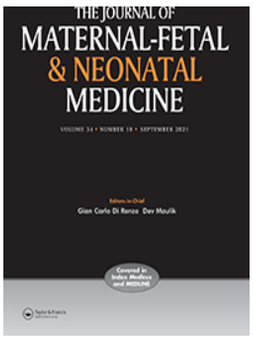

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ORIGINAL ARTICLE

Performance of noninvasive prenatal screening in twin pregnancies: a retrospective study of 5469 twin pregnancies

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ABSTRACT

Objectives: To evaluate the performance of noninvasive prenatal screening (NIPS) for the fetal common aneuploidy screening in twin pregnancies.

Methods: The data of 5469 women with twin pregnancies were collected in this retrospective observational study between January 2017 and December 2018. Patients underwent NIPS as first-line screening or after standard serum screening for fetal aneuploidy. The performance of NIPS was examined, and a regression analysis was performed to investigate testing failure in cases of low fetal fraction.

Results: In this study, 2231 (40.8%) patients opted for NIPS as the primary prenatal screening test, and 3238 (59.2%) opted for serum screening, including 440 patients who opted for NIPS after serum screening. Among the 2671 pregnancies with available NIPS outcomes, 11 cases of aneuploidy were identified, seven of trisomy 21 and four of sex chromosome aneuploidy (SCA). The sensitivity and specificity for trisomy 21 were 100% (95% CI, 56.1–100.0%) and 100% (95% CI, 99.8–100.0%), respectively. The positive predictive value (PPV) for SCA was 40.0% (95% CI, 13.7–72.6%). No false negatives were found, with a negative predictive value (NPV) of 100% (95% CI, 99.8–100.0%) in total. In 32 pregnancies who failed NIPS test without available NIPS outcomes due to low fetal fraction, the regression analysis demonstrated that increasing BMI and assisted reproductive technology treatment were significant independent predictors.

Conclusions: NIPS is a high-performing routine primary prenatal screening test in twin pregnancies, with a high PPV and low false positive rate for detecting trisomy 21. It is also useful to identify common sex chromosome aneuploidies in twin pregnancies, with similar performance to that reported in singleton pregnancy.

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Noninvasive prenatal screening (NIPS); cell-free DNA; twin pregnancy; chromosome aneuploidies; clinical performance

Introduction

In recent years, the incidence of twin pregnancies has greatly increased worldwide due to the use of assisted reproductive technology (ART) treatment and increased average maternal age at delivery. Conventional serum screening is less robust for twin pregnancies than for singletons [1,2], resulting in unnecessary invasive procedures with a theoretically higher rate of iatrogenic miscarriages than in singleton pregnancies [3]. A better alternative prenatal screening test for chromosomal diseases is urgently needed for this enlarging population. This is especially desirable in the context of ART, as 30–50% of twin pregnancies are conceived using an ART procedure. ART leads to a higher rate of chromosomal abnormalities; nevertheless, the fear of procedure-related pregnancy loss deters women from undergoing prenatal testing [4].

Noninvasive prenatal screening (NIPS) is based on the sequencing of cell-free DNA (cfDNA) fragments in the maternal circulation, and it is the most sensitive screening option for traditionally screened aneuploidies (i.e. Patau, Edwards, and Down syndromes) [5,6], with low invasiveness and a lower risk of procedure-related miscarriages [7]. Recently, the international guidelines on aneuploidy screening published by the American College of Obstetricians and Gynecologists (ACOG) and the International Society for Prenatal

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Diagnosis (ISPD) have supported the use of NIPS in twin pregnancies [8,9]. Recent studies also indicated that the performance of NIPS in twin pregnancies is superior to that of conventional prenatal screening, and it is almost as accurate as in singletons [10–14]. Although the application of NIPS in twin pregnancies is promising, there are limited publications describing the actual performance of NIPS as first-line screening in twin pregnancies in a large sample size. Most of the literature presents NIPS as contingent testing on results from the combined serum screening test, a standard for high-risk pregnancies. According to the new ACOG guideline (Practice Bulletin 226), if a patient offered prenatal screening opts to undergo testing, they should only be prescribed one test, either NIPS or serum screening, but should not have multiple tests performed simultaneously [8]. The present study aimed to analyze the feasibility of introducing NIPS as an alternative first-line prenatal screening option with traditional serum screening for the detection of the major trisomies in routine clinical screening in twin pregnancies.

