Am J Transl Res 2026;18(7):6250-6263
www.ajtr.org /ISSN:1943-8141/AJTR0174156

Original Article

Analysis of diagnostic consistency rate and pregnancy outcomes in pregnant women with high-risk NIPT undergoing transabdominal amniocentesis

Xiaojuan Zhang*, Xin Zhao*, Xiaozhi Wu

*Department of Obstetrics, Zunyi First People's Hospital (The Third Affiliated Hospital of Zunyi Medical), Zunyi 563000, Guizhou, China. *Equal contributors.*

Received May 19, 2026; Accepted June 9, 2026; Epub July 15, 2026; Published July 30, 2026

Abstract: Objective: To explore the diagnostic performance and positive predictive value (PPV) of non-invasive prenatal testing (NIPT) for different high-risk subtypes confirmed by amniocentesis, to analyze pregnancy outcomes, and to identify factors influencing true-positive results, thereby providing evidence for clinical genetic counseling and prenatal decision-making. Methods: A total of 342 pregnant women with high-risk NIPT findings and receiving subsequent amniocentesis were retrospectively included. Confirmatory diagnosis rates and PPVs were compared among different NIPT subtypes, and pregnancy outcomes were analyzed across groups. Stratified analyses were conducted according to maternal age, gestational age at amniocentesis and clinical indications. Univariate and multivariate logistic regression analyses were performed to identify independent predictors of true-positive NIPT results. Results: Chromosomal abnormalities were confirmed in 218 of 342 enrolled cases (63.7%). PPV varied substantially among NIPT subtypes. Trisomy 21 (T21) had the highest PPV of 68.5%, followed by sex chromosome anomalies and trisomy 18 (T18), while trisomy 13 (T13) and pathogenic copy number variation (CNV) presented lower PPV. Variants of uncertain significance (VUS) accounted for 70.7% of all pathogenic CNV cases. Pregnancy outcomes differed significantly among high-risk NIPT subgroups. The sex chromosome anomaly group had the highest continued pregnancy rate, whereas trisomy 21 cases showed the highest pregnancy termination rate. All subtypes had higher PPV in women aged 35 years and above than in younger counterparts; statistical comparison was not conducted due to small sample size. No significant PPV difference was found among groups with different gestational age groups ($P>0.05$). Univariate analysis demonstrated that advanced maternal age was correlated with increased PPV. Multivariate logistic regression indicated that NIPT subtype and concurrent abnormal ultrasound soft markers were independent risk factors for true-positive results, while gestational age exerted no independent impact on diagnostic efficiency. The established regression prediction model had moderate discriminative power for true-positive prediction, with an area under the curve (AUC) of 0.695 ($P<0.001$). Conclusion: PPV varies considerably among high-risk NIPT subtypes, and NIPT cannot replace invasive prenatal diagnosis. NIPT subtype classification and abnormal ultrasound soft markers independently affect true-positive results, and advanced maternal age elevates the detection risk of chromosomal anomalies. Stratified genetic counseling should integrate NIPT subtype, maternal age, and ultrasound findings. Early amniocentesis is recommended for high-risk pregnancies with autosomal trisomy, advanced maternal age, or abnormal ultrasound soft markers to facilitate standardized prenatal diagnosis and informed pregnancy decision-making.

Keywords: NIPT, high-risk NIPT, chromosomal aneuploidy, amniocentesis, positive predictive value, pregnancy outcome, genetic counseling

Introduction

With the advent of high-throughput sequencing of cell-free fetal DNA in maternal peripheral blood, non-invasive prenatal testing (NIPT) has been widely adopted as a first-line screening method for common fetal chromosomal aneuploidies, including trisomy 21, trisomy 18, and trisomy 13, owing to its non-invasive nature and high accuracy [1-3]. However, NIPT is essentially a screening method rather than a confirmatory diagnostic tool. All high-risk results need further confirmation of chromosomal abnormalities through invasive prenatal diagnosis such

https://doi.org/10.62347/JUIA1368

as amniocentesis [4, 5]. Clinical studies have demonstrated that the positive predictive value (PPV) varies considerably among different high-risk NIPT subtypes. The PPV for trisomy 21 is higher than that for trisomy 13, sex chromosome abnormalities, and pathogenic copy number variations (CNVs), which have lower PPVs. False-positive results are common in clinical practice [6-8].

Maternal age is an important confounding factor affecting the risk of fetal chromosomal abnormalities and the screening efficacy of NIPT. Advanced maternal age is associated with a higher baseline risk of chromosomal abnormalities, and the true-positive rate increases significantly when combined with high-risk NIPT results [9, 10]. Abnormal ultrasound soft markers, which are important indicators in prenatal screening, are associated with fetal chromosomal abnormalities and pathogenic CNVs [11]. Their presence may further increase the likelihood of true-positive results in pregnant women with high-risk NIPT. Nevertheless, the independent predictive value of ultrasound soft markers remains controversial among existing studies [12, 13]. In addition, no consensus exists regarding the effect of gestational age at amniocentesis on the PPV of NIPT. Large-sample stratified data are still lacking to clarify whether the positive predictive values in the early and late trimesters differ from those in the second trimester [4, 14].

Most existing studies have focused on the screening value of NIPT for common trisomies but lack stratified comparisons and multivariate regression analyses of diagnostic composition, PPV differences, and pregnancy outcomes across various high-risk NIPT subtypes. Furthermore, the effects of maternal age, gestational age, and indications for amniocentesis on PPV have not been thoroughly investigated [15]. This study retrospectively enrolled 342 pregnant women with high-risk NIPT results who underwent amniocentesis for prenatal diagnosis. We systematically analyzed the diagnostic findings, PPVs, and pregnancy outcomes for each high-risk NIPT subtype. Subgroup analyses were performed based on maternal age, gestational age at amniocentesis, and clinical indications. A multivariate logistic regression model was established to identify independent predictors of true-positive NIPT results [13]. This study aimed to provide clinical evidence to guide genetic counseling, determine the optimal timing of prenatal diagnosis, and improve pregnancy decision-making for women with high-risk NIPT results.

