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Impact of Ultrasound Findings on the Positive Predictive Value of NIPT: A 24,263-Case Retrospective Study and a Stratified Prenatal Management Proposal

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Abstract

Objective

This study assessed how ultrasound findings affect NIPT positivity and PPV, aiming to optimize prenatal screening in pregnancies with ultrasound anomalies.

Methods

A retrospective study included 24,263 NIPT cases (3,258 abnormal, 21,005 normal ultrasound). Positivity rates, abnormal type distribution, and PPV were compared; all NIPT-positive cases had invasive confirmation. Subgroup analyses for isolated vs. multiple ultrasound anomalies were performed.

Results

NIPT positivity was significantly higher in the abnormal-ultrasound group (1.78% vs 1.16%, OR = 1.55, 95%CI 1.13–2.11, *P* = 0.006). Abnormal type distribution did not differ significantly (P = 0.058). PPV for Trisomy 21 (90.91% vs 87.88%, *P* = 1.000) and SCAs (41.67% vs 45.56%, *P* = 0.732) were similar. However, PPV for CNVs (microdeletions/microduplications) was markedly higher in the abnormal-ultrasound group (63.64% vs 8.70%, *P* < 0.001; absolute increase 54.94%). Subgroup: positivity was higher with multiple (1.32%) than isolated (0.73%) anomalies. CNVs predominated in isolated anomalies (60.0%), whereas Trisomy 21 predominated in multiple anomalies (71.4%).

Conclusion

Ultrasound anomalies increase NIPT positivity. CNVs dominate isolated anomalies; Trisomy 21 dominates multiple anomalies. An ultrasound-based stratified management is proposed: for multiple anomalies with positive NIPT, prioritize karyotyping; for isolated anomaly with positive NIPT, prioritize chromosomal microarray analysis; for normal ultrasound with positive NIPT, caution for false positives is warranted to guide counseling.

Introduction

Fetal chromosomal abnormalities are one of the leading causes of birth defects, and prenatal screening and diagnosis play a critical role in reducing their incidence. Fetal structural anomalies and positive soft markers detected by ultrasonography are strongly associated with an increased risk of chromosomal abnormalities.[1, 2] Meta-analyses indicate that the prevalence of chromosomal abnormalities is approximately 2.15% in fetuses with isolated positive soft markers, whereas it ranges between 3.66% and 8.57% in those with multiple positive soft markers or structural anomalies.[3, 4] Consequently, pregnancies with positive ultrasonographic findings are considered at high risk for chromosomal

abnormalities. The American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) recommend chromosomal microarray analysis (CMA) as the preferred diagnostic modality for evaluating fetal structural anomalies in prenatal diagnosis[5, 6].

In recent years, given its high sensitivity and specificity, non-invasive prenatal testing (NIPT) has been widely adopted as the first-line screening modality across diverse obstetric populations. The scope of NIPT has expanded significantly beyond the common autosomal trisomies (T21, T18, T13). By employing high-throughput sequencing and sophisticated bioinformatics analyses, NIPT now routinely screens for sex chromosomal aneuploidies (SCAs), such as 45,X and 47,XXY, and detects pathogenic copy number variations (pCNVs) exceeding predefined size thresholds (e.g., > 5–10 Mb), thereby substantially enhancing its clinical utility[7, 8].

However, direct evidence addressing the impact of ultrasonographic abnormalities on NIPT positivity rates remains limited. While previous studies have primarily focused on the detection efficacy of NIPT for specific chromosomal aberrations or have separately examined the association between ultrasound findings and chromosomal abnormalities[3, 9], few have systematically compared the NIPT positivity rates between cohorts with and without abnormal ultrasound findings. This research gap not only precludes a comprehensive risk assessment of NIPT positivity in populations with ultrasonographic abnormalities but also hinders the evidence-based optimization of clinical screening pathways.

