Article

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The Clinical Application and Accuracy Evaluation of Noninvasive Prenatal Testing for Common Trisomy and Sex Chromosome Aneuploidy

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Background: Noninvasive prenatal testing (NIPT) has been widely adopted in prenatal examination for fetal chromosomal aneuploidy. The present study aimed to evaluate the clinical features of NIPT for both common trisomy and sex chromosome aneuploidy (SCA).

Methods: A total of 24,164 pregnant women with NIPT testing from July 2020 to June 2022 were recruited at the Linping Maternity and Child Health Care Hospital.

Results: Ninety cases showed high risk of trisomy 21/18/13 with karyotype results available, and the sensitivity, specificity, and positive predictive value (PPV) were 98.41%, 99.88% and 68.89%, respectively. The three most important reasons for screening were advanced maternal age (AMA, 28.06%), intermediate risk of prenatal screening (20.34%) and Multiple of medium (MoM) abnormality of prenatal screening (17.38%). High risk of NIPT results with Z-score $\geq$15 have a higher PPV when compared to those with 3 $\leq$ Z-score $<$ 10, and 10 $\leq$ Z-score $<$ 15. Meanwhile, 97 pregnant women received positive results for fetal sex chromosome aneuploidy (SCA) in NIPT. In addition, the rate for further diagnostics of SCA was 64.95% and the PPV of SCA was 50.79%.

Conclusions: Our data show that NIPT has a promising future in prenatal screening for genetic abnormalities of the fetus, and that the accuracy of NIPT is closely related to Z-score.

Keywords: noninvasive prenatal testing; common trisomy; sex chromosome aneuploidy; Z-score; positive predictive value

Introduction

Noninvasive prenatal testing (NIPT), a relatively new approach for detecting certain fetal chromosomal abnormalities, can examine both common types of trisomy, including trisomy 21, 18, and13, and sex chromosome aneuploidies, including Turner syndrome (45,X), Klinefelter syndrome (47,XXY), Triple X syndrome (47,XXX) and Jacob syndrome (47,XYY) [1,2]. Unlike traditional prenatal testing methods which involve invasive diagnosis and maternal serological screening combined with nuchal translucency (NT) ultrasound examination, NIPT is a noninvasive procedure which involves analyzing cell-free fetal DNA (cffDNA) obtained from peripheral blood in pregnant women [3]. This approach has several advantages, for example, reducing risk of miscarriage and increasing detection rate for certain genetic abnormalities [4–6]. NIPT has become increasingly popular in recent years, and its utilization rate is likely to grow further as more data are collected and the technology continues to improve.

While NIPT has a high accuracy rate in assessing common autosomal trisomies [7,8], it is important to note that false positive NIPT results occur occasionally, especially in screening for sex chromosome aneuploidies (SCAs). These false positives can not only lead to unnecessary concerns by pregnant women and their family members, but also complicate genetic counseling concerning the NIPT results. Actually, false positives and false negatives can occur, because NIPT is not a diagnostic test, but rather a screening test that provides an indication of the likelihood of a fetal genetic abnormality. If NIPT indicates a high likelihood of fetal chromosomal aneuploidies, further testing by invasive methods is usually recommended to confirm the results [9]. Therefore, it is worthwhile for genetic counselors to seriously consider the proper interpretation of NIPT findings. They must consider some factors which may confound the results, such as confined placental mosaicism (CPM) [10], copy number variations or malignancies in female parent [11] and low fraction of fetal DNA. Furthermore, NIPT results for individual women are typically calculated as Z-score. Specifically, a Z-score of $>$3 or $<$–3 indicates positive results of NIPT for chromosomal abnormalities [12] which is obtained by the ratio of a reporting sample to a group of normal controls [13]. According to recent studies, the accuracy of PPV (positive predictive value) in NIPT for common trisomy was closely related to the Z-score [14,15].

However, similar investigations remain limited [5], because of small sample size and diverse sequencing platforms.

In the current study, we evaluated the clinical features, especially the accuracy of NIPT in simultaneously detecting the three autosomal aneuploidies and SCAs. At the same time, we also evaluated NIPT performance in high-risk pregnancy using these samples by Z-score classification ($3 \leq Z < 10$, $10 \leq Z < 15$ and $Z \geq 15$). Through deeper mining and analyzing the clinical data, we aimed to explore more reasonable use of NIPT.

