Non-invasive prenatal test findings in 41,819 pregnant women: Results from a clinical laboratory in southern China

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Research Article

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Abstract

Background: This paper evaluated the clinical utility of massively parallel sequencing-based non-invasive prenatal testing (NIPT) for detecting trisomy 21 (T21), T18, T13, sex chromosome aneuploidies (SCA), and rare chromosome aneuploides (RCA) among the data collected by a clinical laboratory in southern China.

Methods: In a 3-year period between 1January 2017 and 31 December 2019, over 40,000 pregnant women underwent NIPT clinical screening tests for fetal T21, T18, T13, SCA, and RCA in our laboratory. NIPT samples were processed using the NextSeq CN500 platform. The results were confirmed by karyotype, and chromosomal microarray analysis or CNV sequencing. Details of the pregnancy outcomes were collected via telephone interviews.

Results: NIPT result were available for 41,819 cases; 691positive cases were reported. The overall sensitivity for detection of T21, T18, T13, SCA, and RCA was 99.21%, 100.00%, 100.00%, 98.55%, and 100.00%, and the specificity was 99.95%, 99.94%, 99.98%, 99.69%, and 99.92% respectively. The positive predictive value (PPVs) for detection of T21, T18, T13, SCA, and RCA were 85.62%, 45.24%, 40.00%, 34.17%, and 13.51%, respectively, and those for detection of 45,X, 47,XXY, 47,XXX, 47,XYY, and 46,XY(delX) 20.00%, 59.18%, 28.95%, 61.54%, and 25.00%, respectively. Regarding pregnancy outcomes, 92.38% of the pregnancies with confirmed aneuploidies were terminated, and 91.20% of those identified as having a false-positive result were carried to term. Among 252 unconfirmed cases, 24.60% of the pregnancies were terminated and 38.10% carried to term, while 37.30% unfollow-up.

Conclusions: NIPT is widely used to screen fetal aneuploidies based on its high sensitivity and specificity. However, in this study, the PPVs of NIPT in terms of detecting T18, T13, XO, XXX and RCA were <50%. However more than one-third of NIPT-positive women did not accept the prenatal diagnosis. Confirmatory diagnosis is strongly recommended for women with positive NIPT outcomes before any further decision is made.

Introduction

Cell-free DNA (cfDNA) from the peripheral blood of pregnant women has been widely used to screen for fetal chromosome aneuploidies, including Down syndrome (trisomy 21, T21), Edwards syndrome (trisomy 18, T18), Patau syndrome (trisomy 13, T13), and sex chromosome aneuploidies (SCA). Lo et al., in 1997, were the first to describe fetal cfDNA in the plasma of pregnant women [1], and a prenatal testing method based on cfDNA was introduced in 2008 [2]. Compared with traditional serum screening, cfDNA testing has a higher sensitivity, lower false-positive rate, and higher positive predictive value (PPV) [3, 4]. Both the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics and Genomics (ACMG) have endorsed non-invasive prenatal testing (NIPT) as a routine screening option [5, 6]. The use of NIPT for detecting fetal aneuploidies has rapidly transformed the global prenatal screening landscape, with the test performed in millions of pregnant women worldwide [7,

8, 9]. In China, NIPT has additional value because of the implementation of a two-child policy, such that a sharp rise in high-risk pregnancies is expected [10]. The aim of the present study was to evaluate the performance and accuracy of NIPT, not only for common aneuploides T21, T18 and T13, but also for SCA and rare chromosome aneuploides (RCA), to determine its clinical utility. The knowledge generated from our study could assist clinicians in pregnancy counseling, provide reassurance to pregnant women, and guide further decision-making.

Method And Materials

Ethics statement and sample collection

This retrospective study included pregnant women who underwent NIPT at Nanfang Hospital of Southern Medical University (Guangzhou, Guangdong Province, China) between January 2017 and December 2019. All participants received clinical counseling prior to NIPT and were informed by clinicians about the content, principle, advantages, and limitations of the test. The study was approved by the Institutional Ethics Committee of Nanfang Hospital (approval no. NFEC-2017-035). Written informed consent was obtained from all subjects and their legal guardians.

