

# Two factors affecting the success rate of the second non-invasive prenatal screening after initial no-call result: experience from a single tertiary center in China

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## Abstract

**Background:** One inevitable shortcoming of non-invasive prenatal screening (NIPS)/cell-free DNA (cfDNA) sequencing is the uninterpretable (“no-call”) result, which is mainly caused by an insufficient fetal fraction. This study was performed to investigate the factors associated with a successful second NIPS in these cases and determine the optimal management for women with initial no-call results.

**Methods:** We retrospectively analyzed the data of women who underwent NIPS with initial no-call results due to an insufficient fetal fraction from 2017 to 2019 in our center. We compared these women's maternal and pregnancy information with the data of women who had attained a successful second NIPS result and women who had received no-call results for a second time.

**Results:** Among the 33,684 women who underwent NIPS, 137 with a no-call result underwent a retest. Comparison between the 87 (63.50%) women with a successful retest and the other 50 (36.50%) women showed a significant difference in both the initial fetal fraction and maternal body mass index (BMI), whereas the other factors showed no significant differences. In addition, with an initial fetal fraction of < 2.00%, the retest success rate was very limited.

**Conclusions:** We identified two major factors associated with a successful NIPS retest: the initial fetal fraction and the maternal BMI. These findings suggest the need for specialized management for this subset of women and would be instructional for the counseling for these women.

**Keywords:** Non-invasive prenatal screening; Cell-free DNA; No-call results; Maternal body mass index; Initial fetal fraction

## Introduction

Non-invasive prenatal screening (NIPS), which is based on cell-free DNA (cfDNA) sequencing, has been introduced into clinical practice as an advanced technique for fetal aneuploidy screening in the last decade.<sup>[1,2]</sup> Compared with traditional aneuploidy screening, which is based on serum biochemical assays and fetal nuchal translucency measurements, NIPS/cfDNA sequencing shows a significantly lower false-positive rate and a higher positive predictive value in the general obstetrical population, leading to a reduction in the number of unnecessary invasive procedures.<sup>[3,4]</sup> According to our clinical practice and that in other centers, the sensitivity of NIPS for the detection of common trisomies can reach 97.45% to 100%, and the specificity can reach 99.94% to 99.96%.<sup>[5,6]</sup> Because of its superior performance, NIPS is being evaluated and increasingly adopted as a first-tier

fetal aneuploidy screening test in many countries worldwide.<sup>[7,8]</sup>

An inevitable shortcoming of NIPS is the potential attainment of uninterpretable (“no-call”) results, which may be caused by an insufficient fetal fraction, sampling failure, experimental failure, bioinformatics failure, and other complications. Among these causes, an insufficient fetal fraction is considered the most important, and it reportedly occurs in approximately 0.1% to 6.1% of NIPS cases depending on the particular clinical center and NIPS technical platform used.<sup>[9]</sup>

As the clinical application of NIPS becomes more widespread, it is necessary to assess the risk of aneuploidy in the subgroup of women with a no-call result. One common strategy is to perform a second blood draw and retest, but a portion of these women still receive an

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unsuccessful result.<sup>[10,11]</sup> In addition, an increased risk of trisomy is reportedly associated with these no-call results.<sup>[4,12]</sup> Therefore, the benefit of performing a second blood draw has been questioned.<sup>[13-15]</sup>

To provide optimal management of women who undergo NIPS with an initial no-call result, it is important to evaluate the factors associated with the success rate of a second NIPS. An informative second NIPS result is reportedly associated with several factors, including the initial fetal fraction, gestational age, the time interval between the two blood draws, and maternal body mass index (BMI).<sup>[16-18]</sup> However, these reports were based on limited numbers of cases and different NIPS platforms, and most conclusions were drawn from Caucasian populations. Therefore, it would be beneficial for NIPS providers in China to analyze the factors associated with a successful second NIPS based on a Chinese population and a single NIPS platform.

We conducted the present retrospective case study to analyze the data of women who underwent NIPS and received an initial no-call result from January 2017 to December 2019 in our center. We reviewed the information of 137 women with initial no-call results due to an insufficient fetal fraction and then evaluated the factors associated with a successful second NIPS. The initial fetal fraction and maternal BMI were the two most influential factors in this study, whereas neither the gestational age nor the time interval between the two blood draws was significantly relevant. The difference between the conclusions drawn from our study and those from previous reports also indicates that a specialized strategy for each center will be beneficial for the management of women who undergo NIPS with an initial no-call result.

## Methods

This study was approved by the institutional review board of the Nanjing Maternity and Child Health Care Hospital (No. NFLZ2019-KY-004). All enrolled participants provided written informed consent.