Methods

Study subjects

This study retrospectively analyzed the clinical data of 1540 pregnant women who underwent NIPT at the Prenatal Diagnosis Center of Zunyi First People's Hospital from December 2021 to December 2025. After applying the inclusion and exclusion criteria, 342 pregnant women with high-risk NIPT results were enrolled. All high-risk pregnant women received genetic counseling and were recommended to undergo amniocentesis for prenatal diagnosis. This study was approved by the Ethics Committee of Zunyi First People's Hospital.

Inclusion criteria

(1) Singleton pregnancy; (2) NIPT results indicated high risk of trisomy 21, trisomy 18, trisomy 13, sex chromosome abnormalities, or pathogenic CNV; (3) Complete and traceable clinical data, including maternal age, gestational age, NIPT reports, amniocentesis results, and pregnancy outcomes; (4) No contraindications for amniocentesis.

Exclusion criteria

(1) Multiple pregnancies; (2) Pregnant women complicated with severe internal and surgical diseases (such as severe heart disease, hepatic and renal failure, coagulation dysfunction, etc.) or gestational complications (such as severe preeclampsia, placental abruption, etc.); (3) Incomplete clinical data, unsuccessful prenatal puncture procedure, or loss of follow-up; (4) Cases with unclear or unavailable pregnancy outcomes; (5) Presence of other confounding factors that may affect fetal chromosomal results, such as exposure to teratogenic substances or intake of teratogenic drugs during pregnancy.

NIPT testing and definition of high-risk results

A total of 10 mL of peripheral venous blood was collected from pregnant women at 12-22 weeks of gestation. Cell-free fetal DNA was extracted and analyzed using high-throughput

sequencing technology. In accordance with national health industry standards and published studies, an absolute Z-score (|Z|≥3) was defined as the threshold for high-risk NIPT results [16, 17]. High-risk criteria for trisomy 21, trisomy 18, and trisomy 13 were defined as Z-score ≥3 or a risk ratio of ≥1/270, ≥1/350, and ≥1/270, respectively [16]. The identification of sex chromosome abnormality (SCA) was mainly based on the Z-values of X/Y chromosomes (|Z|≥3), and the positive predictive value (PPV) of SCAs was significantly lower than that of autosomal trisomies [18]. Previous studies have confirmed that the Z-value is positively correlated with PPV. The false-positive rate can reach 69.2% when the Z-score is between 3 and 5, but decreases to 0% when Z>12 [19]. According to the above criteria, cases meeting any high-risk standard were included in the analysis. NIPT high-risk results were classified into five categories: trisomy 21, trisomy 18, trisomy 13, sex chromosome abnormality, and pathogenic CNV. The above groups are not mutually exclusive: the NIPT result of a single pregnant woman may indicate high risks for both autosomal trisomy and pathogenic CNV simultaneously. In such cases, the same individual is counted separately in each relevant group for descriptive purposes. Therefore, the sum of the number of cases in each group may exceed the total number of high-risk cases (342).

Prenatal diagnosis and gold standard

All pregnant women with high-risk NIPT results underwent ultrasound-guided invasive prenatal diagnosis. Transabdominal amniocentesis was the primary procedure, performed between 16 and 26 weeks of gestation. Cordocentesis (percutaneous umbilical blood sampling) was performed in cases where amniocentesis failed or when gestational age was advanced. The samples were subjected to G-banded chro mosomal karyotype analysis (resolution: 400-500 bands), chromosomal microarray analysis (CMA), or low-depth whole-genome sequencing (CNV-seq).

The results of invasive prenatal diagnosis served as the gold standard for final assessment of all high-risk NIPT cases. According to the 2019 ACMG variant classification criteria, the results were divided into three categories: (1) Positive: Confirmed pathogenic or likely pathogenic chromosomal abnormalities, including trisomy 21, trisomy 18, trisomy 13, sex chromosome aneuploidies, pathogenic/likely pathogenic CNVs, and pathogenic variants related to monogenic disorders. (2) Negative: Normal findings in both chromosomal karyotype and CNV detection, without clinically relevant pathogenic variants. (3) Uncertain/Inconclusive: This category included variants of uncertain significance (VUS), benign or likely benign CNVs, isolated abnormal ultrasound soft markers, cases with high-risk NIPT results but no pathogenic abnormalities confirmed by invasive testing, and incomplete test results that could not be definitively classified.

CNV interpretation was performed in accordance with the ACMG/ClinGen guidelines (Riggs et al., 2020) [20], with CNVs classified into five grades: pathogenic, likely pathogenic, VUS, likely benign, and benign. In this study, pathogenic and likely pathogenic CNVs were included in the positive outcome analysis; VUS were counted separately; benign and likely benign CNVs were regarded as false-positive results.

Grouping and definition

To evaluate the effects of different factors on the positive predictive value of NIPT and pregnancy outcomes, the study participants were divided and defined as follows: (1) Age group: Younger maternal age group (<35 years) and advanced maternal age group (≥35 years). (2) Gestational age group: Early group (<18 weeks), mid-trimester group (18-24 weeks), and late group (>24 weeks). (3) Puncture indication grouping: Isolated NIPT group (amniocentesis performed only for high-risk NIPT results), NIPT combined with advanced maternal age group (high-risk NIPT plus maternal age ≥35 years), and NIPT combined with ultrasound abnormality group (high-risk NIPT plus abnormal ultrasound soft markers or structural anomalies). (4) Pregnancy outcomes: Continued pregnancy (live birth), adverse outcomes (termination of pregnancy, spontaneous abortion/fetal demise), and loss to follow-up.