Sample collection and processing

Peripheral blood (10 mL) was collected from each of the pregnant women and stored in tubes from Streck (Irving, TX, USA) or Lakebio (Hefei, China). The blood samples were centrifuged to isolate the plasma, and cfDNA was extracted from the plasma. A Qubit fluorometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to measure the cfDNA concentration, and a cfDNA sequencing library was constructed and purified. The StepOnePlus™ real-time PCR system(Thermo Fisher Scientific, Inc.) was used to quantify the libraries. All libraries were pooled and sequenced using the NextSeq CN500 massive parallel sequencing kit and NextSeq CN500 (Illumina China, Shanghai, China). All steps were performed according to the manufacturer's instructions (Berry Genomics Corp., Beijing, China). The sequencing data were mapped and processed using the Bambino test data analysis system 'RUPA' (Berry Genomics Corp.). Chromosomes with a Z-score between −3 and 3 were defined as disomic (copy number=2) and those with a Z-score ≥3 as trisomic (copy number=3).

Evaluation of the performance of NIPT

Abnormal chromosomal results obtained by NIPT were confirmed by karyotype and chromosomal microarray analysis (CMA) or CNV sequencing after conducting an invasive prenatal diagnostic procedure. All women were followed-up by telephone interviews to document the pregnancy outcomes, miscarriages, terminations, and deliveries were recorded. Cytogenetic or clinical follow-up results were used to calculate the sensitivity and specificity of NIPT. The participants were categorized according to their aneuploidy risk. Those with any of the following factors were classified as high risk: advanced maternal age (AMA; ≥35 years), a positive conventional serum screening test (cut-off of 1/270 for T21 or 1/350 for T18), abnormal ultrasound markers, a family history of aneuploidy, or a history of an abnormal

fetal pregnancy. Participants with none of those factors were defined as low risk. The performance of NIPT for detecting T21/T18/T13 was compared between these two risk groups, and in AMA and young pregnant women. All data analyses were performed using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) and SPSS version 20 software (IBM Corp., Armonk, NY, USA). Differences in proportions were tested for statistical significance using the chi-square test, and a P-value<0.05 was considered significant.

Results

Pregnancy characteristics

A total of 41,819 pregnant women were included in the analysis. The mean maternal age was 31.65 (range: 14–52, median 32) years, and the mean gestational age was 16.67 (range: 9–38, median 16.29) weeks at the time of blood draw. Most (65.04%) of the samples were collected from women < 35 years of age, and the majority (75.80%) were collected during the second trimester. Singleton pregnancies accounted for 96.43% of the cases and twin pregnancies for 1,426 cases (3.61%). The clinical characteristics of the 41,819 women are shown in Table 1 and Figure 1.

Table 1

Clinical characteristic of pregnant women undergoing NIPT

Characteristic	Total (n=41,819)	Ratio (%)
Maternal age, (±SD) years	31.65±5.42	
Range	14~52	
≤24 years	4185	10.01
25-29 years	11364	27.17
30-34 years	11648	27.85
35-40 years	12990	31.06
>40 years	1632	3.90
GA at NIPT (wks)		
First trimester (9-13 wks)	9452	22.60
Second trimester (14-27 wks)	31700	75.80
Third trimester (≥28 wks)	667	1.59
Pregnancy history		
Initial pregnancy	9,262	22.15
Second pregnancy or more	32,557	77.85
Type of Pregnancy		
Singleton pregnancy	40393	96.59
Twin pregnancy	1426	3.41
High-risk factors		
Advanced maternal age	14622	34.96
High serum screen risk	5647	13.50
Personal history of aneuploidy	2861	6.84
Abnormal ultrasound	908	2.17
IVF-ET conception	3291	7.87

IVF, in vitro fertilization/ embryo transfer

NIPT performance for T21, T18, T13, SCA, and RCA

Of the 41,819 samples, positive results were obtained in 691 (1.65%) and negative results in 41,128 (98.35%). Of the 691 positive samples, 196 were positive for T21, 61 forT18, 28 forT13, 316 for SCA, and 90 for RCA. Among the 41,128 cases with negative NIPT results, 2 (one case of T21 and one of 45,X) were later determined to have a false-negative result. Of the 691 women with positive NIPT results, 439

