УДК 618.2-07:575
DOI: https://doi.org/10.17816/JOWD56573

Современное значение неинвазивного пренатального исследования внеклеточной ДНК плода в крови матери и перспективы его применения в системе массового скрининга беременных в Российской Федерации

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В обзоре проведен анализ применения в разных странах мира неинвазивного пренатального скринингового тестирования хромосомных аномалий по внеклеточной ДНК плода в крови матери. Обсуждены диагностические возможности метода, его ограничения, модели применения и этические вопросы, связанные с его использованием. Приведены данные по дискордантным результатам. Представлены преимущества полногеномного варианта анализа внеклеточной ДНК плода и проблемы, связанные с его применением при массовом скрининге. На основе результатов массового комбинированного раннего пренатального скрининга в четырех субъектах Российской Федерации, достигнутых к 2019 г., предложена контингентная модель внедрения данного метода на наиболее частые трисомии (по хромосомам 21, 18 и 13) в систему пренатальной диагностики в России в качестве дополнительного скрининга в группе среднего риска (при отсечках от 1 : 100 до 1 : 500 либо от 1 : 100 до 1 : 1000), сформированной в субъектах по результатам раннего пренатального скрининга. Сформулированы основные требования к внедрению контингентной модели в субъектах Российской Федерации.

Ключевые слова: неинвазивный пренатальный скрининг; неинвазивное пренатальное тестирование; внеклеточная ДНК плода; хромосомные аномалии; ранний пренатальный комбинированный скрининг.

Как цитировать:
Калашникова Е.А., Глотов А.С., Андреева Е.Н., Барков И.Ю., Бобровник Г.Ю., Дубровина Е.В., Жученко Л.А. Современное значение неинвазивного пренатального исследования внеклеточной ДНК плода в крови матери и перспективы его применения в системе массового скрининга беременных в Российской Федерации // Журнал акушерства и женских болезней. 2021. Т. 70. № 1. С. 19–50. DOI: https://doi.org/10.17816/JOWD56573

Рукопись получена: 27.12.2020 **Рукопись одобрена:** 19.01.2021 **Опубликована:** 22.02.2021

DOI: https://doi.org/10.17816/JOWD56573

Current relevance of non-invasive prenatal study of cell-free fetal DNA in the mother's blood and prospects for its application in mass screening of pregnant women in the Russian Federation

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This review article offers an analysis of application of cell-free fetal DNA non-invasive prenatal screening test for chromosome abnormalities in the mother's blood in different countries. The diagnostic capacities of the method, its limitations, execution models and ethical aspects pertinent to its application are discussed. The data for the discordant results is shown and analyzed. The advantages of the genome-wide variant of cell-free fetal DNA analysis and the problems concerning its application in the mass screening are described. The main suggestion is to implement the contingent cell-free fetal DNA testing model for the common trisomies (for the chromosomes 21, 18 and 13) into the prenatal diagnostic screening programs in the Russian Federation. This novel model is based on the results of the mass combined first trimester prenatal screening in four federal subjects of the country completed by 2019 and is offered as an additional screening in the mid-level risk group (with cut-off from 1 : 100 to 1 : 500 or from 1 : 100 to 1 : 1000) defined according to the first trimester prenatal screening results. The basic requirements for the implementation of the contingent model in the Russian Federation are stated.

Keywords: non-invasive prenatal screening; non-invasive prenatal test; cell-free fetal DNA; chromosomal abnormalities; early prenatal combined screening.

To cite this article:
Kalashnikova EA, Glotov AS, Andreyeva EN, Barkov IYu, Bobrovnik GYu, Dubrovina EV, Zhuchenko LA. Current relevance of non-invasive prenatal study of cell-free fetal DNA in the mother's blood and prospects for its application in mass screening of pregnant women in the Russian Federation. *Journal of Obstetrics and Women's Diseases.* 2021;70(1):19–50. DOI: https://doi.org/10.17816/JOWD56573

Received: 27.12.2020 **Accepted:** 19.01.2021 **Published:** 22.02.2021

The team of authors devotes their work to the founder of Russian prenatal diagnostics, teacher, colleague and associate, corresponding member of the Russian Academy of Sciences, Professor, PhD in Medicine Vladislav Sergeevich Baranov to commemorate his anniversary

INTRODUCTION

In the classification of congenital and hereditary human diseases, a special section is dedicated to chromosomal abnormalities (CA) because of their frequency and predominantly the spontaneous nature of occurrence. Being manifested primarily as an error in the process of gametogenesis and early embryogenesis, this group of pathological conditions is a significant cause of morbidity and mortality, affecting public health indicators.

In the diverse range of human chromosomal pathology, 45% of cases are related to the aneuploidies of sex chromosomes and 25% of cases belong to the group of autosomal trisomies, with the most common being trisomies of chromosomes 21, 18, and 13 (Tr21, Tr18, and Tr13). The lack of safe and effective genetic methods for clinical practice and correct hereditary pathology necessitates the improvement of existing and a search for new prenatal testing methods as the primary strategy for the mass prevention of chromosomal diseases.

The methodology of combined early prenatal screening (EPS), developed by the Fetal Medicine Foundation (FMF, headed by Prof. K. Nikolaides, London); ultrasound (US) examination performed by specially trained and licensed specialists; and the study of maternal blood serum markers and calculation of the individual risk of CA at a term of 11–14 weeks of gestation are the most successful and demanded methodology. While calculating the risk, the baseline risk (maternal age and gestational age) and the probability ratios of significant factors, such as a number of indicators of anamnesis and maternal status, US and serum markers (free beta-subunit of chorionic gonadotropin [free β-HCG] and pregnancy-associated plasma protein A [PAPP-A]), are considered [1]. According to the above algorithm, the systematic Cochrane Review analyzed the data of combined prenatal screening for Down syndrome (Tr21) in the first trimester of pregnancy. The review included 152 publications over 31 years (1,604,040 screening results of 8,454 Tr21 cases) and demonstrated a screening sensitivity of more than 90% with 3–5% false positive results (FPR). The effect of this screening model was also manifested in a sharp decrease in the size of the high-risk group of CA and the number of invasive procedures, previously based mostly on maternal age and serum markers [2].

The Laboratory of prenatal diagnostics of Hereditary Diseases of the Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott successfully tested the model of combined screening for the first trimester of pregnancy. This result enabled the recommendation of this technology in 2006 in St. Petersburg for reducing the frequency of birth of children with Down syndrome. By 2011, it reached 70–80% of the detection rate of Tr21 in the group of pregnant women aged 35 and above [3].

In 2009, the Ministry of Health and Social Development of Russia initiated the widespread use of EPS according to the international standards and the phased implementation of a new algorithm in the country's constituent entities from 2010 to 2014. At a joint meeting of the Presidential Council of the Russian Federation on the implementation of national priority projects and demographic policy and interdepartmental working group on the national priority project "Zdorovie" and demographic policy, the support for the formation of a new system of prenatal diagnostics in the country was proposed on February 17, 2010. For the unified interdisciplinary training of health care organizers, obstetricians–gynecologists, geneticists, and US diagnostics specialists (upon the initiative of the Ministry of Health of Russia in 2011), the course "prenatal diagnostics" was organized in the structure of the Russian Medical Academy of Postgraduate Education of the Ministry of Health of Russia as an educational platform for prenatal screening at the Department of Medical Genetics (Order No. 63 of 04/22/2011 of the Russian Medical Academy of Continuing Professional Education). This department was separated in 2018 into an independent subdivision of the pediatric faculty.

To date, within the Program of state that guarantees for the free provision of medical care to Russian citizens in the first trimester of pregnancy, mass prenatal screening is performed, followed by a programmed calculation of the risk of CA and confirmatory (invasive) diagnostics, in the high-risk group. The Astraya software unifies all the subjects, provides the calculation of the CA risk, and facilitates the storage of screening results. Moreover, the software allows a digital audit and assessment of the quality of all EPS activities in the regions. Since 2014, under the authority of the Department of Medical Care for Children and the Obstetrics Service of the Ministry of Health of Russia, the Russian Medical Academy of Postgraduate Education is performing the audit.

By 2019, the EPS coverage in Russia amounted to more than 80% of women registered for pregnancy, with an average value of about 2% representing the high-risk group of CA (the border of the high-risk group is ≥1:100). The detection frequency of frequent CA (by the example of Tr21) increased to 84% compared to 30% and 12% in 2007 and 2004, respectively. The efficiency of prenatal karyotyping in confirming invasive diagnostics in the high-risk group reached 30% as compared to 5–6% in the period 2000–2009 [4].

DOI: https://doi.org/10.17816/JOWD56573

At the same time, the research on the development and use of new technologies is ongoing to increase the efficiency of prenatal detection of CA. In recent years, close attention has been paid to actively developing the technology of non-invasive prenatal testing (NIPT), based on the analysis of free (extracellular) fetal DNA (ecDNA) floating in the blood of a pregnant woman. In less than a decade, the prenatal testing of extracellular DNA to determine the fetus genetic pathology has developed from isolated works implementing the principle of research to the proposals for a global transformation of prenatal medicine [5]. As of the end of 2017, a total of 4–6 million pregnant women underwent plasma ecDNA analysis for fetal aneuploidy [6].

History of NIPT

Much of the NIPT prevalence is attributed to the remarkable progress made in DNA sequencing technology over the past 15 years. The cost of research while maintaining (improving in some cases) the quality of analysis has been decreased by millions of dollars. Moreover, the cost of sequencing the human genome has reached USD 100, and the analysis time has been reduced to several days. The natural result of this progress was the development of non-invasive testing of chromosomal and some gene mutations (along with microdeletions) in the fetus, based on the analysis of trace amounts of free fetal DNA in the blood of a pregnant woman. Such DNA is found in the blood starting from the fifth week of pregnancy. After weeks 9–10, its amount is already sufficient for NIPT. The fetal component of ecDNA in the mother's blood mainly comes from cytotrophoblast cells [7], whose karyotype is analyzed by the invasive cytogenetic study of the so-called direct preparations (or short-term cultures) of chorionic villi [8].

The Russian scientist V.I. Kazakov and the Chinese scientist Dennis Lo obtained the first results on fetal ecDNA in the blood of pregnant women [9, 10]. In 2008, Lo was first to reveal the possibility of using next-generation sequencing (NGS) technology for NIPT, after which many companies got involved in technology development. Soon, the approach was radically improved and became actively used by a number of leading centers for molecular diagnostics in the USA. Already in November 2011, the International Association for prenatal diagnostics (the USA) offered its official support for the method began to be widely used, first, for prenatal screening for Down's disease and, then, for detecting frequent trisomies in other autosomes (18, 13) and abnormalities in different sex chromosomes [11]. After revision, mainly because of an increase in the number of the genome reads, the method has also started to be applied in the diagnostics of chromosomal rearrangements, primarily "deletion syndromes," namely the syndrome of deletion of the chromosome 4 short arm (4p-, Wolf-Hirschhorn syndrome), the syndrome of deletion of the chromosome 5 short arm (5p-, cri du chat disease), and so on.

The non-invasive prenatal test is already widely used in the USA, Western Europe, and China. This technology has appeared relatively recently in Russia, with the Genoanalytica company creating the first Russian analog of international technologies in 2014. Then, its own version was proposed by the V.I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology [12]. In 2018, the original version was developed at the Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott [13]. Other private companies and state research centers are yet to present their inventions.

Nowadays, in Russia, about 30–40 thousand tests are performed using NIPT per year and mainly in private centers and clinics, which, except for two state and five to six private centers, send blood samples of pregnant women to other countries (up to 50% of all tests) and do not bear any responsibility for the quality of diagnostics and interpretation of results.

Non-invasive prenatal examination represents a technologically complex and time-consuming process, primarily involving steps such as taking a blood sample from a patient and obtaining plasma, DNA isolation and sample preparation, sequencing (or another method for determining aneuploidy), bioinformatic analysis, and issuing a report. Any PCR laboratory cannot perform this test. Its implementation requires well-trained specialists not only in laboratory science but also in bioinformatics along with expensive equipment and special conditions. Even though it takes no more than 2.5–3 days to perform all stages of NIPT in the laboratory, the actual time for the analysis is about 5–14 days [14].

The main variants of non-invasive screening for extracellular DNA

In our opinion, the concept of non-invasive prenatal screening (NIPS) as a technology for the mass prenatal examination of pregnant women to detect CA (and other possible genetic defects) in the fetus should be distinguished from the concept of NIPT as a test. NIPT represents the screening of chromosomal and gene mutations for fetal extracellular DNA and is performed using different NIPT variants. The variant 1 is genome-wide. To implement this test, the technology of genome-wide mass parallel DNA sequencing is used. The ratio of copies of DNA fragments of various fetal chromosomes to those of the mother is calculated when the genome of the sample containing DNA of the mother and the fetus is sequenced with low coverage (0.3–0.5×). This test enables the detection of aneuploidy on all chromosomes and rule out some microdeletion syndromes. Its main advantage over other test variants is that it covers up to 98% of all CAs.