Materials and methods

Study patients

A total of 5469 women with twin pregnancies and who opted for prenatal screening at the Prenatal Diagnosis Center of West China Second Hospital of Sichuan University, between January 2017 and December 2018, were included in this study. The clinical phenotypes of chromosomal abnormalities were not found in the parents. The mothers had not received foreign blood transfusion, transplant surgery, cell therapy, or immunotherapy within one year of the pregnancy. No cancer was diagnosed during the course of all pregnancies. All participants received pre- and post-test clinical counseling, and explanations of the contents, principles, and the advantages and limitations of the tests by the clinician. Women with intrauterine fetal demise without fetal karyotype results or lost follow up were excluded from this study. The trisomy status of the pregnancies was determined by pre- or postnatal karyotyping or clinical examination of the neonates. The study was approved by the Medical Ethics Committee of West China Second University Hospital of Sichuan University, as well as the Institutional Ethics Committee of Sichuan University. Written informed consent was obtained from each pregnant woman.

Standard serum screening

A total of 5 ml of elbow venous blood were collected in a BD Vacutainer sample tube (Becton, Dickinson & Co., Franklin Lakes, NJ). Serum markers were detected using 1235 automatic time-resolved fluorescence immunoanalyzer with appropriate reagents (PerkinElmer, Gaithersburg, MD). Screening risks were calculated by Lifecycle4.0 (Finland Wallac Oy Company, Turku, Finland). First trimester combined screening and second trimester screening were offered. First-trimester serum markers included pregnancy-associated plasma protein A (PAPP-A) and free beta subunit human chorionic gonadotropin (βhCG), were used in combination with or without sonographic measurement of fetal nuchal translucency (NT) to formulate the risk score. Second-trimester serum markers included maternal serum alpha-fetoprotein (MSAFP), βhCG, unconjugated estriol (uE3), and inhibin A (inhA). A risk value of $\geq$1 in 270 meant high risk for trisomy 21 and a risk value of $\geq$1 in 350 meant high risk for trisomy 18, a risk value from one in 271 to one in 1000 meant intermediate risk for trisomy 21 and a risk value from one in 351 to one in 1000 meant intermediate risk for trisomy 18. A risk value of $<$1 in 1000 meant low risk for trisomy 21 or trisomy 18.

NIPS

Eight- to 10-ml maternal blood samples were collected in cell-free^{TM} BCT tubes (Streck Inc., Omaha, NE) by venipuncture for all patients. The blood samples were left to stand at room temperature for 30 min, before being stored at 4 °C. Afterwards, cfDNA extraction, library construction, quality control, and pooling followed a previously published method in using the fetal chromosome aneuploidy testing kits (Hangzhou Berry Gene Diagnostic Technology Co., Ltd., Hangzhou, China) [15]. Pooled libraries were then subjected to massively parallel sequencing on the NextSeq CN500 sequencers (Illumina, San Diego, CA) to generate approximately 5 million raw sequencing reads with genomic DNA sequences of 36 bp in length. Sequencing reads were filtered and approximately 3.5 million were uniquely mapped to the hg19 human reference genome, using RUPA extreme speed information analysis method. The sequences of each sample that were mapped to each chromosome were counted, and the guanine-cytosine (GC) content was calculated. To determine the status for all 24 chromosomes, the *Z*-score (normal range, $-3 < Z < 3$) was generated by normalized chromosome representation (NCR) and GC correction. For the X and Y chromosome

evaluations, chromosomal representations are examined as the ratios of normalized chromosome X and Y read counts in the genomic regions vs. the total autosomal read counts. The normalization procedure for SCA and the classification algorithm were completed in accordance with previously published studies [15,16]. The fetal DNA concentration was calculated as a quality control using Y chromosome-based approach [17] and cfDNA size-based approach [18] previously described. For aneuploid samples, fetal DNA fraction was estimated based on the difference of genome percentage of the abnormal chromosome between the test sample and the reference samples [17]. Samples that failed the quality control criteria of cfDNA extraction, library construction, and sequencing as well as fetal DNA concentration (<4%) were removed.