Statistical analysis

Statistical analysis was performed using SPSS 27.0. Continuous variables are presented as mean ± standard deviation (SD), and categori-

Table 1. Demographic and clinical characteristics of 342 pregnant women with high-risk NIPT results

Characteristic	Category	n = 342	Proportion (%)
Maternal age (years)	29.11 ± 4.43	-	-
Age group	<35 years	310	90.6
	≥35 years (advanced maternal age)	32	9.4
Gestational age at puncture (weeks)	19.65 ± 2.03	-	-
Gestational age group	<18 weeks	17	4.9
	18-24 weeks	308	90.1
	>24 weeks	17	4.9
Specimen type	Peripheral blood + amniocentesis	342	100
NIPT high-risk classification	Trisomy 21	200	58.5
	Trisomy 18	26	7.6
	Trisomy 13	13	3.8
	Sex chromosome aneuploidy	96	28.1
Parity	Primipara	2	0.6
	Multipara	340	99.4
Ultrasound soft markers	No abnormal markers	332	97.1
	With abnormal markers (echogenic intracardiac focus, choroid plexus cyst, ventriculomegaly, nasal bone abnormalities, etc.)	10	2.9
Pregnancy outcome	Live birth	80	23.4
	Induced abortion/termination of pregnancy	239	69.9
	Spontaneous abortion/embryonic demise	9	2.6
	Unknown/loss to follow-up	14	4.1

Abbreviations: NIPT, non-invasive prenatal testing; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13; SCA, sex chromosome aneuploidy. Note: Continuous variables are presented as mean ± standard deviation; categorical variables are presented as number (n) and proportion (%).

cal variables as frequencies and percentages (%). Pearson's chi-square test or Fisher's exact test was used to compare PPV and continued pregnancy rates among groups. Pairwise comparisons were performed using the Bonferroni correction when applicable. Univariate analysis was applied to evaluate the influence of each puncture indication on PPV. Multivariate logistic regression analysis was performed including NIPT classification, gestational age at amniocentesis, and clinical indications to identify independent predictors of true-positive NIPT results. All tests were two-tailed, and $P<0.05$ was considered statistically significant.

Results

Baseline characteristics

The study sample consisted of 342 pregnant women with high-risk NIPT results, all of whom underwent NIPT screening and subsequent amniocentesis for prenatal diagnosis. The mean maternal age was 29.11 ± 4.43 years, including 310 women (90.6%) aged under 35 years and 32 women (9.4%) with advanced maternal age (≥35 years). The mean gestational age at amniocentesis was 19.65 ± 2.03 weeks. Among them, 17 cases (4.9%) underwent puncture before 18 weeks of gestation, 308 cases (90.1%) at 18-24 weeks, and 17 cases (4.9%) after 24 weeks. In terms of NIPT high-risk classification, trisomy 21 was the most common subtype with 200 cases (58.5%), followed by sex chromosome abnormalities in 96 cases (28.1%), trisomy 18 in 26 cases (7.6%), and trisomy 13 in 13 cases (3.8%). Most participants were multiparous women, accounting for 340 cases (99.4%). Abnormal ultrasound soft markers were present in 10 cases (2.9%). Regarding pregnancy outcomes, there were 80 live births (23.4%), 239 terminations of pregnancy (69.9%), 9 spontaneous abortions/fetal demises (2.6%), and 14 cases lost to follow-up (4.1%) (**Table 1**).

Diagnostic coincidence rate, positive predictive value and inter-subtype comparison of different NIPT subtypes

Among the 342 pregnant women with high-risk NIPT results, chromosomal abnormalities were confirmed by amniocentesis in 218 cases, yielding a diagnostic confirmation rate

Table 2. Comparison of NIPT classification and prenatal diagnostic results

NIPT classification	Positive by gold-standard	Undetected/Clinically unclear (false positive)	Total high-risk cases	Positive predictive value (%)
Trisomy 21	137	63	200	68.5
Trisomy 18	11	15	26	42.3
Trisomy 13	2	11	13	15.6
Sex chromosome anomaly	44	59	103	42.7
Pathogenic CNV	24	58	82	29.3

Abbreviations: NIPT, non-invasive prenatal testing; CNV, copy number variation; VUS, variant of uncertain significance; PPV, positive predictive value. Note: Undetected/Clinically unclear cases included variants of uncertain significance (VUS), benign variants, and NIPT high-risk results without confirmed pathogenic findings in prenatal diagnosis. The same pregnant woman with a high-risk NIPT result may meet the criteria for multiple high-risk categories simultaneously (e.g., high risk for trisomy 21 combined with high risk for pathogenic CNV). Therefore, the sum of cases across all subgroups (424 cases) exceeds the total number of high-risk cases (342 cases). The positive predictive value (PPV) was calculated separately for each category, reflecting the diagnostic confirmation rate for that specific indicator.

of 63.7%. The number of cases in the following subgroups overlap because some pregnant women's NIPT results showed high risks for both autosomal trisomy and pathogenic CNV simultaneously, resulting in a total count exceeding 342 cases. There were significant differences in positive predictive value (PPV) among various NIPT subtypes: trisomy 21 had the highest PPV at 68.5% (137/200), followed by sex chromosome abnormalities (42.7%, 44/103) and trisomy 18 (42.3%, 11/26). Trisomy 13 (15.4%, 2/13) and pathogenic CNVs (29.3%, 24/82) showed relatively lower PPV. In the pathogenic CNV group, variants of uncertain significance (VUS) accounted for 70.7% (58/82), indicating that the screening performance of NIPT for CNVs is limited.

Overall comparison revealed statistically significant differences in PPV among subtypes (χ^2 = 50.21, P<0.0001). Pairwise comparison showed that the PPV of trisomy 21 was significantly higher than that of all other subtypes (all P<0.01), with the most remarkable differences compared with trisomy 13 and pathogenic CNVs (P<0.0001) (**Tables 2**, **3**).

