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Clinical indications and Z-score-assisted NIPT testing: a new perspective in prenatal screening

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ABSTRACT

Objective We aim to explore positive predictive value (PPV) in non-invasive prenatal testing (NIPT)-positive cases and investigate the impact of diverse clinical indications and Z-scores on PPV performance.

Methods From January 2021 to June 2024, 37 891 pregnant women underwent NIPT screening for fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) at our laboratory using the NextSeq CN500 platform. Positive results were verified through prenatal diagnostic karyotype analysis and fluorescence in situ hybridisation (FISH) techniques.

Results The sensitivity, specificity and PPV were 95.24%, 99.95%, 67.80% for T21; 100%, 99.97%, 56.00% for T18; and 100%, 99.97%, 16.67% for T13. Across clinical indications, PPV ranged from 0% to 100% for T21 and T18 and 0% to 28.57% for T13. In the T21 group, the predominant proportion of pregnant women (45.76%) exhibited Z-scores between 5 and 10, accompanied by a PPV of 77.78%. For those with Z-scores above 10 (23.73%), the PPV was 85.71%. Pregnant women with Z-scores between 3 and 5 exhibited a PPV of 16.67%. In the T18 group, the majority of women (52.00%) exhibited Z-scores ranging from 3 to 5, with a PPV of 33.85%. In the T13 group, all women had Z-scores between 5 and 10, with a PPV of 40.00%.

Conclusions NIPT exhibits elevated PPVs for T21 and T18. Moreover, the detection efficacy of NIPT differs across several clinical indication categories. The PPV performance of NIPT for T21/T18/T13 is associated with Z-scores. These results provide valuable guidance for clinicians in prenatal consultation and interpretation of NIPT results.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Non-invasive prenatal testing (NIPT) has been integrated into clinical practice and is now extensively used to screen for trisomy 21, 18 and 13.

WHAT THIS STUDY ADDS

⇒ We collected NIPT data from 37 891 pregnant women and diagnostic results from 96 high-risk pregnant women. We grouped pregnant women with high-risk results to explore the correlation between NIPT and clinical screening indications as well as Z-scores, providing data and information for clinical genetic counselling and prenatal diagnosis.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We suggest that increased caution should be exercised in the administration of NIPT to high-risk pregnant women, including those of advanced maternal age, those with ultrasonic structural abnormalities or those with positive serum screening results and the Z-score values of NIPT can assist clinicians in interpreting NIPT results and providing prenatal consultation.

INTRODUCTION

In 1997, Professor Dennis Lo of the Chinese University of Hong Kong identified the existence of cell-free fetal DNA (cffDNA) in maternal peripheral plasma which remains stable throughout gestation and swiftly diminishes postdelivery. This substance is regarded as the optimal choice for non-invasive prenatal diagnosis, hence propelling the advancement of non-invasive prenatal testing (NIPT).[1] Since 2011, NIPT has been integrated into clinical practice[2] and is now extensively used to screen for trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13), having demonstrated superiority as the most precise screening tool for common fetal aneuploidies. NIPT provides superior sensitivity and specificity in identifying T21, T18 and T13 relative to traditional biochemical and ultrasound testing.[3] A multitude of studies has demonstrated that NIPT can diminish the occurrence of superfluous invasive procedures.[4] Overall, NIPT, as an accurate screening instrument, enhances aneuploidy detection capabilities while diminishing dependence on invasive diagnostic methods. NIPT is recognised as the premier non-invasive technique for detecting common fetal aneuploidies; nonetheless, it functions solely as a screening tool since cffDNA predominantly derives from the placenta which may exhibit genetic discrepancies compared with the fetus.[5] Therefore, false-positive and false-negative results may

arise. In actual testing, false-positive results from NIPT are more prevalent than false-negative ones, frequently heightening anxiety levels throughout pregnancy.