(63.5%) underwent prenatal diagnosis, and 252 (36.5%) either did not or refused the subsequent follow-up telephone interview. Among the 439 positive cases that underwent prenatal diagnosis, true-positive aneuploidies (TP) were confirmed in 223 (50.8%) and false-positive (FP) results in 216 (49.2%). The flowchart is shown in Figure 2. Overall, the sensitivities of NIPT for detecting T21, T18, T13, SCA, and RCA were 99.21%, 100.00%, 100.00%, 98.55%, and 100.00% and the specificities 99.95%, 99.94%, 99.98%, 99.69%, and 99.92%, respectively. The PPVs of NIPT for detecting T21, T18, T13, SCA, and RAT were 85.62%, 45.24%, 40.00%, 34.17%, and 13.51%, respectively (Table 2). The incidences of T21, T18, T13, SCA, and RCA were 0.30%, 0.05%, 0.01%, 0.16%, and 0.01%, respectively. Of the RCA, trisomy 7 was the most frequently detected (n=40), followed by trisomy 8 (n=11) and trisomy 16 (n=7). The 5 RCA TP cases consisted of 1 case each of trisomy 15, trisomy 16, uniparental disomy (UPD) on chromosome 6, trisomy 9 (karyotype 47,XN,+der(9)del(9)(q34)/46,XN[46]) and monosomy 18 (karyotype 46,XN,der(18)del(18)(p11.2)del(18)(q21.3)).

Table 2

Performance of non-invasive prenatal testing (NIPT) in detecting trisomies (T) 21, 18 and 13 , SCA and RCA

Aneuploidies	**TP**	**FP**	**FN**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**Incidence (%)**
T21	125	21	1	99.21	99.95	85.62	0.30
T18	19	23	0	100.00	99.94	45.24	0.05
T13	6	9	0	100.00	99.98	40.00	0.01
SCA	68	131	1	98.55	99.69	34.17	0.16
RCA	5	32	0	100.00	99.92	13.51	0.01

Among 1426 women with twin pregnancies, 1414 (99.16%) had negative NIPT results and 12 had positive NIPT results, which included 3 for T21, 1 for T18, 1 for T13, 5 for SCA, and 2 for RCA. 7 cases underwent prenatal diagnosis, and 5 cases either did not or refused the subsequent follow-up telephone interview. TP were confirmed in 3 cases, 2 cases of T21 and 1 case XXY.

NIPT performance on different SCA

The results of a detailed analysis of the 316 SCA detected by NIPT are shown in Table 3. There were 149 cases (47.2%) of XO (monosomy X or Turner syndrome), 76 cases (24.1%) of XXY (Klinefelter syndrome), 64 cases (20.2%) of XXX (triple X syndrome), 19 cases (6.0%) of XYY (Jacob syndrome), and 8 cases (2.5%) of 46,XY (delX, deletion on X). Among the 316 cases, there were 199 (63.0%) confirmed NIPT results, 68 (34.2%) were TPs and 131 (65.8%) were FPs. Of the 68 TP SCA cases, 19 were XO, 29 XXY, 11 XXX, 8 XYY, and 1 was 46,XY(delX). The PPVs of XO, XXY, XXX, XYY, and 46,XY(delX) were 20.00%, 59.18%, 28.95%, 61.54%, and 25.00%, respectively. The XO abnormality accounted for most of the

positive NIPT results, but the PPV was the lowest (20.00%). XYY (61.54%) detection was associated with the highest PPV, followed by XXY (59.18%). The incidences of XXY, XO, XXX, XXY, and 46,XY (delX) were 0.07%, 0.05%, 0.03%, 0.02%, and <0.001%, respectively.

