



Experience in the Application of Non-invasive Prenatal Screening for the Detection of Down Syndrome in Russia: A Retrospective Cohort Study

Elena V. Kudryavtseva^{1*}, Vladislav V Kovalev¹, Igor I Baranov², Andrew A Dektyarev³

Abstract

Objectives: Assessment the effectiveness of non-invasive prenatal screening (NIPS) for identifying pregnant women at high risk of giving birth to a child with Down syndrome.

Materials and Methods: The retrospective cohort study included 25798 pregnant women who underwent NIPS in Russia from January 2013 to December 2018. 21 042 and 4756 participants underwent tests from the Natera laboratory, USA (non-invasive prenatal test 1, NIPT-1) and the Genomed laboratory, Russia (non-invasive prenatal test 2, NIPT-2), respectively.

Results: A high risk of trisomy 21 (T21) was detected in 544 cases (2.59%). The mean age in women at high risk of T21 was 37.3 years. According to the NIPT results, in patients who revealed a low risk of T21, the mean age was 33.8 years ($P < 0.001$). In 535 cases, invasive prenatal diagnosis was performed. In 7 (1.3%) cases, the presence of T21 in the fetus was not confirmed. In 528 (97.3%) cases, T21 was confirmed by fetal karyotyping. Among women who revealed a low risk of T21 ($N = 25\,086$), in 4 cases (0.015%), fetal trisomy 21 was missed. Thus, the indicators of the effectiveness of NIPS in Russia in relation to T21 are as follows: sensitivity - 99.25%, specificity - 99.96%, PPV - 98.7%, NPV - 99.98%. In the first trimester, in the presence of fetal T21, the level of the fetal fraction of free-DNA is significantly lower.

Conclusions: NIPS has good prospects for implementation in pregnancy management programs and increasing the effectiveness of prenatal detection of T21. The level of the fetal fraction is associated with the presence of fetal T21.

Keywords: NIPT, Non-invasive prenatal screening, Prenatal diagnosis, Down syndrome, Trisomy 21, Prenatal care, Genetic screening

Introduction

Chromosomal abnormalities (CA) are one of the leading causes of perinatal, infant, and child mortality. Among all prenatally identified CA, the most common is trisomy 21 (T21) (1, 2). According to audit data in the Russian Federation (RF), the prenatal detection rate of fetuses with Down syndrome does not exceed 80%-85%.

Researchers all over the world have high hopes for non-invasive prenatal screening (NIPS), designed to identify pregnant women at high risk for the presence of CA in the fetus (3,4). The term "NIPS" refers to the isolation of fragments of maternal and placental DNA (traditionally called "fetal" DNA) from maternal plasma to identify major fetal aneuploidies. In some countries, NIPS has been used since 2011 (2,5), in RF - since 2013 (6).

Certainly, the most effective method for prenatal detection of T21 is cytogenetic karyotyping or chromosomal microarray analysis of biological materials obtained during an invasive procedure. However, given a certain level of possible complications (including spontaneous abortion), referral of pregnant women to invasive procedures should be carried out only through

the reasonable formation of high-risk groups of CA in the fetus (7).

In some works illustrating the value of NIPS, it is noted that today the main drawback of this research method is its high cost and the lack of its compensation by insurance companies (6,8). NIPS has some advantages over conventional first-trimester screening (the possibility of its use at an earlier stage, higher sensitivity, and specificity). Nevertheless, at the same time, a number of authors note that the commercialized and actively promoted into practice technology has significantly outstripped the necessary scientific research confirming its effectiveness (9). Therefore, we consider it appropriate to carry out a scientific analysis of the effectiveness of NIPS at the population level.

This study aims to evaluate the effectiveness of NIPS for identifying pregnant women at high risk of giving birth to a child with Down syndrome (T21).

Materials and Methods

Setting

The retrospective cohort study was conducted based

Received 22 October 2021, Accepted 11 May 2022, Available online 22 August 2022

¹Ural State Medical University, department of Obstetrics and Gynecology, Transfusiology, Ekaterinburg, Russian Federation. ²National Medical Research Center, Obstetrics, Gynecology, and Perinatology the name of Academician V.I. Kulakov, Moscow, Russian Federation. ³Clinical Institute of Reproductive Medicine, Ekaterinburg, Russian Federation.