After the prenatal screening test, all participants would be given a general test report showing the estimated fetal risk (positive or negative) of trisomies 13, 18, and 21. For NIPS, a suspected risk of SCA was reported in the form of a supplementary report. Qualified clinical geneticists offered post-test clinical counseling. When a screen positive test result, and/or a supplementary report of a suspected risk of SCA, or a test failure of NIPS is obtained, a diagnostic testing and a comprehensive ultrasound evaluation were recommended to confirm results. Following the results, pregnant women could agree or refuse to further invasive prenatal diagnosis by amniocentesis. Amniocentesis was performed by puncturing the amnion using a needle to aspirate 20–25 ml of amniotic fluid. A majority of the procedures were performed in the late second or early third trimester, in accordance with the current practice in China [19]. Karyotyping, chromosome microarray analysis, or copy number variation sequencing was used to identify fetal chromosomes.

Statistical analysis

Descriptive data are presented as numbers and percentages for categorical variables and in median (interquartile range, IQR) for continuous variables. Comparisons between outcome groups were performed using the Mann–Whitney *U*-test for continuous variables and the χ^2-test or Fisher's exact test for categorical variables. Multivariate logistic regression analysis was used to determine the significant predictors of failed NIPS results with low fetal fraction after second sampling in twin pregnancies. The statistical software package SPSS 26.0 (SPSS Inc., Chicago, IL) was used to analyze the data.

Results

A total of 5617 twin pregnancies were enrolled between 1 January 2017 and 31 December 2018. Out of these participants, 148 of them were excluded because karyotype was not obtained ($n = 81$; pregnancy ended in termination with severe twin-to-twin transfusion syndrome or fetal growth restriction ($n = 26$), selective embryonic reduction ($n = 4$), stillbirth ($n = 12$), intrauterine fetal death ($n = 39$)), cfDNA test did not provide a result ($n = 34$), or there were lost follow-ups ($n = 33$). Finally, 5469 participants were analyzed for demographic characteristics with the available neonatal clinical data and/or fetal karyotypes, as illustrated in Figure 1. The demographic and pregnancy characteristics of the 5469 patients are shown in Table 1. The demographic and pregnancy characteristics of the 5469 patients are shown in Table 1. The median maternal age and gestational age were 28.4 (IQR, 26.0–31.0) years and 16.8 (IQR, 15.9–17.8) weeks, respectively; a total of 5.6% (308/5469) patients had an advanced maternal age (AMA) of 35 years or more. Among them, 3305 (60.4%) pregnancies were conceived naturally, and 2164 (39.6%), via ART treatment. Regarding test choice, 3238 (59.2%) opted for serum screening, and 2231(40.8%), for NIPS as the first-line prenatal screening test. After serum screening, 21.8% (705/3238) women opted for follow-up testing, included 13.6% (440/3238) for NIPS and 8.2% (265/3238) for confirmatory tests. A total of 48.8% (2671/5469) ultimately underwent NIPS. Compared with women who underwent serum screening, women who underwent NIPS had significantly higher maternal and gestational ages, lower prevalence of ART pregnancies, and lower prevalence of invasive procedures (1.6% *vs.* 8.2%; $p < .05$).

Among the 3238 pregnancies who opted for serum screening as a first-line prenatal screening test, 197 (6.1%), 542 (16.7%), and 2499 (77.2%) were classified as being at high risk, intermediate risk, and low risk, respectively. No trisomy 21 or 18 was detected, and none of the follow-up tests indicated trisomy 21 or 18. The overall false positive rate (FPR) was 6.1% (197/3238). The invasive testing rate in women with high-risk results was higher than that in women with intermediate-risk results (30.3%, 60/197 vs. 9.7%, 53/542).