Materials and Methods

Patients

Women who opted for prenatal screening and diagnosis during pregnancy in Linping Maternity and Child Health Care Hospital from July 2020 to June 2022 were recruited. A qualified clinician explained the purpose, accuracy, and limitations of the prenatal test, before patients signed written informed consent. The inclusion criteria were as follows: (1) Gestational age $\geq$12 weeks; and (2) strong desire to use NIPT. The exclusion criteria were as follows: (1) Pregnant women who received allogeneic blood transfusion, transplantation surgery, cell therapy or other possible treatments that would interfere with NIPT testing and (2) documented chromosomal abnormalities in one member of the couple. Finally, 24,164 pregnant women selected NIPT testing and received effective results. Several maternal characteristics were recorded simultaneously, including nuchal translucency, serum biochemical screening risk, ultrasonic examination and advanced maternal age (AMA, $\geq$35 years).

Noninvasive Prenatal Testing

We conducted NIPT testing in accordance with the manufacturer's instructions (Capitalbio Co., Ltd., Dongguan, Guangdong, China, catalog#S10020). As a first step, a cell-free BCT tube (Streck, Omaha, NE, USA, catalog#230253) was used to collect five mls of maternal peripheral blood. Using two centrifugations (1600 g for 10 min and 16,000 g for 10 min) at 4 °C within 72 hours, we separated the upper plasma from whole blood. Then, the Extraction and Purification kit (Capitalbio Co., Ltd., Dongguan, Guangdong, China) was utilized to extract cffDNA from 400 μL plasma. After that, cffDNA libraries were constructed by filling the ends and ligating the adapter. A qPCR analysis was then conducted in order to verify the quality and concentration of the cffDNA libraries. Finally, after adding the Fetal Aneuploidies Detection kit (Capitalbio Co., Ltd., Dongguan, Guangdong, China, catalog#320020), massive parallel sequencing was carried out on eligible libraries using a BioelectronSeq 4000 platform (Capitalbio Co., Ltd., Dongguan, Guangdong, China). Z-score was used to determine the test result (normal range, $-3 < Z < 3$) which indicated as high triploid risk if Z-score >3 and high haploid risk if Z-score <-3. There were also individual reports on the estimated fetal risk of Trisomy 21/18/13 for each participant. In cases of high risk of SCA, a supplementary report was simultaneously given.

Karyotype Analysis

Those with suspected risk after NIPT testing were recommended for consultation with professional geneticists and further provided with ultrasound-guided amniotic fluid puncture. After amniotic fluid cell culture, fetal karyotypes were analyzed using Giemsa-banding techniques. A minimum of 20 metaphase cells were analyzed in each sample with an average resolution of over 400 bands. Furthermore, chromosomal microarray analysis (CMA) and fluorescence in situ hybridization (FISH) were also used to evaluate fetal chromosomal abnormalities.

Follow-Up

We followed up by telephone or medical records with diagnostic selection once the NIPT reports were available and with pregnancy outcomes three months after delivery.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) software version 22 (SPSS Inc., Chicago, IL, USA) to analyze the data. Data were presented in the form of n (%). For each group, we calculated the performance of NIPT, including sensitivity, specificity and positive predictive value (PPV). ROC curves were plotted to determine the optimal cutoff value for the prediction of fetal chromosome aneuploidy. The area under the curve (AUC) was used to quantify the ability of NIPT to identify fetal aneuploidy.

Results

Basic Characteristics

In all, 24,164 pregnant women accepted NIPT with effective results from July 2020 to June 2022. The ranges of their physiological age and gestational age when samples enrolled were 18–50 years old and 12–34 weeks, respectively. The main three indications for selecting NIPT testing were AMA (28.06%), intermediate risk of prenatal screening (20.34%), and MoM abnormality of prenatal screening (17.38%). The specific purpose for undergoing NIPT is shown in Table 1.

Screening Efficiency for T21/T18/T13

The results of NIPT were positive for T21/T18/T13 in 90 cases, including fifty-nine T21 cases, fourteen T18 cases and seventeen T13 cases, which finally received invasive prenatal diagnosis by amniocentesis and karyotype analysis. According to Table 2, their prenatal diagnosis results were as follows. The sensitivity, specificity and PPV were 98.04%, 99.96% and 84.75% for T21; 100%, 99.98% and 57.14% for T18; and 100%, 99.95% and 23.53% for

Table 1. Different indications to select NIPT testing in 24,164 samples.