*Corresponding Author: Elena V. Kudryavtseva, Tel +79226164012, Email: elenavladpopova@yandex.ru



Key Messages

- The present study demonstrates the effectiveness of non-invasive prenatal screening in identifying a high-risk group of trisomy 21 (Down syndrome) in the fetus.

on the Department of Obstetrics and Gynecology, Transfusiology of the Ural State Medical University (Ekaterinburg, RF) and the “Genomed” Medical Genetic Center (Moscow, RF). The study included 25 798 women who underwent the procedure of NIPS in RF in the period from January 2013 to December 2018. The blood plasma of a pregnant woman taken from a vein was used to conduct non-invasive prenatal tests. The blood was collected in special tubes (Streck, USA) with an extracellular DNA stabilizer. The clinical material was delivered to the laboratory in compliance with the transportation rules. Extracellular DNA can originate from cells (extravillous trophoblast) and trophoblastic microvesicles/exosomes, which are actively released into the maternal space, a significant portion originating from the remnants of syncytiotrophoblasts as a result of apoptosis and the release of multicellular fragments (10).

In the NIPT-1 targeted test, fetal extracellular DNA is isolated using the single nucleotide polymorphism (SNP) sequencing method and a proprietary algorithm from Natera. The patient's blood was taken at the “Genomed” center, the official distributor of Natera in Russia.

When carrying out the whole genome test NIPT-2 in the laboratory, blood was centrifuged to obtain plasma. Special kits were used to isolate extracellular DNA, performed amplification, followed by sequencing. As a result of high-throughput sequencing (next-generation sequencing, NGS) of extracellular DNA, about 5-10 million fragments were obtained. Each fragment was analyzed for belonging to a particular chromosome using bioinformatics analysis. The number of extracellular DNA fragments was determined. In the case of fetal trisomy 21, an increase in the total number of fragments of this chromosome is observed in comparison with the norm.

Participants

The average age of the women was 33.57 (29; 38) years. 21 042 and 4756 participants underwent tests from the Natera laboratory, USA (non-invasive prenatal test 1, NIPT-1) and the Genomed laboratory, Russia (non-invasive prenatal test 2, NIPT-2), respectively.

Inclusion criteria for the study: singleton, clinically confirmed pregnancy more than 9 weeks old when using the NIPT-1 test and more than 10 weeks when using the NIPT-2 test, informed consent of the patient for the study and for conducting the test. Exclusion criteria: multiple pregnancies (including those with spontaneous reduction of one of the fetuses), cancer in the mother, the presence of karyotype abnormalities in one of the parents, pregnancy

resulting from IVF using a donor egg, surrogacy. The exclusion criterion from the study was the refusal to perform the test before the result was obtained.

Variables

Determination of the presence or absence of trisomy 21 in the fetus was carried out using invasive prenatal diagnostics and prenatal karyotyping (in high-risk patients). Patients of the low-risk group underwent examination of the child after birth; in the presence of phenotypic signs of Down's syndrome, a cytogenetic study was carried out. The results of the examination and the cytogenetic study were compared with the results of the NIPS.

Data Sources/Measurement

Data collection was carried out using a medical electronic system and by analyzing the patient's medical records.

Bias

To avoid reporting bias, we report both positive and negative results in our study. The results were compared with similar studies conducted in other countries. Authors did not receive funding from laboratories where NIPTs were performed

Sample Size

The sample size was not pre-calculated and was determined by the planned study time.

Quantitative Variables

To examine if the a variable is normally we conducted Shapiro-Wilk test. The median and interquartile ranges (Me (Q1, Q3)) are indicated to assess quantitative indicators.

Data Analysis

Mathematical data processing was carried out using the Microsoft Excel 7.0 and Statistica 10.0 software. Qualitative indicators are presented in absolute and relative values (%). The analysis of qualitative signs (the number of high-risk T21 in the fetus in different groups) was carried out using contingency tables using the χ^2 test. The Mann-Whitney test was used to assess the statistical significance of differences between patient groups. Differences were considered statistically significant at $P < 0.05$. Sensitivity, specificity, positive and negative predictive values were determined based on the number of true-positive, false-positive, true-negative, and false-negative results. The likelihood ratio was calculated to indicate the 95% confidence interval (CI).