The data of women who underwent NIPS in the Prenatal Diagnosis Center of Nanjing Maternity and Child Health Care Hospital from January 2017 to December 2019 were reviewed. For accurate fetal fraction calculation, the inclusion criterion for this study was a single pregnancy with complete clinical information, and the exclusion criterion was a pregnancy without complete follow-up information.

We conducted a retrospective case analysis based on the NIPS results. Women with an initial no-call result were further selected for analysis. Among these cases, we collected data regarding the maternal and pregnancy characteristics of women who underwent a second NIPS, including maternal age, maternal BMI (recorded at first sampling), method of conception, parity, history of using heparin, gestational age at the first and second samplings, the time interval between the two samplings, a fetal fraction at the first and second NIPS, and NIPS results. The detailed NIPS procedure has been previously

described.<sup>[5,19]</sup> In brief, 5 mL of peripheral blood was collected from the pregnant woman. Libraries were generated using a Beijing Genomics Institute (BGI) protocol and sequenced on a BGISEQ-500 platform (BGI, Beijing, China). The fetal fraction in the cfDNA was calculated as previously described.<sup>[19]</sup> The minimal fetal fraction for a reportable result was 3.5%. Women with a no-call result were recommended to undergo a second blood draw and retest, and no additional fee was charged for the retest. Women who failed to receive a reportable result for a second time received a test failure report with a refund and were referred to specialists for genetic counseling.

Continuous variables are expressed as median with interquartile range (IQR) or mean  $\pm$  standard deviation (SD). Categorical variables are expressed as numbers and percentages. Continuous variables, including maternal age, maternal BMI, gestational age at the first samplings, the time interval between the two samplings, and fetal fraction at the first NIPS, were compared between the NIPS failure group and the NIPS success group after a second draw using the Mann-Whitney *U* test, and categorical variables, including method of conception, parity, and history of using heparin, were compared using the  $\chi^2$  test or Fisher exact test. Multivariate logistic regression analysis was used to further evaluate factors identified as significant by univariate analysis. All statistical analyses were carried using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant with a two-sided *P* value threshold of 0.05, and Bonferroni correction was used for multiple comparisons.

## Results

In total, 33,684 women with singleton pregnancies were enrolled in this study. Of these women, 244 (0.72%) did not have a reportable result at the initial test, including 142 (0.42%) due to an insufficient fetal fraction and 102 (0.30%) due to other causes. Among the 142 women with an insufficient fetal fraction, five refused to undergo a retest and were excluded from further analysis. A second NIPS was performed in the remaining 137 (96.48%) women, and the median time interval between the two blood draws was 9 days (IQR, 8.00–12.50). The fetal fraction was significantly higher at the time of redraw than at the initial blood draw ( $4.12\% \pm 1.85\%$  vs.  $2.90\% \pm 0.49\%$ , respectively;  $P < 0.001$ ). The detailed maternal and pregnancy characteristics of the 137 women are summarized in Table 1.

After the second NIPS, 87 (63.50%) women obtained an informative result, whereas the other 50 (36.50%) women failed to obtain an informative result for a second time because of an insufficient fetal fraction [Figure 1]. Our follow-up information confirmed no pregnancies with fetal trisomies in either group. We then compared the available maternal and pregnancy characteristics between the two groups. The results showed that the initial fetal fraction was significantly higher in women with than without a successful retest ( $3.13\%$  vs.  $2.72\%$ , respectively;  $P < 0.001$ ) and that the fetal fraction gain (change in the fetal fraction) at the second NIPS was also significantly

**Table 1: Baseline maternal and pregnancy characteristics of the 137 women who underwent a second NIPS test because of a low fetal fraction.**

Maternal and pregnancy characteristics	Results
<i>n</i>	137
Maternal age (years), Median (IQR)	31.00 (28.00–35.00)
Gestational age at first blood draw (weeks), Median (IQR)	17.00 (15.00–18.00)
Maternal BMI (kg/m <sup>2</sup> ), Median (IQR)	26.17 (22.90–29.09)
Parity	
Nulliparous	77
Parous	60
Conception	
IVF	10
Spontaneous	126
Unknown	1
History of using heparin	
Yes	5
No	132
Initial fetal fraction (%), Mean ± SD	2.90 ± 0.49
Time between draws (days), Median (IQR)	9.00 (8.00–12.50)
Second fetal fraction (%), Mean ± SD	4.12 ± 1.85

BMI: Body mass index; IQR: Interquartile range; IVF: *In vitro* fertilization; NIPS: Non-invasive prenatal screening; SD: Standard deviation.

success rate of the second NIPS. The results showed that the initial fetal fraction was significantly associated with the success rate of a second NIPS ( $P < 0.001$ ); however, the association of the BMI with the success rate of a second NIPS was marginal ( $P = 0.063$ ) [Table 3].