To address this gap, the present large-scale retrospective study was designed to: (1) compare the NIPT positivity rates between pregnant women with abnormal versus normal ultrasound findings; (2) evaluate whether the presence of abnormal ultrasound findings constitutes an independent risk factor for a positive NIPT result; and (3) analyze the differential distribution of specific chromosomal abnormalities detected by NIPT between these two cohorts. The overarching goal is to provide evidence-based guidance for the risk-stratified management of pregnancies with ultrasonographic abnormalities and the optimization of prenatal screening strategies.

Methods

Study Population, Ethics Statement, and Sample Collection

A total of 24,263 pregnant women who underwent both NIPT and ultrasonography at Jiangmen Central Hospital from May 2017 to August 2025 were retrospectively enrolled in this study. All participants provided written informed consent. The study protocol was approved by the Institutional Review Board (IRB) of Jiangmen Central Hospital (Approval No. 2025004) and strictly complied with institutional guidelines for patient data confidentiality.Peripheral blood samples (5 mL) were collected from each participant using Streck Cell-Free DNA BCT® tubes (Streck, La Vista, NE, USA). Plasma DNA extraction was performed within three days of sample collection.

Ultrasound Screening

Ultrasound examinations were performed by experienced obstetric sonographers in accordance with standardized guidelines for fetal anomaly screening[10, 11]. The sonographers were blinded to the NIPT results to minimize ascertainment bias. Ultrasound follow-up was performed throughout pregnancy or until pregnancy termination, including first-trimester screening (6–8 weeks of gestation), nuchal translucency (NT) scanning (11–13 weeks), second-trimester anomaly scan for major structural defects (20–24 weeks), third-trimester scan for minor or late-onset anomalies (28–32 weeks), and a final prenatal examination.

Screening for major fetal anomalies targeted conditions such as anencephaly, severe encephalocele, severe open spina bifida, severe abdominal wall defects with evisceration, single ventricle, lethal achondroplasia, and limb reduction defects. Screening for minor fetal anomalies aimed to detect abnormalities including hydrocephalus, hydronephrosis, polycystic kidney disease, duodenal atresia, and congenital pulmonary airway malformation (CPAM). Mid-trimester reassessment and detection of delayed-onset anomalies were also included.

A positive ultrasound result was defined as the detection of any fetal structural anomaly or soft marker at any ultrasound examination during the follow-up period.

NIPT Procedures and Bioinformatic Analysis

The NIPT methodology has been described in detail previously[12, 13]. Briefly, blood samples were centrifuged at 1,600 × g for 10 min at 4°C to separate plasma. Cell-free DNA (cfDNA) was extracted from the plasma and subjected to library preparation. Library quantity and quality were assessed by quantitative PCR (qPCR) and fragment size distribution analysis. Only qualified libraries were sequenced, and the resulting data were analyzed using the NIFTY® algorithm to detect Trisomy 21 (T21), Trisomy 18 (T18), Trisomy 13 (T13), SCAs, and rare autosomal trisomies (RATs), as described previously[12]. Genome-wide detection of large fetal deletions/duplications was performed using fetal copy number analysis of maternal plasma sequencing (FCAPS)[13].

Confirmation of Fetal Chromosomal Abnormalities by Karyotyping and Chromosomal Microarray Analysis

All NIPT-positive cases underwent invasive prenatal diagnostic testing for confirmation. Amniotic fluid samples were obtained via ultrasound-guided amniocentesis and subjected to both conventional G-band karyotyping and chromosomal microarray analysis (CMA) .

G-band karyotyping was performed with a resolution of approximately 400–550 bands. CMA was performed using the Affymetrix CytoScan 750K array (Thermo Fisher Scientific, USA), comprising approximately 750,000 probes covering known pathogenic copy number variant (CNV) regions genome-wide. Data were analyzed with reference to the GRCh37 (hg19) human genome assembly. Pathogenicity

assessment of CNVs was conducted in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines[14, 15].

Both true-positive NIPT results (i.e., NIPT high-risk confirmed by invasive testing) and false-positive NIPT results (i.e., NIPT high-risk with normal invasive findings) were included in the subsequent calculation of the positive predictive value (PPV) for each abnormality type.

