UDC 618.29-074/-079-056.7
DOI https://doi.org/10.32782/2226-2008-2024-3-11

Yu. I. Bazhora[1] https://orcid.org/0009-0005-5006-7339
M. M. Chesnokova[1] https://orcid.org/0009-0008-5644-5515
A. V. Shevelenkova[1] https://orcid.org/0009-0009-0038-7587
S. V. Matviyuk[2] https://orcid.org/0000-0002-2023-1709
K. V. Ostapchuk[1] https://orcid.org/0009-0002-5972-6466

NON-INVASIVE PRENATAL TESTING (NIPT) AS A MORDEN METHOD OF PRENATAL SCREENING (A LITERATURE REVIEW)

[1] Odesa National Medical University, Odesa, Ukraine
[2] Regional Children's Clinical Hospital, Odesa, Ukraine

UDC 618.29-074/-079-056.7

Yu. I. Bazhora[1], M. M. Chesnokova[1], A. V. Shevelenkova[1], S. V. Matviyuk[2], K. V. Ostapchuk[1]
NON-INVASIVE PRENATAL TESTING (NIPT) AS A MORDEN METHOD OF PRENATAL SCREENING (a literature review)
[1] Odesa National Medical University, Odesa, Ukraine
[2] Regional Children's Clinical Hospital, Odesa, Ukraine

Introduction. Non-invasive prenatal testing (NIPT), which is based on the examination of cell-free fetal DNA (cffDNA) in the blood of a pregnant woman, is an important addition to prenatal screening tests for chromosomal abnormalities. When used correctly, the test increases the probability of detecting chromosomal pathology in the fetus and, at the same time, reduces the number of invasive tests.

The research purpose is to summarize literature data on the principle of cffDNA testing for prenatal screening of chromosomal pathology.

Methods. Analysis of scientific publications in international electronic scientometric databases over the last 10 years.

Research and discussion. The analyses of characteristics of cffDNA, the principles of a non-invasive prenatal test (NIPT), its sensitivity, specifics, and the expected positive predictive value in relation to chromosomal diseases were performed. The algorithms for using NIPT in different countries were analysed. Testing of cffDNA is recommended from the 10th week of gestation throughout pregnancy. NIPT is used as a screening test and requires conformation of positive results by invasive methods. In a number of countries, it is included in state prenatal screening programs as a first- or second-line test. In Ukraine, the test is offered by private laboratories at the patients' own expense. The NIPT use requires the elaboration of own or adaptation of existing guidelines following the algorithm of the method's application and interpretation of test results.

Key words: cell-free fetal DNA, non-invasive prenatal test, NIPT, prenatal screening for chromosomal aneuploidy, NIPT implementation.

УДК 618.29-074/-079-056.7

Ю. І. Бажора[1], М. М. Чеснокова[1], А. В. Шевеленкова[1], С. В. Матвиюк[2], К. В. Остапчук[1]
НЕІНВАЗИВНЕ ПРЕНАТАЛЬНЕ ТЕСТУВАННЯ (NIPT) – СУЧАСНИЙ МЕТОД ПРЕНАТАЛЬНОГО СКРИНІНГУ (ОГЛЯД ЛІТЕРАТУРИ)
[1]Одеський національний медичний університет, Одеса, Україна
[2]КНП «Обласна дитяча клінічна лікарня» Одеської обласної ради, Одеса, Україна

Неінвазивний пренатальний тест (NIPT) базується на дослідженні позаклітинної ДНК плода в крові вагітної жінки. Він є важливим доповненням до пренатальних скринінгових тестів на хромосомні аномалії. Тест збільшує імовірність виявлення хромосомної патології у плода й одночасно зменшує кількість необхідних інвазивних тестів. NIPT використовується як скринінговий тест і потребує за позитивних результатів підтвердження діагнозу інвазивними методами. У ряді країн NIPT передбачений у державних програмах пренатального скринінгу як тест першої або другої лінії. В Україні тест пропонується приватними лабораторіями за власні кошти пацієнтів. Використання NIPT потребує створення власних або адаптації наявних рекомендацій щодо алгоритму застосування методу, інтерпретації результатів тесту.

Ключові слова: позаклітинна ДНК плода, неінвазивний пренатальний тест, NIPT, пренатальний скринінг на хромосомні анеуплоїдії, імплементація NIPT.

Introduction. The main task of prenatal screening is to select women at high risk of having children with congenital and hereditary pathology for further clarification of this diagnosis in the fetus. First of all, it concerns congenital malformations and chromosomal pathology.

In most countries of the world, including Ukraine, the following is used for prenatal screening: 1) combined screening in the first trimester at 11 weeks + 0–13 weeks + 6 days, 2) in II trimester, ultrasound at 18–22 weeks of gestation for a detailed assessment of the anatomical structure of the fetus, excluding malformations of later terms, 3) determination of maternal serum markers at 16–20 weeks ("double test" – determination of AFP and free β-chorionic gonadotropin (β-hCG) or "triple test" – AFP + free β-hCG + free estriol, "quadruple test" – AFP + free β-hCG + free estriol + inhibin A), 4) non-invasive prenatal screening (NIPT) [1, 2].

Combined screening of the first trimester includes ultrasound screening to clarify the gestational age, assess fetal anatomy, search for early malformations and early markers of chromosomal abnormalities, such as nuchal translucency (NT) scan and visualisation of the nasal bones. If the fetal anatomy is normal and NT < 3.5 mm, biochemical

© Yu. I. Bazhora, M. M. Chesnokova, A. V. Shevelenkova et al., 2024
Стаття поширюється на умовах ліцензії CC BY

screening is performed simultaneously to determine serum markers: free β-hCG, pregnancy-associated plasma protein-A (PAPP-A), and placental growth factor (PlGF) [2]. Combined screening is aimed at determining the individual risk of fetal trisomy 21, 18, 13 chromosomes and predicting the risk of pre-eclampsia. The calculation takes into account the results of the examination, the mother's age, body weight, ethnicity, smoking, diabetes mellitus and family genetic history.