For clinicians and patients, the positive predictive value (PPV) is more informative than conventional sensitivity and specificity measures.[6] PPV indicates the proportion of patients with a positive NIPT result who genuinely possess the illness, functioning as an effective metric for genetic counselling. A low PPV of 10–20% indicates that more than 80% or 90% of abnormal results are likely false positives.[7] Recent studies indicate significant variability in PPV values, with reported ranges of 65%–94% for T21, 47%–85% for T18 and 12%–62% for T13.[8] In fact, the PPV performance of NIPT may be influenced by various factors, including fetal fraction, confined placental mosaicism, maternal copy number variations, testing procedures, classification algorithms and sequencing platforms.[9] In addition to these factors, the clinical indications of pregnant women are also an important consideration in determining PPV. Xiang *et al* pointed out that among seven clinical screening indications, the PPV for T21 ranged from 50.62% to 73.09%, for T18 from 20.00% to 58.33% and for T13 from 4.17% to 47.37%.[10] Other studies have demonstrated statistically significant variations in PPV for different clinical screening indications of T21, T18 and T13, with the highest positive rates and PPV for NIPT detection indications linked to ultrasonic anomalies, particularly structural abnormalities and elevated nuchal translucency (NT).[11 12] Consequently, clinical indications for NIPT should be strictly controlled. Furthermore, PPV is also closely related to the Z-score obtained from NIPT testing. The Z-score in NIPT refers to the value obtained by comparing the test sample with a normal diploid control sample during the NIPT testing process. The Z-score represents the risk value in NIPT and serves as an important indicator for assessing whether the fetus is at high risk for chromosomal aneuploidy and is the predominant approach employed for screening fetal chromosomal aneuploidies.[9] Numerous researches have reported a significant correlation between Z-scores and PPV. The most recent research report indicates that Z-scores are closely related to NIPT accuracy, with positive NIPT results at $Z \geq 9$ exhibiting more accuracy than those at $5 \leq Z < 9$ and $3 \leq Z < 5.4$.[13] Therefore, laboratories providing NIPT must assess and evaluate the precision of Z-scores to guarantee the reliability of testing and the clinical utility of result interpretation for individual patients. Nonetheless, information about the evaluation of the precision of Z-scores for NIPT of T21, T18 and T13 is still scarce.

In this study, we collected NIPT data from 37 891 pregnant women and diagnostic results from 96 high-risk pregnant women between January 2021 and June 2024 to assess the performance of NIPT. Additionally, we grouped pregnant women with high-risk results to explore the correlation between NIPT and clinical screening indications as well as Z-scores, providing data and information for clinical genetic counselling and prenatal diagnosis.

MATERIALS AND METHODS

Study population

From 2021 to June 2024, a total of 37 891 pregnant women participated in NIPT with informed consent. According to the requirements of Document No. 45 issued by the National Health and Family Planning Commission of the People's Republic of China in 2016, the appropriate gestational age for this study was 12+0 to 22+6 weeks. Pregnant women beyond 22 weeks and 6 days must sign additional requirements of the informed consent form. 96 pregnant women underwent prenatal cytogenetic diagnosis. All participating pregnant women received prenatal genetic counselling and subsequently voluntarily signed the informed consent form issued by the General Office of the National Health and Family Planning Commission.

Non-invasive prenatal testing

The NIPT procedure, including DNA extraction, library construction, whole-genome sequencing and data analysis, was conducted following protocols published elsewhere. 10 mL of peripheral blood from each pregnant woman was collected using an EDTA anticoagulant tube. In order to separate the plasma into an Eppendorf tubes, 1600 g of peripheral blood must be centrifuged at 4°C for 10 min, followed by another 10 min of centrifugation at 16 000 g. Total cell-free DNA (cfDNA) was extracted using a plasma cfDNA extraction kit (magnetic bead method) (Berry Genomics, Beijing) and the Invitrogen Qubit 3.0 (Thermo Fisher Scientific, USA) was used to measure the DNA concentration. The library was subsequently constructed and purified using a library preparation kit and purification kit (Berry Genomics, Beijing). The KAPA Library Quantification Kit (Illumina, USA) was employed to evaluate the quality control of the sequencing library. The NextSeq CN500 sequencer was employed to conduct DNA sequencing. The above steps were performed according to Berry instructions. The Z-score was used to assess the risk of chromosomal aneuploidy, with a defined range of −3 to 3. If the Z-score>3 or $Z < -3$, the sample was classified as high risk for chromosomal aneuploidy. Conversely, if the Z-score was ranged from −3 to 3, the sample was classified as low risk. Based on the Z-score values, we have divided the NIPT-positive cases into three groups: $3 \leq Z < 5$, $5 \leq Z < 10$ and $Z \geq 10$. The formulation of this classification standard has taken into account the currently available sample size, the range of sample selection and the information provided by previously conducted relevant studies.[9 14]