Clinical Follow-up

Participants with low-risk NIPT results were advised to continue routine prenatal care. Serial ultrasound examinations were performed to monitor fetal growth and detect potential anomalies. If fetal abnormalities were identified, genetic counseling was offered. For participants who declined pregnancy termination, postpartum follow-up was conducted via telephone interview at three months after delivery. Data collected included ultrasound reports, final pregnancy outcomes, infant sex, and neonatal physical examination findings.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were compared using the independent samples *t*-test where applicable. Categorical data were expressed as numbers (percentages) and compared using Fisher's exact test. A two-sided P-value < 0.05 was considered statistically significant.

Results

Baseline Demographic Characteristics

A total of 24,263 pregnant women who underwent both NIPT and ultrasound examinations at Jiangmen Central Hospital from May 2017 to August 2025 were retrospectively enrolled. The cohort was stratified into two groups based on ultrasound findings: the abnormal ultrasound group (n = 3,258) and the normal ultrasound group (n = 21,005). All NIPT-positive cases underwent invasive diagnostic confirmation via amniocentesis, including both karyotyping and chromosomal microarray analysis (CMA). The study flowchart is presented in Fig. 1.

Baseline characteristics of the two groups are summarized in Table 1. The mean maternal age was 28.5 ± 3.06 years in the abnormal ultrasound group and 28.8 ± 3.35 years in the normal ultrasound group, with no statistically significant difference between groups (t = -1.851, P = 0.065). The mean body weight was 54.53 ± 6.71 kg in the abnormal ultrasound group and 54.4 ± 7.02 kg in the normal ultrasound group, also showing no significant difference (t = 0.072, P = 0.943). Notably, the mean gestational age at NIPT sampling was significantly higher in the abnormal ultrasound group compared to the normal ultrasound group (15.23 ± 2.63 weeks vs. 14.64 ± 1.91 weeks; t = 3.972, P < 0.001).

Table 1
Basic characteristics of pregnant women enrolled in this study

crowd characteristics	Abnormal ultrasound group (n = 3258)	Normal ultrasound group (n = 21005)	*t*	*P* value
Age (years)	28.5 ± 3.06	28.8 ± 3.35	-1.851	0.065
Pregnancy Week (weeks)	15.23 ± 2.63	14.64 ± 1.91	3.972	<0.001
weight (kg)	54.53 ± 6.71	54.4 ± 7.02	0.072	0.943

Data are presented as mean ± standard deviation. Abbreviations: NIPT, non-invasive prenatal testing. *P* values were calculated using independent samples *t*-test.

NIPT Positivity Rates and Stratified Analysis by Ultrasound Findings

The NIPT positivity rate was significantly higher in the abnormal ultrasound group compared to the normal ultrasound group (1.78%, 58/3,258 vs. 1.16%, 243/21,005; $\chi^2 = 7.70$, $P = 0.006$), corresponding to a 55% higher risk (OR = 1.55, 95% CI: 1.13–2.11).

Stratified analysis revealed that this difference was primarily driven by SCAs and T21. Specifically, the positivity rate for SCAs was significantly higher in the abnormal ultrasound group than in the normal ultrasound group (0.74%, 24/3,258 vs. 0.43%, 90/21,005; $P = 0.014$). Similarly, the positivity rate for T21 was elevated in the abnormal ultrasound group compared to the normal ultrasound group (0.34%, 11/3,258 vs. 0.16%, 33/21,005; $P = 0.026$).

However, the distribution of NIPT-detected abnormalities did not differ significantly between the abnormal ultrasound group and the normal ultrasound group (Fisher's exact test, $P = 0.058$). In pairwise comparisons of individual abnormality types, no significant differences were observed between groups for any type except T13 (all $P > 0.05$).

Specifically, SCAs were the most frequently detected abnormality in both groups, accounting for 41.38% (24/58) of NIPT-positive cases in the abnormal ultrasound group and 37.04% (90/243) in the normal ultrasound group ($\chi^2 = 0.389$, $P = 0.533$). Similarly, no significant between-group differences were observed for T21 (18.97% vs. 13.58%, $P = 0.285$), microdeletion/microduplication syndromes (MMS) (18.97% vs. 18.93%, $P = 0.995$), RATs (15.52% vs. 19.75%, $P = 0.454$), or T18 (5.17% vs. 3.70%, $P = 0.706$) (Table 2).