Indications	n	Constituent ratio, %
Advanced age women	6780	28.06
Intermediate risk of prenatal screening	4916	20.34
MoM abnormality of prenatal screening	4200	17.38
High risk of prenatal screening	2941	12.17
Voluntary structural abnormality	2761	11.43
Ultrasonic abnormality	980	4.06
Assisted reproductive conception	920*	3.81
Twins	521*	2.16
Others	302	1.25
Total	24,164	100

NIPT, noninvasive prenatal testing.

*One hundred and fifty-seven cases with twins were assisted reproductive conception.

T13, respectively. In addition, ROC curve analysis was applied to assess NIPT efficiency and calculate the optimal Z-score cutoff (Fig. 1). It showed that the areas under the curve (AUC) for detecting T21, T18 and T13 were 0.949 (95% CI 0.856–1.042), 0.875 (95% CI 0.685–1.065) and 0.750 (95% CI 0.394–1.106) with the optimal cutoff values of 9.261, 10.849 and 12.368, respectively. Follow-up revealed one case as a false negative NIPT result. Nevertheless, it was detected with structural abnormalities by prenatal ultrasound scan and was finally diagnosed as positiveby umbilical cord blood analysis. At the same time, 28 cases were NIPT false positive, resulting in a false positive rate (FPR) of 0.12%.

Screening Efficiency for SCA

Additionally, the NIPT results also revealed that 97 cases might have fetal sex chromosome aneuploidy (SCA), including 58 cases of Turner syndrome (45,X), six cases of XXX syndrome (47,XXX), 16 cases of Klinefelter syndrome (47,XXY) and 17 cases of XYY syndrome (47,XYY). After informed consent, 63 pregnancy with high risk of NIPT outcomes accepted amniocentesis and karyotype analysis to confirm the results. The further diagnostic rate of SCA was 64.95%. As shown in Table 3, 32 women were eventually proved to be true positives, and 31 were false positives. Therefore, NIPT for SCA had a total PPV of 50.79%. Among which, the PPV was the lowest (29.73%) in screening for Turner syndrome. In addition, ROC curve analysis was applied to assess the NIPT efficiency, and calculate the optimal cutoff of Z-score (Fig. 1). It showed that the area under the curves (AUC) for detecting (45,X), (47,XXY) and (47,XYY) were 0.514 (95% CI 0.300–0.728), 0.944 (95% CI 0.797–1.092) and 0.815 (95% CI 0.487–1.142), with optimal cutoff values of –5.621, 39.745 and 70.292, respectively.

Accuracy of Z-score in T21/T18/T13

We divided the NIPT positive cases according to Z-score. Table 4 shows the distribution of Z-scores in 90 positive cases of T21/T18/T13 by groups (Group 1: $3 \leq Z < 10$, Group 2: $10 \leq Z < 15$, Group 3: $Z \geq 15$). In all, 20 high risk of NIPT cases were included in Group 1. Amniotic fluid puncture and karyotype analysis were used to validate three true positive cases, with a PPV of only 15%. Meanwhile, Group 2 comprised 21 cases having abnormal NIPT results. Twelve cases were proved true positive by amniotic fluid puncture and karyotype analysis while nine false positive cases were verified. The PPV was 57.14%. Furthermore, there were 49 cases in Group 3. Invasive prenatal diagnosis detect only two false positive cases, but found 47 true positive cases, resulting in a PPV of 95.92%. There could have been a small region of chromosome duplication in sample N2110749 and N2201972 that led to the false positive NIPT results.

Discussion

The NIPT screening test has been extensively used to detect fetal T21, T18, and T13 because of its advantages of high sensitivity, specificity, easy operation, and noninvasive processing. Meanwhile, despite its relatively inferior performance for SCAs, American College of Medical Genetics and Genomics (ACMG) still advocates expanding the NIPT testing scope for this kind of chromosomal disease based on fully informed consent during pre-test counseling [16]. According to the current study, NIPT detected 90 cases with T21/T18/T13 positive results and 97 cases with SCA positive results. The invasive rate was 64.95% for SCA. The results were similar to many other studies [17,18]. The PPV was 84.75% for T21, 57.14% for T18 and 23.53% for T13 respectively. Furthermore, the PPV was 29.73% for Turner syndrome (45,X), 100% for Triple X syndrome (47,XXX) and 81.82% for Klinefelter syndrome (47,XXY) and 75.00% for Jacob syndrome (47,XYY). It showed that NIPT may be more effective in detecting sex chromosome trisomy compared to monosomy X, as often reported [19].