Pregnancy outcomes and subgroup analysis of pregnant women with high-risk NIPT

Among the 342 enrolled pregnant women with high-risk NIPT results, 328 (95.9%) had definitive pregnancy outcomes, while 14 cases (4.1%) were lost to follow-up. Pregnancy outcomes were dichotomized into "continued pregnancy" and "adverse outcomes" (including termination of pregnancy, spontaneous abortion/intrauterine fetal death, and loss to follow-up). Pearson's chi-square test showed that the rate of continued pregnancy differed significantly among different high-risk subtypes (χ^2 = 140.00, df = 3, P<0.0001). Stratified outcomes of each subtype were as follows: in the trisomy 21 group (200 cases), the continued pregnancy rate was 3.5% and the pregnancy termination rate was 89.0%; in the trisomy 18 group (26 cases), the continued pregnancy rate was 19.2% and the pregnancy termination rate was 73.1%; in the trisomy 13 group (13 cases), the continued pregnancy rate was 15.4% and the pregnancy termination rate was 84.6%; in the sex chromosome abnormality group (103 cases), the continued pregnancy rate was 64.1% and the pregnancy termination rate was 30.1%. The pathogenic CNV group (24 cases) was not included in the tables due to classification overlap.

Subgroup comparison indicated that the sex chromosome abnormality group had the highest continued pregnancy rate, which was significantly higher than that of all autosomal trisomy groups (all P<0.001). Overall, pregnancy decisions varied markedly across different abnormal subtypes, and the trisomy 21 group had the highest termination rate (89.0%) (**Tables 4**, **5**).

Age-stratified analysis

Of the 342 pregnant women with high-risk NIPT results, 32 cases (9.4%) were of advanced maternal age (≥35 years), and 310 cases (90.6%) were of non-advanced maternal age (<35 years). The overall diagnostic confirmation rate was 75.0% (24/32) in the advanced

Table 3. Comparison of positive predictive values among different NIPT classifications

Comparison	χ^2 value	*P* value	Significance
Overall (5 groups)	50.21	<0.0001	****
Pairwise comparisons (vs. Trisomy 21)			
Trisomy 21 vs. Trisomy 18	6.984	0.0082	**
Trisomy 21 vs. Trisomy 13	15.19	<0.0001	****
Trisomy 21 vs. Sex chromosome anomaly	18.79	<0.0001	****
Trisomy 21 vs. Pathogenic CNV	36.54	<0.0001	****

Abbreviations: NIPT, non-invasive prenatal testing; CNV, copy number variation. Note: Overall comparison was performed using Chi-square test (2 × 5 contingency table). Pairwise comparisons between trisomy 21 and each other classification were performed using Chi-square test with Bonferroni correction (significance threshold set at P<0.0125).

Table 4. Pregnancy outcomes of different NIPT high-risk types

NIPT classification	Continued pregnancy (n)	Termination of pregnancy (n)	Spontaneous abortion/ Fetal demise (n)	Lost to follow-up (n)	Total (n)
Trisomy 21	7	178	4	11	200
Trisomy 18	5	19	0	2	26
Trisomy 13	2	11	0	0	13
Sex chromosome anomaly	66	31	5	1	103
Total	80	239	9	14	342

Abbreviations: NIPT, non-invasive prenatal testing. Note: Data are presented as number of cases (n). Pregnancy outcomes include continued pregnancy, termination of pregnancy, spontaneous abortion/fetal demise, and lost to follow-up.

Table 5. Continued pregnancy rates among women with different types of NIPT high-risk results (n = 342)

NIPT high-risk type	Total n	Continued pregnancy, n (%)	Adverse outcomes, n (%)	χ^2 value	df	*P* value
Overall	342	80 (23.39)	262 (76.61)	140.00	3	<0.0001
Trisomy 21	200	7 (3.50)	193 (96.50)	136.40	1	<0.0001
Trisomy 18	26	5 (19.23)	21 (80.77)	16.87	1	<0.0001
Trisomy 13	13	2 (15.38)	11 (84.62)	11.28	1	0.0008
Sex chromosome anomaly	103	66 (64.08)	37 (35.92)	-	-	Reference

Abbreviations: NIPT, non-invasive prenatal testing. Note: Adverse outcomes include termination of pregnancy, spontaneous abortion/fetal demise, and loss to follow-up. Pairwise comparisons were performed using the Pearson χ^2 test, with the sex chromosome anomaly group as the reference. The overall difference across the four groups was analyzed using the Pearson χ^2 test (χ^2 = 140.00, df = 3, P<0.0001). Bonferroni correction was applied to control for multiple comparisons, with a corrected significance level of α = 0.0167.

age group, compared with 54.8% (170/310) in the non-advanced age group. Stratified analysis by subtype showed: (1) Trisomy 21: In the advanced age group, 14 of 17 cases were confirmed (PPV = 82.4%); in the non-advanced age group, 123 of 183 cases were confirmed (PPV = 67.2%). (2) Trisomy 18: In the advanced age group, 1 of 1 case was confirmed (PPV = 100.0%); in the non-advanced age group, 10 of 25 cases were confirmed (PPV = 40.0%). (3) Trisomy 13: In the advanced age group, 1 of 3 cases was confirmed (PPV = 33.3%); in the non-advanced age group, 1 of 10 cases was confirmed (PPV = 10.0%). (4) Sex chromosome abnormalities: 8 of 12 cases were confirmed in the advanced age group, with a PPV of 66.7%; 36 of 91 cases were confirmed in the non-advanced age group, with a PPV of 39.6%. Overall, the PPV of each abnormal subtype was higher in the advanced maternal age group than in the non-advanced group, which was consistent with the trends reported in previous literature. Due to the relatively small sample size of the advanced age group (only 32 cases),