DOI: https://doi.org/10.17816/JOWD56573

The second variant of the test is targeted and can be implemented using both NGS technology (200–1000× with high coverage) and other technologies, such as microchips, real-time polymerase chain reaction (PCR) (digital PCR), the rolling circle technology, and so on [15]. Within this test, certain aneuploidies and the corresponding syndromes, namely Tr21 (Down's syndrome), Tr18 (Edwards syndrome), Tr13 (Patau's syndrome), X monosomy (Shereshevsky–Turner syndrome), Klinefelter's syndrome, and some others, are detected. The advantage of the test is its higher specificity, but no more than 80–85% of all CAs of the fetus are determined with this variant. Tests for the three main trisomies comprise even more unpretentious characteristics, not to mention testing only for Down's syndrome. We believe that the latter version of NIPT is not only ineffective from a diagnostic point of view (it enables to detect no more than 65% of all abnormal fetuses), but actually misleads the patient while intentionally distorting the essence of screening for chromosomal mutations.

Value of determining the fetal fraction. Bioinformatics

An essential criterion for the quality of NIPT is determining the so-called fetal fraction (FF), which is the proportion of fetal DNA among the entire ecDNA of maternal blood. The FF appears in the mother's blood already starting from the fourth week of pregnancy. It is determined beginning from week 7–8. At weeks 9–10, its level is sufficient for the accurate detection of CA. The FF value is determined by different methods: real-time PCR, comparison of methylation patterns, and bioinformatic analysis. The latter approach is used most often. In 1–6% of cases, it is not feasible to obtain a result with primary NIPT due to the low levels of FF (<4%). The quality of NIPT depends on the quality of FF determination. First, only the presence of FF indicates that a blood sample of a pregnant woman is being tested. Second, the detection of FF serves as a criterion for the quality of the test itself and gives the clinician faith to interpret the results, which establishes the reliability of the screening result [16].

The assurance of the test results largely depends on the methods of bioinformatics analysis. Because NIPT uses different sequencing platforms (Illumina, Thermo Scientific, BGI), different types of tests (whole genome, targeted) are performed, and testing is conducted in diverse populations, there is an urgent need in clinical practice to make those decisions that are validated on the results of own control and blind samples.

The tasks of bioinformatics are careful processing (filtering) of data, multiple quality control (for contamination, for the quality of sequencing, etc.), and the filtering of non-specific areas (determining the sex of the fetus). Experts use different variants for determining FF for male and female fetuses, correction of outliers and verification of the sample compliance with the control sample, which provides high sensitivity and specificity along with the detection of mosaic variants and other anomalies.

Sensitivity and specificity of NIPT

While anticipating a review of data on using NIPT at a global scale, it should be noted that NIPT is generally accepted as a screening rather than a diagnostic method. A positive test result enables to classify a pregnant woman in the high-risk group for the CA under investigation, but it is by no means a final diagnosis. Accordingly, a negative test indicates a low risk of CAs, but does not rule them out completely.

As for the terminology, NIPT or NIPS, based on the foregoing about the screening purpose of this test, it seems logical that there is no significant difference between these definitions. Foreign recommendations [17] usually use the terms "screening test for extracellular DNA" or NIPS because they unambiguously define the assignment of this test as a screening test, whereas, the term NIPT has become more widespread in scientific, popular literature and the internet. In the future, we will use the term NIPT, which is more familiar to the Russian-speaking audience.

A lot of research with NIPT results is present in the literature; therefore, it is advisable to focus on only a few major meta-analyses, in which data on the sensitivity and specificity of the method are combined and evaluated. All meta-analyses concluded that NIPT employing the ecDNA analysis in the maternal plasma is a highly effective screening method for frequent CA, trisomies 21, 18, and 13 [18–21] in both singleton and twin pregnancies [21, 22]. Testing is also used to screen for fetal sex chromosome abnormalities and determine its sex (with insufficient validation data) [20, 23]. The NIPT method can also be successfully used for screening the presence of a specific set of submicroscopic repeated (not unique) microdeletions associated with potentially severe clinical phenotypes [24, 25].

Frequent trisomies

Table 1 presents data from several meta-analyses on the sensitivity and FPR of NIPT based on the study of fetal ecDNA. Meta-analyses design, work exclusion criteria, and statistical data processing methods were different.

Table 1 shows that an analysis of a huge pool of published works demonstrating that NIPT using fetal ecDNA exhibits a very high sensitivity for Down syndrome and a somewhat lower sensitivity for Edwards and Patau syndromes in singleton pregnancies. These indicators are lower in case of twins [22]. Due to the low percentage

Table 1. Data from meta-analyses on the sensitivity and false positive results of non-invasive prenatal testing based on the analysis of fetal extracellular DNA

Nosological forms	Sensitivity, %	False positive results, %	Sources
Trisomy 21	99.2	0.09	[18] (37 peer-reviewed publications over 2011–2015)
Trisomy 18	96.3	0.13	
Trisomy 13	91.0	0.13	
Monosomy X	90.3	0.23	
Sex chromosome aneuploidy (others)	93.0	0.14	
Trisomy 21	99.7	0.04	[19] (35 of 7759 publications over 2011–2016)
Trisomy 18	97.9	0.04	
Trisomy 13	99.0	0.04	
Monosomy X	95.8	0.14	
Sex chromosome aneuploidy (others)	100	0.004	
Trisomy 21	99.3	–	[21] (41 of 2012 publications over 2007–2015), combined calculations
Trisomy 18	97.4	–	
Trisomy 13	97.4	–	
Trisomy 21	95.9	0.09	[21] Calculations for the sample-free obstetric population per 100,000 pregnancies
Trisomy 18	86.5	0.15	
Trisomy 13	77.5	0.04	
Trisomy 21	97	0.03	[21] Calculations for high-risk population per 10,000 pregnancies
Trisomy 18	93	0.03	
Trisomy 13	95	0.007	
Trisomy 21	99.4		[20] (117 of 4,433 publications over 1997–2015)
Trisomy 18	97.7		
Trisomy 13	90.6		
Monosomy of chromosome X	92.9		
Trisomy 21	98.2	0.05	[22] (8 publications over 2011–2016) Data on multifetal pregnancies (twins)
Trisomy 18	88.9	0	
Trisomy 13	66.7	0.20	

of FPR, there was a high specificity of NIPT in the vast majority of publications ranging from 98% to 99.9% (not presented in Table 1).

Gil et al. note that screening for trisomy 21 using fetal ecDNA assay in maternal blood is superior to all other traditional screening methods with higher sensitivity and lower false positive rates [18]. At the same time, the screening efficiency for trisomies 18 and 13 and aneuploidies for sex chromosomes is significantly lower than the screening efficiency for trisomy 21.

An updated meta-analysis [19], including additional publications, based on more stringent inclusion criteria (data on clinical validation of a new method or on the introduction of NIPT in an aneuploidy screening algorithm, wherein data on pregnancy outcome were provided in more than 85% of the study population) showed an increase in the sensitivity of detecting frequent CA and a significant decrease in the number of FPR. The number of reported cases of sex chromosome aneuploidies was too small to assess the screening efficiency accurately.

The meta-analysis of studies on using NIPT in the general (non-sampled) obstetric population reveals significantly lower sensitivity indicators for the first trimester of pregnancy [21].

Positive predictive value (PPV), or prognostic value of a positive result as the probability of having a disease with a positive test result, holds a great importance in screening, especially for rare diseases, including CAs. Taylor–Philips et al. calculated generalized PPV values for the NIPT of frequent trisomies. They turned out to be different for the non-sampled obstetric population and for the population of high-risk pregnant women (82% and 91% for Tr21 and Tr37, respectively; 84% for Tr18; and 49% and 87% for Tr13, respectively) [21]. Maki et al. noted that they did not provide PPV values in their meta-analysis due to differences in disease prevalence among the populations included in the

study [20]. Lower PPV values for the general population are given in later or prospective studies, for example, 53% for Tr13 [26], 33% for Tr13, and 77% for Tr18 [27].

Sex chromosome aneuploidy

Chitty et al. [28] present NIPT data on sex chromosomes. Significant fluctuations in sensitivity (50%–100%) have been reported for 45,X, 47,XXX, 47,XXY, and 47,XYY, but the true sensitivity remains unknown because negative cases are not karyotyped after birth [19, 29, 30]. The proportion of FPR for the imbalance of sex chromosomes is 0.12–1.1% and also different in different studies. The PPV is from 9% to 40% for 45,X and from 7% to 90% for other sex chromosome aberrations [23, 28, 31].

NIPT as screening

The successful commercialization and great popularity of NIPT over the recent years among patients and obstetricians–gynecologists have generated a long and heated professional debate about the place of NIPT in prenatal diagnostics and provided an opportunity to make a diagnosis based on its results. As already mentioned, at present, the discussion has actually ended with the agreement that the analysis of fetal ecDNA for CA is a screening and not a diagnostic method.

First, it is impractical to ignore the findings of individual studies and meta-analyses of biased presentation of material in many published works, proving the high and even unique diagnostic value of NIPT. Hence, Maki et al. were unable to assess the compliance of some of the data presented in the literature with quality criteria, because this was not clearly reported in most papers. They emphasize that false positive and false negative results and non-responding data (test failures) are poorly represented in most of the analyzed articles. In 84 of 117 studies, the authors considered a non-sampled obstetric population, whereas in 28 of 117 studies, pregnant women were enrolled randomly from high-risk groups of fetal CA [20]. Taylor–Philips et al. noted that the mathematical assessment of the quality of work in the meta-analysis revealed a high-risk of subjectivity in the included studies, and the graphs obtained indicated signs of publication bias. There was a lower sensitivity of NIPT in studies on the general (non-sampled) obstetric population and the first trimester of pregnancy as well as in cohort studies with prospective history taking. It was concluded that the results of these studies should be interpreted with caution [21].

Gil et al. [19] noted that most of the selected studies were classified as having a high-risk of bias because: (1) it was not explicitly stated whether the samples were taken sequentially or randomly; (2) some studies did not explicitly state that the NIPT result was obtained without prior knowledge of the fetal karyotype or pregnancy outcome; (3) studies with assumptions about the absence of CA on sex chromosomes based on the clinical examination of newborns, and not karyotyping, cannot be considered in most cases because newborns with sex chromosome aneuploidies, in contrast to situations with trisomies 21, 18, and 13, are often phenotypically normal; and (4) in most studies, either not all pregnancy outcomes were presented or the methods for determining the outcomes were not the same in all cases.

In a systematic review of the Cochrane Library, a group of Canadian authors [32] revealed similarity between meta-assay results in the sensitivity and specificity of NIPT, but emphasized that the combined sensitivity, specificity, and associated predictive values of the assay cannot be used as evidence that a particular patient sample will definitely have pathology with a positive result or will not have it with a negative result. It is important that before the clinical implementation of a laboratory-developed NIPT, the method was fully validated in accordance with recognized clinical laboratory molecular diagnostic methods. The authors noted the generally poor methodological quality of the studies with a high-risk of bias, especially in terms of patient selection, study description, timing of recording, and confirmation of results.

Second, the presence of discordant results when compared with the karyotype of the fetus or newborn one of the main reasons why NIPT is recognized only as a screening method.

DISCORDANT RESULTS

The most common causes of false positive and false negative results cited in many studies are confirmed or suspected low FF; confirmed chromosomal mosaicism of the fetus or mother; maternal copy number variants of DNA regions (CNV); an technical and human factors. Moreover, the cause could not be identified in a significant number of cases.

Hartwig et al. published a systematic literature review including 22 papers for the period 2013–2016 with a detailed description of false positive and false negative NIPT results for autosomal aneuploidies [33]. In total, 206 cases of discrepancy between NIPT results and the karyotype of the fetus or newborn were collected and analyzed. Consequently, 182 (88%) of the 206 cases were false positive and 24 (12%) cases were false negative. There were biological, technical (human factor, technology and reagents, bioinformatics), and unknown causes of discrepancy between NIPT and the karyotype of the fetus. Biological ones are the most common of them. These include placenta-limited mosaicism, maternal mosaicism and chimerism, vanishing twin, maternal CNV, and maternal cancer (Fig. 1).