Table 3

Performance of non-invasive prenatal testing for detecting sex chromosome aneuploidies (SCA)

Aneuploidies	TP	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	Incidence (%)
XO	19	76	1	95.00	99.82	20.00	0.05
XXY	29	20	0	100.00	99.95	59.18	0.07
XXX	11	27	0	100.00	99.94	28.95	0.03
XYY	8	5	0	100.00	99.99	61.54	0.02
46,XY(delX)	1	3	0	100.00	99.99	25.00	<0.001

PPV according to pregnancy characteristics

Among the 41,819 samples, 21,164 (50.61%) were from women in the high-risk group and the remaining 20,655 from women in the low risk group. The performance of NIPT in detecting T21/T18/T13 was compared between the two risk groups, AMA and young women (Table 4).T21 showed 92.45% PPV in the high-risk group versus 67.50% in the low-risk group (P<0.001). T18 had the PPV 70.00% versus 5.88% in high and low-risk groups respectively (P<0.001). Higher PPVs were showed in AMA for T21/T18/T13, and there was a significant difference in T18 (81.25% versus 23.08%), not in T21 and T13.

Table 4

Performance of non-invasive prenatal testing (NIPT) in detection of T21/T18/T13 in high-risk, low-risk pregnancies, AMA and young women

Aneuploidy	PPV in high risk group (%)	PPV in low risk group (%)	PPV in AMA (%)	PPV in young women (%)
Trisomy 21	92.45 (98/106)	67.50 (27/40)	90.00 (63/70)	81.58 (62/76)
Trisomy 18	70.00 (18/25)	5.88 (1/17)	81.25 (13/16)	23.08 (6/26)
Trisomy 13	54.55 (6/11)	0 (0/4)	66.67 (4/6)	22.22 (2/9)

Clinical outcomes of NIPT positive cases

The outcomes of the 691 NIPT-positive pregnancies are shown in Table 5. Among the 223 women with TP results, 97.35% T21/T18/T13 and 100% RCA aneuploidies chose to terminate pregnancy, while 80.88% of SCA-affected women terminated pregnancy. For the FP result, there was 6 cases of terminate pregnancy with some other abnormalities. Of the 252 unconfirmed cases, accounting for 36.47% of all positive NIPT results, the pregnancy was terminated in 62 (24.60%), and live births were recorded in 96 cases (38.10%), while 37.30% (94/252) with unknown outcome. In the study, we found that 43 (52.44%) women with unconfirmed T21/T18/T13 NIPT positive result chose terminate pregnancy, 33 (40.24%) women un-follow up, and only 6 cases (7.32%) of live birth. For 170 cases of unconfirmed SCA and RCA NIPT positive cases, there were 90 cases liver birth, and 19 terminated pregnancies including 3 cases of spontaneous abortion and 1 cases of fetal ultrasound abnormal.

Table 5

Clinical outcomes of pregnancy women with NIPT positive results

NITP with confirmed results or unknown	NIPT results	Cases	Live birth	Terminate pregnancy	Unfollow-up
TP (n=223)					
	T21/T18/T13	150	**1**[a]	146 (97.33)	3
	SCA	68	**10**[b]	55 (80.88)	3
	RCA	5	0	5 (100.00)	0
	Total	223	11	206 (92.38)	6
FP (n=216)					
	T21/T18/T13	53	50	0 (0.00)	3
	SCA	131	**117**	**5**[c] **(3.82)**	9
	RCA	32	30	0 (0.00)	2
	Total	216	197	5 (3.82)	13
Unknown (n=252)					
	T21/T18/T13	82	6	**43**[d] (52.44)	33
	SCA	117	**61**[e]	**14**[f](11.97)	42
	RCA	53	29	**5**[g] (9.43)	19
	Total	252	96	62 (24.60)	94

a. this case is a mosaicism T21; b. including 2 cases of mosaicism XO, 2 cases of XXX, 1 case

XXY, and 5 cases of XYY; c. including 1 case miscarriage, 1 case soft marker abnormal, 1 case T21 (while NITP showed ChrX-, 1 case with a pathogenic CNV on 22q11.21 and 1 case 46,XN,inv(9)(p11q13); d. including 3 cases of spontaneous abortion and 4 cases of fetal ultrasound abnormal; e. including 1 case of death after born, NIPT result ChrX+Y(Mat); f. including 2 cases of spontaneous abortion and 1 cases of fetal ultrasound abnormal; g. including 1 cases of spontaneous abortion.