Table 6. Comparison of diagnostic performance between advanced age (≥35 years) and non-advanced age (<35 years) groups

NIPT classification	Advanced age group (≥35 years, n = 32)	Non-advanced age group (<35 years, n = 310)
Trisomy 21		
High-risk cases (n)	16	183
True positive (n)	14	123
PPV (%)	87.5	67.2
Trisomy 18		
High-risk cases (n)	1	25
True positive (n)	1	10
PPV (%)	100.0	40.0
Trisomy 13		
High-risk cases (n)	3	10
True positive (n)	1	1
PPV (%)	33.3	10.0
Sex chromosome anomaly		
High-risk cases (n)	12	91
True positive (n)	8	36
PPV (%)	66.7	39.6

Abbreviations: NIPT, non-invasive prenatal testing; PPV, positive predictive value. Note: Data are presented as number of cases (n) and positive predictive value (%). Advanced age was defined as maternal age ≥35 years at the time of NIPT. Due to the small sample size in the advanced age group (n = 32), statistical comparisons were not performed between the two groups.

Table 7. Gestational age-stratified analysis of NIPT performance

Gestational age group	Total high-risk cases (n)	True positive (n)	PPV (%)
<18 weeks	17	13	76.5
18-24 weeks	308	174	56.5
>24 weeks	17	7	41.2
Total	342	194	56.7

Abbreviations: NIPT, non-invasive prenatal testing; PPV, positive predictive value. Note: Data are presented as number of cases (n) and positive predictive value (%). Gestational age was calculated as completed weeks at the time of amniocentesis. The overall difference among the three groups was not statistically significant (χ^2 = 1.177, df = 2, P = 0.5551).

no statistical comparison was performed between the two groups (**Table 6**).

Stratified analysis by gestational age at amniocentesis

According to gestational age at amniocentesis, the 342 pregnant women with high-risk NIPT results were divided into three groups: early group (<18 weeks, n = 17, 4.9%), mid-trimester group (18-24 weeks, n = 308, 90.1%), and late group (>24 weeks, n = 17, 4.9%). The positive predictive value (PPV) of the three groups was 76.5% (13/17), 56.5% (174/308), and 41.2% (7/17), respectively. The chi-square test showed no statistically significant overall difference in PPV among the three groups (χ^2 = 1.177, df = 2, P = 0.5551). Although the early pregnancy group had a higher PPV (76.5%) than the mid-trimester group (56.5%), and the late pregnancy group showed a lower PPV (41.2%) than the mid-trimester group, none of the differences reached statistical significance (**Table 7**).

This finding may be attributed to the small sample size of the early and late pregnancy groups (17 cases each), resulting in insufficient statistical power. Further studies with enlarged sample sizes in extreme gestational age subgroups are needed to verify the effect of gestational age on the diagnostic performance of NIPT.

Stratified analysis by puncture indication (univariate analysis)

To evaluate the influence of different puncture indications on the positive predictive value of NIPT, the 342 pregnant women with high-risk NIPT results were divided into three groups: isolated NIPT group (n = 301), NIPT combined with advanced maternal age group (n = 31), and NIPT combined with ultrasound abnormality group (n = 10). The PPV of the three groups was 54.8%, 74.2%, and 50.0%, respectively.

Univariate analysis showed that the PPV of the NIPT with advanced age group was significantly higher than that of the NIPT alone group (χ^2 = 4.296, P = 0.038), with an OR of 2.37 (95% CI: 1.04-5.38), suggesting that advanced maternal age is a predictor of true-positive NIPT results. The difference in the NIPT combined

Table 8. Comparison of PPV among different referral indication subgroups

Group	Total cases (n)	True positive (n)	PPV (%)	OR (95% CI)	*P* value
NIPT alone	301	165	54.8	Reference	-
NIPT + advanced age	31	23	74.2	2.37 (1.04-5.38)	0.038
NIPT + ultrasound anomaly	10	5	50.0	0.82 (0.23-2.90)	0.760

Abbreviations: NIPT, non-invasive prenatal testing; PPV, positive predictive value; OR, odds ratio; CI, confidence interval. Note: Data are presented as number of cases (n) and positive predictive value (%). Advanced age was defined as maternal age ≥35 years. Ultrasound anomalies included NT thickening, absent nasal bone, choroid plexus cyst, echogenic intracardiac focus, pyelectasis, abnormal amniotic fluid volume, and other structural abnormalities. The NIPT alone group (no advanced age, no ultrasound anomalies) served as the reference category. *P* values were calculated using Chi-square test or Fisher's exact test as appropriate. Statistical significance was set at P<0.05.

Table 9. Multivariate logistic regression analysis of factors associated with true positive NIPT results

Variable	B	SE	Wald χ^2	OR	95% CI	*P* value
Gestational age (continuous)	-0.078	0.062	1.607	0.925	0.819-1.044	0.205
NIPT classification (ref: T21)			29.757			<0.001
T18 vs. T21	-1.076	0.429	6.290	0.341	0.147-0.790	0.012
T13 vs. T21	-2.800	0.825	11.522	0.061	0.012-0.306	<0.001
SCA vs. T21	-1.144	0.259	19.489	0.318	0.192-0.529	<0.001
Indication (ref: NIPT alone)			7.131			0.028
NIPT + ultrasound anomaly	1.268	0.475	7.130	3.554	1.401-9.016	0.008
NIPT + advanced age	0.082	0.696	0.014	1.086	0.278-4.247	0.906
Constant	2.230	1.211	3.389	9.296	-	0.066

Abbreviations: NIPT, non-invasive prenatal testing; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13; SCA, sex chromosome aneuploidy. Note: Omnibus test: $\chi^2 = 43.54$, df = 7, P<0.001; Nagelkerke $R^2 = 0.160$; Hosmer-Lemeshow test: $\chi^2 = 13.22$, df = 8, P = 0.104. Overall classification accuracy: 66.7%. The subgroup of patients with concomitant ultrasound anomalies had a very small sample size (n = 10), which may lead to low statistical power and unstable estimates in the multivariate logistic regression model. The results for this variable should be interpreted with caution.

with ultrasound abnormality group did not reach statistical significance in univariate analysis (P = 0.760) due to its small sample size (only 10 cases).