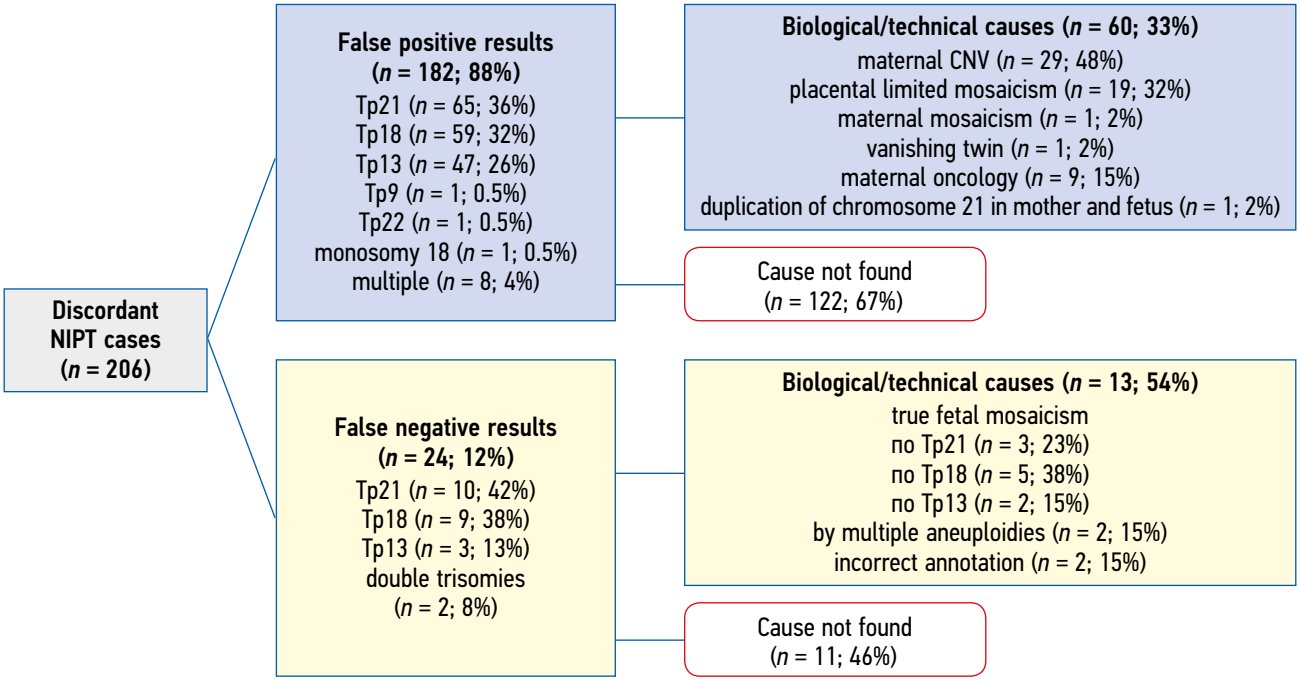

Fig. 1. Discordant results of non-invasive prenatal testing (cited from [33]). NIPT — non-invasive prenatal testing; CNV — copy number variations of DNA regions; Tr21, 18, 13, 9, 22 — trisomies for chromosomes 21, 18, 13, 9, and 22, respectively

Russian data on discordant results are illustrated by the analysis of NIPT data of the female patients of the Center for Family Planning and Reproduction of the Department of Health of Moscow (CFPR DH of Moscow) for 2017–2019 (tests Panorama, Prenetix, Verasity, Veragene; laboratories Genomed, Genetiko, Genoanalytica, Natera, NIPD Genetics, Medicalgenomics). In total, 120 cases of non-coincidence of positive NIPT results for 5 chromosomes (Tr21, Tr18, Tr13, sex chromosomes) and fetal karyotype were analyzed during subsequent invasive diagnostics with chromosomal analysis of mainly amniotic fluid cells. Importantly, 15 (12.5%) of 120 cases turned out to be false positive (Table 2). The PPV was also calculated, which varied greatly depending on the chromosomal abnormality that is important for post-test counseling. False negative results could not be traced because of the lack of data on pregnancy outcomes in women without high NIPT risk.

Wilkins-Haug et al. analyzed the biological causes for the discrepancy between ecDNA results and fetal karyotypes, and studied samples of the mother, newborn, and placenta using digital droplet PCR. The causes included kidney transplantation, vanishing twin, cancer disease, cases of limited placental and true fetal mosaicism. They concluded that the supposed biological causes for discordant NIPT based on ecDNA can be identified in more than half of the cases, including in the studies of biosamples and the clinical history of the mother [34].

Italian cytogenetics studied the problem of placental chromosomal mosaicism in detail [35–38]. Malvestiti et al. presented the study results of 60,347 samples of chorionic villi (CV) karyotypes over 14 years of research. For each biopsy sample, both layers of the placenta were analyzed (direct analysis for cytotrophoblast or with daily cultivation, and mesenchymal cells under conditions of long-term

Table 2. Discordant results of non-invasive prenatal testing and fetal karyotype according to the Moscow Family Planning and Reproductive Center of the Moscow Department of Health for 2017–2019

Nosological forms of chromosomal abnormalities	Number of inconsistencies in NIPT positive results	PPR, %
Tp21	4/89 (4.5%)	96
Tp18	2/11 (18%)	82
Tp13	2/5 (40%)	60
Sex chromosome aneuploidy, including monosomy of X-chromosome	7/15 (46.7%) 6/8 (75%)	53 25
Total	15/120 (12.5%)	88

Note. NIPT — non-invasive prenatal testing; PPR — prognostic value of a positive result; Tr21, Tr18, Tr13 — trisomies for chromosomes 21, 18, and 13, respectively.

culture). There were 1317 cases of CV mosaicism (2.18%). In these cases, a subsequent amniocentesis was performed (the analysis of the karyotype of amniocytes was possible in 1001 out of 1317 cases of mosaicism). The incidence of true fetal mosaicism among 1001 mosaic samples was 13%, that of placenta-limited mosaicism was 87%, and that of uniparental disomy was 2.1%. The incidence of placenta-limited mosaicism depended on the CA type. It was less common for Tr21 (2%) and Tr18 (4%), and more common for Tr13 (22%) and monosomy X (59%). Considering these data, the authors formulated a number of recommendations for the method of invasive diagnostics after a positive NIPT. Thus, for cases with Tr21 and Tr18, a biopsy of CV is recommended (with a 2–4% chance of detecting placenta-limited mosaicism), whereas for Tr13 and monosomy X, if there are no signs of pathology on US, then amniocentesis is used to determine the true karyotype of the fetus [38].

Discordant results are most often registered for sex chromosomes [35, 36, 38]. Chitty et al. [28] indicated the causes for the inconsistent results of NIPT on sex chromosomes, including limited placental and true fetal mosaicism on sex chromosomes, maternal sex chromosome abnormalities (in number and structure), exponential loss of the X chromosome with an increase in maternal age. They showed that all these factors cause false positive screening results for ecDNA and increase the number of unnecessary invasions.

Maternal aneuploidies are a common cause of false positive X chromosome monosomy results in NIPT. If all cells of the mother are aneuploid, then it is not difficult to distinguish maternal aneuploidies with the involvement of the X chromosome from the fetal ones because of the abnormally high number of fragments of the X chromosome detected in the study of extracellular DNA of blood plasma. However, mosaic monosomies on the X chromosome are often observed in the mother. In such cases, aneuploidies of maternal origin detected during a non-invasive study may be indistinguishable from aneuploidies of fetal origin. An increase in the study accuracy can be achieved using an additional analysis algorithm if, according to the NIPT data, a high-risk of aneuploidies on the X chromosome is detected. This algorithm is based on a mismatch in the distribution of the lengths of the fetal and maternal DNA, which differ from each other, as the DNA fragments of the fetal origin are usually shorter than the maternal ones. While selecting DNA fragments of a shorter length for analysis, in case of aneuploidy of fetal origin, the "effective" proportion of fetal DNA increases. However, if aneuploidy is of maternal origin in the sample, then the proportion of fetal DNA remains unchanged. An additional stage of analysis enables to identify cases when the results of DNA screening are attributed to the characteristics of the mother's karyotype and significantly increase the PPV of the study [39].

Rare CA, major partial CA, and microdeletions/microduplications. Pros and cons of genome-wide NIPT

A retrospective analysis of a large set of recommendations, as well as a number of fundamental and clinical studies on using NIPT suggested that this testing can be extended to a wide range of other CAs, in addition to the most frequent trisomies, which was done with the introduction of genome-wide NIPT (gwNIPT) into practice.

The specified range of CAs includes unbalanced chromosomal rearrangements, rare autosomal trisomies (rAT), supernumerary marker chromosomes, as well as microdeletions and microduplications. Each of these abnormalities is rare, but collectively they are relatively common, especially in prenatal diagnostic samples [40]. These abnormalities can clinically manifest as spontaneous fetal loss, fetal malformations, or pregnancy complications.

The clinical significance of such results is the subject of scientific debate, and there are no generally accepted recommendations for monitoring high-risk patients for "rare" CAs. Although some authors argue that rare autosomal aneuploidies may indicate an increased risk of fetoplacental disease and be beneficial for pregnancy management, others believe that pregnancy outcomes with an increased risk of rare autosomal trisomies are not so unfavorable as expected. Nevertheless, there is evidence that only the gwNIPT results enabled the identification of prenatal risk of genetic pathology in a large number of cases. An example is the case of determining the risk of Prader–Willi syndrome, which was further confirmed using invasive prenatal diagnostics. In this case, according to gwNIPT results, at week 13 of pregnancy, a high-risk of trisomy on chromosome 15 was established in a patient with a low risk of frequent aneuploidies. According to the results of prenatal diagnostics, mosaicism on chromosome 15 in the placenta and the presence of uniparental disomy on this chromosome in the fetus itself, which was noted with a normal karyotype in a standard cytogenetic study, were revealed [41]. Another example is the case when screening results for ecDNA revealed CA in the fetus on chromosomes 4 and 12, which cannot be performed with non-invasive screening only for frequent aneuploidies. During karyotyping, the mother revealed a balanced translocation with the involvement of chromosomes 4 and 12, which was the cause of the unbalanced karyotype in the fetus [42].

However, many CAs found in gwNIPT may be clinically insignificant because the abnormal cell line may be limited to the placenta and be present with an insignificant frequency in the fetus tissues or in a phenotypically normal parent, or cytogenetic rearrangement does not cause a gene imbalance that affects significantly the phenotype of the fetus or the child. In addition, the detection of rare cytogenetic

abnormalities limited to the placenta is often associated with complex genetic counseling, additional follow-up invasive testing, and uncertain pregnancy outcomes [36]. In this regard, the clinical feasibility of gwNIPT to identify all additional chromosomal imbalances seems to be debatable [43–45].

Based on the assumption that gwNIPT is completely equivalent to cytotrophoblast karyotyping (assuming the absence of false positive or false negative cases), F.R. Benn and P. Grati [46] considered a set of rare CAs detected in the course of standard karyotyping of CV samples. The authors tried to make a prognosis of the necessary additional studies of the mother and the fetus, the problems of clinical interpretation and counseling that may arise in the detection of rare CA in gwNIPT. Previously published cytogenetic results of 41,782 analyses of CV samples performed in the same laboratory (Toma Laboratory, Varese, Italy) on women in the first trimester of pregnancy were combined with an overlapping set of 45,867 CV samples from the same laboratory, which documented chromosomal mosaicism in cytotrophoblast and/or mesenchyme and confirmed in amniotic fluid [38, 47]. Only those cytogenetically visible abnormalities were considered that were not included in the current standard NIPT protocols (i.e., excluding trisomies 21, 18, and 13 and sex chromosome aneuploidy).

The additional detection rate of rare CAs was 0.8%, including approximately 0.5% of cases with rAT and 0.3% of cases with segmental aneuploidy. It is predicted that approximately 0.1% of cases would be associated with an early fetal loss because of non-mosaic rAT. In about 0.7% of cases of the remaining ones, which required amniocentesis, only 0.06% would be attributed to an unambiguous diagnosis of fetal impaired development (non-mosaic unbalanced chromosomal rearrangements or clinically significant homogeneous disomias). All other cases would be caused by mosaic CA with a highly variable risk of malformation or associated with an unconfirmed result that would still involve some degree of residual risk, even after amniocentesis [46].

Chromosomal mosaicism, as a major cause of uncertainty, is a common finding in cytogenetic analysis after chorionic biopsy and is widely recognized as highly problematic in terms of genetic counseling. For example, data were obtained on the variability of the karyotype of biopsy samples from different parts of the placenta [48, 49]. It should be considered that NIPT analyzes the totality of DNA fragments of many apoptotic trophoblast cells in maternal blood, whereas cytogenetic analysis of trophoblast examines the karyotype of cells in a specific sample obtained by means of an invasive procedure.

It is substantially evidenced that mosaic placental trisomy of chromosome 16 can lead to fetal growth retardation and preeclampsia. For other mosaic rATs, the association is plausible, but not proven. Even when an invasive procedure confirms a true fetal mosaicism after a positive NIPT, it is not possible to predict the clinical outcome, as in the cases of localized placental mosaicism (except for limited placental mosaicism for trisomy 16) [37].