Prenatal diagnosis

Prenatal genetic counselling was provided to pregnant women with high-risk NIPT results. Patients at high risk for NIPT were confirmed by karyotype analysis and fluorescence in situ hybridisation (FISH) (Guangzhou LBP Medicine Science & Technology, Guangzhou). Invasive prenatal diagnosis was performed with the informed consent of the pregnant women, adhering to conventional laboratory protocols, which encompassed

cell culture, preparation and karyotype analysis. FISH test used Gene Specific Probe (GSP) 13/GSP 21 and Centromere Specific Probe (CSP) X/CSP Y/CSP 18 probes manufactured by Guangzhou LBP Medicine Science & Technology. These probes target the 13q14.2 region of chromosome 13, the 18p11.1-q11.1 region of chromosome 18 and the 21q22.13 region of chromosome 21, respectively. True positive cases were defined as those with karyotypes or FISH results consistent with the NIPT results.

Statistical analysis

All statistical analyses were conducted using SPSS V.18.0 software. Comparisons between groups were performed using the χ^2 test or Fisher's exact test and a p value of <0.05 was defined as statistically significant.

RESULTS

Patient characteristics

From 2021 to June 2024, a total of 37 891 pregnant women underwent NIPT testing at our institution, resulting in 96 individuals identified as high-risk for T21, T18 and T13, comprising 59 cases of T21, 25 cases of T18 and 12 cases of T13. The ages of the pregnant women with abnormal results ranged from 23 to 43 years, with a mean age of 32.3 years. 52.98% (50/96) of the mothers were under the age of 35. The majority of the pregnant women, 98.96% (95/96), had singleton pregnancies and 94.79% (91/96) conceived naturally. At the time of NIPT testing, the gestational age of the pregnant women ranged from 12 to 25 weeks, with an average gestational age of 16W+6. The 96 high-risk pregnant women were categorised according to clinical indications. The basic information and proportions of different clinical indications are as follows: 54 pregnant women (56.25%) were of advanced maternal age, 30 (31.25%) requested testing voluntarily, 4 (4.17%) had high-risk serum screening results, 4 (4.17%) had borderline-risk serum screening results, 1 (1.04%) had NT thickening, 1 (1.04%) had abnormal soft markers and 2 (2.08%) had twin/in vitro fertilisation-embryo transfer (IVF-ET) pregnancies. Table 1 presents the fundamental information and distribution of various clinical indications.

Table 1 The basic clinical characteristics of pregnant women with 96 cases T21/T18/T13 for NIPT

Characteristics	Number (n)	Percentage (%)
Maternal age (years)		
<35	50	52.08
≥35	46	47.92
Number of fetus		
Singleton	95	98.96
Twins	1	1.04
Method of conception		
Natural conception	5	5.21
Assisted reproduction	91	94.79
Indications		
Advanced maternal age	54	56.25
High-risk serum screening	4	4.17
Borderline-risk serum screening	4	4.17
NT thickening	1	1.04
Abnormal ultrasound soft markers	1	1.04
Twin/IVF-ET pregnancies	2	2.08
Voluntary screening	30	31.25
Gestational age (weeks)		
<14	13	13.54
14–27	83	86.46

IVF-ET, in vitro fertilisation-embryo transfer; NIPT, non-invasive prenatal testing; NT, nuchal translucency; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21.

Clinical performance of NIPT in testing T21, T18, T13

Of the 96 pregnant women identified with high-risk results for T21, T18 and T13, there were 59 cases of T21, 25 cases of T18 and 12 cases of T13 (figure 1). The screening positive rate was 0.25% (96/37 891). Following up, we identified two false-negative results. The overall sensitivity and specificity were 96.55% (56/58) and 99.89% (37 793/37 833), respectively.

Table 2 shows the clinical performance of NIPT in testing T21, T18 and T13. Of the 59 women diagnosed with T21, 40 were confirmed as true positives, resulting in a PPV of 67.80%. The sensitivity and specificity for T21 were 95.24% (40/42) and 99.95% (37 830/37 849), respectively. In a cohort of 25 women with T18, 14 were identified as true positives, yielding a PPV of 48.00%. The sensitivity and specificity for T18 were 100% (25/25) and 99.97% (37 866/37 877), respectively. Among the 12 women diagnosed with T13, 2 were confirmed as true positives, leading to a PPV of 15.38%. The sensitivity and specificity for T13 were both 100% (12/12) and 99.97% (37 879/37 889), respectively. The PPVs of T21, T18 and T13 had significant differences (χ^2=26.272, p<0.001).