The American College of Obstetricians & Gynecologists (ACOG) recommends screening all pregnant women for genetic disorders during pregnancy, regardless of maternal age. However, routine prenatal screening is not recommended for women over 35 years of age in China. Due to the risk of puncture, only a small percentage of this group of women accepts traditional prenatal screening. Therefore, in our study, advanced age women were the most frequent group for NIPT application. Furthermore, women with intermediate risk of prenatal screening were the second largest group accepting NIPT. Additionally, our results indicated that women at intermediate risk of serological testing benefited from NIPT screening. Otherwise, a missed diagnosis is likely to occur based on the traditional screen-

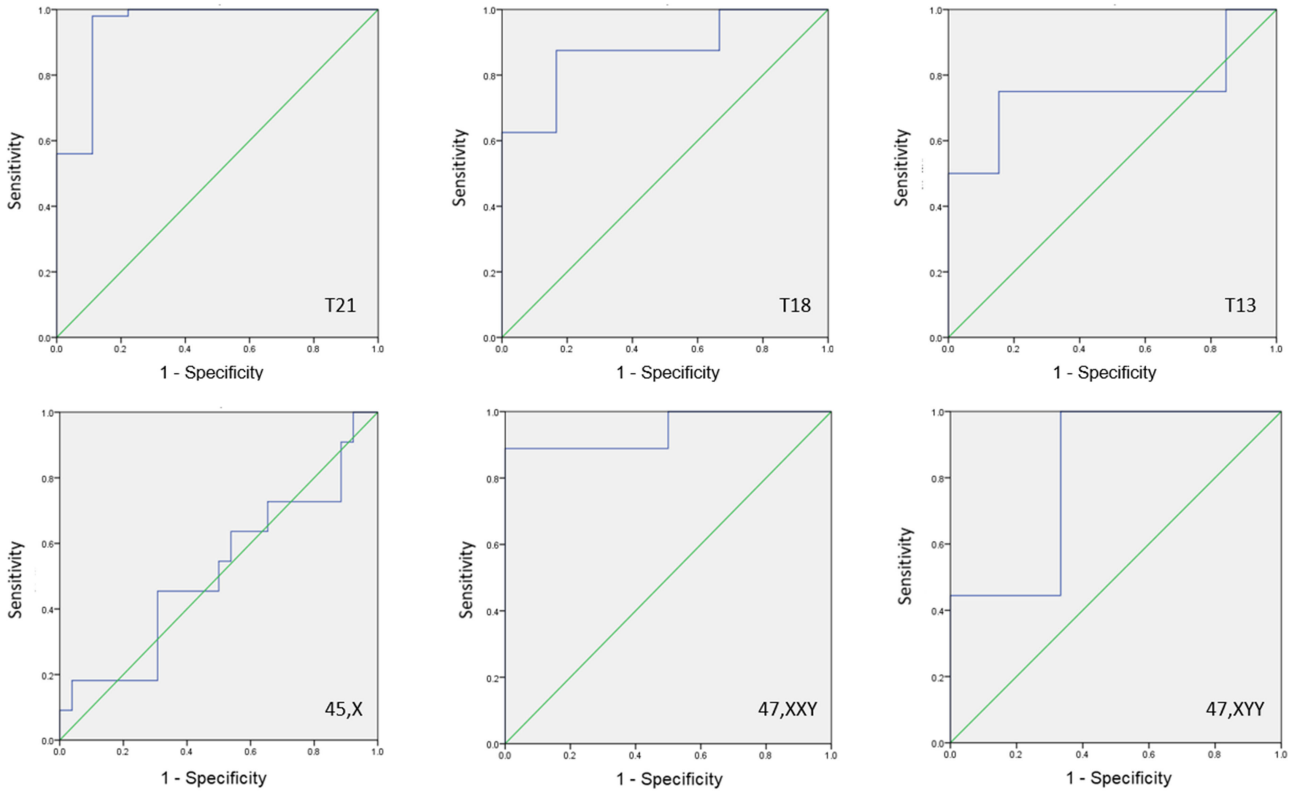

Fig. 1. ROC curve analysis of NIPT-positive cases with common trisomy and SCA.

Table 2. The clinical performance of NIPT in screening for T21/T18/T13.