Benn et al. analyzed the cases of tAT in 10 recently published studies. Positive test results were confirmed by karyotyping CV samples. The authors found that the clinical outcome of cases with a positive gwNIPT analysis for rAT included the birth of an outwardly normal baby in 40% of cases or miscarriage/fetal loss in 27% of cases. In the study population, there was a low association between the presence of rAT and pregnancy complications such as fetal growth retardation and fetal malformations [50].

A review [51] of 89,817 gwNIPT detected 0.4% of cases of chromosomal imbalance, in addition to those associated with aneuploidies of chromosomes 21, 18, 13, X, or Y. Pregnancy outcomes were only available for 57 cases, and 24 of them were associated with the termination of pregnancy (miscarriage, silent miscarriage, or intrauterine fetal demise). The remaining 33 cases included 4 cases of copy number variation (CNV) without the description of phenotypes, 1 result with impaired ploidy (not confirmed later), 5 rATs with true fetal mosaicism confirmed after amniocentesis (pregnancy outcomes or phenotypes were not described), 1 case with uniparental disomy (Prader–Willi syndrome), as well as 1 case of congenital anomaly at birth (trisomy 9), and 2 cases of intrauterine growth retardation.

Genome-wide NIPT can improve the detection of additional maternal chromosomal imbalances, some of which may be clinically significant. These include constitutive and acquired anomalies that can be caused by malignant neoplasms [33, 52].

It can be expected that gwNIPT, compared with targeted NIPT, will lead to additional FPR for a limited set of chromosomes. These FPR may also be related to the presence of a vanished twin. Given the high frequency of conception of twins and the early loss of aneuploid conception, the number of such cases can be significant [53].

With gwNIPT, the probability of false negative results should not be underestimated. These may be cases of mosaicism, with an abnormal cell line in the fetus, but which is not found in the cytotrophoblast (type 5 true fetal mosaicism) or low level mosaicism underrepresented in the cytotrophoblast that can be detected using modern technologies (types 4 and 6 true fetal mosaicism).

Researchers are interested in the possibility of detecting clinically significant microdeletion/microduplication syndromes using gwNIPT, which, according to some estimates, occur in more than 1% of pregnancies regardless of maternal age [54]. There are limited data on the clinical efficacy

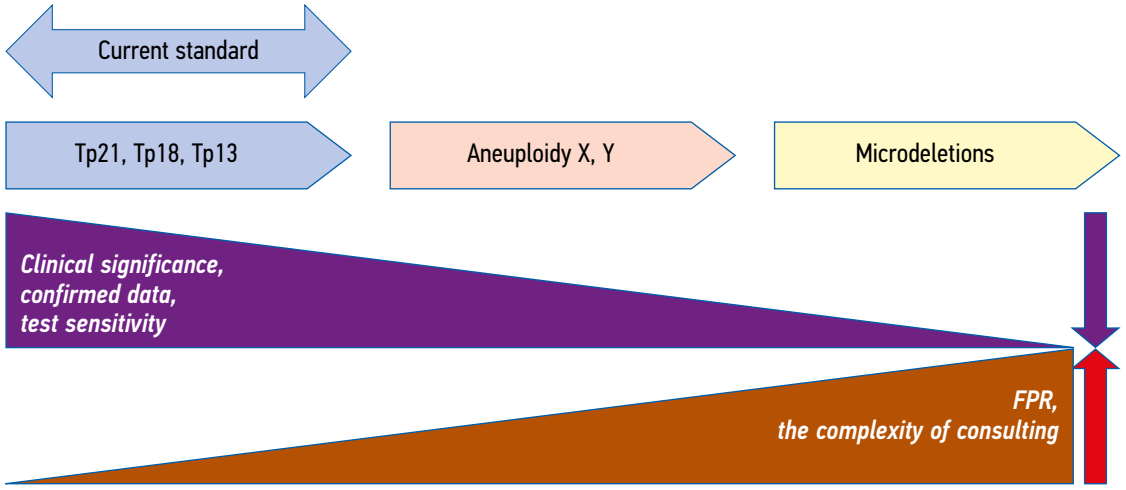

Fig. 2. Clinical significance, confirmed data, sensitivity of screening tests. Tr21, Tr18, and Tr13 — trisomies for chromosomes 21, 18, and 13, respectively; FPR — false positive results

of genome-wide screening for these syndromes [55–57]. In review papers [28, 55], the authors present the following observations:

1) most of the results were obtained on small samples;
2) the sensitivity is extremely variable (14–97%): PPV ranges from 3.8 to 17% (depending on the syndrome) for pregnant women in the general population (low risk) and from 50 to 97% for high-risk pregnancies or with US signs of fetal abnormalities;
3) the number of invasions with uncertain indications and the degree of parental concern are increasing;
4) there is a high residual risk of undetected microdeletions along with the complexity of post-test counseling;
5) there are no data on the clinical efficacy of screening tests for ecDNA for microdeletion syndromes in large populations; and
6) most microdeletions and duplications are rare and their prevalence has not yet been determined; moreover, clinical symptoms cannot be reliably predicted in the prenatal period.

Fig. 2 schematically presents the consequences of including frequent trisomies of other chromosomal aneuploidies and microdeletion syndromes in the standard ecDNA screening model [28].

With the expansion of NIPT after the inclusion of microdeletions, sex chromosome abnormalities and rare autosomal trisomies, the screening sensitivity indicators will decrease from about 99% (for Tr21) to 60% for all CAs. The decrease in sensitivity will be accompanied by an increase in FPR from about 0.14 to greater than 1.5% (Fig. 2). Because most of the evidence for the effectiveness of NIPT to date is received from studies in high-risk pregnancies, the sensitivity of screening is even lower when offered to all pregnant women [28]. The reviewers warn of declining clinical relevance, diminishing or lack of validation data, complicating counseling, and an increase in parental anxiety and the incidence of invasions with uncertain clinical indications. The position of researchers who recommend conducting a greater number of clinical trials before the introduction of gwNIPT into a widespread clinical practice appears to be justified [43, 58–60].

An authoritative group of authors from nine European countries published a collective opinion on the clinical use of gwNIPT [61]. Evidence suggesting that ecDNA testing in maternal blood provides an effective prognosis for the presence of Tr21 and, to a lesser extent, Tr18 and Tr13 in the fetus in both singleton and twin pregnancies [18, 19, 22] has led to the clinical use of the test in some countries, as a rule, in women from the high and moderate risk groups, who are pre-selected using combined screening in the first trimester. However, in Belgium and the Netherlands, ecDNA testing is offered to all pregnant women as an alternative to early combined screening. In this case, the test was used in a genome-wide variant to identify, in addition to screening the three main trisomies and aneuploidies of sex chromosomes, clinically significant rare autosomal trisomies and additional segmental (partial) chromosomal aneuploidies. The analysis results of the first year of gwNIPT testing in the Netherlands (TRIDENT-2 study) included 56,818 pregnant women. In 207 (0.4%) women, the test was positive for rAT (n = 101), partial chromosomal aneuploidies (n = 95), or complex CAs (n = 11) [26].

Among the 101 rATs, 6 (6%) were subsequently confirmed, but only 1 of them was associated with an abnormal phenotype. A total of 29 (35%) of 95 partial chromosomal aneuploidies were confirmed. Because US examination was not performed, the number of structural abnormalities of the fetus was not described. In another seven cases associated with malignant or precancerous neoplasms in the mother, the clinical feasibility of a positive test result has not been determined. However, a positive test result inevitably caused anxiety, the need for additional examinations for the fetus

and mother, and led to a decision to terminate the pregnancy before the end of the examination in some cases.

Thus, according to the authors [61], the TRIDENT-2 study shows that at present, the benefits of genome-wide screening for all genetic imbalances do not appear to outweigh the potential negative aspects. Moreover, clinical implementation may be questionable from an ethical point of view, even for research purposes.

A study of gwNIPT conducted in Belgium with 3,373 women, in addition to frequent cases, identified other CAs in 28 (0.8%) cases. These cases included 4 sex chromosome aneuploidies, 6 rATs, and 1 rare autosomal monosomy; none of which were confirmed in the fetus or newborn, as well as 17 major or submicroscopic partial CAs, 3 of which were confirmed in amniocytes [62]. In all 28 cases, no clinical pathology was traced.

Discussing the experience of large-scale application of gwNIPT [61], the authors formulated an agreed position and stated the following:

1) an increase in the number of screening positive pregnant women, even though NIPT was originally intended to reduce the risk group of frequent CA, and an increase in the frequency of invasive intervention for cases of unknown clinical significance, which may remain uncertain in terms of diagnosis establishment even after an invasive procedure;
2) the uncertainty regarding both the clinical significance of the heterogeneous pool of CAs and further recommendations in the case of a positive result;
3) heterogeneity of laboratory protocols for mass parallel sequencing (lack of standardization);
4) the need to resolve ethical and legal issues while consulting parents before they give their informed consent because the information is not unambiguous. In fact, women are already undergoing gwNIPT without clear information about its limitations and drawbacks, and clinical decisions are made based on results with uncertain clinical significance. There are also ethical concerns about the increased incidence of voluntary termination of pregnancy due to positive NIPT results even after the confirmation of the normal karyotype of the fetus and with normal US results;
5) gwNIPT contradicts the screening principles of the World Health Organization [63, 64, 59].

In conclusion, the reviewers noted that although research should always be encouraged, the advantages and disadvantages of introducing gwNIPT screening should be carefully assessed. Health care providers and subsidizing bodies have a responsibility to provide more robust evidence and organizational strategies before obtaining the approval of screening algorithms that include gwNIPT in the national reimbursable screening programs [61].

Thus, the question remains unresolved: if there is a clinical benefit in gwNIPT, can gwNIPT provide clinical conclusions for the prognosis of pregnancy complications? Obviously, this screening test could potentially reveal some additional clinically significant unbalanced CAs that could not otherwise be detected, except for invasive test results or possibly fetal abnormalities revealed by means of US. However, this must be referred to the much greater number of cases in which the diagnosis is uncertain, even if further invasive tests and US are offered. This situation is fundamentally different from the proposed NIPT technology for frequent autosomal trisomies and recurrent microdeletions, in which the phenotypes are explicit and subsequent CV biopsy or amniocentesis almost always provides a definitive diagnosis.

Even though the prospects for using gwNIPT as a total screening for CA remain highly controversial, a review of this technology's possibilities for future screening programs for pregnant women is extremely important. Because the sequencing of all extracellular DNA occurs within the framework of this technology, the whole genome "version" of NIPT has certain additional capabilities. In particular, the blood plasma of a pregnant woman contains not only fetal DNA but also individual fragments of the mother's DNA, including those of an oncological nature, mitochondrial DNA, and fragments of the genomes of viruses and bacteria. And if the effectiveness of using gwNIPT in the detection of bacterial load or as a "zero" point in early oncological screening is questionable, then other areas of "non-core" use of this technique, such as the detection of mutations in mitochondrial DNA, viral load, risk assessment of major obstetric problems based on the analysis of individual loci of maternal DNA (preeclampsia, gestational diabetes mellitus, fetal growth retardation, and macrosomia), are very promising [65]. Such solutions, of course, should be tested in the framework of randomized scientific studies. The introduction of new technologies (methods of processing gwNIPT data) should be the responsibility of state institutions that use not only NIPT, but also other technologies necessary for testing using invasive prenatal diagnostics methods (karyotyping, FISH, aCGH, etc.), and have a wide range of specialists such as geneticists, molecular geneticists, obstetricians–gynecologists, and licensed US specialists.

Apparently, any version of NIPT as a screening assumes the presence of state-accredited reference centers that would be engaged in the external assessment of the method quality and supervise the results of research projects in this field because FPR can cause the erroneous termination of a normal pregnancy.

Limitations and contraindications for NIPT

Contraindications to NIPT are malignant neoplasms in the mother; organ and tissue transplantation in the mother; radio or immunotherapy in the mother (including treatment

with hematopoietic stem cells); silent miscarriage for three months before the study; and vanishing twin syndrome (pregnancy with twins with the loss of one of the fetuses). Relative contraindications include the treatment with low molecular weight heparins (heparin drugs must be discontinued for three to four days or blood sampling for NIPT is made before the next administration of heparin), treatment with antiretroviral therapy drugs (blood sampling is required before the next intake of an antiretroviral drug), blood transfusion over the last six months, and surgical interventions or trauma less than three months ago, for example, an abortion.

NIPT also has its own limitations, which can lead to the absence of a response, its unreliability, or difficulty in interpretation. These include low FF (below 4%), multifetal pregnancy (two or more fetuses), fetal or maternal mosaicism, balanced structural chromosomal rearrangements (rearrangements without changing the amount of genetic material are not manifested externally and cannot be determined by this study), and minor unbalanced structural rearrangements of chromosomes (triploidy and tetraploidy, monogenic and other genetic diseases not associated with aneuploidy) [66].