Table 2
Distribution of different types of chromosomal abnormalities in the two groups

Type of chromosomal abnormality	Abnormal ultrasound group (n = 3,258)	Normal ultrasound group (n = 21,005)	Statistic	*P* value	OR (95% CI)
SCAs	24 (0.74%)	90 (0.43%)	$\chi^2 = 5.99$	0.014	1.72 (1.09–2.70)
T21	11 (0.34%)	33 (0.16%)	$\chi^2 = 4.96$	0.026	2.13 (1.07–4.24)
MMS	11 (0.34%)	46 (0.22%)	$\chi^2 = 1.74$	0.187	1.55 (0.80–2.99)
RATs	9 (0.28%)	48 (0.23%)	$\chi^2 = 0.27$	0.605	1.21 (0.59–2.47)
T18	3 (0.09%)	9 (0.04%)	Fisher accurate	0.216	2.15 (0.58–7.95)
T13	0 (0.00%)	17 (0.08%)	Fisher accurate	0.049	—

P values were calculated using chi-square test or Fisher's exact test as appropriate. For T13, no NIPT-positive cases were identified in the abnormal ultrasound group; therefore, OR could not be calculated.

PPV of NIPT Confirmed by Invasive Diagnostic Testing

All NIPT-positive cases underwent invasive diagnostic confirmation via amniocentesis. The PPV for each abnormality type were calculated and are summarized in Table 3.

For T21, the PPV was 90.91% (10/11) in the abnormal ultrasound group and 87.88% (29/33) in the normal ultrasound group ($P = 1.000$), demonstrating consistently high PPV in both groups. For SCAs, the PPV were 41.67% (10/24) and 45.56% (41/90), respectively ($\chi^2 = 0.117$, $P = 0.732$).

In contrast, for CNVs, the PPV was significantly higher in the abnormal ultrasound group than in the normal ultrasound group (63.64% [7/11] vs. 8.70% [4/46] ; $P < 0.001$). The risk difference was 54.94% (95% CI, 26.58–83.30), and the odds ratio was 18.38 (95% CI, 4.10–82.33), highlighting the substantially enhanced PPV of NIPT for CNVs in the presence of ultrasound abnormalities.

For T18, the PPV were 0.00% (0/3) in the abnormal ultrasound group and 22.22% (2/9) in the normal ultrasound group ($P = 0.519$). T13 was detected only in the normal ultrasound group, with a PPV of 11.76% (2/17). For RATs, the PPV was 0.00% in both groups, indicating that all NIPT-detected RATs were false positives.

Table 3
Positive predictive values for different types of abnormalities in the two groups and intergroup comparisons

Abnormality type	PPV (95% CI) for the group with abnormal ultrasound findings	Normal ultrasound group: PPV (95% CI)	*P* value	Risk differential(RD) (95% CI)	Odds Ratio (OR) (95% CI)
T21	90.91% (10/11) [58.72–99.77]	87.88% (29/33) [71.80–96.59]	1.000	3.03% (-18.98 to 25.04)	1.38 (0.14–13.76)
SCA	41.67% (10/24) [22.11–63.36]	45.56% (41/90) [35.05–56.35]	0.732	-3.89% (-26.39 to 18.61)	0.85 (0.34–2.12)
CNV	63.64% (7/11) [30.79–89.07]	8.70% (4/46) [2.41–20.81]	*P* < 0.001	54.94% (26.58 to 83.30)	18.38 (4.10–82.33)
T18	0.00% (0/3) [0.00–70.76]	22.22% (2/9) [2.81–60.01]	0.519	-22.22% (-73.36 to 28.92)	—
T13	— (0/0)	11.76% (2/17) [1.46–36.44]	—	—	—
RATs	0.00% (0/9) [0.00–33.63]	0.00% (0/48) [0.00–7.42]	—	—	—

95% confidence intervals are shown in square brackets. For T13, no NIPT-positive cases were identified in the abnormal ultrasound group, precluding statistical comparison. For RATs, no true-positive cases were observed in either group, rendering PPV comparison unfeasible.