Results

All pregnant women included in the study lived in the RF and were Slavic. The largest number of participants decided on the need for NIPS in the period of conventional first-trimester screening - 11-14 weeks of gestation. This

is probably because when receiving an unsatisfactory or questionable result of standard screening, the women were informed about the availability of an alternative method. However, some of the women underwent this procedure at many later stages of pregnancy, including in the third trimester. As a result, the average gestational age of the study group women was 14 weeks and 6 days (median - 13 weeks and 4 days).

A high risk of T21 was detected in 544 cases (2.59%): 402 cases were determined using the NIPT-1 test, 142 using the NIPT-2 test. Comparison of patients with high and low risk of trisomy 21 in the fetus is presented in Table 1. Statistically significant differences were obtained only for the age of the participants. According to their mass and height characteristics, the participants of both groups are clinically comparable.

The average gestational age at which a high risk of T21 was identified was 14 weeks, and 83 (15.2%) participants with a high risk of T21 based on NIPT results underwent this screening up to 11 weeks.

Invasive prenatal diagnosis was recommended for all pregnant women with a high risk of T21 based on the NIPS results. A number of specialists believe that if the result of NIPS is obtained before 12 weeks and a high risk of T21 is identified, it would be possible to recommend the patient to terminate the pregnancy, which will be formalized as an artificial abortion at the request of the patient. However, we consider this approach unacceptable because, despite the fact that NIPS has high sensitivity and specificity (as we wrote about earlier) (6), it cannot be considered a diagnostic test and is just a screening study.

Among the participants with high T21 risk according to the NIPS results, in 4 (0.7%) cases, a missed abortion was diagnosed even before the NIPS result was obtained; a genetic study of the fetus was not carried out. There is no information about two women. Whether they underwent invasive prenatal diagnostics is unknown. In 3 cases, NIPS, which revealed a high risk of Down syndrome, was already performed in the third trimester of pregnancy. An invasive diagnosis was not performed, but after delivery, Down syndrome was confirmed clinically and by karyotyping in all three newborns. In 535 cases, invasive prenatal diagnosis was performed. In 7 (1.3%) cases, the presence of T21 in the fetus was not confirmed - a normal

karyotype was determined. In 528 (97.3%) cases, trisomy 21 was approved by fetal karyotyping.

Among participants who, according to the results of NIPS, revealed a low risk of T21 (N=25 086), in 4 cases (0.015%), trisomy 21 was missed in the fetus. In two of them, morphological abnormalities (fetal malformations), markers of chromosomal abnormalities CA were revealed by ultrasound examination in the 18-21-week gestation period. Therefore, the women, despite the NIPS result, were referred for cordocentesis. Moreover, T21 was detected in the fetus in both cases, and the pregnancy was terminated. In two cases, children were born with verified Down syndrome.

In 17 026 cases, pregnancy ended in the birth of a child without clinical signs of Down syndrome. In 98 cases, antenatal fetal death occurred, in 7962 cases, there is no information on pregnancy outcomes (however, they did not report the birth of a child with Down syndrome). Thus, the number of false-negative results was 0.02%.

All false-positive and false-negative results were obtained with the NIPT-1 test, and with the NIPT-2, there were no reports of false-positive or false-negative results. At the same time, we consider it incorrect to compare the sensitivity and specificity of these tests since the Russian test NIPT-2 has been performed to date in a much smaller number of participants than the test NIPT-1. Therefore, we calculated the indicators of the effectiveness of NIPS in relation to the detection of trisomy 21 in total for both tests (Table 2). We calculated these indicators only among participants who underwent invasive prenatal diagnostics, and the karyotype of the fetus was determined, or the outcome of pregnancy is known (the birth of a healthy child or a child with a chromosomal pathology).