The relatively high frequency of unsuccessful attempts at NIPT (1.4–5.4%) is caused by the low level of fetal ecDNA in the blood of a pregnant woman, especially in women with obesity [67]. This is attributed to the large volume of circulating blood and simultaneously the increased level of ecDNA of the pregnant woman herself because of the excessive breakdown of adipose cells. However, pregnant women whose body mass index is elevated because of an increased muscle mass have higher ecDNA values of fetal origin as compared to women with obesity.

NIPT offers a wide range of FPR in patients with tumor diseases, both malignant and benign. Most often, these manifestations are in the form of detected multiple CA.

Serious difficulties of NIPT occur with the mosaicism/chimerism of chromosomes in the mother or fetus, with twins, consanguineous marriage, or false paternity. The reliability of NIPT results in the case of mosaicism depends on the level of aneuploid cells. According to the experience of Russian authors, NIPT enables the detection of trisomy on chromosome 21 with mosaicism over 40% [67].

It should be noted that during NIPT, the sex of the fetus can be determined. However, we believe that, except for cases of sex chromosome abnormalities and sex-linked diseases, the sex of the unborn child can be reported only after week 12 of pregnancy to avoid the induced termination of pregnancy in accordance with Article 56 of Federal Law No. 323-FZ of 11/21/2011 "On fundamental healthcare principles in the Russian Federation" [68].

Ethical considerations for NIPT

The active implementation of NIPT obliges to consider a large list of conditions necessary for large-scale or selective clinical implementation of the technology. It includes the legal status, standardization of the method with the definition of diagnostic capabilities, the availability of diagnostic characteristics for the entire range of tested diseases, the provision of open and continuously updated information on the predictive value of positive and negative results, external independent quality control of laboratory tests, monitoring of results, as well as an assessment of clinical effectiveness of NIPT by specialists, expert groups, councils, and professional communities.

The interaction between the consulting doctor and the pregnant woman in the examination process cannot be determined by the commercial interest of the contractor and/or the financial capacity of the patient. It is the responsibility of the physician to provide reliable and accurate information on the proposed test variants and their results, and the family must assess the need for testing as a part of their free choice and informed consent or refusal. Special attention should be paid to the extended versions of NIPT, in which findings with unknown clinical significance come in the sight of post-test counseling, which complicates decision making about the fate of the unborn child.

All of these and many other topics outline the main layer of ethical problems that exist in parallel with the advancement of NIPT into clinical practice and which should be solved by every civilized society with a socially oriented state policy in the health care sector, including in the field of protecting the interests and health of the patients.

Experts from the European and American Societies of Human Genetics, because of debates and discussions on the use of NIPT, formulated a joint position back in 2015, in which they focused on prenatal pre- and post-test counseling of pregnant women [69]. The authors did not recommend expanding the method for screening structural abnormalities and sex chromosome abnormalities because of the high probability of false positive and false negative results, which complicate counseling and lead to an increase in the number of invasive procedures. The consultant should notify about the possibility of obtaining additional information and about other findings at NIPT on Tr21, Tr18, and Tr13, but not use it in post-test counseling without the patient's request.

In the NIPT Use Guidelines, developed and published by the American College of Medical Genetics and Genomics (ACMG) in 2016, special attention is paid to medical genetic counseling for women, including the possibilities, restrictions, and conditions of testing [57]. As a part of the pre-test consultation, it is recommended to provide information on various screening tests for detecting CA with a higher sensitivity of NIPT for Tr21, Tr18, and Tr13 as compared with other screening tests about the possibility

of NIPT for screening aneuploidies by sex chromosomes and the presence of NIPT variants for clinically significant pathologies (microduplications and microdeletions), as well as the need for an invasive diagnostic examination with a positive screening result. ACMG does not recommend the use of NIPT for screening sex chromosome aneuploidies and genome-wide CNV analysis, but if additional findings with unclear clinical significance are identified, then it suggests additional genetic counseling and the possibility of additional testing. The Guidelines define the laboratory's obligations to provide all additional information required by the consulting doctor to interpret the results in the protocol, including an indication of the proportion of fetal ecDNA and indicators characterizing the predictive value of positive and negative results.

In 2018, a team of researchers evaluated 10 commercial laboratories (BioReference Laboratories' ClariTest, LabGenomics' Determine 10, Roche's Harmony [formerly Ariosa Diagnostics], Integrated Genetics' InformaSeq [part of LabCorp], NxGen's Informed Prenatal Test, Sequenom's MaterniT21 Plus [part of LabCorp], PathGroup's NIPS, Natera's Panorama, Counsyl's Prelude [now part of Myriad Genetics], Quest Diagnostics' QNatal) offering NIPTs in the USA to meet the eight Guidelines outlined in the Guidelines. After reviewing the samples of study reports from these laboratories' websites, as well as patient materials and responses to direct inquiries, the authors expressed concern about the level of compliance by laboratories with the recommendations for using NIPT in clinical practice and noted that "none of them follows all the ACMG recommendations" [70]. Thus, no laboratory provided information on the sensitivity, specificity, and predictive value of tests in laboratory reports and promotional materials, and only a few laboratories had information resources for patients and "health care providers" about the test. The fetal ecDNA fraction was reported by 9 out of 10 laboratories, and only 8 laboratories followed the recommendation not to screen for autosomal aneuploidies other than Tr13, Tr18, and Tr21.

The authors of the article believe that this situation "can lead to confusion and inappropriate counseling, and laboratories should not offer screening if they do not follow all ACMG recommendations," and the study materials "will help doctors and future parents trying to determine the quality of the proposed NIPT in modern market." According to one of the authors, B. Skotko, Co-Director of the Down Syndrome Program at the Harvard University Massachusetts General Hospital, the article should be "an incentive for laboratories to revise their reports, include patient resources and test metrics that will enable clinicians and obstetricians to help their patients make informed decisions based on reliable information."

Following the article, the researchers published a spreadsheet of the above ratings at https://prenatalinformation.org/table/, which was updated on June 19, 2020, noting that some of the 2016 ACMG Guidelines may be outdated and downgrade laboratories on the criteria published in the Guidelines. The authors wrote, "In some cases, reporting on all test metrics is meaningless." Therefore, it makes no sense to report on the predictive value of a positive result in the case of its negative result, and the true predictive value cannot be determined in some cases. Experts pointed out that in most cases of sex chromosome aneuploidies, it is difficult to determine the truth of a positive result immediately at birth and it is impractical to follow the patients for years.

In other publications, B. Skotko noted that the study did not include academic laboratories but began with commercial ones, which occupied the largest share of NIPT sales in the US market [71]. In the future, the group of authors plans to update the rating of 10 companies presented in the article, as well as to conduct an analysis of new commercial companies and academic laboratories. "Laboratories should follow the most important ACMG recommendation of providing a PPV because parents have the right to know the probability that their test result will be truly positive and the parent's response after a positive result will be determined by understanding its predictive value. It is also very important for the informed choice of future families that laboratories support the needs of parents for educational materials by providing access to quality resources and information, such as those recommended by the ACMG," the author added.

In a scientific review published in 2017 [72] and focused on European and American recommendations for using NIPT in clinical practice, Russian authors assessed the benefits of experience gained in other countries and focused on the "urgent need for professional discussion" to assess the possibilities and restrictions of dynamically developing NIPT technology, including legal, ethical, and educational issues, such as the current legislation regulating medical activity, commercialization of NIPT, and attitude of doctors and patients toward it. The work noted the absence of recommendations on medical and genetic counseling of pregnant women, an important clinical and ethical component in the use of NIPT, including in the only so far published Russian document on non-invasive testing of fetal aneuploidies [12].

The following key points of medical consultation before the prescription of NIPT have been proposed [5, 73]:

- state that testing is optional;
- clarify that this is a screening test, not a diagnostic test;
- describe the test restrictions (i.e., what the test is not intended for);
- analyze the clinical aspects and variability of the conditions studied;
- briefly introduce the test methods and laboratory report formats;
- determine the positive and negative predictive values of the method and their clinical significance;

- recommend that all positive screening tests be confirmed by a diagnostic test to determine the karyotype of the fetus or newborn;
- tell about the possibility of accidental findings related to maternal health; and
- refer the patient to a medical geneticist for the clarification of unusual test results.

The role of the social and cultural environment is no less important for the observance of ethical principles in NIPT implementation. Numerous studies to assess the impact of demographic, ethnic, religious, socioeconomic, and other spheres of society on the preferences of prenatal testing methods have revealed a number of aspects. The results of a survey of 2,707 women and 1,275 doctors in 9 countries (Great Britain, Denmark, Israel, Iceland, Italy, Canada, the Netherlands, Portugal, and Singapore) showed a preference for a safe test and the possibility of obtaining information about the risk of additional CAs besides Tr21. For doctors, the sensitivity indicators of the test results in early pregnancy were of a greater importance. At the same time, the residents of Israel and the Netherlands were more likely to refuse any screening option for Down's syndrome than other countries' residents [74].

A study in the USA with 3,164 respondents showed that women in the older age group, with higher education, income, and insurance, as well as those who are already familiar with genetic testing, are more likely to choose NIPT. At the same time, women who indicated their sufficient religiosity (regardless of the type of religion) and who belong to the indigenous peoples of the North American continent are not ready to use the NIPT. Women of religious groups (Protestants, Mormons) and African Americans were more likely to exclude the possibility of termination of pregnancy if an unfavorable result was obtained, which affected their attitude toward NIPT [75].

There are interesting results of motivation of refusal from NIPT as an additional test, obtained on a sample of 6,782 pregnant women included in the intermediate-risk group based on combined screening results, when 8.5% of women refused because of satisfaction with the results, 26% of patients refused because of rejection of the option of termination of pregnancy for medical indications in any result of NIPT, 10% of patients did not want to undergo NIPT as an experimental test, and 2% of women did not agree with sending a sample for research in another country (USA) [76].

A group of Russian specialists tried to determine the preferences of doctors and pregnant women while choosing prenatal tests with different characteristics through questionnaires. While analyzing the answers, they determined that the choice is influenced by the information content (the possibility of obtaining additional information about the fetus' health) and the cost of the test. Moreover, the choice is not affected by the sensitivity and the time of obtaining the result. Doctors aged below 35 years preferred to use NIPT only for screening Down syndrome [77].

In the analysis of 800 pregnant women from a non-selective group from 16 regions of the Russian Federation, the overwhelming majority of respondents (90.2%) defined EPS as a mandatory examination method for each pregnant woman and 84.7% of women preferred tests with the possibility of obtaining a result about fetal health as early as possible (90.97%) and paid by the state, including insurance funds (75.6%). Before the survey, 63.4% of pregnant women did not know about NIPT, regardless of place of residence and level of education [78].

The results of all the cited studies underscore the need for countries to consider specific social issues while defining the framework within which ethically sound NIPT implementation should be ensured. In addition, the medical professional training of counselors remains paramount to ensure an informed choice of the family.

Models of application of NIPT by countries of the world

The introduction of NIPT into screening programs around the world is happening in different ways. With a centralized version (Western European countries such as Holland, Belgium, Switzerland, Denmark, and others, as well as Australia and China), the introduction of this technology into practice is regulated by the state. These countries use different algorithms for prenatal screening. In other countries, patients are given the right to choose and pay for the test (e.g., the USA), and the countries where NIPT is implemented only in some regions (Finland, Italy, Czech Republic, Singapore, etc.) stand apart.

The question of whether NIPT should be offered as a first-line screening or as a part of a contingent model, where ecDNA analysis is limited to a group of patients selected based on EPS results, is constantly under discussion [79, 80]. The advantage of the first approach comprises a greater screening sensitivity for the entire population of pregnant women with respect to Tr21. Such an algorithm was implemented in Belgium [81]. The disadvantage of this approach, based only on ecDNA analysis, is that often no anatomical US assessments of the fetus are performed, and congenital malformations are omitted during screening. Cost is also a bigger challenge with this approach, and practically few countries can afford it. Another problem with this model is in the large number of FPR that require invasive prenatal diagnostics (massive gwNIPT in the Netherlands) [26, 82].

For this reason, many countries have now chosen to start with a contingent screening model. Within this model, NIPT is performed on pregnant women who are at risk for CA based on EPS results. There are usually high-, intermediate-, and low risk groups. NIPT is offered

to high-risk and intermediate-risk pregnant women. In the high-risk group, where invasive diagnostics is indicated, NIPT is used to reduce the number of invasions in cases of a negative ecDNA result. In the intermediate-risk group, NIPT is performed to identify those CAs that could not be detected in EPS within the high-risk range. The main problem of this approach is the definition of the intermediate-risk boundaries. Different countries use different numbers. For example, in England, a cut-off risk greater than 1:150 is taken, it is 1:250 or higher in Norway, 1:50–1:250 in Spain, 1:1000 or higher in Switzerland, from 1:50 up to 1:1000 in Sweden, 1:301–1:1000 in Denmark, and 1:10 to 1:1000 in Australia.