Subgroup Analysis of Chromosomal Abnormalities by Number of Ultrasound Anomalies

Within the abnormal ultrasound group, a subgroup analysis stratified by the number of ultrasound anomalies was performed (Table 4). The isolated ultrasound anomaly subgroup comprised 2,729 cases, including 20 NIPT true-positive cases(0.73%, 20/2,729). The multiple ultrasound anomaly subgroup comprised 529 cases, including 7 NIPT true-positive cases (1.32%, 7/529). The true-positive rate did not differ significantly between subgroups ($P = 0.155$); however, the distribution of abnormality types among confirmed cases differed markedly.

In the isolated anomaly subgroup, CNVs were the most frequent finding (60.0%, 12/20), followed by SCAs (30.0%, 6/20) and Trisomy 21 (T21) (10.0%, 2/20). In the multiple anomaly subgroup, T21 predominated (71.4%, 5/7), whereas SCAs and CNVs each accounted for 14.3% (1/7) .

The 12 CNV cases involved chromosomal regions including 18p, 18q, 13q, 16p, 6p, 4q, 10q, 11q, and 9q. Associated ultrasound findings included mega cisterna magna, ectopic kidney, aberrant right subclavian artery (ARSA), bronchopulmonary sequestration, intestinal dilatation, congenital talipes equinovarus

(clubfoot), Blake's pouch cyst, ventriculomegaly, and caudal regression anomaly (see Online Resource 1 for details).

Table 4
Subgroup analysis of isolated ultrasound abnormalities versus multiple ultrasound abnormalities

Subgroup	Total cases, *n*	NIPT-positive, *n*	True-positive (CMA confirmed), *n*	PPV,%	True-positive rate,% (*n*/Total)	Distribution of abnormality types in true-positive cases, *n* (%)
Isolated ultrasonic abnormality	2729	42	20	47.6% (20/42)	0.73% (20/2729)	T21: 2 (10.0%), SCA: 6 (30.0%), CNV: 12 (60.0%)
Multiple ultrasound abnormalities	529	21	7	33.3% (7/21)	1.32% (7/529)	T21: 5 (71.4%), SCA: 1 (14.3%), CNV: 1 (14.3%)

True-positive rate was calculated as the number of CMA-confirmed cases divided by the total number of cases in each subgroup. PPV was calculated as the number of CMA-confirmed cases divided by the number of NIPT-positive cases. $P = 0.155$ for comparison of true-positive rates between subgroups (Fisher's exact test).

Discussion

In this large cohort study of 24,263 pregnant women, we compared NIPT positivity rates, PPV, and the distribution of abnormality types between women with abnormal versus normal ultrasound findings, with further subgroup analysis of isolated and multiple ultrasound anomalies.

We observed a significantly higher NIPT positivity rate in the abnormal ultrasound group, driven primarily by elevated rates of SCAs and T21. T21 and SCAs showed comparable PPV between the two groups, whereas pathogenic CNVs had a substantially higher PPV in the context of abnormal ultrasound findings. Subgroup analysis further revealed distinct patterns in the true-positive spectrum of chromosomal abnormalities between isolated and multiple ultrasound anomalies.

We further compared the PPV of NIPT-detected chromosomal abnormalities between the abnormal and normal ultrasound groups. No significant differences in PPV were observed for T21 or SCAs between groups (both $P > 0.05$), suggesting that the association between ultrasound abnormalities and these aneuploidies reflects increased prevalence rather than altered test performance.

This finding is consistent with the study by Wang et al.[16], which reported PPV variations among abnormality types in women with ultrasound anomalies (T21, 86.21%; SCAs, 46.43%), but did not directly compare PPV for the same abnormality between ultrasound-stratified groups. Our findings extend this

observation by confirming that ultrasound status does not significantly modify the PPV for T21 or SCAs, indicating stable positive predictive values for these aneuploidies irrespective of ultrasound findings.