Nevertheless, after adjusting for NIPT classification in multivariate logistic regression analysis, NIPT combined with ultrasound abnormality emerged as an independent risk factor for true-positive results (OR = 3.554, 95% CI: 1.401-9.016, P = 0.008), indicating that the additive effect of ultrasound abnormalities became evident after controlling for confounding factors (**Tables 8**, **9**).

Multivariate logistic regression analysis

To evaluate the independent effects of various factors on true-positive NIPT results, gestational age, NIPT classification, and clinical indications were included in the multivariate logistic regression model. The overall model was statistically significant (Omnibus test: $\chi^2 = 43.54$, df = 7, P<0.001), with a Nagelkerke R^2 of 0.160. The Hosmer-Lemeshow test indicated good model fit (P = 0.104), and the overall classification accuracy was 66.7%. For NIPT classification, using T21 as the reference group, the probability of a true-positive result was significantly lower for T18 (OR = 0.341, 95% CI: 0.147-0.790, P = 0.012), T13 (OR = 0.061, 95% CI: 0.012-0.306, P<0.001), and SCAs (OR = 0.318, 95% CI: 0.192-0.529, P<0.001). Regarding clinical indications, NIPT with ultrasound abnormality was an independent risk factor for true-positive results. Using the NIPT alone group as the reference, the NIPT plus ultrasound abnormality group showed a significantly higher probability of true positivity (OR = 3.554, 95% CI: 1.401-9.016, P = 0.008), whereas no significant difference was observed between the NIPT combined with advanced maternal age group and the isolated NIPT group (OR = 1.086, P = 0.906). For gestational age, the probability of true positivity decreased by approximately 7.5% per additional gestational

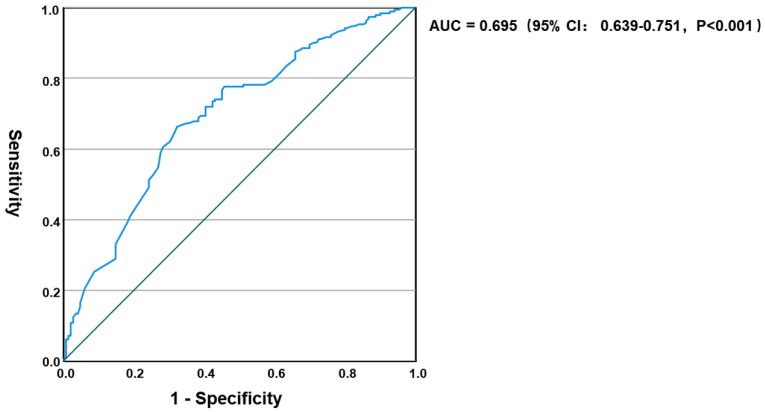

Figure 1. Receiver operating characteristic (ROC) curve of the predictive model for true positive outcomes in high-risk NIPT cases. The area under the curve (AUC) was 0.695 (95% confidence interval: 0.639-0.751, P<0.001), indicating a moderate predictive performance of the model.

week, though the difference was not statistically significant (OR = 0.925, P = 0.205) (**Table 9**).

The AUC of the prediction model established by multivariate logistic regression was 0.695 (95% CI: 0.639-0.751, P<0.001), suggesting that the model has moderate predictive value for true-positive outcomes in pregnant women with high-risk NIPT results (**Figure 1**).

Discussion

This study systematically analyzed the amniocentesis confirmation results, positive predictive value (PPV), and pregnancy outcomes of 342 pregnant women with high-risk NIPT results, and explored the effects of maternal age, gestational age, and puncture indications on true-positive NIPT findings. The results showed that the screening performance of NIPT varied significantly across different chromosomal abnormality subtypes, and abnormal ultrasound soft markers served as an important independent predictive factor for true-positive results. These findings can provide valuable evidence for clinical genetic counseling and the formulation of prenatal diagnosis strategies.

In the present study, the PPV of NIPT for trisomy 21 was 68.5%, which was generally consistent with findings reported in published studies. Cai et al. [6] reported a PPV of 78.9% for trisomy 21 among 52,855 pregnant women, while Pang et al. [7] reported a PPV of 91.67%. The slightly lower value in our study may be attributed to differences in baseline risk across study populations. The proportion of women with advanced maternal age was only 9.4% in this cohort, whereas a higher proportion was included in the above studies (19.5% in the research by Cai et al.). This indicates that the PPV of NIPT is affected by the prevalence in the target population, which conforms to the basic principle of Bayes' theorem. According to Bayes' theorem, the positive predictive value is jointly determined by the sensitivity and specificity of the screening method as well as the prior probability of the target disease in the screened population [21]. Since the prevalence of trisomy 21 increases exponentially with maternal age, the prior disease probability is higher in older pregnant women. Accordingly, under equivalent NIPT diagnostic performance, the PPV is inherently higher in advanced-age populations than in younger ones [22]. This phenomenon has been validated in multiple studies. For instance, nationwide NIPT data analysis from Japan demonstrated that demographic differences among age groups can substantially influence the actual clinical performance of PPV.