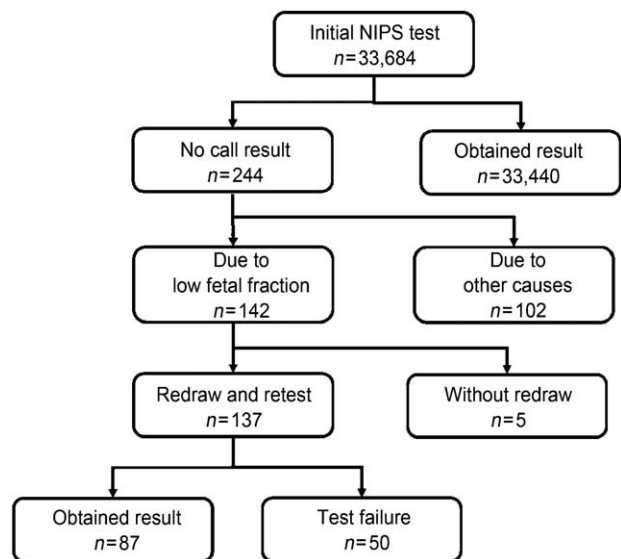
To further explore the effect of the initial fetal fraction on the success rate of a second NIPS, the women were classified into four different groups based on their initial fetal fraction:  $< 2.00\%$ ,  $2.00\text{--}2.49\%$ ,  $2.50\text{--}2.99\%$ , and  $\geq 3.00\%$ . The results showed that the proportion of women with a successful second NIPS in each of these groups was 0, 47.40%, 56.10%, and 80.90%, respectively, suggesting a positive correlation between the initial fetal fraction and the success rate of a retest [Figure 2A]. Notably, the percentage of women with a successful retest was significantly higher among those with an initial fetal fraction of  $\geq 3.00\%$  than in those with an initial fetal fraction of  $< 2.00\%$  ( $P < 0.001$ ),  $2.00\text{--}2.49\%$  ( $P = 0.003$ ), and  $2.50\text{--}2.99\%$  ( $P = 0.002$ ). However, there was no significant difference in the initial fetal fraction between the  $< 2.00\%$  and  $2.00\text{--}2.49\%$  groups ( $P = 0.026$ ) or between the  $2.00\text{--}2.49\%$  and  $2.50\text{--}2.99\%$  groups ( $P = 0.397$ ).

To examine the relationship between the maternal BMI and the success rate of a second NIPS, we classified the women into three groups by maternal BMI based on the World Health Organization obesity classification system: normal weight ( $< 25.00\text{ kg/m}^2$ ), overweight ( $25.00\text{--}29.99\text{ kg/m}^2$ ), and obese ( $\geq 30.00\text{ kg/m}^2$ ). The success rate of a retest in each of these groups was 76.40%, 53.80%, and 56.70%, respectively, with a negative correlation between the maternal BMI and the success rate of a retest. There was a significant difference in the retest success rate between the normal weight group and the overweight group ( $P = 0.014$ ), but no significant difference was identified in the other groups [Figure 2B].

**Discussion**

In this study, we retrospectively analyzed 137 women who underwent NIPS with an initial no-call result due to an insufficient fetal fraction in our center, among whom 87 (63.50%) women obtained a successful result after a second blood draw. As expected, a successful second NIPS was found to be positively correlated with the fetal fraction at the initial test and negatively correlated with the maternal BMI. However, other factors, including the time interval between the two blood draws and the gestational age, showed little relevance. The main strength of this study is that all women were from a single tertiary center in east China, which assures that the conclusions were drawn based on the same technical platform and the same population. In this way, our conclusion is potentially informative to other centers in China and may allow them to optimize their strategies for a second NIPS.