Based on the years of testing, we conducted an in-depth analysis of the PPV values for NIPT. Table 3 shows the results. The peak PPV occurred in 2021 at 71.43%, followed by 2022 at 64.29%, 2024 at 52.63% and the lowest PPV was recorded in 2023 at 46.43%. The trend in PPV values for pregnant women with T21 corresponded to the overall results, declining from 92.31% in 2021 to 68.75% in 2022, 70.00% in 2024 and reaching a low of 50.00% in 2023. The PPV values for T18 were highest in 2022 at 75.00%, followed by 2021 and 2023, both at 50.00%, with the lowest value

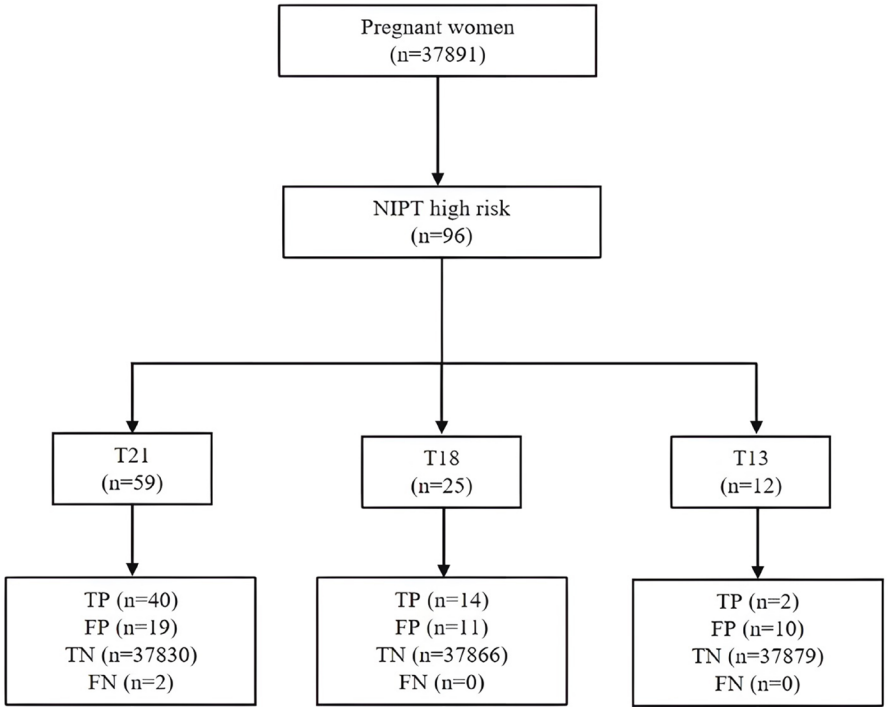

Figure 1 Flowchart of NIPT results of pregnant women undergoing screening for fetal chromosome disorders between 2021 and June 2024. FN, false negative; FP, false positive; NIPT, non-invasive prenatal testing; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TN, true negative; TP, true positive.

recorded in 2024 at 40.00%. In T13, true positives were identified solely in 2022 and 2024, yielding a PPV of 25.00%.

False-negative cases

We identified two pregnant women with false negative NIPT results. In Case 1, NIPT testing was conducted based on the clinical indication of advanced maternal age (≥35 years). Mid-pregnancy ultrasound screening revealed abnormalities, including a short fetal nasal bone and a potential ventricular septal defect. Consequently, prenatal diagnosis was conducted through umbilical vein puncture, yielding a result of T21. In Case 2, the NIPT testing indication was voluntary screening. During subsequent ultrasound screening, fetal duodenal obstruction and polyhydramnios were observed. Prenatal diagnosis was subsequently performed, confirming the presence of T21.