In 1181 (4.6%) participants, during the NIPS, the first analysis was ineffective due to a low level of fetal fraction (FF) of cell-free DNA, or the DNA quality control was not passed (Figure 1). The participants were re-analyzed after 7-14 days. The second time the result was obtained for 809 (80%) participants who agreed to repeat the study. Among the participants who received the result on the second attempt, a high risk of T21 was detected in 24 (3%), on the third attempt - in 6 (7.2%). Among all women with initially unsuccessful NIPS, the incidence of high-risk T21 was 2.54%. Still, if we do not consider the participants who refused to repeat the analysis at various

Table 1. Comparison of Groups of Patients With Low and High Risk of Trisomy 21 in the Fetus

	High Risk Women Me (Q1; Q3)	High Risk Women Me (Q1; Q3)	P Value ^a
Age (y)	33.8 (29.9; 38)	37.3 (33; 40.6)	<0.001
Height (cm)	164.5 (162; 170)	163.9 (162.2; 169)	0.345
Weight (kg)	63.4 (57.25-74.15)	63.8 (58.05; 73.95)	0.563
BMI (kg/cm ²)	23.57 (21.06; 27.42)	23.41 (21.06; 27.42)	0.788

BMI, body mass index.

^a Mann-Whitney test.

Table 2. Indicators of the Effectiveness of NIPS in Relation to the Detection of Trisomy 21

Index	Value, % (95% CI)
Sensitivity	99.25 (98.1-99.8)
Specificity	99.96 (99.92-99.98)
Positive predictive value	98.7 (97.34-99.48)
Negative predictive value	99.98 (99.94-99.99)

CI, confidence interval.

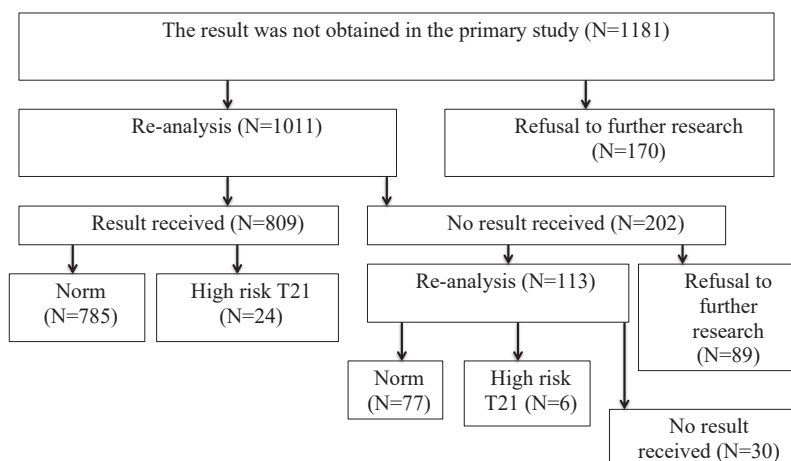


Figure 1. The Study Flowchart.

stages and did not receive the result, the final figure rises to 3.38%. Among 24617 participants who received the result 1 time, 514 cases of high risk of T21 (2.09%) were identified. Statistically significant differences were obtained compared to participants in whom the analysis was twice ineffective ($\chi^2 = 5.58, P=0.02$).

In retrospect, we compared the mean fetal fraction (proportion of fetal DNA among the total cell-free DNA) in the 1st and 2nd trimesters in participants with fetal T21 and in participants who eventually gave birth to a healthy baby. The results are presented in Table 3 and Figure 2.

In the first trimester, in the presence of T21, the level of the fetal fraction was significantly lower when using both tests. In the second trimester, such a pattern was not obtained.

Discussion

In RF, NIPS is used most often as a second-tier test. According to the pregnancy management standard, in RF patients with a risk level of fetal CA (>1:100), calculated during the conventional first-trimester combined screening (ultrasound, PAPP-A, β -hCG), should be directed to invasive diagnostics. But in a significant part of cases, patients refuse this procedure, fearing possible complications, and are looking for alternative screening options, the main of which is NIPS. NIPS decreases unnecessary invasive procedures and enables people to

know whether the fetus is affected and prepare without risking miscarriage (11–13).