K. Nikolaides et al. calculated that using EPS as the main screening for Tr21 and NIPT for pregnant women with a risk of more than 1:3000 (1:2–1:3000) would provide 97% sensitivity with the value of the risk group for Tr21, formed as a result, considering the results of NIPT, in 0.4% of cases [83], that is, invasive diagnostics would be indicated for only 0.4% of patients and would have to reveal 97% of Tr21 cases. In the RAPID study, all patients at risk for Tr21 ≥1:1 were offered NIPT based on either the EPS or the quadruple screening test. Invasive testing was recommended to patients at risk ≥1:150. The results coincided completely with the predicted mathematical modeling [84]. The authors make a similar conclusion that lowering the risk threshold because of the possibility of a second screening with NIPT increases the number of detected cases of Down syndrome while reducing the number of invasive tests and associated miscarriages; however, the financial costs also increase significantly. Miltoft et al. from Denmark reported 6,449 women who underwent combined screening for Tr21 [85]. In this study, women at risk ≥1:1000 underwent additional NIPT. The authors compared routine combined screening followed by invasive diagnostics at risk greater than 1:300 (high-risk group in Denmark) with a contingent screening model in which NIPT was offered to pregnant women at risk of 1:100 to 1:1000. Sensitivity was 100% in both screening variants; the number of FPR was reduced from 3% in the usual combined screening to 1.2% in the contingent variant, which can lead to a significant reduction in the number of invasions in the high-risk group.

Nevertheless, even with a significant increase in the intermediate-risk group, approximately 1.5–5% of pregnant women with Tr21 in fetuses remain in the low risk group and are omitted at screening [83, 86–89].

Kagan et al. conducted a retrospective study of 21,052 pregnant women who underwent EPS in Dusseldorf (Germany) [88]. The sensitivity of EPS or NIPT was assessed separately, comparing them at the same cut-offs of risk groups. Additionally, the sensitivity of a two-stage approach was assessed, in which NIPT was performed in the "intermediate" risk group, calculated after EPS for all pregnant women, with cut-offs from 1:50 to 1:1000 and from 1:150 to 1:500. In total, in the general group, 127, 34, 13, and 15 pregnancies with Tr21, Tr18, Tr13, and sex chromosome abnormalities, respectively, were detected. Other CAs with an increased risk of adverse outcome, which were not detected by NIPT, were revealed in 23 fetuses. The remaining 20,840 pregnancies were classified as normal, because pre- or postnatal examinations revealed no signs of clinically significant CAs. Calculations have shown that EPS enable to detect 81% of aneuploidies with a cut-off risk of 1:50 and 91% of all aneuploidies with a cut-off of 1:250. With NIPT and the same cut-offs, 88% of the corresponding CAs can be detected. In the case of a two-stage approach of EPS + NIPT with cut-off boundaries from 1:50 to 1:1000, 94% of all aneuploidies can be detected. With another "intermediate" risk range from 1:150 to 1:500, the detection rate is 93%. The authors concluded that the two-step contingent screening principle with EPS for all patients and NIPT in the intermediate-risk group results in a high detection rate for all aneuploidies. In their subsequent work [90], German authors admit the option of screening with the possibility of replacing maternal serum markers with NIPT with mandatory US examination at a term of 11–14 weeks with the measurement of the nuchal fold thickness (NFT) in the fetus according to the FMF algorithm and US assessment of the fetus state. Invasive diagnostics is offered to pregnant women with NFT of 3.5 mm or greater, when fetal malformations are detected and with the risk of CA according to NIPT.

Another study investigated the incidence of atypical chromosomal and submicroscopic abnormalities, as well as structural fetal abnormalities detected by US in the first trimester of pregnancy in fetuses with NFT greater than 99th percentile to assess the suitability of NIPT as the only screening test [91]. In a retrospective cohort study of 226 fetuses with NFT greater than 99th percentile at a term of 11–14 weeks of gestation, the authors evaluated the theoretical yield of two ecDNA testing models, namely standard targeted NIPT (chromosomes 21, 18, and 13) and extended NIPT (chromosomes 21, 18, 13, and sex chromosomes), and compared it with the results of cytogenetic testing and US assessment in trimesters I, II, or III. In 226 fetuses, according to the cytogenetic analysis, 84 (37%) CAs were found, including 68 frequent aneuploidies (involving chromosomes 13, 18, or 21), 6 sex chromosome aneuploidies (4 cases of monosomy X and 2 cases of trisomy X), 3 clinically significant rare CAs (1 trisomy 22, 1 mosaicism by trisomy 21, and 1 unbalanced translocation), 5 submicroscopic pathogenic variants, and 2 cases with Noonan syndrome. In the case of standard and extended NIPT, at least 12% (10/84) and 19% (16/84) of genetic abnormalities would be omitted, which would amount to 4.4% and 7.1% of fetuses with increased NFT, respectively. Finally,

out of 142 fetuses in which no genetic abnormalities were detected by laboratory methods, significant malformations were registered in 15 (10.6%) cases with early US scanning and in 19 (13.4%) cases with US in the second and third trimesters. The authors question the suitability of NIPT as a screening test in patients with increased NFT. The conclusion was that 11–13-week US scan should be offered to all pregnant women to assess NFT and early anatomy, regardless of the screening method. If the 11–13-week scan is performed earlier than the NIPT, then an NFT value greater than 99th percentile should interfere using subsequent testing by this method.

It should be noted that structural abnormalities in fetuses (including fatal congenital malformations) in the general population are more common than CAs; therefore, any decision on the algorithm for the clinical use of NIPT should include an expert US at the first trimester (including assessment of NFT) with the definition of pregnancy term, fetal viability, the presence of multifetal pregnancy, fetal structural abnormalities (including the assessment of heart defects), and the risk of pregnancy loss [20, 80, 92, 93]. It should be kept in mind that combined prenatal screening enables to form high-risk groups of pregnant women for the development of preeclampsia, fetal growth retardation, and preterm delivery.

It is becoming increasingly evident that with an integrated approach to screening in the first trimester of pregnancy, "genotyping should be performed following the description of the clinical phenotype" [92]. Bardi et al. described 1,901 pregnancies with the NFT of 95th percentile and greater in the period 2010–2016. Pregnancies with unknown outcomes were excluded. The results of detailed US, karyotyping, genotyping, pregnancy and neonatal outcomes, consultation with a clinical geneticist, and postmortem research data were collected. At least one structural abnormality was registered in 43% of the fetuses out of a total of 821 cases. The incidence of abnormalities was 21% for fetuses with NFT of the 95th–99th percentiles and 62% for fetuses with NFT of 99th percentile or higher. In this cohort, the incidences of monogenic disorders, submicroscopic and chromosomal abnormalities were 2%, 2%, and 30% (24% of trisomy 21, 18, and 13, and 5.4% of other CAs), respectively. Isolated structural abnormalities, for which no genetic defects were found, were noted in 9%. The authors concluded that NIPT was superior in sensitivity to combined screening (especially for detecting trisomy 21), but ecDNA testing is not combined with fetal US, including the measurement of NFT, and 34% of congenital anomalies may remain unnoticed in the first trimester of pregnancy. In the case of a normal karyotype of fetuses with high rates of NFT and/or structural abnormalities, additional molecular genetic studies and increased attention to the management of pregnancy are required [92].

Nowadays, the current cost of NIPT limits its use as a first-line screening. In 2016, K. Nikolaides et al. revealed the price ratios of prenatal screening protocols for Tr21, including NIPT [94]. While using NIPT as the first-line of screening, there was a three- to fourfold increase in the average cost of detecting one case of birth with Tr21. However, this is most probably a temporary constraint, as the price of NIPT has been decreasing since its introduction and is expected to continue to decrease.

Nshimyumukiza et al. analyzed the quality of the economic assessment of NIPT in 16 studies published in the period 2009–2016, in which NIPT was compared with the current screening practice (determination of biochemical markers with or without US and/or maternal age) [95]. It is concluded that at the 2018 price level, contingent NIPT offers the best value for money factor for publicly funded screening programs. As a first-line test, NIPT has been economically ineffective in most studies. The most common uncertain variables were NIPT costs, risk cut-offs for current screening practices, screening coverage rates, and the frequency and costs of invasive diagnostic procedures. The overall quality of the papers included in the analytical review was adequate. Given the potential falling prices and the ongoing expansion of NIPT to the genome-wide variant, further research is required to explore the potential cost-effectiveness of introducing non-invasive screening as a first-line test.

The calculation of the economic efficiency of NIPT is presented in a Russian publication in 2016 [96]. The authors tried to assess the feasibility of using NIPT for prenatal screening of CA in the fetus in a simulated situation based on the results of prenatal examination of pregnant women in the Tomsk region. According to the authors, the preferred option, both clinically and economically, is using NIPT in combination with invasive prenatal diagnostics in high-risk groups for CA, which are formed according to the routine combined screening results with an increase of up to 48.3% in direct screening costs.

In this work, we do not focus on the financial aspects and issues of economic feasibility of NIPT because this topic needs to be analyzed separately in detail; there are practically no Russian works on it and the extrapolation of data from international publications seems to be incorrect.

Prospects for NIPT in the prenatal screening system in Russia: modeling based on our own results

Based on the data that the universal NIPT for ecDNA increases the screening sensitivity for Down's syndrome and reduces the frequency of invasive tests [89, 93, 97], we tried to assess the possibility of introducing NIPT into the mass EPS system in Russia. Based on combined screening results, the strategy of additional screening, the so-called

Vol. 70 (1) 2021

DOI: https://doi.org/10.17816/JOWD56573

Table 3. Results of combined early prenatal screening in four regions of the Russian Federation in 2018

Boundaries (cut-offs) of risk groups (total CA, *n* = 668)	Tp21 (*n* = 388)		Tp18 + 13 (*n* = 128)		Tp21 + 18 + 13 (*n* = 516)		Other CA (*n* = 152)		Pregnant women included in various risk groups (*n* = 143,834)		Normal karyotype/ phenotype in fetus and newborn (FPR) (*n* = 143,166)	
	n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
≥1:10 (*n* = 390)	236	60.8 (57.2; 64.3)	89	69.5 (65.6; 76.3)	325	63.0 (59.8; 67.5)	65	42.8 (38.7; 48.9)	1112	0.8 (0.6; 0.9)	722	0.5 (0.6; 0.7)
≥1:50 (*n* = 489)	286	73.7 (71.2; 77.2)	113	88.3 (84.0; 92.0)	399	77.3 (75.6; 80.5)	90	59.2 (55.8; 61.7)	2274	1.6 (1.3; 1.9)	1785	1.2 (0.9; 1.5)
≥1:100 (*n* = 557)	320	82.5 (81.9; 83.0)	121	94.5 (91.6; 96.6)	441	85.5 (85.2; 85.9)	116	76.3 (70.6; 83.7)	3415	2.4 (1.8; 2.9)	2858	2.0 (1.5; 2.6)
≥1:300 (*n* = 598)	351	90.5 (84.8; 92.9)	126	98.4 (97.3; 100)	477	92.4 (89.4; 93.8)	121	79.6 (73.8; 85.9)	6607	4.6 (3.5; 5.7)	6009	4.2 (3.1; 5.3)
≥1:500 (*n* = 610)	357	92.0 (87.5; 94.2)	128	100	485	94.0 (91.2; 95.5)	125	82.2 (77.3; 87.0)	9338	6.5 (5.0; 8.0)	8728	6.1 (4.6; 7.6)
≥1:1,000 (*n* = 624)	367	94.6 (92.9; 95.7)	–	–	495	95.9 (94.9; 96.8)	129	84.9 (77.5; 91.5)	15 094	10.5 (8.3; 12.7)	14 470	10.1 (8.0; 12.3)
less than 1:1,000 (*n* = 44)	21	5.4 (4.3; 7.1)	–	–	21	4.1 (3.2; 5.1)	23	15.1 (11.9; 22.8)	128 740	89.5 (87.3; 91.7)	128 696	89.9 (87.7; 90.0)
≥1:2,000 (*n* = 635)	374	96.4 (93.9; 97.7)	–	–	502	97.3 (95.7; 98.2)	133	87.5 (80.9; 94.5)	24 897	17.3 (14.3; 20.3)	24 262	16.9 (13.9; 19.9)
≥1:2,500 (*n* = 639)	376	96.9 (94.2; 98.2)	–	–	504	97.7 (96.0; 98.5)	135	88.8 (82.1; 95.6)	29 077	20.2 (17.0; 23.4)	28 438	19.9 (17.3; 23.7)
less than 1:2,500 (*n* = 29)	12	3.1 (1.8; 5.8)	–	–	12	2.3 (1.5; 4.0)	17	11.2 (4.4; 17.9)	114 757	79.8 (76.6; 83.0)	114 728	80.1 (76.3; 82.7)
≥1:3,000 (*n* = 640)	377	97.2 (94.2; 98.5)	–	–	505	97.9 (96.0; 98.8)	135	88.8 (82.1; 95.6)	33 417	23.2 (20.0; 26.5)	32 777	22.9 (19.7; 26.2)
≥1:3,500 (*n* = 646)	380	97.9 (96.5; 98.7)	–	–	508	98.4 (97.6; 98.7)	138	90.8 (86.2; 96.7)	37 232	25.9 (21.8; 30.1)	36 586	25.6 (21.5; 29.8)
less than 1:3,500 (*n* = 22)	8	2.1 (1.3; 3.5)	–	–	8	1.6 (0; 8.4)	14	9.2 (3.3; 13.8)	106 602	74.1 (69.9; 78.2)	106 580	74.4 (70.2; 78.5)

Note. CA — chromosomal abnormalities; Tr21, Tr18, Tr13 — trisomies for chromosomes 21, 18, and 13, respectively; FPR — false positive results. This table presents the number of diagnosed CAs, the number of pregnant women who underwent early prenatal screening, and the number of FPRs in each risk group with the corresponding cut-offs in absolute values and the proportion of the total. Percentages are indicated with 95% confidence intervals (CIs)

contingent model, was analyzed. This approach retains the main advantages of NIPT, increases sensitivity, and reduces the number of false positive screening results for CAs, but at a significantly lower cost than while using NIPT for the entire population. Standard US in the first trimester ensures the detection of congenital malformations, and the determination of serum markers of the mother ensures the early prediction of pregnancy complications, such as preeclampsia, fetal growth retardation, preterm delivery, with the potential for the preventive treatment and follow-up of pregnant women in the formed risk groups.