Correlation between clinical indications and PPV for T21, T18 and T13

The positive rate, detection rate and PPV of NIPT among pregnant women diagnosed with T21/T18/T13 vary across different clinical indications (table 4, table 5). Pregnant women of advanced maternal age exhibit the highest positive rate (56.25%), followed by those who voluntarily requested testing (31.25%) and those with borderline or high-risk serum screening results (4.17%). The PPV is highest in the group with NT thickening (100.00%), followed by individuals with high-risk serum screening (75.00%), whereas the PPV is lowest for those with ultrasound soft marker abnormalities and twin/in vitro fertilization-embryo transfer (IVF-ET) pregnancies. In pregnant women with T21, although the positive rates are high for screening indications of advanced maternal age and voluntary request, the detection rates for these two indications are lower than those for other indications

Table 2 Clinical performance of NIPT in testing T21, T18, T13

	Number (n)	TP	FP	TN	FN	Sensitivity	Specificity	PPV	P value of PPV
T21	59	40	19	37 830	2	95.24%	99.95%	67.80%	0.005
T18	25	14	11	37 866	0	100%	99.97%	48.00%	
T13	12	2	10	37 879	0	100%	99.97%	15.38%	

FN, false negative; FP, false positive; NIPT, non-invasive prenatal testing; PPV, positive predictive value; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TN, true negative; TP, true positive.

Table 3 PPV performance of T21, T18 and T13 in pregnant women in different years

	High-risk			T21			T18			T13		
Year	Number (n)	TP	PPV	Number (n)	TP	PPV	Number (n)	TP	PPV	Number (n)	TP	PPV
2021	21	15	71.43%	13	12	92.31%	6	3	50.00%	2	0	0
2022	28	18	64.29%	16	11	68.75%	8	6	75.00%	4	1	25.00%
2023	28	13	46.43%	20	10	50.00%	6	3	50.00%	2	0	0
2024	19	10	52.63%	10	7	70.00%	5	2	40.00%	4	1	25.00%

PPV, positive predictive value; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive.

due to the presence of false-negative cases. Furthermore, the NIPT clinical indications with the highest PPV (100%) include serum screening intermediate risk and NT thickening. The detection rate for pregnant women with T18 is 100% for all NIPT clinical indications, with the highest PPV of 57.14% noted in the advanced maternal age group. Likewise, for pregnant women with T13, the detection rate remains at 100% for all NIPT clinical indications, with the highest PPV of 28.57% observed in the advanced maternal age group. Additionally, there were no significant differences in the PPVs of all clinical indications for T21, T18 and T13.

Correlation between Z-scores and PPV for T21, T18 and T13

The distribution of Z-scores in NIPT-positive cases may provide additional valuable information for clinical consultation. Therefore, we further categorised Z-scores into three ranges: $3 \le Z < 5$, $5 \le Z < 10$ and $Z \ge 10$, and calculated the PPV for each range. As shown in table 6, in the T21 group, most pregnant women (45.76%) had Z-scores ranging from 5 to 10, with a PPV for T21 of 77.78%. Merely 23.73% of pregnant women had Z-scores over 10 and the PPV for this group was 85.71%. The PPV for pregnant women with Z-scores ranging from 3 to 5 was merely 16.67%. A χ^2 test indicated a significant association ($p<0.001$) between the Z-scores and true positive results for T21. Analogous to T21, in the T18 group, the predominant proportion of pregnant women (52.00%) exhibited Z-scores ranging from 3 to 5, accompanied by a PPV of 33.85%. Only two pregnant women had Z-scores greater than 10, but the PPV for this group was as high as 100.00%. In the T13 group, all pregnant women had Z-scores less than 10, with 41.67% displaying Z-scores ranging from 3 to 5 and the PPV for this group was 40.00%. For T18 and T13, there was no significant difference in PPVs among the three Z-score groups ($p=0.123$ and 0.152).

DISCUSSION

NIPT is a non-invasive and highly reliable screening test. NIPT, in contrast to invasive techniques, does not pose a

Table 4 Positive rates of T21, T18 and T13 in pregnant women with diverse clinical indications undergoing NIPT

	High-risk			T21			T18			T13		
Indications	Number (n)	TP	PR	Number (n)	TP	PR	Number (n)	TP	PR	Number (n)	TP	PR
Advanced maternal age	54	35	56.25%	33	24	55.93%	14	9	56.00%	7	2	58.33%
High-risk serum screening	4	3	4.17%	2	1	3.39%	2	2	8.00%	0	0	0
Borderline-risk serum screening	4	2	4.17%	2	2	3.39%	1	0	4.00%	1	0	8.33%
NT thickening	1	1	1.04%	1	1	1.69%	0	0	0	0	0	0
Abnormal ultrasound soft markers	1	0	1.04%	1	0	1.69%	0	0	0	0	0	0
Twin/IVF-ET pregnancies	2	0	2.08%	1	0	1.69%	1	0	4.00%	0	0	0
Voluntary screening	30	15	31.25%	19	12	32.20%	7	3	28.00%	4	0	33.33%
Total	96	56	100%	59	40	100%	25	14	100%	12	2	100%

IVF-ET, in vitro fertilisation-embryo transfer; NIPT, non-invasive prenatal testing; NT, nuchal translucency; PR, positive rate; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive.