Discussion

This study was based on the clinical data from 41,819 pregnant women who underwent NIPT at our center, including detailed pregnancy outcome data for all cases with positive NIPT results. Detection sensitivity and specificity were high for the common aneuploidies T21/T18/T13 and RAT, but lower for detection of SCA, as shown in other studies [7–9, 11–14].The PPVs for detection of T18, T13, SCA, and RCA were low, as also reported by Chen [13]. Our results regarding the performance of NIPT for detecting different types of SCA were similar to those of previous studies [9, 11, 12, 14–19].XO accounted for the majority of the positive NIPT results, but the PPV for detection of aneuploidy was the lowest. The highest PPV was that for XYY, although we encountered few NIPT-positive cases. XXY was the most common abnormality, and exhibited the second highest PPV, followed by XXX, consistent with the findings of several other retrospective clinical studies [9, 12, 14–16], although the PPV was higher for XXX than XXY in some works [11, 17–19].Circulating cfDNA consists of both maternal and fetal DNA, but the proportion of fetal DNA is only 3–6%. Hence, abnormal maternal chromosomes affect NIPT results. There is a small percentage of healthy fertile women with maternal SCA mosaicism or occasionally a full SCA, such as XO or XXX, but nonetheless they may have healthy euploid fetuses. and in this situation, NIPT presented a FP result [20]. Maternal X chromosome CNVs have been found in association with discordant fetal SCA detected by NIPT [21]. Moreover, the gradual and preferential loss of chromosomes occurs in some AMA women, such that their blood karyotype might change from XX to XO/XX mosaic [22].However, the maternal chromosomal complement was not investigated in this study, because it was not part of the informed consent procedure; this is a limitation of the study. For RCA, the study showed that trisomy 7 was the most frequently detected, followed by trisomy 16 and trisomy 8, which is consistent with other studies, while PPVs ranged from 6–52.5% [7, 23–25].

We also compared NIPT performance for detecting T21/T18/T13 in women with high- and low-risk pregnancies, AMA and young women; Significant higher of PPVs were presented in high risk group and AMA. The lower PPV in the low-risk group was attributable to the lower prevalence of chromosomal abnormalities in the population, emphasizing that NIPT is only a screening test such that confirmation via invasive testing is essential. As described by AGOG, PPV oscillates between 38–80% and 91–99% for T21, between 11–41% and 66–92% for T18, and between 5–13% and 45–71% for T13, respectively, at 20 and 40 years old [26].

The pregnancy outcomes of all women were evaluated in this study. Among the 691 cases with positive NIPT results, 252 (36.47%) were not confirmed by invasive prenatal diagnosis, which similar to the other study [27]. In this study, we found that the terminate pregnancies were more higher in T21/T18/T13 NIPT positive cases without confirmed results. Pregnancy termination without confirmation by invasive diagnostic testing can be prevented by adequate pre- and post-NIPT counseling, but apparently there were many women didn't get detail and knowledge counsel by the study. In our follow-up phone interviews, some women stated that their clinician at a local hospital had not informed them about invasive testing to confirm their RCA results, some women mentioned that they could not afford confirmatory testing or have limited access to a diagnostic center (often remotely located), so they chose to ignore the results or determine the pregnancy without prenatal diagnosis confirmation. By the study we found that women in economically developed areas are likely to undergo confirmatory tests, while women in rural areas are more unlikely to undergo confirmatory testing. However, clinicians should inform women that diagnostic tests are important, and it is essential to confirm a high risk NIPT result, so as to prevent the disaster of wrongful pregnancy termination. National recommendations or clear guideline and continuing educational support are essential for ensuring that providers and clinicians are appropriately trained in this rapidly evolving field.

Conclusion

In conclusion, our study supports the integration of NIPT into current clinical practice, but the accuracy of detecting T13, XO and RCA is still relatively poor, especially in low risk and young women. We urge that clinicians inform women with positive NIPT results to undergo further prenatal diagnosis before making any decision. Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing.

Declarations

Data Availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

FL and QC conceived the analysis. FL drafted the manuscript. SL, YX reviewed NIPT results. RW, WC, FH performed the validation experiments. QC, FY, BJ, LL and AY performed clinical diagnosis, communication with patients. FL followed the pregnancy outcome. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee of Nanfang Hospital (approval no. NFEC-2017-035) and all methods were carried out in accordance with relevant guidelines and regulations. Written Informed consent was obtained from all subjects and their legal guardians.