Notably, the PPV of NIPT for trisomy 13 was only 15.4% in this study, and the PPV for pathogenic CNVs was 29.3%, with VUS accounting for as high as 70.7% in the CNV high-risk group. These findings are consistent with those of previous studies. Chen et al. [8] reported a PPV of 13.79% for trisomy 13 and 28.99% for CNVs among 42,910 singleton pregnancies, while Pang et al. [7] documented a CNV PPV of 32.14%. The limited performance of NIPT in CNV screening is mainly attributed to the following factors: the proportion of cell-free fetal DNA in maternal plasma is relatively low (usually 5%-20%), and CNV detection requires a higher signal-to-noise ratio. Meanwhile, confined placental mosaicism and maternal CNV interference may lead to false-positive results [4, 5]. Furthermore, authoritative guidelines from organizations including ACMG and SMFM

currently do not recommend the routine application of NIPT for genome-wide CNV screening [3]. Therefore, in the clinical interpretation of CNV-related high-risk NIPT results, patients should be fully informed of the possibility of false positives, and confirmation by amniocentesis combined with chromosomal microarray analysis (CMA) is strongly recommended. In the present study, the PPV for sex chromosome abnormalities was 42.7%, falling within the range of 30.8% to 66.7% reported in the published literature [23-25]. In addition to placental mosaicism, the relatively low PPV for sex chromosome abnormalities is also related to the interference of maternal X chromosome abnormalities (such as Turner syndrome mosaicism and trisomy X) on cell-free fetal DNA detection [4].

Maternal age is a key factor affecting the screening efficacy of NIPT and the risk of chromosomal abnormalities. This study showed that the overall diagnostic confirmation rate was significantly higher in the advanced maternal age group (≥35 years) (75.0%) than in the non-advanced age group (55.0%), and the PPV for each subtype was also higher in the advanced age group, consistent with trends reported in previous studies [26, 27]. Lu et al. [28] confirmed that the risk of abnormal chromosome segregation increases in advanced-age pregnant women due to oocyte aging, leading to a substantially higher probability of true-positive results when NIPT indicates high risk. This suggests that age stratification is important for guiding clinical genetic counseling. Although the sample size of the advanced age group was relatively small in this study (32 cases), and no statistical comparison was performed between groups, stratified analysis revealed that the PPV of all subtypes was higher in the advanced age group. The findings indicate the clinical value of advanced maternal age as a potential influencing factor for true-positive NIPT results, which merits further verification in larger cohorts.

Abnormal ultrasound soft markers serve as an important indication for amniocentesis, and their independent predictive value for true-positive NIPT results was verified in this study. Univariate analysis showed that the PPV of the NIPT combined with ultrasound abnormality group (50.0%) was not significantly different from that of the NIPT alone group (54.8%). However, after adjusting for confounding factors in multivariate regression analysis, ultrasound abnormality emerged as an independent risk factor for true-positive outcomes (OR = 3.554, P = 0.008), which was consistent with the findings of Zhao et al. [17], Liu et al. [29], and other related studies. Abnormal ultrasound soft markers (such as increased NT thickness and absent nasal bone) are closely associated with fetal chromosomal abnormalities. They may synergistically increase the probability of true-positive results among high-risk NIPT populations by affecting the release of cell-free fetal DNA or by directly indicating abnormal fetal development. Clinically, attention should be paid to the additive effect of ultrasound abnormalities, and invasive prenatal diagnosis should be preferentially recommended for such high-risk pregnant women [30].

This study explored the influence of gestational age at amniocentesis on the screening performance of NIPT. The results showed that the PPV of the early pregnancy group (<18 weeks) was 76.5%, which was higher than that of the mid-trimester group (56.5%) and the late pregnancy group (41.2%). However, no statistically significant difference was detected among the groups, which may be attributed to the small sample size of the extreme gestational age subgroups (only 17 cases in each) and insufficient statistical power. Li et al. [4] found that the false positive rate of NIPT increases with advancing gestational age, reaching 25%-60% after 17 weeks of gestation. The underlying mechanism may involve a higher proportion of cffDNA in early pregnancy and an elevated risk of placental-fetal discordance in late pregnancy. The decreasing trend of PPV with gestational age observed in this study is consistent with the above finding, suggesting that gestational age may affect the accuracy of NIPT. Further verification with an expanded sample size is required in subsequent research.

Pregnancy outcome analysis revealed significant differences in the continued pregnancy rate among different NIPT high-risk subtypes. The continued pregnancy rate in the sex chromosome abnormality group was 64.1%, which was markedly higher than that of autosomal trisomy groups (3.5%-19.2%). The T21 group had the highest pregnancy termination rate at

89.0%, reflecting distinct clinical decision-making tendencies for pregnancies with different chromosomal abnormalities. SCA (such as 47, XXY and 47, XXX) are generally associated with relatively mild phenotypes, and many affected individuals can achieve normal fertility. In contrast, autosomal trisomies including T21 and T18 are often accompanied by severe congenital malformations and poor fetal prognosis, which constitute the primary reasons for clinical pregnancy termination [31, 32]. These findings provide real-world evidence for pregnancy outcome prediction and clinical decision-making during genetic counseling, and help optimize doctor-patient communication and pregnancy management.

Multivariate logistic regression analysis further identified the independent influencing factors of true-positive NIPT results. The model showed good fit with a Hosmer-Lemeshow test value of $P = 0.104$ and a Nagelkerke $R^2 = 0.160$, indicating acceptable explanatory power. T21 as the reference group, the probabilities of true positivity were significantly lower for T18, T13, and SCA, which was consistent with the inter-subtype differences in PPV. Zhao et al. [17] also confirmed that the PPV of T21 and T18 was positively correlated with the Z-value, with a higher Z-value corresponding to a lower false-positive rate. A systematic review by Marton et al. [33] pointed out that although different NIPT assays showed no significant differences in sensitivity and specificity for detecting common trisomies, their PPV varied greatly. This further highlights the essential role of invasive prenatal diagnosis in confirming positive screening results. In terms of puncture indications, only NIPT combined with ultrasound abnormality remained an independent risk factor after adjustment, while the effect of advanced maternal age was attenuated, suggesting the central role of ultrasound abnormalities in predicting true-positive outcomes. Consistent with the present findings, multivariate analysis in the study by Lian Zeying et al. verified that fetal ultrasound abnormalities were an independent risk factor for fetal chromosomal abnormalities (OR = 2.294) [34]. Similarly, Tu Haoyan et al. [35] observed in a twin NIPT cohort that concomitant abnormal ultrasound soft markers could affect detection performance, and PPV exhibited stratified differences across gestational age and maternal age. Each additional week of gestational age was associated with a 7.5% reduction in the probability of true positivity. Although this trend did not reach statistical significance, it still implies that the interpretation of NIPT results in late pregnancy should be more cautious to avoid overreliance on screening findings alone.