Notably, for pathogenic CNVs, the PPV was markedly higher in the abnormal ultrasound group than in the normal ultrasound group (63.64% vs. 8.70%; $P < 0.001$). This finding has important clinical implications: in the setting of abnormal ultrasound findings, a positive NIPT result for CNVs warrants a high index of suspicion, and CMA should be strongly recommended.

Consistent with our findings, Chen et al.[17] reported that combining NIPT with ultrasonography significantly improves the detection of microdeletion/microduplication syndromes, and that ultrasound combined with NIPT-Plus achieved the highest sensitivity (88.24%) for SCAs, RATs, and MMS. Yuan X et al.[18] further demonstrated that CMA identifies additional CNVs beyond conventional karyotyping, thereby improving the detection of chromosomal structural abnormalities, and that the PPV of NIPT was highest in women of advanced maternal age for Trisomy 21 and SCAs ($P = 0.001$). Wang et al.[19] also reported that pathogenic variants were detected in 42.21% (320/758) of the NIPT high-risk group, and that combining CMA with other modalities increased the overall diagnostic yield to 20.36%, representing a 2.11% absolute increase compared with karyotyping alone.

We further stratified the abnormal ultrasound group into isolated and multiple anomaly subgroups. The true-positive rate was numerically higher in the multiple anomaly subgroup than in the isolated anomaly subgroup (1.32% vs. 0.73%; $P = 0.155$). Despite the lack of statistical significance, the distribution of abnormality types differed markedly: the isolated anomaly subgroup was predominantly characterized by pathogenic CNVs (60.0%, 12/20), involving chromosomal regions including 18p, 18q, 13q, 16p, 6p, 4q, 10q, 11q, and 9q, with diverse ultrasound phenotypes; in contrast, the multiple anomaly subgroup was predominantly characterized by T21 (71.4%, 5/7), with highly consistent ultrasound features including absent nasal bone, cardiac anomalies, thickened nuchal fold, tricuspid regurgitation, and ventriculomegaly[20, 21], mirroring the classic prenatal phenotypic spectrum of Down syndrome.

These findings are consistent with prior reports on fetuses with nasal bone anomalies, which have repeatedly demonstrated a substantially higher incidence of chromosomal abnormalities in the non-isolated group than in the isolated group. For instance, Moczulska et al.[22] reported total chromosomal abnormality rates of 7.1% and 57% in isolated and non-isolated fetal nasal bone hypoplasia, respectively, while Shi et al.[23] showed that even in isolated cases with a normal karyotype, CMA could detect an additional 3.2% of pathogenic CNVs. Our study extends these observations to all types of ultrasound anomalies, confirming a higher true-positive rate in the multiple anomaly subgroup, with a predominance of T21, whereas the isolated anomaly subgroup was predominantly characterized by pathogenic CNVs.

Notably, the isolated CNV cases in this study involved multiple chromosomal regions, with several cases demonstrating rare, highly specific genotype–phenotype associations, including 4q22 deletion with congenital talipes equinovarus (clubfoot), 10q11 deletion with Blake's pouch cyst, 11q25 duplication with caudal regression anomaly, and 13q21 deletion with bronchopulmonary sequestration. These associations have been rarely documented in the literature, suggesting that certain isolated ultrasound

anomalies may directly point to specific microdeletion/microduplication syndromes, thereby further supporting the preferential use of CMA in the diagnostic workup of isolated anomalies.

In the normal ultrasound group, the NIPT positivity rate was 1.16% (243/21,005). After invasive diagnostic confirmation, the PPV were 87.88% for T21 and 45.56% for SCAs, whereas the PPV for pathogenic CNVs, T18, T13, and RATs were all below 25%, with RATs showing a PPV of 0%. These findings indicate that NIPT-positive results are associated with a considerable false-positive risk in pregnancies with normal ultrasound findings, particularly for pathogenic CNVs, RATs, and T18/T13.