Consent for publication

Not applicable.

Competing Interests

The authors report no declarations of interest.

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Figures

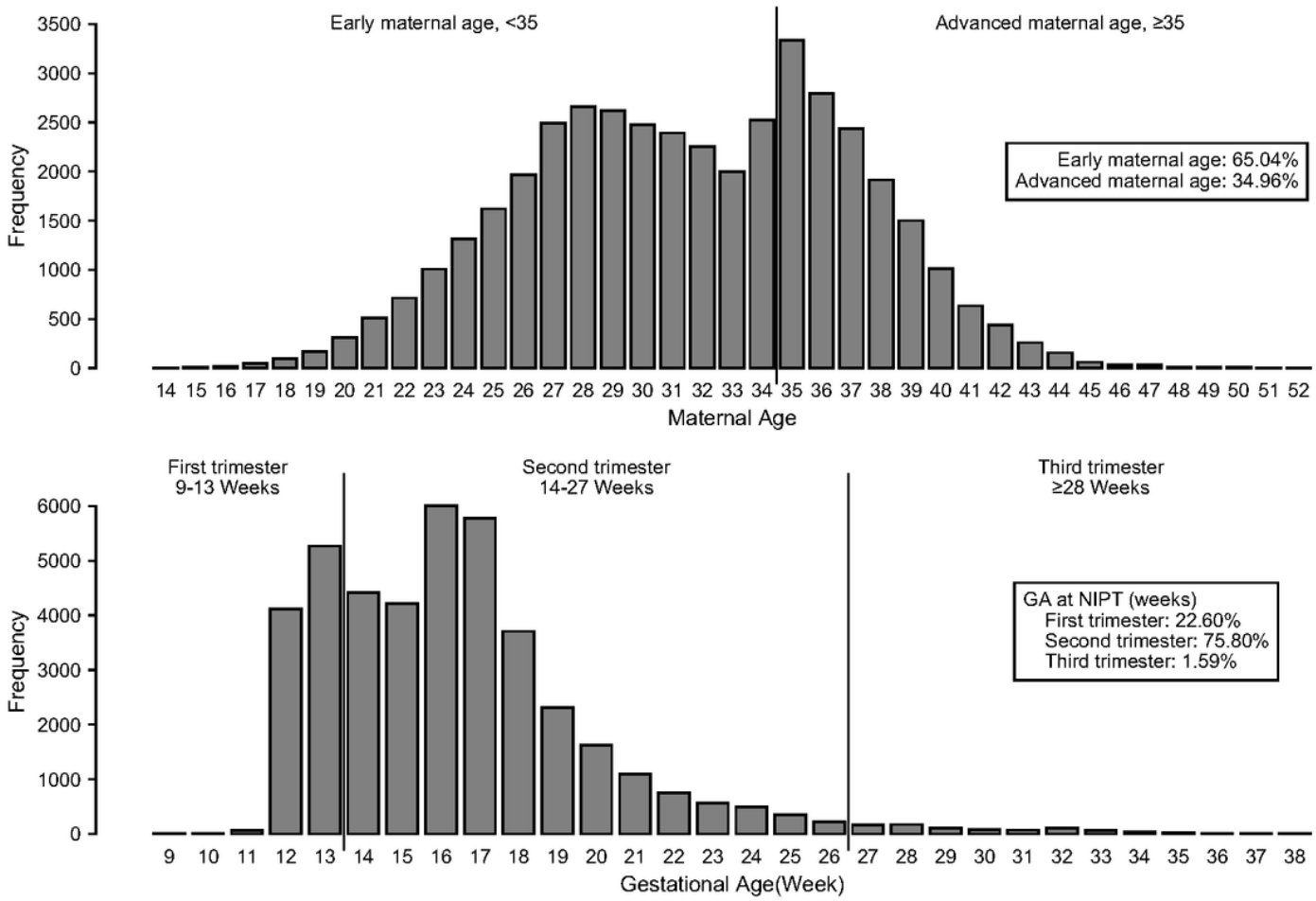