Table 5 Detection rates and PPV performance of T21, T18 and T13 in pregnant women with diverse clinical indications undergoing NIPT

	High-risk			T21			T18			T13		
Indications	DR	PPV	P value of PPV	DR	PPV	P value of PPV	DR	PPV	P value of PPV	DR	PPV	P value of PPV
Advanced maternal age	96.77%	64.81%		95.23%	72.73%		100%	64.29%		100%	28.57%	
High-risk serum screening	100%	75.00%		100%	50.00%		100%	100%		/	/	
Borderline-risk serum screening	100%	50.00%		100%	100%		100%	0		100%	0	
NT thickening	100%	100%	0.285	100%	100%	0.340	/	/	0.339	/	/	0.576
Abnormal ultrasound soft markers	100%	0		100%	0		/	/		/	/	
Twin/IVF-ET pregnancies	100%	0		100%	0		100%	0		/	/	
Voluntary screening	93.75%	50.00%		92.31%	63.16%		100%	42.86%		100%	0	
Total	96.55%	58.33%		95.24%	67.80%		100%	56.00%		100%	16.67%	

DR, detection rate; IVF-ET, in vitro fertilisation-embryo transfer; NIPT, non-invasive prenatal testing; NT, nuchal translucency; PPV, positive predictive value; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21.

danger of miscarriage or injury to the fetus.[15] While NIPT is extensively used for identifying fetal anomalies, its outcomes fluctuate among various populations and there is insufficient data regarding the PPV of NIPT screening in different cohorts. This study presents an in-depth analysis of high-risk samples that underwent NIPT at our institution from 2021 to June 2024. The aim was to gain a deeper understanding of the detection capabilities of NIPT and to specifically focus on the PPV values of NIPT across various population subgroups, to provide valuable data and information for clinical genetic counselling and prenatal diagnosis.

This study, involving 37 891 pregnant women, determined that the overall sensitivity and specificity of NIPT were 96.55% and 99.89%, respectively. The PPVs of NIPT for detecting T21, T18 and T13 were 67.80%, 56.00% and 16.67%, respectively. Recent research data indicate that the sensitivity and specificity of NIPT for detecting T21, T18 and T13 exceed 98%, with associated PPVs of 86.81%, 56.81% and 18.18%, respectively. In our study, the corresponding PPVs showed a decreasing trend compared with these values, which may be attributed to a decrease in the incidence of these three conditions.[16] Due to the fact that our data showed that the PPV was at its lowest in 2023, which was only 50.00%, it is possible that the low PPV in that year was a contributing factor to the overall fall in PPV. On reviewing our data, we found that nearly 80.00% of the high-risk samples in 2023 had Z-scores within the grey zone of 3–5 which presumably contributed significantly to the low PPV problem. Despite the relatively low PPV for T21,[17] NIPT still has a superior detection rate and specificity for T21 compared with serum screening,[18] making it a more accurate screening test in clinical practice.

Table 6 The PPV performance of NIPT positive results by classifying Z score as 3≤Z< 5, 5≤Z<10 and Z≥10

Z score	T21				T18				T13			
	Number (n) (%)	TP	PPV	P value of PPV	Number (n) (%)	TP	PPV	P value of PPV	Number (n) (%)	TP	PPV	P value of PPV
3–5	18 (30.51)	3	16.67%		10 (40.00)	3	30.00%		7 (58.33)	0	0.00%	
5–10	27 (45.76)	22	81.48%	<0.001	13 (52.00)	8	61.54%	0.123	5 (41.67)	2	40.00%	0.152
>10	14 (23.73)	13	92.86%		2 (8.00)	2	100%		0 (0.00)	0	0.00%	
Total	59 (100.00)	38	64.41%		25 (100.00)	13	52.00%		12 (100.00)	2	16.67%	

NIPT, non-invasive prenatal testing; PPV, positive predictive value; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive.