To illustrate a possible model of contingent screening, pooled EPS data for 2018 in four regions of the Russian Federation are presented, in which consistently high results have been noted over the past several years [4], namely in the Moscow region, the Republic of Tatarstan, Sverdlovsk, and Tomsk regions (54,064, 37,770, 42,504, and 9,496 patients examined by the EPS Program, respectively). The defining characteristics for selecting regions were the indicators of prenatal detection of congenital malformations and CA (the proportion of all pregnant women who underwent EPS) more than 0.35%, the sensitivity of EPS for the prenatal detection of Down's syndrome in the fetus in the first trimester of more than 80%, and the completeness of data entry of more than 85% (e.g., Tr21) to the database.

The algorithm for screening and calculating the CA risk in constituent entities was unified and based on a combination of the main markers, namely maternal and gestational ages (baseline risk) within 11–13.6 weeks, anamnestic data, free β-HCG and PAPP-A, NFT and heart rate in the fetus, as well as additional US markers, such as the nasal bone, pulse index in the venous duct, blood flow over the tricuspid valve. The characteristics of pregnant women by age in 2018 are as follows. In the Moscow region, the median age was 30 years (average age: 29.7 ± 0.04 years). In the Republic of Tatarstan, the median age was 29 years (average age: 28.9 ± 0.05 years) whereas in the Sverdlovsk Region, the median age was 30 years (average age: 29.5 ± 0.05 years). In the Tomsk region, the median age was 29 years (average age: 29.3 ± 0.11 years).

Table 3 presents generalized data on the number of CAs revealed through combined EPS in four regions of the Russian Federation in 2018. The table data include the number of CAs diagnosed pre- and postnatally by pregnancy outcomes and recorded in the EPS database (Astraya software), the number of pregnant women who underwent EPS, and the number of so-called FPR (fetuses and newborns with normal karyotype/phenotype) in each risk group with corresponding cut-offs.

The high-risk group in the range of 1:2–1:100 (≥1:100) was 2.4% of all the examined pregnant women. It accounted for 83% of Tr21, 95% of Tr18, and Tr13 in total and 76% of other cases (monosomies of the X chromosome, triploidy, rare CAs, aneuploidies for sex chromosomes, unbalanced CAs, etc.) of the total number of identified CAs for each anomaly, respectively (Table 3). The number of most detected frequent trisomies (Tr21, Tr18, and Tr13) decreases sharply from the cut-off risk of 1:50 for Tr18 and Tr13, that is, from 1:100 for other CAs and from 1:300 for Tr21 (Fig. 3).

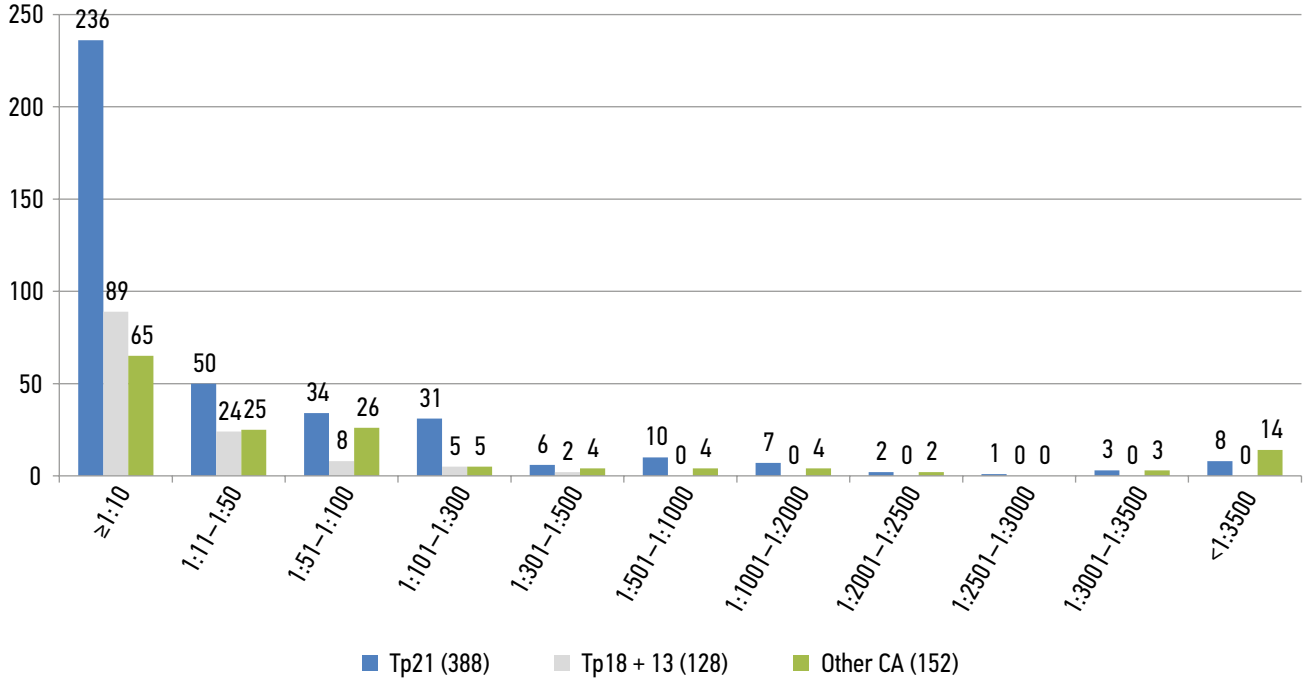

Fig. 3. The number of chromosomal abnormalities revealed during early prenatal screening within the designated risk groups in four constituent entities of the Russian Federation in 2018. The abscissa presents the intervals of risk groups between the borders. CA — chromosomal abnormalities; Tr21, Tr18, and Tr13 — trisomies for chromosomes 21, 18, and 13, respectively

Table 4. Distribution of pregnant women by risk groups based on the results of early prenatal screening (own data and literature data [83, 89, 93, 98, 99, 100])

Boundaries (cut-off) of the risk groups	Four regions of the Russian Federation 2018 (*n* = 143,834)		[83] (*n* = 87,241)	[98] (*n* = 1,005)	[99] (*n* = 11,692)		[89] (*n* = 108,982)		[93] (*n* = 688)	[100] (*n* = 12,327)	
	RG, %	FPR, %	FPR, %	FPR, %	RG, %	FPR, %	RG, %	FPR, %	RG, %	RG, %	FPR, %
≥1:10	0.8	0.5	–	0.5	1.0	0.5	1.4	0.8	–	1.1	0.7
≥1:50	1.6	1.2	–	1.8	2.3	1.8	3.4	2.7	–	1.7	1.2
≥1:100	2.4	2.0	2.1	3.4	3.9	3.4	5.3	4.6	2.5	–	–
≥1:300	4.6	4.2	–	–	–	–	11.1	10.4	–	4.9	4.3
≥1:500	6.5	6.1	7.2	11.6	12.1	11.6	15.3	14.7	–	–	–
≥1:1000	10.5	10.1	11.9	18.8	19.3	18.8	24.3	23.7	13.9	13.4	12.8
≥1:2000	17.3	16.9	19.0	29.5	30.0	29.5	–	–	–	23.3	22.7
≥1:2500	20.2	19.9	21.8	33.9	34.3	33.9	–	–	–	–	–
≥1:3000	23.2	22.9	24.3	38.3	38.7	38.3	–	–	–	30.8	30.2
≥1:3500	25.9	25.6	26.6	42.2	42.5	42.2	–	–	–	–	–

Note. Causes of risk groups and false positive results (the number of pregnant women as a percentage of the number of examined pregnant women who underwent EPS) within the corresponding calculated boundaries (cut-offs). FPR — false positive results, RG — risk group.

Table 5. Sensitivity of early prenatal screening for Down syndrome (detectability) with different cut-offs of risk groups (own data and literature data [83, 85, 89, 99, 100])

Boundaries (cut-offs) of risk groups	Four regions of the Russian Federation, 2018	[83]	[99]	[89]	[85]	[100]
Tp21 (*n*)	388	324	47	432	22	42
≥1:10	60.8	–	64.0	75.7	–	64.3
≥1:50	73.7	–	81.0	87.3	–	76.2
≥1:100	82.5	85.2	87.0	92.1	95.0	–
≥1:300	90.5	–	–	96.3	100.0	83.0
≥1:500	92.0	92.9	98.0	97.0		–
≥1:1000	94.6	95.3	98.0	98.4	100.0	88.0
≥1:2000	96.4	97.1	98.0	–	–	95.0
≥1:2500	96.9	97.5	98.0	–	–	–
≥1:3000	97.2	97.9	98.0	–	–	97.0
≥1:3500	97.9	98.1	100.0	–	–	–

Note. Tr21 — trisomy 21 syndrome.

While analyzing the size of the risk group, the proportion of FPR and the sensitivity of EPS for Down's syndrome at different cut-offs obtained in this study, the comparability of the indicators with international data was noted (Tables 4 and 5). An increase in the cut-off (from 1:100 to 1:300, 1:500, 1:1,000, etc.) leads to an increase in the detection rate of CA, that is, the sensitivity of screening, but at the same time to an increase in FPR (Tables 3–5).