Eighteen (0.7%) cases were classified as NIPS positive among 2671 pregnancies, included seven cases of trisomy 21, one of trisomy 13, and 10 of SCAs. Eleven

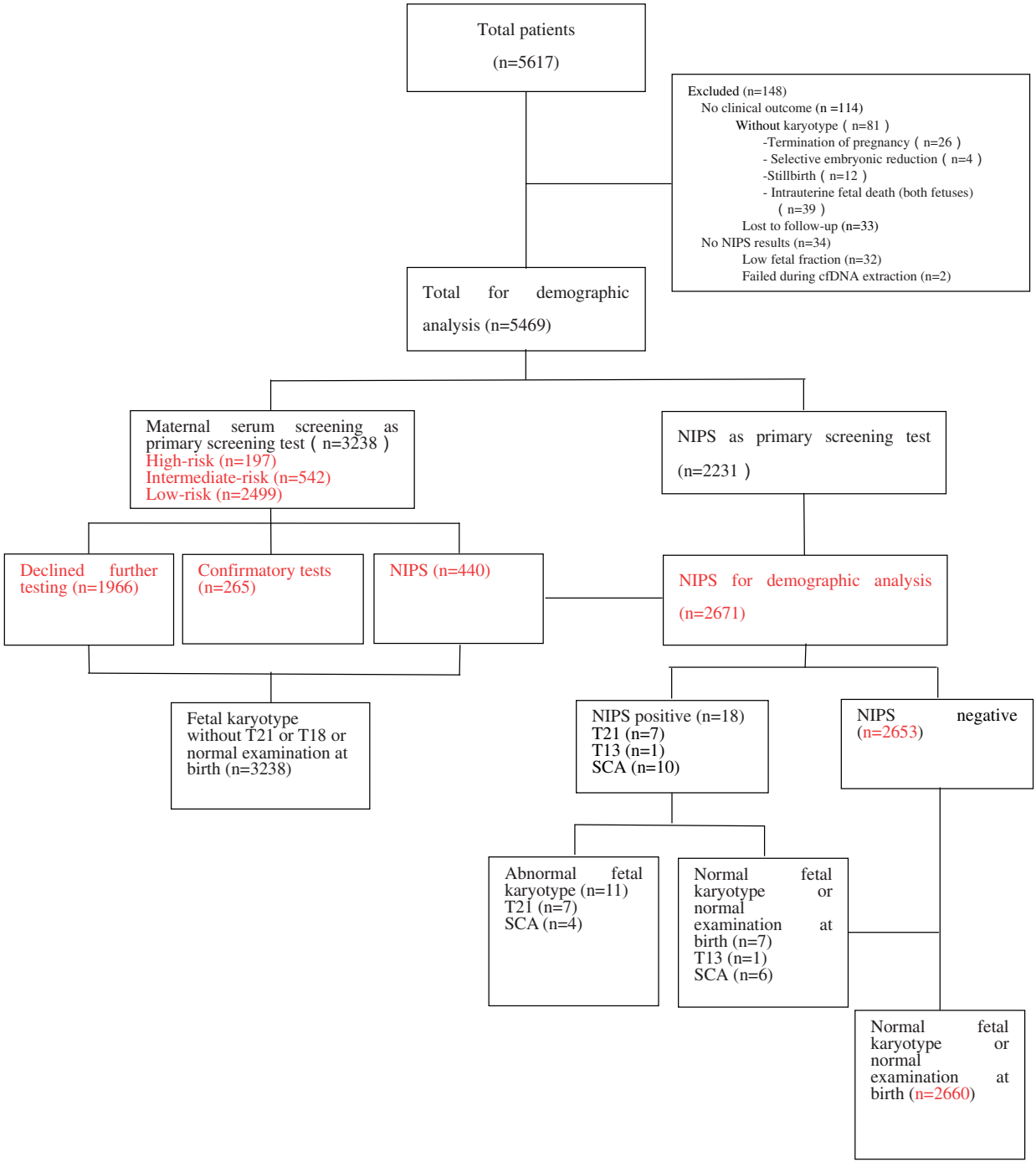

Figure 1. Flowchart of the research process.

