally abnormal fetuses but requires more expensive equipment and reagents. Thus, selecting a screening method that can maximize the benefit of fetal chromosome aneuploidy screening is of great interest to clinicians. In the present study, health economic analysis was performed through a simulation of the operation of different screening strategies in 100,000 pregnant women: NIPT was able to avoid 133.88 births of infants with chromosomal aneuploidies, while serologic screening only avoided 63.90 births; thus, NIPT was significantly superior to serologic screening in detecting chromosomal aneuploidies. Comparison of the safety between the 2 screening methods showed that NIPT prevented 133.88 births of infants with chromosomal aneuploidies while causing 44.99 abortions of normal fetuses due to amniocentesis, yielding an SI of 0.34; serologic screening prevented 63.90 births of infants with chromosomal aneuploidies while causing 80.42 abortions of normal fetuses due to amniocentesis, yielding an SI of 1.26. Therefore, NIPT is a safer screening method. In terms of ICER, the cost of NIPT in avoiding the births of infants with chromosomal aneuploidies was 1,394,356.31 RMB, while the cost of serological screening was 604,554.67 RMB. However, serologic screening caused the birth of 69.98 infants with chromosomal aneuploidies, and the average lifetime cost of each new case from the family and societal perspectives were 390,600 and 450,000 RMB, respectively; therefore, the additional social cost of serological screening was RMB 58,825,188, and the cost-effectiveness ratio of serological screening strategy was RMB 15,25,137. Therefore, NIPT has better accuracy, safety, and economic impact than does traditional serologic screening and is feasible for pregnant women.

We also analyzed the correlation between ultrasound soft markers and fetal chromosomal abnormalities and found that the positive rate of abnormal karyotypes was 7.55% in the presence of 1 soft marker on prenatal ultrasound, 12.50% in the presence of 2 ultrasound soft markers, and 16.67% in the presence of 3 or more soft markers. Thus, the risk of abnormal fetal karyotypes raises with the increase in the number of combined soft markers. Some of the isolated soft markers (e.g., single umbilical artery and bright ventricular spots) do not increase the risk of fetal chromosomal abnormalities if the risk of chromosomal abnormalities is assessed as low by NIPT, and further invasive prenatal diagnosis due to the presence of soft markers is not recommended. However, the risk of chromosomal abnormality is relatively high in pregnant women with several ultrasound soft markers. The increased risk is related to the combination of soft markers, such as NT thickening, renal pelvis dilation, single umbilical artery, nasal bone hypoplasia, strong intestinal echogenicity, and short femur, which are not only related to fetal chromosomal abnormalities but also suggest that other fetal developmental abnormalities may exist. Therefore, the fetus should be carefully investigated for other structural abnormalities.

T21 is the most frequent chromosome aneuploidy. In our current study, newborns with T21 had the highest incidence rate of abnormal nasal bone and ventral bright spots, which were seen in 27.78% of the newborns with T21; NT/NF thickening was the second most common feature, accounting for 16.67%. We also found 2 cases of polyhydramnios and 3 cases of abnormal postures of the

foot or hand, suggesting that abnormal limb posture may be associated with T21 and that polyhydramnios may be associated with duodenal atresia. Although ultrasound is effective in detecting T21-related structural abnormalities, research has demonstrated that the incidence rates of ultrasound structural abnormalities in T21 are lower than those in other chromosome diseases, and ultrasound screening alone is not sufficient to diagnose all T21 cases (29). Similarly, in our current study, no obvious abnormalities were detected on prenatal ultrasound in 9 (50%) T21 fetuses, but the NIPT results of these fetuses showed high risk for T21, indicating that NIPT combined with ultrasound screening can avoid or reduce false negatives caused by unclear ultrasonic manifestations.

Ultrasonically, T18 and T13 often manifest as multiple structural abnormalities including ventricular septal defect, overlapping fingers, craniocerebral malformation, and facial malformation (30-32). Although NIPT has high sensitivity in detecting T18 and T13, the presence of false positives reduces the accuracy of the screening. In our study, NIPT had a specificity of 91.24%, a sensitivity of 60%, and a FNR of 40% in detecting SCAs in 15 fetuses. Its screening efficiency for SCAs was lower than those for T21, T18, and T13. In addition, most fetuses had abnormal ultrasound markers such as cervical hygroma, ventricular bright spots, ventricular septal defect, NF thickening, and polyhydramnios.

Prenatal ultrasonography and NIPT have their distinct advantages and limitations in screening for chromosomal abnormalities. Ultrasonography can help estimate the risk of chromosomal abnormalities through the detection of soft markers and structural abnormalities and has the advantages of being noninvasive, accurate, and rapid. The more the number of fetal ultrasound structural abnormalities and ultrasound soft indexes, the higher the risk of fetal chromosome karyotype abnormalities. In some pregnant women with false-negative NIPT results due to placental chimerism, the true-positive rate can be increased by ultrasonography. However, ultrasonography can be affected by the physician's skill and machine resolution and cannot offer an accurate diagnosis for cases that do not show structural abnormalities or for those with unclear structural abnormalities. In addition, NIPT has good screening efficacy for chromosomal aneuploidies and has high accuracy and applicability. Therefore, NIPT has attracted extensive attention in prenatal detection, and can compensate for the limitations of ultrasound. All told, ultrasound and NIPT are complementary, and the combined techniques can improve the screening of fetal chromosomal abnormalities, providing clinicians with a greater range of diagnostic information.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of the Second Hospital of Hebei Medical University (No. 2017-R208) and informed consent was taken from all the patients.