To date, it is unclear whether the complete replacement of the conventional first-trimester combined screening with NIPS will become cost-effective in RF (on the one hand, the number of newborns with Down syndrome may decrease, on the other hand, the cost of detecting 1 additional case of trisomy 21 will increase) - further research is required. If we use the so-called contingent screening (that is, send only patients with a certain level of risk calculated during conventional first-trimester screening, or in the presence of certain risk factors). In that case, it is necessary with the help of further studies, including economic ones, to accurately determine a group of patients who require the second-tier study (1, 13, 14). In many countries, such studies have already been carried out, for example, in China (13, 15), the United Kingdom (14), Australia (16), Turkey (17). The Netherlands launched a nationwide implementation study on non-invasive prenatal testing as a first-tier test offered to pregnant women (2). Most researchers agree that NIPS leads to very high costs despite its high effectiveness (17). Implementation of NIPS within a public sector Down syndrome screening program can improve quality of care, and contingent screening may be an appropriate strategy to balance the effectiveness and cost factors of the new technology (13, 14). At the same time, technology

Table 3. Average Level of Fetal Fraction

Test/Trimester of pregnancy	Fetal Fraction, % (Me (Q1; Q3))		P Value ^a
	T21+	T21-	
NIPT-1/1st trimester	9.1 (6.6; 11.9)	9.65 (7.47; 12.1)	0.044
NIPT-1/2nd trimester	11.01 (8.03; 13.5)	10.3 (7.5; 12.8)	0.689
NIPT-2/1st trimester	7.71 (5.3; 10.2)	8.95 (6.23; 11.7)	0.037
NIPT-2/2nd trimester	9.4 (6.4-12.31)	8.65 (6.33; 12.1)	0.311

T21+: participants with confirmed fetal trisomy 21; T21-: participants without fetal trisomy 21; 1st trimester: 9-14 weeks of pregnancy; 2nd trimester: 14.1-22 weeks of pregnancy.

^a Mann-Whitney test.

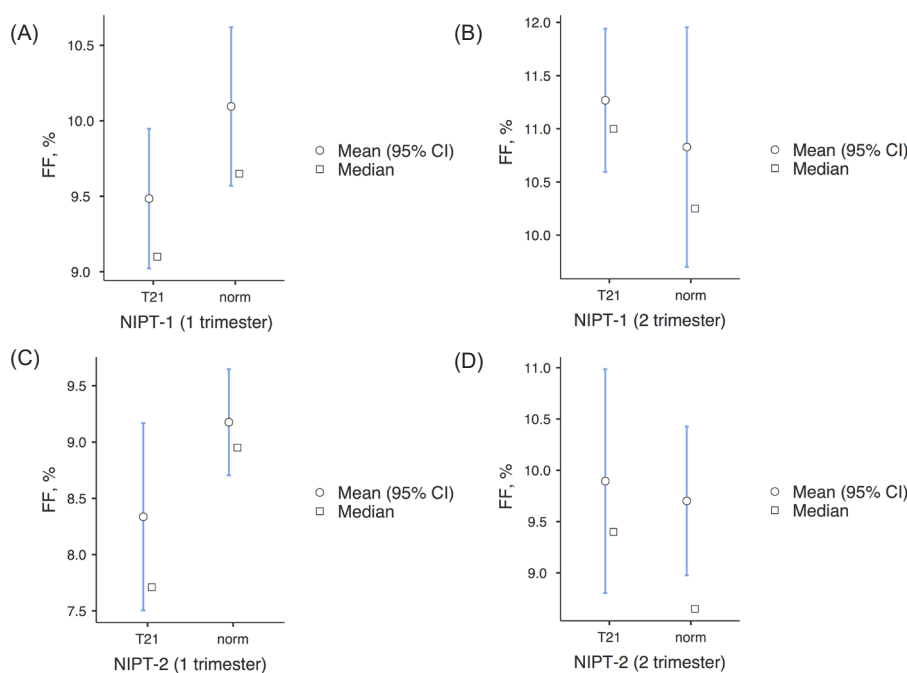


Figure 2. The Level of the Fetal Fraction, %. (A) NIPT-1, 1st trimester; (B) NIPT-1, 2nd trimester; (C) NIPT-2, 1st trimester; (D) NIPT-2, 2nd trimester.

develops further, and the cost of sequencing falls (18). The introduction of NIPS would reduce the number of invasive diagnostic procedures and procedure-related fetal losses (16). Nevertheless, it can already be argued that NIPS is an effective tool for identifying high-risk pregnant women for the presence of fetal T21. Therefore, patients should be informed about this study and the advantages and limitations of the method (11). In case of revealing a high risk of CA based on the results of NIPS, invasive prenatal diagnostics must be carried out, which must be reported to the patients at the stage of pretest counseling. NIPS is a screening, not a diagnostic method (19, 20).