(0.4%) cases of common chromosome aneuploidies were confirmed by karyotyping, included seven cases of trisomy 21 and four of SCAs (one 47, XYY, two 47, XXY, and one 47, XXX), at a positive predictive value (PPV) of 61.1% (95% CI, 36.1–81.7%) (Table 2). No false negatives were found at an negative predictive value (NPV) of 100% (95% CI, 99.8–100%). The PPVs for trisomy 21 and SCA were 100% (95% CI, 56.1–100%) and 40.0% (95% CI, 13.7–72.6%), respectively. NIPS for screening trisomy 21 had a sensitivity of 100%, specificity of 100%, and no false positives. The proportion of SCAs in the overall aneuploid chromosomal anomalies was 36.4% (4/11). For SCA, the sensitivity was 100.0% (95% CI, 39.6–100%), and specificity, 99.8% (95% CI, 99.5–99.9%). Table 3 summarizes the clinical details of all 11 cases of common chromosome aneuploidies. There were nine (81.8%) DCDA and two (18.2%) MCDA pregnancies; five (45.4%) pregnancies were conceived naturally and six (54.5%) were conceived by ART treatment. Based on NIPS and

Table 1. Characteristics of the study population.

Characteristic	Standard screening ($n = 3238$)[a]	NIPS ($n = 2671$)[b]	Total ($n = 5469$)
Maternal age, years	28.0 (26.0–31.0)*	29.0 (26.0–32.0)*	28.4 (26.0–31.0)
AMA, *n* (%)	49 (1.5%)*	273 (10.2%)*	308 (5.6%)
Gestational age, weeks	16.3 (15.0–17.1)*	16.9 (15.7–18.3)*	16.8 (15.9–17.8)
9–13 weeks	987 (30.5%)*	129 (4.8%)*	388 (7.1%)
14–27 weeks	2979 (92.0%)*	2536 (94.9%)*	5075 (92.8%)
≥28 weeks	0 (0%)*	6 (0.2%)*	6 (0.1%)
Mode of conception			
Spontaneous	2044 (63.1%)*	1536 (57.5%)*	3305 (60.4%)
ART	1194 (36.9%)*	1135 (42.5%)*	2164 (39.6%)
Patient choice for further testing	1194 (36.9%)*	970 (43.3%)*	
NIPS	440(13.6%)	–	455 (8.3%)
Invasive test	265 (8.2%)*	36 (1.3%)*	301 (5.5%)
No further testing	2533 (78.2%)*	2635 (98.7%)*	4713 (86.2%)

AMA: advanced maternal age; ART: assisted reproductive technology; NIPS: noninvasive prenatal screening.
Data are given as *n* (%) or median (interquartile range, IQR). Comparisons between groups were performed using Mann–Whitney's *U*-test for continuous variables and the χ^2 or Fisher's exact test for categorical variables, with *post hoc* Bonferroni's correction with adjusted *p* value of <.05 (*).
[a]Seven hundred and twenty-eight women underwent integrated screening, whose' samples were obtained in the first and second trimesters.
[b]Four hundred and forty pregnancies opted for NIPS for further testing after serum screening.

Table 2. Clinical test performance of NIPS.

Variable	T21 ($n = 2671$)	SCA ($n = 2671$)	Total ($n = 2671$)
Having fetal aneuploidy			
Test positive for aneuploidy	100.0 (7/7)	100.0 (4/4)	100.0 (11/11)
Test negative for aneuploidy	0.0 (0/7)	0.0 (0/4)	0.0 (0/11)
Not having fetal aneuploidy			
Test positive for aneuploidy	0.0 (0/2664)	0.2 (6/2667)	0.3 (7/ 2660)
Test negative for aneuploidy	100.0 (2664/2664)	99.8 (2661/2667)	99.7 (2653/2660)
Sensitivity	100.0 (56.1–100)	100.0 (39.6–100)	100.0 (56.1–100)
Specificity	100.0 (99.8–100)	99.8 (99.5–99.9)	99.7 (99.4–99.9)
Positive predictive value	100.0 (56.1–100)	40.0 (13.7–72.6)	61.1 (36.1–81.7)
Negative predictive value	100.0 (99.8–100)	100.0 (99.8–100)	100.0 (99.8–100)

T21: trisomy 21; SCA: sex chromosome aneuploidy.
Data are in percentages with raw numbers shown in parentheses. Statistical analysis shows 95% confidence intervals in parentheses.

confirmatory results, pregnancy decisions were discussed with the pregnant women during the post-test genetic counseling. The sensitivities and specificities could not be calculated for trisomy 18 and 13 because of insufficient cases. All seven mothers with a false-positive NIPS result delivered two healthy babies each, with normal findings during physical examinations at term (Table 4).