	NIPT+	TP	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)
T21	59	50	9	1	98.04 (88.21–99.90)	99.96 (99.93–99.98)	84.75 (72.51–92.37)
T18	14	8	6	0	100 (59.77–100)	99.98 (99.94–99.99)	57.14 (29.65–81.19)
T13	17	4	13	0	100 (39.58–100)	99.95 (99.91–99.97)	23.53 (7.82–50.24)
Total	90	62	28	1	98.41 (90.32–99.92)	99.88 (99.83–99.92)	68.89 (58.14–78.00)

NIPT, noninvasive prenatal testing; NIPT+, NIPT positive result; TP, true positive; FP, false positive; FN, false negative; PPV, positive predictive value.

Table 3. The detection accuracy of NIPT in screening for SCAs.

	NIPT+	TP	FP	No diagnosis	PPV, %
45,X	58	11	26	21	29.73
47,XXX	6	3	0	3	100
47,XXY	16	9	2	5	81.82
47,XYY	17	9	3	5	75.00
Total	97	32	31	34	50.79

SCA, sex chromosomal aneuploidies; NIPT, noninvasive prenatal testing; NIPT+, NIPT positive result; TP, true positive; FP, false positive; PPV, positive predictive value.

ing and diagnosis programs for pregnancies. Consequently, we believe that managing pregnant women with intermediate risk of serological testing will be important to reduce the occurrence of birth defect.

Below are possible explanations for the false positive rate, the discordance between NIPT and invasive examination results. First of all, confined placental mosaicism (CPM) is an important factor contributing to the discordant results. Due to the fact that cffDNA is mainly produced by cytotrophoblast cells [20], the NIPT results are not always representative of the fetal situation. Actually, CPM occurs in approximately 2% of pregnant women [21]. Another common cause of discordant SCA results is maternal SCA [22,23]. There are other factors that may contribute, including low cffDNA fraction and maternal copy number variations.

As mentioned above, the existence of false positive rates by NIPT make after-test counseling not only important, but also necessary. Positive NIPT results should never be used to decide to terminate a pregnancy. Women receiving positive NIPT results must receive genetic counseling and a prenatal diagnosis to confirm the fetal kary-

Table 4. The distribution of Z-scores in 90 NIPT positive cases of T21/T18/T13 by groups.

Group 1: $3 \leq Z < 10$				
No.	Sample ID	Z-scores	NIPT results	Fetal karyotype
1	N2008396	9.182	T13	46,XN
2	N2011201	6.745	T18	46,XN
3	N2012535	3.479	T21	46,XN
4	N2100099	7.931	T21	46,XN
5	N2103259	7.392	T21	47,XN+21
6	N2103757	4.202	T21	Xq11.1q11.2(62,950,823-63,831,851)x3
7	N2108782	6.172	T18	46,XN
8	N2108814D	4.584	T13	46,XN
9	N2108798	3.315	T21	46,XN
10	N2109264	8.488	T13	45,XX,rob(13;14)(q10;q10)
11	N2111862	3.208	T21	46,XN
12	N2112731	9.347	T18	46,XN
13	N2112780	5.063	T21	46,XN
14	N2112926	3.613	T18	46,XN
15	N2200457	6.288	T13	47,XN+13 mos
16	N2201536	5.629	T18	47,XN+18
17	N2202032D	5.747	T13	46,XN
18	N2204025	4.581	T18	46,XN
19	N2204408	5.121	T21	46,XN
20	N2205301	4.469	T21	46,XX,del(21)(q22)
Group 2: $10 \leq Z < 15$				
No.	Sample ID	Z-scores	NIPT results	Fetal karyotype
1	N2007307	12.218	T13	46,XN
2	N2008823	11.474	T21	47,XN+21
3	N2010787	14.347	T18	47,XN+18
4	N2011518	10.258	T13	46,XN
5	N2011650	11.5	T21	47,XN+21
6	N2011819	13.464	T13	46,XN
7	N2100714	12.552	T13	46,XN
8	N2101924	14.489	T21	47,XN+21
9	N2102775	14.118	T21	47,XN+21
10	N2103007	10.442	T13	46,XN
11	N2103245	13.137	T21	47,XN+21
12	N2103374	12.517	T13	47,XN+13
13	N2105777	11.659	T21	47,XN+21
14	N2107450	10.314	T13	46,XN
15	N2108299	11.201	T13	46,XN
16	N2110430	10.657	T13	46,XN
17	N2110745	10.603	T21	47,XN+21
18	N2112156	13.625	T21	47,XN+21
19	N2201917	12.351	T18	47,XN+18
20	N2203202	10.591	T21	47,XN+21
21	N2205471	10.022	T13	46,XN

Table 4. Continued.