In Russia, EPS has a lower sensitivity, provides smaller sizes of each of the risk groups, and lower FPR within each cut-off, which, in our opinion, may be due to a younger population of pregnant women (Tables 4, 5). Thus, in Nicolaides et al., the median age of pregnant women in the groups of examined female patients with Tr21 was 37.9 years [83], that without Tr21 was 31.2 years. According to Santorum et al., the median ages were 37.9 and 31.5 years for the category of pregnant women with pathological and normal fetal karyotype, respectively [89]. In the works of Gil et al., the median age for the general population of examined pregnant women was 36.7 [98] and 31.0 years [99], 31.0 years in works by Miltoft et al. [85], and 33.9 years in works by Kagan et al. [93]. Cotarelo-Pérez

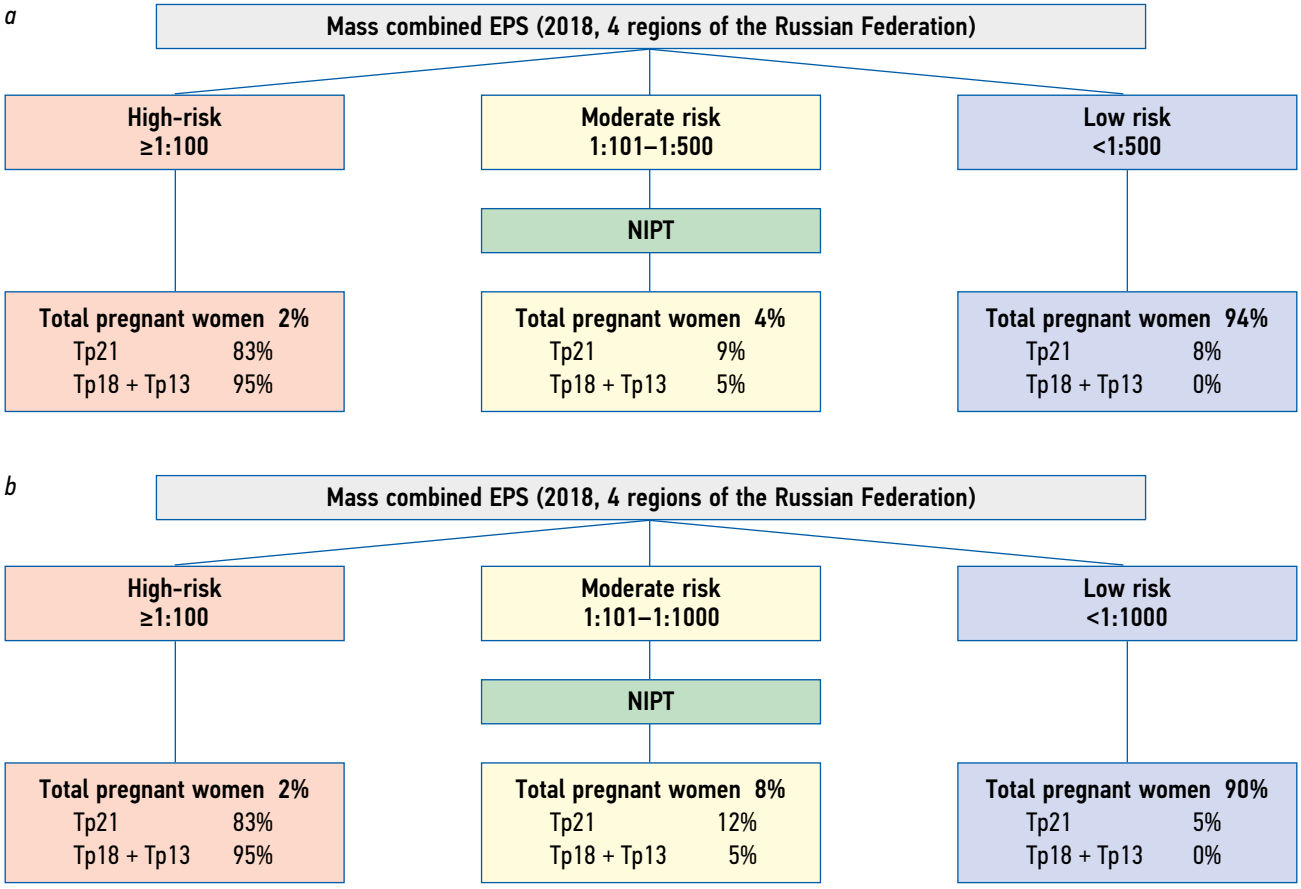

Fig. 4. Possible cohort models of the use of non-invasive prenatal testing (NIPT) in early prenatal screening: *a* — in the moderate risk group 1:101–1:500; *b* — in the moderate risk group 1:101–1:1,000

et al. determined the median age of women with a risk of CA higher than 1:300 as 36.9 years, and as 31.1 years with that lower than 1:300 [100].

The data on EPS presented in Table 3 and Figure 3 show that a certain number of CAs belong to the risk group less than 1:101 (17% for Tr21, in total 5% for Tr18 and Tr13, 24% for other CAs) and may not be detected in the first trimester because only a group of high-risk pregnant women is subject to IPD for the diagnosis of CA based on invasive material. It is assumed that with the use of NIPT, CAs can be detected in these groups. If we predict the use of a contingent model of NIPT for common trisomies (Tr21, Tr18, and Tr13) to the "moderate" risk group in the range from 1:101 to 1:500, assuming that non-invasive screening will reveal 100% of common trisomies in this interval, then NIPT with coverage of an average of about 4% of the population of pregnant women should cause the detection of 92% of trisomy 21 cases (of 17% of cases remaining undetected in the risk boundary of 1:100) and all cases of trisomies on chromosomes 18 and 13. If the risk range is increased from 1:101 up to 1:1000, then about 8% of pregnant women will be selected for screening by the NIPT method. As a result, 95% of cases of Tr21 will be recorded and only 5% will remain undetected (Tables 1 and 5; Fig. 4).

The data presented in Table 3 show that a further increase in the sensitivity of Tr21 screening to 97% and 98% would necessitate NIPT for approximately 18% of pregnant women of all patients who underwent EPS (risk in the range from 1:101 to 1:2,500) and up to 23.5% (risk from 1:101 to 1:3,500). This, undoubtedly, greatly increases both the overall costs of screening in the middle ranges and the costs of each prevented birth of a child with frequent CAs, but does not enable to reach a sensitivity of 100%. According to other authors, even with a significant expansion of the intermediate-risk group, approximately 1.5–5% of pregnant women with Tr21 in fetuses are in the low risk group [83, 86–89, 93, 98–100] and Table 5.

In some countries and private clinics, NIPT is used in both moderate- and high-risk groups (with different risk cut-offs). This is aimed to reduce the number of invasive procedures. Various studies have demonstrated a significant decrease in the proportion of pregnant women at high-risk for Tr21 based on the results of combined EPS, subject to invasive diagnostics, if they underwent NIPT as a second screening [83–85, 97]. However, while using the contingent NIPT model for frequent trisomies (Tr21, 18, 13) in the high-risk group, the possibility of omitting other CAs must be understood. A number of authors object to the use of NIPT in the high-risk group because although the incidence of fetal pathologies

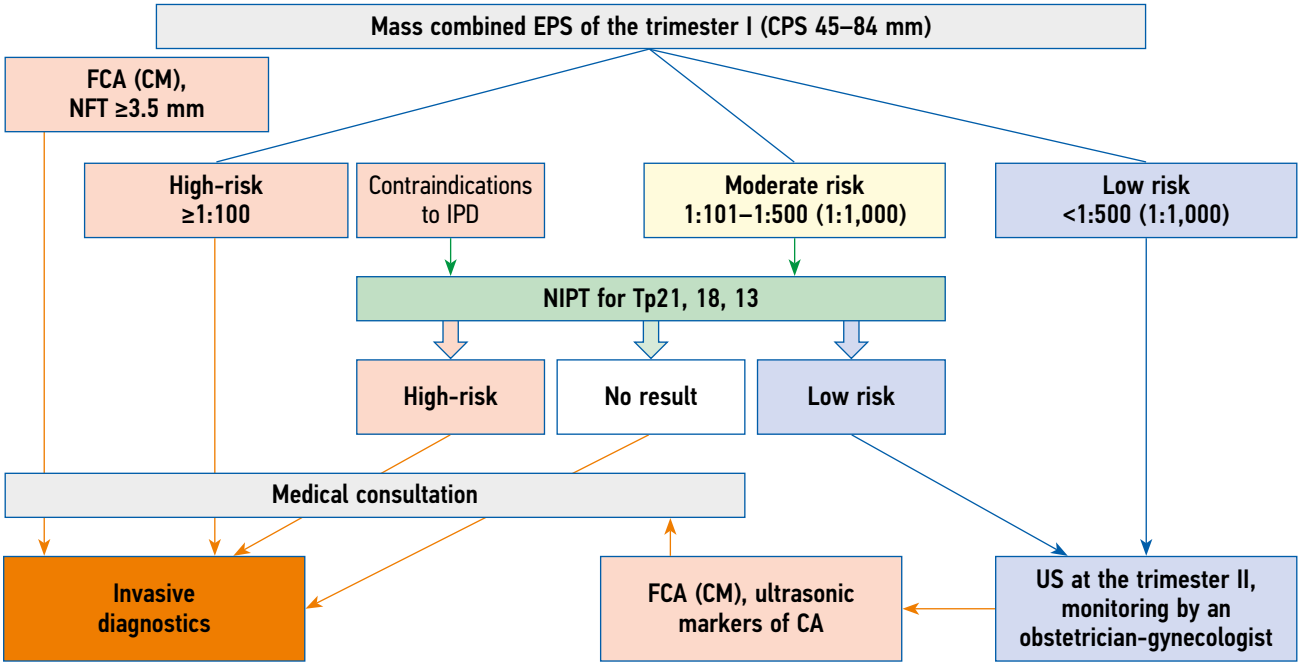

Fig. 5. Schematic model of contingent prenatal screening of chromosomal abnormalities with the technology of non-invasive prenatal testing. NIPT — non-invasive prenatal testing; CPS — coccygeal-parietal size; FCA (CM) — fetal congenital abnormalities (congenital malformations); NFT — nuchal fold thickness; IPD — invasive prenatal diagnostics; CA — chromosomal abnormalities; US — ultrasound examination

caused by microdeletions/microduplications is significant for this group, following modern gwNIPT protocols, they cannot be detected with high clinical sensitivity and specificity. For this reason, ACOG and SMFM have not yet recognized as genome-wide ecDNA screening as clinically validated [101]. Only when NIPT based on the ecDNA [44, 45] or DNA of fetal cells in the mother's blood [102] can correspond to a level comparable to the chromosomal microarray analysis of fetal material, gwNIPT can become the method of choice [103, 104].

In Russia, pregnant women at high-risk for EPS (≥1:100) are referred for invasive diagnostics [105]. In the regions of the Russian Federation selected for the analysis in 2018, the number of high-risk pregnant women who refused IPD averaged 34%, whereas this indicator exceeded 50% in half of the regions of Russia. It is permissible to offer NIPT to selected high-risk pregnant women because of combined screening only if there are medical contraindications for invasive prenatal diagnostics. The use of NIPT as an additional screening in this group at the request of the patient is possible on the condition of highly qualified medical and genetic counseling with an explanation of all residual risks of genetic pathology and congenital malformations. At the same time, the consulting physician should pay attention to the importance of invasive diagnostics in the high-risk group because of the high efficiency of prenatal karyotyping in identifying CAs other than 3 frequent aneuploidies, which is 76.3% in this sample (Table 3, Fig. 3).

Thus, the model of contingent prenatal screening for CA, possible for Russia, assumes the following conditions (Fig. 5).

1. The introduction of NIPT on fetal ecDNA for the most frequent trisomies (Tr21, Tr18, and Tr13) as an additional screening in the moderate risk group is from 1:100 to 1:500 or from 1:100 to 1:1000, formed according to the results of EPS in each region of the Russian Federation.
2. Conducting invasive prenatal diagnostics with the determination of the fetus karyotype for all pregnant women from the high-risk group for EPS (≥1:100), as well as pregnant women with positive NIPT results from the moderate risk group and with uninformative NIPT results.

The main requirements for the implementation of the NIPT contingent model in the constituent entity of the Russian Federation are the following:

1) performing EPS at a high-quality level to ensure reliable data on female patients of different risk ranges;
2) a high level of laboratory diagnostics (including a wide range of molecular genetic methods) and bioinformatic analysis; and
3) a high level of quality of consultation by the doctors of various specialties at all stages of screening.

Modern invasive prenatal diagnostics is impossible without molecular genetic methods, in particular, comparative genomic hybridization or chromosomal microarray analysis. It is extremely important to exclude microdeletion syndromes in fetuses from the group of pregnant women with a high-risk on EPS with NFT of more

than 3.5 mm or with congenital malformations, given that the karyotypes in these fetuses were normal. Under certain clinical indications, in case of detecting fetal US pathology, laboratory diagnostics of the parental and fetal material ("trio") is possible using new genomic sequencing methods.

The genome-wide variant of NIPT, which is widely introduced in a number of countries, undoubtedly increases the detection frequency of all types of chromosomal pathology but, today, compliance with all the necessary requirements for its implementation in different regions in Russia is problematic for various reasons. That is why, to use gwNIPT, the scale of clinical trials must be expanded, including the organization of pilot research projects in major centers with high professional and technical levels.

CONCLUSION

There is an undeniable value of NIPT in the detection of CA from the perspective of science and medical practice; however, its application in the system of already existing mass prenatal screening is associated with the solution of a large number of problems. In this regard, the position of the authors coincides with the recommendations of international professional medical communities presented in the review and the opinion of most researchers in accordance with the screening principles of the World Health Organization [63].

The proposed contingent model of NIPT still undoubtedly needs to be discussed. In the Russian Federation, NIPT currently does not have a state status in the public health system, that is, there is no national statutory regulation and it is offered mainly by laboratories under voluntary medical insurance at the expense of the patient. The decision to include NIPT in the system of state-guaranteed prenatal care should be based on the obvious medical significance and economic feasibility of additional complex costs, for which studies should be conducted for comparative assessment of the cost and efficiency of the current system of prenatal screening and screening with the inclusion of new technology.

It should be emphasized once again that a crucial aspect of any new prenatal care algorithm is highly qualified pre- and post-test counseling that is aiming to provide pregnant women and her family with detailed information about the proposed examination so that they can make an informed decision with respect to all possible subsequent additional medical interventions and procedures, which are associated with the organization of educational training of doctors and information adaptation of society.

Conflict of interest. The authors declare no conflict of interest.

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