Additionally, 1.3% (34/2671) samples failed the cfDNA test. Thirty-two (1.2%) failed because of low fetal fraction for accurate evaluation even after redrawing, and two (0.1%) failed during cfDNA extraction. There were no clear biologic reasons for testing failures in the 32 patients with low fetal fraction; 18.8% (6/32) of the patients chose to proceed with invasive testing and subsequently obtained results of normal fetal karyotypes. The median maternal age was 29.5 (IQR, 27.0–33.0) years and the median gestational age was 17.7 (IQR, 16.0–18.9) weeks; the median BMI was 24.8 (IQR, 22.2–28.1). A total of 71.9% (23/32) patients conceived by ART treatment. All 32 patients with previously failed testing with low fetal fraction had live births with normal findings during physical examinations. Multivariate logistic regression analysis demonstrated that the risk of test failure with low fetal fraction significantly increased in ART (OR = 2.89, 95% CI, 1.32–6.29%) in comparison to natural conception, and increased with increasing BMI (OR = 1.17, 95% CI, 1.07–1.29%) (Table 5). Maternal age, gestational age, parity, pregnancy loss history, and maternal tumor history did not result in a higher no-result rate.

Discussion

We presented a clinical application of providing NIPS for prenatal screening in twin pregnancies, as recommended by the international guidelines [8,9], and evaluated its performance in twin pregnancies. Ultimately, 48.8% of patients underwent NIPS for fetal aneuploidy screening. Conventional methods of aneuploidy screening, mostly for trisomy 21, rely exclusively on biochemical and sonographic measurements in the first and second trimesters which have lower sensitivity and specificity in twin pregnancies than in singleton pregnancies [1,2]. Theoretically, this is responsible for an unnecessary increase in subsequent invasive procedures and often cannot provide results for trisomy 18 or trisomy 13. In the present study, the

Table 3. Clinical details of the 18 cases with fetal trisomies with NIPT-positive results.

Samples	NIPS results	MA (years)	GA (weeks)	Chorionicity	Conception	Ultrasound examination	Number of affected fetuses	Cytogenetic confirmation	Prenatal decision
Case 1	T21	40	21w + 5	DCDA	SP	Abnormal NT of 3.2 mm/normal	1	AF: 47, XX, +21/46, XX	Continue pregnancy
Case 2	T21	30	16w + 2	DCDA	ART	Both normal	1	AF:47, XY, +21/46, XY	Selectively terminated
Case 3	T21	33	17w + 5	DCDA	SP	Abnormal NT of 2.9 mm, persistent left superior vena cava with double coronary sinus, hydramnios/normal	1	AF: 47, XY, +21/46, XX	Selectively terminated
Case 4	T21	36	16w + 6	DCDA	ART	Absent fetal nasal bone and complete atrioventricular septal defect/normal	1	AF: 47, XY, +21/46, XX, inv (9) (p12, q12)	Selectively terminated
Case 5	T21	37	15w + 2	DCDA	ART	Ventricular septal defect/normal	1	AF: 47, XY, +21/46, XX,22pss	Selectively terminated
Case 6	T21	28	15w + 1	DCDA	ART	Abnormal NT of 3.8 mm, strephenopodia/normal	1	NB: normal/T21	Continue pregnancy
Case 7	T21	28	14w + 6	MCDA	SP	Both normal	2	AF: T21/T21	Stillbirth/terminated
Case 8	SCA	30	15w + 5	DCDA	ART	Both normal	1	AF: 47, XYY/46, XX	Continue pregnancy
Case 9	SCA	27	18w + 0	DCDA	SP	Both normal	1	AF: 47, XXY/46, XX	Continue pregnancy
Case 10	SCA	35	19w + 5	DCDA	ART	Both normal	1	AF: 47, XXY/46, XX	Continue pregnancy
Case 11	SCA	23	17w + 3	MCDA	SP	Both normal	2	AF: 47, XXX/47, XXX	Continue pregnancy

T21: trisomy 21; SCAs: sex chromosome aneuploidies; MA: maternal age; GA: gestational age; DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; SP: spontaneous; ART: assisted reproductive technology; NT: nuchal translucency; AF: amniotic fluid; NB: neonatal blood.

higher rate of invasive testing in women with results indicating high risk than in women with results indicating intermediate risk (30.3% vs. 9.7%) insinuated that the rate of invasive testing increases with increasing estimated risk for trisomies, and this was consistent with the finding of a previous study [20].