Figure 1

Maternal age and gestational distributions at NIPT sampling

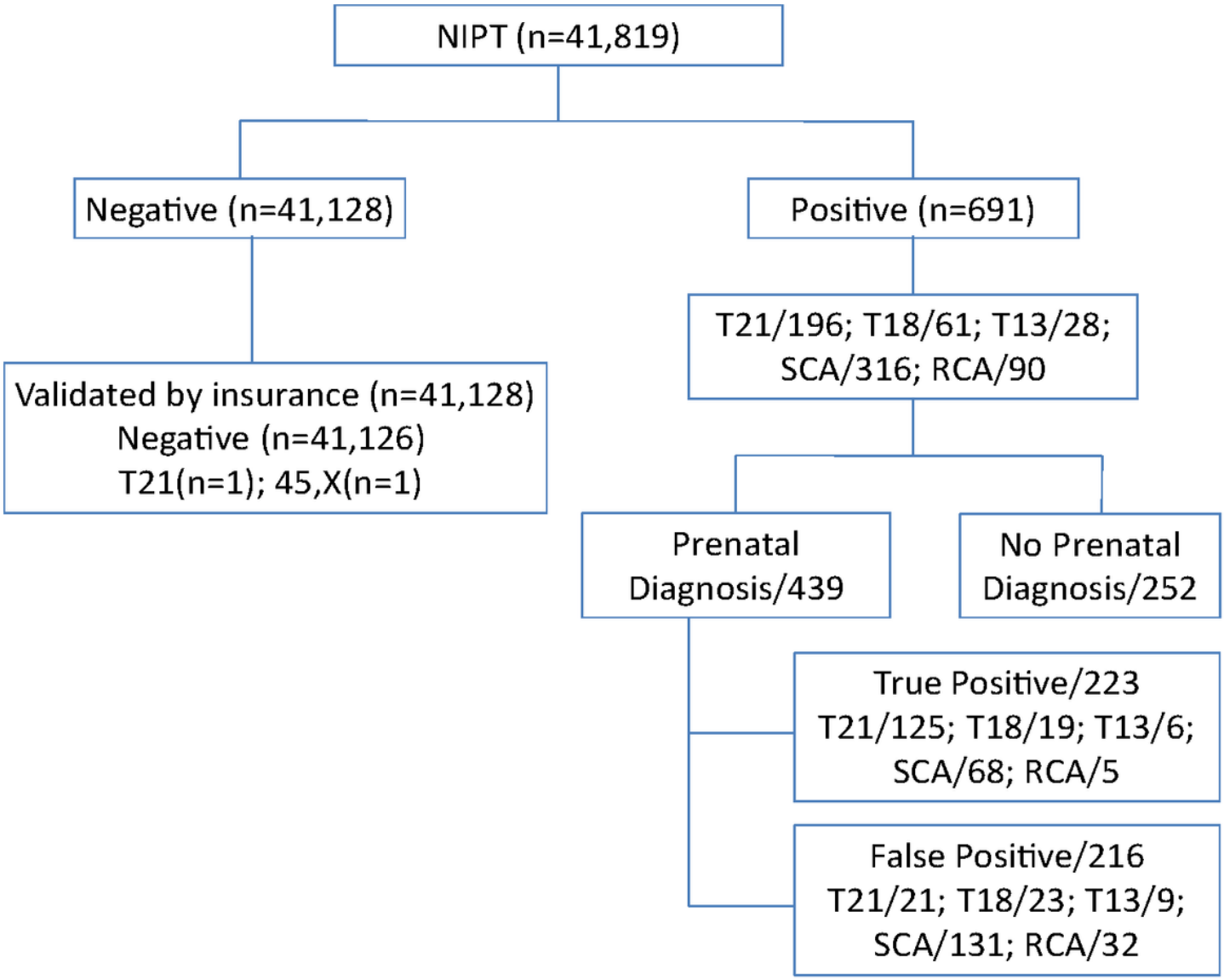

Figure 2

Flowchart of non-invasive prenatal test (NIPT) results and clinical outcome of pregnant women