This study has several limitations. First, comparison with published large-scale studies. The core findings of this study are largely consistent with those of a previously published large multicenter study in southern China (Cai M, et al., 2023). We acknowledge that our single-center design and smaller sample size (n = 342) represent methodological disadvantages compared to that large-scale study (n = 52,855). However, our study is not intended to replicate but to complement those findings by providing the first real-world NIPT data from the under-represented southwestern China (Guizhou province), offering valuable geographical validation of NIPT performance across different populations and healthcare settings.

Second, small subgroup sample sizes and low statistical power. As the reviewer noted, the sample sizes of the advanced maternal age group (32 cases, 9.4%), the NIPT combined with ultrasound abnormality group (10 cases, 2.9%), and the extreme gestational age subgroups (17 cases each, 4.9%) were relatively small. This limitation may have led to insufficient statistical power to detect genuine differences in subgroup comparisons and multivariate regression analyses. Therefore, we have adopted a predominantly descriptive approach for these subgroup analyses and caution against over-interpretation of the statistical comparisons.

Third, loss to follow-up. The loss-to-follow-up rate in this study was 4.1% (14/342), which may compromise the external validity of the pregnancy outcome analysis, particularly for the pathogenic CNV subgroup (70.7% lost to follow-up).

Fourth, study design and generalizability. This was a single-center retrospective study, and the generalizability of the conclusions needs to be verified by multicenter prospective studies.

Fifth, absence of key NIPT quality control parameters. Quality control parameters of the

NIPT platform (such as cell-free fetal DNA concentration and Z-value stratification) were not included in the analysis, while these factors are known to independently affect the positive predictive value (PPV). This limits our ability to explore the underlying mechanisms for the observed PPV differences among different NIPT subtypes. Future studies should incorporate these parameters to enable more refined risk stratification.

Sixth, limitations in CNV interpretation. Variants of uncertain significance (VUS) accounted for a high proportion (70.7%, 58/82) in the CNV group; however, restricted by the completeness of chromosomal microarray analysis (CMA) data, further interpretation of the functional and clinical significance of VUS was not performed in this study.

Future directions. Future research should establish expanded multicenter prospective cohorts and enroll more high-risk pregnant women with advanced maternal age, ultrasound abnormalities, and extreme gestational age. Meanwhile, laboratory indicators including cell-free fetal DNA concentration and Z-value, as well as clinical parameters such as ultrasonic structural anomalies and soft markers, should be integrated to construct an individualized risk stratification model for pregnant women with high-risk NIPT results. This will provide more sufficient evidence-based references for precise genetic counseling and clinical decision-making.

Conclusions

Based on a comprehensive analysis of clinical data, amniocentesis results, and pregnancy outcomes among 342 pregnant women with high-risk NIPT findings, this study drew the following conclusions: The positive predictive value (PPV) differed significantly across NIPT high-risk subtypes. Trisomy 21 had the highest PPV, followed by trisomy 18 and sex chromosome abnormalities, while trisomy 13 and pathogenic CNVs showed relatively low PPVs. Variants of uncertain significance (VUS) accounted for a large proportion among pathogenic CNVs. These findings further confirm that NIPT is only a screening tool and cannot replace invasive prenatal diagnosis such as amniocentesis. Pregnancy outcomes and clinical decision-making varied markedly among different NIPT high-risk subtypes. Women with high-risk sex chromosome abnormalities showed a higher rate of continued pregnancy, whereas those with high-risk trisomy 21 had the highest proportion of pregnancy termination. Both the overall and subtype-specific PPVs were higher in women of advanced maternal age than in younger women, suggesting that age stratification can serve as an important reference for clinical genetic counseling. Gestational age at amniocentesis was not associated with a statistically significant difference in the positive predictive value of NIPT. Although the PPV tended to be higher in early pregnancy and lower in late pregnancy, this trend requires further verification with larger sample sizes due to the limited cases in extreme gestational age subgroups. Multivariate logistic regression analysis indicated that NIPT classification and concomitant abnormal ultrasound soft markers were independent influencing factors for true-positive NIPT results. After adjusting for confounding factors, concurrent ultrasound soft marker abnormalities significantly increased the risk of confirmed chromosomal abnormalities, whereas gestational age exerted no independent effect on diagnostic performance. In clinical practice, stratified genetic counseling should integrate high-risk NIPT subtype, maternal age, gestational age at amniocentesis, and ultrasound findings. For NIPT high-risk pregnancies with autosomal trisomy, advanced maternal age, or coexisting abnormal ultrasound soft markers, invasive prenatal diagnosis should be recommended as early as possible. This approach may provide clinical evidence to optimize prenatal counseling and guide pregnancy decision-making.

Disclosure of conflict of interest

None.

Address correspondence to: Xiaozhi Wu, Department of Obstetrics, Zunyi First People's Hospital (The Third Affiliated Hospital of Zunyi Medical), No. 98 Fenghuang North Road, Zunyi 563000, Guizhou, China. Tel: +86-0851-23231371; E-mail: wuxiaozhi0621@163.com

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