In our study, NIPS detected all 11 (0.4%) cases of common chromosome aneuploidies among the 2671 twin pregnancies, including seven (0.3%) of trisomy 21 and four (0.1%) of SCA, all confirmed by karyotyping. For trisomy 21, NIPS showed high sensitivity and specificity of 100%. Similarly, in a recent systematic review of 997 twin pregnancies, the pooled weighted detection rate (DR) and FPR for trisomy 21 were 98.2% (95% CI, 83.2–99.8%) and 0.05% (95% CI, 0.01–0.26%), respectively [14]. The performance of cfDNA test is superior in terms of a higher DR and a substantially lower FPR, consistent with published literature, thereby demonstrating a similar performance in terms of accuracy between twin and singleton pregnancies [10–13], in addition to the reduction of unnecessary invasive procedures and iatrogenic fetal loss. In our study, compared with patients who underwent serum screening, although those who underwent NIPS had higher maternal age and higher rate of AMA with a higher rate of high-risk pregnant women theoretically, as the incidence of fetal chromosomal abnormalities increases as a woman ages, NIPS showed a considerably lower invasive testing rate (8.2% vs. 1.3%). This is consistent with the finding of a previous study, which showed that NIPS reduced the number of invasive tests [7]. In addition, despite a significantly lower FPR in comparison to that with serum screening, the ACOG recommends that patients with a positive NIPS result for fetal aneuploidy still undergo genetic counseling, a comprehensive ultrasound evaluation, and possibly a follow-up diagnostic testing such as amniocentesis to confirm the preliminary results [8]. Furthermore, women whose NIPS results are unreported, indeterminate, or uninterpretable (a no-call test result) should receive further genetic counseling and undergo comprehensive ultrasound evaluation and further diagnostic testing [21,22].

Following trisomy 21, the second most common chromosome aneuploidy is SCA among high-risk pregnant women. In our study, NIPS also represented a high proportion (36.4%, 4/11) of SCA in twin pregnancies, with a PPV of 40.0% (95% CI, 13.7–72.6%) based on cytogenetic confirmation, that was similar to that in our previous study of 32.4% in singletons [23], and 48.4% in Porreco RP's study mixed singleton and twin pregnancies [5]. Cell-free DNA is the only laboratory

Table 4. Clinical details of the eight cases with normal fetuses with NIPT-positive results.

Samples	NIPS results	MA (years)	GA (weeks)	Chorionicity	Conception	Ultrasound examination	Number of affected fetuses	Cytogenetic confirmation/ neonatal phenotype	Prenatal decision
Case 12	T13	25	17w + 3	MCDA	SP	Both normal	0	AF: 46, XY/46, XY	Continue pregnancy
Case 13	SCA	23	16w + 3	MCDA	SP	Both normal	0	Both normal	Continue pregnancy
Case 14	SCA	36	16w + 1	Unknown	SP	Both normal	0	Both normal	Continue pregnancy
Case 15	SCA	32	17w + 4	DCDA	ART	Both normal	0	AF: 46, XX/46, XY	Continue pregnancy
Case 16	SCA	30	19w	DCDA	ART	Both normal	0	Both normal	Continue pregnancy
Case 17	SCA	33	16w	DCDA	ART	Both normal	0	AF: 46, XX/46, XY	Continue pregnancy
Case 18	SCA	31	15w	DCDA	ART	Both normal	0	Both normal	Continue pregnancy

T21: trisomy 21; SCAs: sex chromosome aneuploidies; MA: maternal age; GA: gestational age; DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; SP: spontaneous; ART: assisted reproductive technology; NT: nuchal translucency; AF: amniotic fluid; NB: neonatal blood.

screening test to identify sex chromosome aneuploidies. The American College of Medical Genetics and Genomics (ACMG) recommends that all pregnant women should be informed of the extended use of screening for SCAs as part of the pretest counseling for NIPS [22]. Our data remind us that the performance of NIPS for SCAs in twin pregnancies is currently similar to that in singleton pregnancies, and therefore can be used to screen for SCAs in twin pregnancies. However, it should also be emphasized that the poor PPV for SCA could lead to a relative increase in the number of invasive procedures [5,24]. The very high NPV of NIPS for SCA also reflects the very low prevalence rate of these abnormal karyotypes. Of note, because most SCAs have a normal neonatal phenotype and will not be detected by clinical exam, but karyotyping was not performed on all pregnancies in our study, the sensitivity for SCA only represents the current follow-up results. The performance of NIPS for sex chromosome aneuploidy (SCA) in twin pregnancies needs more large studies to be assessed.