References

1. Sun LY, Xing QH, He L. Retrospect and prospect of the genetic research on birth defects in China. Yi Chuan

2018;40:800-13.
2. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. Obstet Gynecol 2007;110:1459-67.
3. Wu QJ, Yan XP, Chen CL, et al. The value of ultrasonography in prenatal diagnosis of pregnant women. Imaging Science and Photochemistry 2021;39:124-7.
4. Cai M, Lin N, Chen X, et al. Evaluation of chromosomal abnormalities and copy number variations in fetuses with ultrasonic soft markers. BMC Med Genomics 2021;14:19.
5. Ma MX, Liao GQ. Clinical significance of fetal ultrasound soft markers in prenatal diagnosis of chromosome abnormalities. Shanxi Medical Journal 2020;49:2577-9.
6. Huang H, Li XY, Lu J, et al. A comparative analysis on prenatal ultrasonography findings of fetuses with trisomy 13 syndrome, trisomy 18 syndrome, and trisomy 21 syndrome. Maternal & Child Health Care of China 2017;32:4199-202.
7. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
8. Ministry of Health, PRC. National Stocktaking Report Birth Defect Prevention. 2012.
9. Yang GY, Yang XD, Li XZ. Application of serological screening and fetal ultrasonography in prenatal diagnosis of trisomy 18 and trisomy 13. Journal of Clinical Medical Literature (Electronic Edition) 2018;5:125.
10. Xu Y, Ming J, Li N, et al. Health economic analysis of noninvasive prenatal gene testing technology screening strategy. Chinese Journal of Obstetrics and Gynecology 2017;52:53-6.
11. Liu XH, Yang Y. Comparative analysis of the results of noninvasive prenatal DNA testing and serological prenatal screening. Chinese Community Doctors 2020;36:115-7.
12. Akolekar R, Beta J, Picciarelli G, et al. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;45:16-26.
13. Jiang F, Ren J, Chen F, et al. Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. BMC Med Genomics 2012;5:57.
14. Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol 2015;45:530-8.
15. Badeau M, Lindsay C, Blais J, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. Cochrane Database Syst Rev 2017;11:CD011767.
16. Grati FR, Malvestiti F, Ferreira JC, et al. Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. Genet Med 2014;16:620-4.
17. Snyder MW, Simmons LE, Kitzman JO, et al. Copy-number variation and false positive prenatal aneuploidy screening results. N Engl J Med 2015;372:1639-45.
18. Lenaerts L, Jatsenko T, Amant F, et al. Noninvasive Prenatal Testing and Detection of Occult Maternal Malignancies. Clin Chem 2019;65:1484-6.
19. Grömminger S, Yagmur E, Erkan S, et al. Fetal Aneuploidy Detection by Cell-Free DNA Sequencing for Multiple Pregnancies and Quality Issues with Vanishing Twins. J Clin Med 2014;3:679-92.
20. Wang E, Batey A, Struble C, et al. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat Diagn 2013;33:662-6.
21. Pang K, Feng L, Jiao HP, et al. Effect analysis of color Doppler ultrasound in prenatal diagnosis of fetal malformation for pregnant woman. China Medical Equipment 2020;17:92-5.
22. Zhang P. Clinical value of neonatal hearing combined with deafness gene screening. China Continuing Medical Education 2020;12:109-11.
23. Cheng XF, Lyu HL. Jaw-winking syndrome. Beijing Medical Journal 1988;6:340-2.
24. Qian XQ. Locus mapping and mutation detecting for blepharophimosis ptosis epicanthus inversus syndrome (BPES) and a study of the candidate gene to schizophrenia. Graduate School of Chinese Academy of Sciences (Shanghai Academy of Life Sciences), 2005.
25. Wang Y, Wang LH, Qu Y, et al. Pigment incontinence: a case report and literature review. Chinese Journal of Leprosy Dermatology 2016;32:626-7.
26. Tang ZP, Zhao T, Zhang F, et al. Advances in genetic studies of congenital ichthyosis. Journal of Diagnosis and Therapy on Dermatovenereology 2013;20:447-51.
27. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. Obstet Gynecol 2016;127:e123-37.
28. Qi QW. Interpretation of relevant guidelines on clinical application of noninvasive prenatal testing. Journal of Developmental Medicine (Electronic Version) 2019;7:161-7.
29. Chen F, Zhou K. The role of the second trimester

ultrasound in screening of trisomy 21 and trisomy 18. Chinese Journal of Birth Health & Heredity 2014;22:48-9.
30. Pitukkijronnakorn S, Promsonthi P, Panburana P, et al. Prenatal ultrasonographic findings in "trisomy 13". J Med Assoc Thai 2008;91:1651-5.
31. Watson WJ, Miller RC, Wax JR, et al. Sonographic findings of trisomy 18 in the second trimester of pregnancy. J Ultrasound Med 2008;27:1033-8; quiz 1039-40.
32. Deng FL, Duan LM, Guo YL, et al. Ultrasonography analysis of fetus structure abnormality with trisomy 18 syndrome at middle pregnancy. Chongqing Medicine 2019;48:2373-6.

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