This finding is consistent with the study by Zhang et al.[24], which reported overall PPV of 45.45% and 48.65% for NIPT-Plus in low-risk and high-risk groups, respectively, with no significant difference between them; however, both false-positive and false-negative results were observed. Similarly, Zhao et al.[25] reported that the composite PPV in high-risk populations (including those with abnormal ultrasound soft markers) was 85.2%, significantly higher than the 59.2% observed in non-high-risk populations ($P < 0.01$), consistent with the trend toward higher PPV in the ultrasound-abnormal group observed in our study.

Strengths of this study include: (1) a large sample size (24,263 pregnancies), which ensures robust statistical power; (2) confirmation of all NIPT-positive cases by invasive prenatal diagnosis (the gold standard), thereby minimizing verification bias; (3) systematic comparison of NIPT positivity rates, abnormality distributions, and PPV between women with abnormal versus normal ultrasound findings, with further subgroup analysis of isolated versus multiple anomalies, thereby providing refined, evidence-based clinical guidance.

This study has several limitations. First, there was a significant difference in gestational age between groups (15.23 weeks vs. 14.64 weeks; $P < 0.001$), likely because ultrasound abnormalities are predominantly detected during the mid-trimester anomaly scan. Although Liehr et al.[26] has suggested that gestational age at NIPT sampling may influence the false-positive rate—reporting that false-positive results are less frequent the earlier the screening is performed—the observed difference in our study was only approximately 0.6 weeks, and its impact on NIPT performance is likely limited.

Second, we did not include potential confounders such as maternal age and body mass index (BMI) in a multivariable regression analysis; future studies should adjust for these factors to further validate our findings. Third, the sample sizes for the isolated and multiple anomaly subgroups were relatively small, and some subgroup comparisons did not achieve statistical significance; however, the distinct patterns of abnormality types observed remain of clinical importance. Fourth, the retrospective design of this study may have introduced selection bias.

Conclusion

In conclusion, the presence of abnormal ultrasound findings significantly increases the likelihood of a positive NIPT result and exerts an important modifying influence on the clinical interpretation of such findings. Furthermore, isolated ultrasound anomalies are predominantly associated with pathogenic

CNVs, whereas multiple anomalies are most frequently linked to Trisomy 21, underscoring the need for differentiated diagnostic strategies based on the number of ultrasound findings.

Based on these findings, we propose an Ultrasound-Based Stratified Management Strategy for NIPT-Positive Results: (1) for multiple ultrasound anomalies with positive NIPT, karyotyping should be prioritized; (2) for isolated ultrasound anomalies with positive NIPT, CMA should be prioritized; and (3) for normal ultrasound findings with positive NIPT, a high index of suspicion for false-positive results is warranted, and the decision to proceed with invasive diagnostic testing should be made cautiously.

This strategy is expected to optimize the allocation of prenatal diagnostic resources, preserve diagnostic yield, and reduce unnecessary invasive procedures. Future prospective multicenter studies are warranted to incorporate additional confounding variables and to further delineate the refined genotype–phenotype correlations between specific ultrasound anomaly subtypes and individual CNVs.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval and consent to participate:

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committees of Jiangmen Central Hospital (Approval No: [2025]004). Given the retrospective nature of the study and the use of fully anonymized clinical data, the requirement for written informed consent was waived by the Ethics Committees of Jiangmen Central Hospital.

Consent to publish:

This is a retrospective study. All patient data included in this manuscript were fully anonymized. No individual person's data in any form (including individual details, images, or videos) that could identify a specific individual are contained in this article. Therefore, no consent for publication was required.

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Author Contribution

Conceptualization: H.L., Q.Z.Data curation: H.L., M.Z., Z.L., Z.P., L.L.Formal analysis: H.L., Q.Z., L.L.Methodology: H.L., M.Z., Z.L., Z.P., L.L.Validation: H.L.Writing – original draft: H.L.Writing – review & editing: H.L., Q.Z.Supervision: H.L., Q.Z.Note: H.L.: Huahua Li; Q.Z.: Qiang Zhao; M.Z.: Miaolian Zhang; Z.L.: Zhongwei Li; Z.P.: Zhuoyi Pan; L.L.: Lihua Li.

Data Availability

The data that support the findings of this study are available from Jiangmen Central Hospital, but restrictions apply to the availability of these data, which were used under institutional authorization for the current study and so are not publicly available. The data are, however, available upon reasonable request and with the permission of Jiangmen Central Hospital.