Prenatal diagnosis is the gold standard for chromosomal abnormalities. Some pregnant women assert that a low Z-score from NIPT for aneuploidy negates the need to undergo further examination.[19] However, in this study, we identified two cases of false negatives, where NIPT results indicated low risk, yet prenatal diagnosis confirmed T21. Previous research indicates that low fetal DNA concentration, fetal cellular and chromosomal abnormalities, mosaicism, maternal copy number variations and statistical fluctuations in Z-scores during detection may contribute to false negatives in NIPT.[20–23] Among these, low fetal DNA concentration and placental mosaicism are the two principal contributors.[24 25] False negatives are more probable when fetal DNA concentration is<2%. Hu *et al* successfully increased the average concentration of cfDNA from 10% to 20% using cfDNA enrichment technology, significantly reducing the incidence of false negatives.[26] Our analysis of the two pregnant women who experienced false negative results in this study, along with their baseline information and the original NIPT data, revealed that both of the missed samples met the quality control criteria for detection with a cffDNA concentration of>3%. Nevertheless, they were still missed, prompting the hypothesis that placental mosaicism could be responsible for these two false-negative cases. CffDNA originates from the placenta and discrepancies between the placental karyotype and the fetal karyotype can exist. Analysing the placenta could help verify this hypothesis. Furthermore, in examining the causes of false negatives, it is crucial to not only analyse the raw data and retest the original samples but also to ascertain the authenticity of the specimens; specifically, to confirm that the specimens originated from the child's mother rather than another pregnant woman. Both false-negative cases subsequently showed abnormal ultrasound findings, highlighting the significance of prenatal ultrasound. Given the constraints of NIPT, the interpretation and communication of NIPT results should be more comprehensive to guarantee that both pregnant women and healthcare providers fully understand the limitations and potential risks of NIPT, facilitating more reasonable medical decisions. Consequently, performing comprehensive clinical consultations prior to testing is essential.

Our research findings demonstrate that the PPV of NIPT for detecting T21, T18 and T13 exhibits significant variability across pregnant women with diverse clinical indications. The results demonstrate that the highest positive rates for NIPT screening indications for T21, T18 and T13 correlate with advanced maternal age and Xiang *et al* reported a maximum PPV of 73.09% for T21 in "advanced age" pregnancies.[10] In addition, Cai *et al* also showed that the PPV of common trisomy was significantly higher in "advanced age" pregnant women.[27] The results indicate that advanced maternal age is associated with an increased likelihood of chromosomal abnormalities in fetuses, necessary for diagnostic testing. The PPV escalates with maternal age due to the heightened occurrence of fetal chromosomal aneuploidy, which arises during mitosis or meiosis, as maternal age increases. Nevertheless, certain studies have demonstrated that the PPV for T21, T18 and T13 does not exhibit a substantial increase throughout specified age categories (5-year intervals), indicating that positive results from NIPT should be regarded with similar importance by both providers and patients, irrespective of maternal age.[5] In high-risk pregnant women, NT thickening has a PPV of 100%, consistent with previously reported data by Wang *et al*.[12] As NIPT testing is conducted after NT screening, this serves as a reminder to NIPT testers to exercise heightened caution in pregnant women exhibiting ultrasonic structural abnormalities. Additionally, other studies have found that the highest positive rate and PPV for NIPT indicators are associated with ultrasonic abnormalities, particularly significant structural abnormalities and NT thickening.[11] The findings suggest that pregnant women exhibiting aberrant ultrasound results have a higher likelihood of carrying fetuses with chromosomal abnormalities. Despite low-risk NIPT results, subsequent prenatal examinations, such as the comprehensive abnormality scan, should meticulously assess fetal condition. Among pregnant women at high risk for T21, T18 and T13, the clinical indication of "voluntary request" has the second-highest positive rate following advanced maternal age. For pregnant women at high risk for T21 and T18 with the clinical indication of "voluntary request", the PPVs are 63.16% and 42.86%, respectively. Despite pregnant women with the screening indication of "voluntary request" being classified as low-risk, conducting NIPT screening in low-risk populations may diminish the sensitivity and PPV of NIPT. Nevertheless, our research findings indicate that this group warrants attention. Furthermore, for pregnant women with abnormal serum screening, Li *et al's* study found that among all screening indications, the abnormal maternal serum screening group had the highest prevalence of chromosomal abnormalities.[28] Nevertheless, owing to the limited number of individuals in this group within our study, we were unable to reach the same conclusion. Wei *et al* demonstrated that the probability of chromosomal abnormalities in twins is higher than in singletons.[29] However, in our study, we only observed two cases of screening-positive twin pregnancies, indicating a low efficacy of screening for abnormalities in twin pregnancies. Compared with a previous study,[11] there was no significant difference in PPV values between different clinical indications in our study which may be due to a small number of NIPT-positive cases. Therefore, in future research, it is necessary to increase the sample data size in order to obtain more in-depth results.