In contrast to our results, several previous studies showed that the time interval between two blood draws was positively related to the success rate of a second NIPS.<sup>[16,18]</sup> A possible explanation for this discrepancy is that in the present study, more than half of the women underwent



**Figure 1:** Flow diagram of all the 33,684 women who underwent NIPS. NIPS: Non-invasive prenatal screening.

different between the two groups (1.59% vs.  $-0.10\%$ , respectively;  $P < 0.001$ ) [Table 2]. In addition, the women who underwent a successful NIPS retest had a significantly lower maternal BMI than those who underwent failed retests ( $25.39\text{ kg/m}^2$  vs.  $27.24\text{ kg/m}^2$ ;  $P = 0.022$ ). Notably, there was no significant difference in maternal age, gestational age, time between draws, parity, method of conception, or history of using heparin between the two groups. We then performed a multivariate logistic regression analysis to further evaluate the factors affecting the

**Table 2: Comparison of characteristics between women who underwent NIPS and achieved a successful retest result and those who underwent a failed second test.**

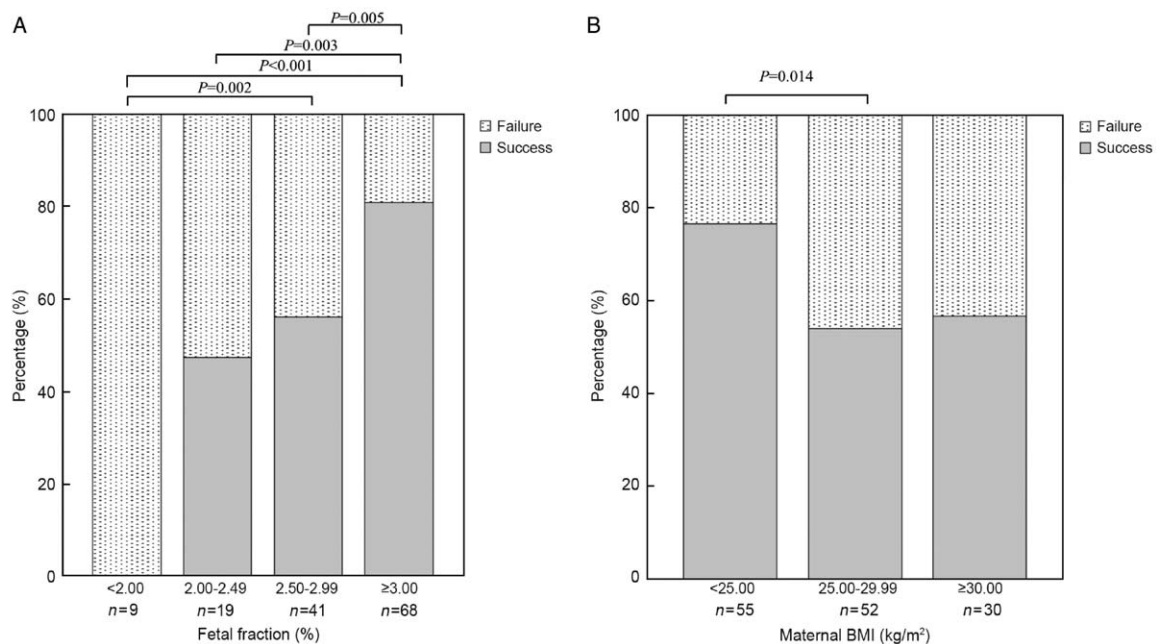
Maternal and pregnancy characteristics	Successful retest (n = 87)	Test failure (n = 50)	P values
Maternal age (yr), Median (IQR)	31.00 (28.00–35.00)	33.00 (27.00–35.00)	>0.999
Gestational age at first blood draw (weeks), Median (IQR)	16.00 (15.00–18.00)	17.00 (15.00–19.00)	0.165
Maternal BMI (kg/m <sup>2</sup> ), Median (IQR)	25.39 (22.58–28.49)	27.24 (24.90–30.96)	0.022*
Parity			0.452
Nulliparous	51	26	
Parous	36	24	
Conception			>0.999
IVF	6	4	
Spontaneous	80	46	
Unknown	1	0	
History of using heparin			>0.999
Yes	3	2	
No	84	48	
Initial fetal fraction (%), Median (IQR)	3.13 (2.85–3.37)	2.72 (2.24–3.05)	<0.001*
Time between draws (days), Median (IQR)	10.00 (8.00–14.00)	9.00 (8.00–11.00)	0.244

The two groups were compared using the Mann–Whitney *U* test for continuous variables and the  $\chi^2$  test or Fisher exact test for categorical variables. BMI: Body mass index; IQR: Interquartile range; IVF: *In vitro* fertilization; NIPS: Non-invasive prenatal screening. \**P* < 0.05.

**Table 3: Multivariate logistic regression analysis of factors affecting the success rate of the second NIPS after initial no-call result.**

Parameters	$\beta$	SE	<i>P</i>	OR (95% CI)
Initial fetal fraction	2.112	0.493	<0.001*	8.261 (3.145–21.701)
Maternal BMI	–0.103	0.052	0.063	0.902 (0.814–1.000)

\**P* < 0.05. BMI: Body mass index; CI: Confidence interval; NIPS: Non-invasive prenatal screening; OR: Odds ratio; SE: Standard error.