In some cases, during the first blood test, NIPS is ineffective. In the overwhelming majority of cases, NIPS is not effective due to the low level of the fetal fraction. It raises the question of harms related to increased uncertain and unknown results (21). One of the possible reasons for a decrease in the level of the fetal fraction may be the presence of CA in the fetus (22). According to our data, if the fetus has T21, the level of the fetal fraction in the 1st trimester is lower than normal. However, in 80% of cases in our study, the reanalysis was successful, and the frequency of high-risk T21 was slightly higher than that among patients who received the first result. If retesting is not successful, then the chances of getting a result with retesting are reduced, and the risk of having trisomy 21 in the fetus increases. Therefore, we believe that in the event that NIPS was twice unsuccessful, the issue of invasive prenatal diagnosis should be resolved. Further research is required to assess the cost-effectiveness of NIPS. It is planned to study the assessment of the effectiveness of NIPS not only in relation to T21 but also in relation to

other frequent and rare CA.

Limitation

The study was conducted on Slavic women, in women of a different race and nationality, the results may differ, the effectiveness of NIPS was assessed only in relation to T21.

Conclusion

NIPS has good prospects for implementation in pregnancy management programs in RF and for increasing the efficiency of prenatal detection of T21 due to its high sensitivity and specificity. The level of the fetal fraction is associated with the presence of trisomy 21 in the fetus: if it is present, the average level of the fetal fraction is lower. With repeated studies of NIPS, the likelihood of detecting T21 increases significantly. In the case of two ineffective NIPS studies, the following attempts are inappropriate, and the patient should be referred for invasive diagnostics.

Authors' Contribution

EVK was responsible for conceptualization and methodology, EVK designed the study and led the conduction of the research. VVK took part in investigation and formal analysis of the results. IIB and AAD contributed in validation and analyses of the obtained results of the study. All authors contributed in writing—Original Draft Preparation and Review and Editing; all authors approved the final manuscript and take responsibility for the integrity of the data.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Issues

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Ural State Medical University (protocol №2, 28.02.2020).

Financial Support

Research and publication of the article were carried out at the personal expense of the group of authors.

Acknowledgments

We acknowledge Prof. Korostelev S.A., Dr. Kievskaya Yu.K., Dr. Kanivets I.V. (Genomed LTD, Russia) for their help in collecting data.