The data from our study indicate that in the T21 and T18 groups, an increase in the Z-score corresponds with an increase in the PPV, signifying a substantial correlation between PPV and Z-score. Similar to previous research data, the performance of PPV is closely related to the Z-score.[30] The PPV for T21, T18 and T13 in the high Z-score group is significantly higher than that in the low Z-score group.[14 25] In the study by Junhui *et al*, receiver operating characteristic curve analysis showed

that the optimal cut-off Z-scores for fetal T13, T18 and T21 were 6.889, 7.574 and 6.612, respectively. At these cut-off values, the sensitivity for T21 and T18 was 96.8% and 88.9%, respectively, and the specificity was 90% and 92.6%, respectively,[9] This finding also supports our study's result that T21 and T18 have the highest PPV when the Z-score exceeds 10. However, due to the limited number of samples for T13 in our study, there were no high-risk samples with Z>10, necessitating further investigation in the future. These data suggest that a higher Z-score is associated with a greater likelihood of a true positive result for aneuploidy. Additionally, a retrospective analysis of the Z-scores of false-positive cases for T13, T18 and T21 revealed that false-positive cases primarily had Z-scores<5 which falls within the grey zone according to the kit manual. In our study, when the Z-score ranged from 3 to 5, the PPV for high-risk T21 populations was less than 20%. Theoretically, the likelihood of a false positive is greater than 80%. Studies have shown that the occurrence of placental mosaicism may slightly increase the NIPT Z-score to above 3 but below 5, thereby affecting the accuracy of NIPT.[13] Therefore, for pregnant women with Z-scores between 3 and 5, they can be informed of the possibility of a false positive to alleviate anxiety. Given the substantial link between Z-score and PPV, Yang *et al*[25] conducted a rank correlation analysis between Z-score and maternal age, gestational age, fetal fraction and body mass index, revealing that the concentration of cffDNA significantly increases with the Z-scores of T21, T18 and T13. Thus, when a case has a fetal DNA concentration of about 10% and a Z-score of about 10, it suggests a potential true positive case; if the sample shows a fetal DNA concentration higher than 10% but a lower Z-score, around 5, it may be suspected as a false-positive case. Consequently, we advocate for the incorporation of the fetal DNA fraction value in the NIPT result report to furnish clinicians with additional information.

Our research further supports that NIPT is an effective screening technique to detect aneuploidy disorders and signifies a groundbreaking progression in prenatal diagnosis. Nevertheless, our study also possesses certain limitations. First, with the development of assisted reproductive technologies and the growing percentage of older pregnant women, the incidence of twin pregnancies has significantly risen.[31 32] NIPT is an advanced prenatal screening method for twin pregnancies, demonstrates excellent sensitivity and specificity in detecting fetal aneuploidy.[33] However, there is limited research data on the performance of NIPT in twin pregnancies. Our investigation identified only two cases of twin pregnancies with positive screening results, necessitating future research to assess the efficacy of NIPT in twin pregnancy detection. Second, all cases in our study were from a tertiary hospital in Beijing and data from more medical institutions are required to better clarify the prognostic efficacy of NIPT. Third, due to the limited incidence of T13, more cases need to be studied to assess the efficacy of NIPT in detecting T13.

Conclusion

Our research indicates that NIPT serves as a highly effective prenatal screening method, exhibiting elevated sensitivity and specificity in identifying T21, T18 and T13. We also provide evidence that the PPV of NIPT for fetal trisomy of chromosomes 13, 18 and 21 is closely related to the screening indications of pregnant women and their Z-scores. Based on our findings, we suggest that increased caution should be exercised in the administration of NIPT to high-risk pregnant women, including those of advanced maternal age, those with ultrasonic structural abnormalities or those with positive serum screening results. Furthermore, the Z-score values of NIPT can assist clinicians in interpreting NIPT results and providing prenatal consultation.

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