Group 3: Z $\geq$ 15				
No.	Sample ID	Z-scores	NIPT results	Fetal karyotype
1	N2007725	20.819	T21	47,XN+21
2	N2008409	28.992	T21	47,XN+21
3	N2008569	18.492	T21	47,XN+21
4	N2009019	16.502	T21	47,XN+21
5	N2009135	38.9	T21	47,XN+21
6	N2009286	30.421	T18	47,XN+18
7	N2010714	16.143	T21	47,XN+21
8	N2012790	17.861	T21	47,XN+21
9	N2100821	22.123	T21	47,XN+21
10	N2101171	15.141	T21	47,XN+21 mos
11	N2101673	24.486	T21	47,XN+21
12	N2102034	22.631	T21	47,XN+21
13	N2103394	24.237	T21	47,XN+21
14	N2103555	21.394	T21	47,XN+21
15	N2104108	16.999	T21	47,XN+21
16	N2104557	15.588	T21	47,XN+21
17	N2104706	20.693	T21	47,XN+21
18	N2104847	21.858	T21	47,XN+21
19	N2104856	20.837	T21	47,XN+21
20	N2105190	15.813	T21	47,XN+21
21	N2105548	20.558	T21	47,XN+21
22	N2106021	19.736	T21	47,XN+21
23	N2106410	19.322	T21	47,XN+21
24	N2106789	15.583	T21	47,XN+21
25	N2107499	15.695	T21	47,XN+21
26	N2107697	27.069	T21	47,XN+21
27	N2110054	15.852	T21	47,XN+21
28	N2110170	28.351	T18	47,XN+18
29	N2110749	17.779	T18	46,XN
30	N2110765	21.844	T21	47,XN+21
31	N2112916	29.68	T18	47,XN+18
32	N2113083	21.746	T21	47,XN+21
33	N2200230	28.908	T21	47,XN+21
34	N2200891	23.435	T21	47,XN+21
35	N2201710	26.639	T21	47,XN+21
36	N2201972	18.326	T21	46,XN
37	N2202031	18.525	T13	47,XN+13
38	N2202389	20.872	T21	47,XN+21
39	N2202565	20.164	T21	47,XN+21
40	N2202753	16.122	T21	47,XN+21
41	N2203093	19.758	T13	47,XN+13
42	N2203600	24.177	T18	47,XN+18
43	N2203781	29.632	T21	47,XN+21
44	N2203833	23.297	T21	47,XN+21
45	N2205005	22.415	T18	47,XN+18
46	N2205047	35.73	T21	47,XN+21
47	N2205059	19.434	T21	47,XN+21
48	N2205124	16.84	T21	47,XN+21
49	N2205421	23.137	T21	47,XN+21

otype in further detail. At the same time, negative NIPT results do not guarantee a normal fetus, there may be a possibility of false negatives. Consequently, the characteristics of different prenatal screening and diagnostic techniques (in relation to advantages and disadvantages) need to be fully disclosed to pregnant women and their families before they make their choice accordingly. Furthermore, our data showed that there was a strong correlation between the PPV of NIPT test and the Z-score, screening for samples with Z-score $\geq$15 was more accurate than those with 3 $\leq$ Z-score $<$ 10 and 10 $\leq$ Z-score $<$ 15. It should be taken into account when conducting genetic counseling.

Based on our results, we considered that NIPT may be effective for prenatal screening for both common trisomy and SCA. However, there were several limitations in the study. For example, we could not eliminate false positive or false negative NIPT results caused by some potential factors which were currently unclear. On the other hand, we also could not get 100% follow-up rate after NIPT test or fetal delivery, some participants with high-risk NIPT results refused to undergo karyotype analysis while a small number of women receiving negative NIPT results were lost to follow-up.

Conclusions

In conclusion, our data showed that NIPT has a promising future in prenatal screening for genetic abnormality (both common trisomy and SCA) of the fetus. We should optimize the use of NIPT based on the present analysis of clinical data, especially in explaining the test results estimated by Z-score.

Availability of Data and Materials

The data for this article are not publicly available because of privacy concerns. Requests to access these datasets should be directed to the corresponding author.

Author Contributions

YW and JY designed the research study. YW and YS performed the research. YS and JY provided help and advice on the data acquisition. YW analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study design was approved by the ethics committee of Linping Maternity and Child Health Care Hospital and the ethical approval number was LLSC-KYKT-2023-0004-A. The patients provided their written informed consent to participate in this study.

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Conflict of Interest

The authors declare no conflict of interest.

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