References

- Zhang W, Mohammadi T, Sou J, Anis AH. Cost-effectiveness of prenatal screening and diagnostic strategies for Down syndrome: a microsimulation modeling analysis. *PLoS One*. 2019;14(12):e0225281. doi:10.1371/journal.pone.0225281
- van der Meij KRM, Sistermans EA, Macville MVE, et al. TRIDENT-2: national implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in the Netherlands. *Am J Hum Genet*. 2019;105(6):1091-1101. doi:10.1016/j.ajhg.2019.10.005
- Allyse M, Minear MA, Berson E, et al. Non-invasive prenatal testing: a review of international implementation and challenges. *Int J Womens Health*. 2015;7:113-126. doi:10.2147/ijwh.s67124
- Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn*. 2013;33(6):575-579. doi:10.1002/pd.4103
- Donner C, Daelemans C, Ceyssens G. (Prenatal screening: the example of Down's syndrome screening). *Rev Med Brux*. 2015;36(4):207-211.
- Kudryavtseva EV, Kanivets IV, Kievskaya Yu K, Baranov II, Kovalev VV, Korostelev SA. Noninvasive prenatal testing in Russia: a population study. *Obstet Gynecol*. 2019;12:30-35. doi:10.18565/aig.2019.12.30-35
- American College of Obstetricians and Gynecologists. Committee opinion no. 640: cell-free DNA screening for fetal aneuploidy. *Obstet Gynecol*. 2015;126(3):e31-e37. doi:10.1097/aog.0000000000001051
- Tetruashvili NK, Kim LV, Parsadanyan NG, et al. Noninvasive prenatal DNA test as a screening procedure for women from different risk groups: a view on the problem. *Obstet Gynecol*. 2016;8:24-28. doi:10.18565/aig.2016.8.24-28
- Kascheeva TK, Kuznetzova TV, Baranov VS. New technologies and trends of prenatal diagnostics. *J Obstet Womens Dis*. 2017;66(2):33-39. doi:10.17816/jowd66233-39
- Del Gobbo GF, Konwar C, Robinson WP. The significance of the placental genome and methylome in fetal and maternal health. *Hum Genet*. 2020;139(9):1183-1196. doi:10.1007/s00439-019-02058-w
- van Schendel RV, Kater-Kuipers A, van Vliet-Lachotzki EH, Dondorp WJ, Cornel MC, Henneman L. What do parents of children with Down syndrome think about non-invasive prenatal testing (NIPT)? *J Genet Couns*. 2017;26(3):522-531. doi:10.1007/s10897-016-0012-4
- Seror V, L'Haridon O, Bussi eres L, et al. Women's attitudes toward invasive and noninvasive testing when facing a high risk of fetal Down syndrome. *JAMA Netw Open*. 2019;2(3):e191062. doi:10.1001/jamanetworkopen.2019.1062
- Xu Y, Wei Y, Ming J, et al. Cost-effectiveness analysis of non-invasive prenatal testing for Down syndrome in China. *Int J Technol Assess Health Care*. 2019;35(3):237-242. doi:10.1017/s0266462319000308
- Chitty LS, Wright D, Hill M, et al. Uptake, outcomes, and costs of implementing non-invasive prenatal testing for Down's syndrome into NHS maternity care: prospective cohort study in eight diverse maternity units. *BMJ*. 2016;354:i3426. doi:10.1136/bmj.i3426
- Poon CF, Tse WC, Kou KO, Leung KY. Uptake of noninvasive prenatal testing in Chinese women following positive Down syndrome screening. *Fetal Diagn Ther*. 2015;37(2):141-147. doi:10.1159/000365811
- O'Leary P, Maxwell S, Murch A, Hendrie D. Prenatal screening for Down syndrome in Australia: costs and benefits of current and novel screening strategies. *Aust N Z J Obstet Gynaecol*. 2013;53(5):425-433. doi:10.1111/ajo.12136
-  okem ZG,  org ul G, Kasnakoglu BT,  akar M, Beksa c MS. Economic analysis of prenatal screening strategies for Down syndrome in singleton pregnancies in Turkey. *Eur J Obstet Gynecol Reprod Biol*. 2017;219:40-44. doi:10.1016/j.ejogrb.2017.09.025
- Drury S, Hill M, Chitty LS. Cell-free fetal DNA testing for prenatal diagnosis. *Adv Clin Chem*. 2016;76:1-35. doi:10.1016/bs.acc.2016.05.004
- Belloin C, Jacquemard F, Bernab e-Dupont C, Viot G, Lohmann L, Grang e G. (The noninvasive prenatal testing for Down's syndrome. Retrospective study of 8821 patients). *J Gynecol Obstet Biol Reprod (Paris)*. 2016;45(9):1127-1132. doi:10.1016/j.jgyn.2016.01.007
- Mackie FL, Hemming K, Allen S, Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG*. 2017;124(1):32-46. doi:10.1111/1471-0528.14050
- D'Ambrosio V, Squarcella A, Vena F, et al. Update in non-invasive prenatal testing. *Minerva Ginecol*. 2019;71(1):44-53. doi:10.23736/s0026-4784.18.04306-x
- Suciu ID, Toader OD, Galeva S, Pop L. Non-invasive prenatal testing beyond trisomies. *J Med Life*. 2019;12(3):221-224. doi:10.25122/jml-2019-0053

  2022 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.