Moreover, we found that the significant predictors of cfDNA test failure with low fetal fraction were increasing BMI and ART treatment in twin pregnancies, that is consistent with several previous analyses [10,13,25–27]. Due to an increased turnover of adipocytes in obese pregnant women, there is an increase in the amount of maternal cfDNA and hence a decrease in the cfDNA fraction [28]. Qiao et al. [29] found that fetal fraction was reduced by 0.541% for every BMI increase of 1 kg/m^2. Additionally, as the serum PAPP-A concentration is decreased by 10–25% [25] and the incidence of pre-eclampsia is increased [30] in *in vitro* fertilization (IVF) pregnancies, an impaired placentation also could be associated with NIPS test failure. Although Lambert-Messerlian et al. found no difference in the cfDNA level in ART and naturally conceived pregnancies, they identified a small, but statistically significant, reduction in *Z*-scores among euploid ART pregnancies [31].

The major strength of our study was the description of SCA screening of NIPS in twin pregnancies. Our relatively large sample size of routine clinical samples reflected a true clinical setting, providing an informative experience for the clinical application of NIPS in twin pregnancies. However, there are several limitations. First, we could not obtain the information about maternal or placental mosaicism that may contribute to the false positive results for NIPS [8]. Second, our participants are mainly the second trimester twin pregnancies. Due to the increasing incidence and risk of maternal complications in twin pregnancies, there

Table 5. Regression analysis for the prediction of failed NIPS because of low fetal fraction.

	Univariate analysis		Multivariate analysis	
Independent variable	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Maternal age (years)	1.032 (0.958, 1.111)	.406	–	
Gestational age	1.007 (0.987, 1.027)	.522	–	
BMI	1.188 (1.083, 1.302)	<.0001*	1.174 (1.065, 1.293)	.001*
Fertilization				
SP pregnancies	(Reference)			
ART pregnancies	3.316 (1.528, 7.199)	.002*	2.893 (1.324, 6.292)	.008*
Parity				
Nulliparity	(Reference)			
Multiparity	0.743 (0.332, 1.662)	.469	–	
Pregnancy loss history				
Non pregnancy loss history	(Reference)			
Pregnancy loss history	2.037 (0.957, 4.335)	.065	–	
Maternal tumor history				
Non-maternal tumor history	(Reference)			
Maternal tumor history	3.058 (0.911, 10.269)	.071	–	

CI: confidence interval; BMI: body mass index; SP: spontaneous; ART: assisted reproductive technology; USMs: ultrasonographic soft markers.
*$p<.025$.

is a higher risk of miscarriage with increasing gestational age. It would be preferable to offer NIPS in early pregnancy as a first-line prenatal screening method in twin pregnancies, which is possible to perform with the present technology; thus, this will be a further research direction. Lastly, less information was available for screening twin pregnancies for trisomies 18 and 13, which was mainly due to their lower prevalence, though both can be well identified using ultrasound measurements. As such, further clinical studies including more of such cases on twin pregnancies are required.

Conclusions

Our study confirmed that NIPS is an accurate advanced screening test for twin pregnancies, with extremely high sensitivity and specificity for trisomy 21 (>99%), and with high specificity but somewhat less sensitivity for SCA, similar to that reported in singleton pregnancy. This may indicate that NIPS might be reliably used as an indication for further prenatal diagnosis in twin pregnancies. Due to the paucity of reported studies in twins; however, more rigorous research on large samples is needed to confirm the accuracy of NIPS in twin pregnancies. Moreover, it is important to establish the standards of NIPS for twin pregnancies to benefit this population and to expand the clinical application of NIPS.

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Disclosure statement

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