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Figures

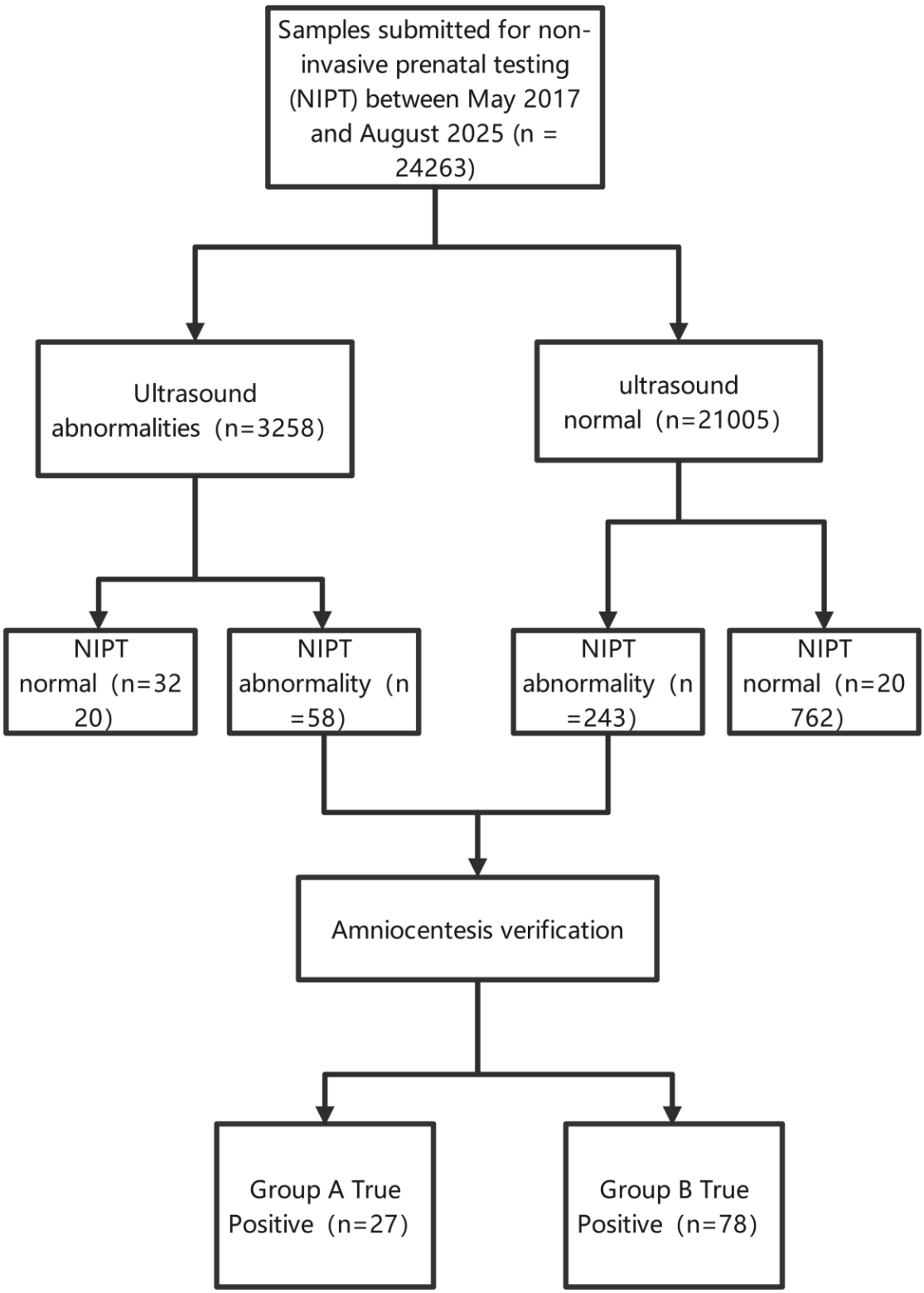


Figure 1

Flowchart of this study

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