**Figure 2:** Effect of initial fetal fraction and maternal BMI on a successful second NIPS test. (A) The women were classified by their initial fetal fraction as follows: < 2.00%, 2.00–2.49%, 2.50–2.99%, and ≥3.00%. *P* values of < 0.008 were considered significant after Bonferroni correction. (B) The women were classified by maternal BMI according to the World Health Organization obesity classification system as follows: normal weight (<25.00 kg/m<sup>2</sup>), overweight (25.00–29.99 kg/m<sup>2</sup>), and obese (≥30.00 kg/m<sup>2</sup>). *P* values of < 0.0167 were considered significant after the Bonferroni correction. BMI: Body mass index; NIPS: Non-invasive prenatal screening.



their first NIPS in the second trimester, which is in accordance with the NIPS guideline in China. In contrast, most women in previous reports underwent their first NIPS in the first trimester. As a result, the time interval between the two blood draws was not a key factor for a successful second NIPS in our study, and this probably holds true throughout China.

The fetal fraction is a key factor for obtaining a reliable NIPS result, and an insufficient fetal fraction has been considered the most important cause of a no-call result in NIPS. The initial fetal fraction has consistently been found to be the most relevant factor determining a successful redraw.<sup>[18]</sup> Interestingly, we found that when the initial fetal fraction was  $< 2.00\%$ , the chance of a successful retest was very limited ( $0.00\%$ ,  $n = 9$ ). Our result indicates that avoidance of a second blood draw would be a proper management strategy for this subset of women.

Obesity is closely associated with the risk of NIPS test failure, and maternal BMI has been suggested to be an important predictor of test failure caused by an insufficient fetal fraction.<sup>[18,20,21]</sup> In addition, higher maternal weight was been reported to be associated with a low success rate of a second NIPS.<sup>[17,18,22]</sup> Our study showed a similar trend in Chinese women undergoing NIPS, although our multiple regression analysis revealed a marginal association, which was probably due to the small number of patients in this study. In this way, we reconfirmed the negative relationship between obesity and a successful second NIPS, and we thus recommend the use of the corresponding obesity classification standard during the consultation with women undergoing NIPS in clinical practice.

The major task in aneuploidy screening is to detect high-risk cases for further diagnosis with reasonable cost and sensitivity. Therefore, ideal management of the subset of women with failed NIPS results would minimize the chance of missing fetal aneuploidies and would help to avoid unnecessary invasive procedures. The beneficial role of a second NIPS remains controversial. Previous studies have revealed that the risk of fetal aneuploidy is higher in women with NIPS test failures.<sup>[23,24]</sup> Therefore, when a second NIPS fails, this subset of women with a high risk of fetal aneuploidy may miss the optimal time period for other possible screening methods. In 2016, the American College of Medical Genetics and Genomics stated that a repeat blood draw is not appropriate when women whose initial test results are not reported and that diagnostic testing should be offered.<sup>[15]</sup> However, with the increasing number of NIPS being performed, several recent studies have indicated that a no-call result is not necessarily associated with an increased risk of fetal aneuploidy.<sup>[22,25]</sup> In the present study, we found no fetal aneuploidies in the women who obtained no-call results. Thus, skipping a second NIPS for women with an average aneuploidy risk would only add unnecessary invasive procedures. Nevertheless, it would be beneficial to provide a specific recommendation according to the estimated success rate of a second NIPS.<sup>[18]</sup> For example, a previous study suggested that a retest would benefit women with a lower mean BMI and later gestational age.<sup>[26]</sup> In agreement with

this finding, our results have led us to propose a potential management strategy for women who undergo NIPS with an initial no-call result in our center. In brief, a retest would be recommended after NIPS test failure if the initial fetal fraction was  $> 2.00\%$ , and there would be limited benefit to elongate the time interval between the two blood draws [Supplementary Digital Content, Figure 1, <http://links.lww.com/CM9/A567>]. However, this strategy will be optimized continuously as more women undergo NIPS.

To conclude, we retrospectively analyzed factors affecting the achievement of a successful second NIPS in women with initial no-call results due to an insufficient fetal fraction. Our data showed the initial fetal fraction to be the most influential factor, followed by the maternal BMI. Our results suggest the need for a specialized management strategy for this subset of NIPS cases based on the factors affecting the success rate of a second NIPS in each center.

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### Conflicts of interest

None.

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