Combined first-trimester screening can identify more than 90% of trisomies, with a screening-positive rate of 5% of the general population, most of which are false positives [3]. The sensitivity of the test (detection rate) for trisomy 21 is 87% at 11 weeks of gestation, 85% at 12 weeks and 82% at 13 weeks [3].

Determination of serum markers in the second trimester also allows calculating the individual risk of chromosomal abnormalities and, in addition, the risk of the neural tube defects [2]. The sensitivity of the second-trimester screening for chromosomal abnormalities is lower than that of the first trimester, with a quadruple test of 81% for trisomy 21 [4].

If the chromosomal abnormality risk score is higher than the cut-off value, the result is considered positive or "high risk". For Down syndrome, the cut-off risk in the first trimester can optionally be 1/150–1/250, and in the second trimester, the recommended cut-off risk is 1/250 [4]. Screening tests can be false positive, so to confirm the diagnosis of chromosomal pathology, it is necessary to perform invasive prenatal diagnostics – cytogenetic analysis of cells to determine the fetal karyotype. In the first trimester, chorionic villi biopsy (chorionocentesis) is most often performed, and in the second trimester, amniocentesis is performed. A possible complication of invasive diagnostics is pregnancy termination with a probability of 0.5%–1.2% for chorionocentesis and 0.5% for amniocentesis, but in recent years, a decrease in this risk to 0.06%-0.13% has been reported [5].

Non-invasive prenatal testing (NIPT), which is based on the study of cell-free fetal DNA (cffDNA) in the blood of a pregnant woman, is an important complement to prenatal screening tests for chromosomal abnormalities. When used correctly, NIPT increases the posssibility of detecting chromosomal abnormalities in the fetus while reducing the number of invasive tests required [6].

The aim of the study is to summarise the literature data on using cfDNA for prenatal screening of chromosomal pathology.

Methods. We analysed scientific publications in the international electronic scientometric databases PubMed (https://pubmed.ncbi.nlm.nih.gov/), ScienceDirect (https://www.sciencedirect.com/) and Google Scholar (https://scholar.google.com/) using keywords.

Research results and their discussion

Characteristics of cffDNA. Cell-free nucleic acids released into the plasma/serum by various tissues through apoptosis, necrosis, and secretion are always circulating in the human blood. They are a mixture of single- or double-stranded nucleic acids and include DNA fragments, RNA, microRNA, long non-coding RNA, and mitochondrial DNA/RNA. Cell-free nucleic acids can be used as potential biomarkers in various clinical conditions [7].

The presence of cffDNA fragments in the blood plasma and serum of a pregnant woman was reported by Lo YM et al. in 1997 [8]. To identify fetal DNA, women carrying male fetuses were examined. The SRY gene, located in the Y-chromosome, was used as a genetic marker. The source of cffDNA circulating in the maternal blood is mainly placental cells that undergo a programmed cell death [9]. Later, Lo YM et al. demonstrated that the fetal fraction (the percentage of all cell-free fetal DNA in the maternal blood/plasma/serum) is higher in the maternal plasma than in the serum. This is due to the greater amount of background maternal DNA in the serum compared to plasma as the maternal DNA the release during the blood clotting process [10].

Cell-free fetal DNA can be detected as early as the first trimester from the 14th day after conception [11]. The fetal fraction increases at a rate of 0.10% per week between 10 and 21 weeks, and then at a rate of 1% per week [12]. For example, in one study, in the first (12–14 weeks), second (17–22 weeks) and third (38–39 weeks) trimesters of pregnancy, the fetal fraction was 9.7%, 9.0% and 20.4% of the total maternal plasma cfDNA concentration, respectively [13]. The increase in the cffDNA concentration in the maternal blood with the progression of pregnancy is explained by increased placental apoptosis [14].

After delivery, cell-free fetal DNA rapidly disappears from the maternal blood. The half-life of cell-free DNA is 16 minutes. In most women, it cannot be detected within 2 hours after delivery [15]. Purification of the mother's blood from fetal DNA after childbirth eliminates the possibility of false positive results of prenatal diagnosis in subsequent pregnancies.

The size of cfDNA fragments is determined by the way the DNA molecule is packaged in the cell. In chromosomes, DNA is joined to histone proteins to form nucleosomes. Nucleosomes are not closely spaced. The linker DNAs with an average length of 20 base pairs (bp) (varies from several to 80) are between them. During apoptosis, nuclear endonucleases cut chromosomal DNA along the linker DNA into fragments, so the size of cfDNA fragments circulating in the blood is a multiple of the nucleosomal DNA size (147 bp) [16]. It is interesting that fetal DNA molecules in the mother's bloodstream are shorter than maternal DNA molecules. Massive parallel sequencing (next generation sequencing (NGS) data showed that most maternal cfDNAs are 166 bp in size, followed by a pronounced peak of 143 bp and accompanied by a series of smaller peaks with a frequency of 10 bp at size below 143 bp [17]. Cell-free fetal DNA is predominantly 143 bp or smaller. It is assumed that the 166 bp peak represents nucleosomal DNA plus a portion of linker DNA. The elevation of the 143 bp peak for fetal cfDNA may be due to truncation of linker DNA.

It has been established that the size of cell-free DNA fragments in blood plasma positively correlates with the level of its methylation [18]. It is believed that unmethylated regions are more accessible for cutting by endonucleases. Maternal somatic cell DNA is hypermethylated compared to placental DNA. This leads to increased nucleosome stability and a longer average length of maternal cfDNA fragments compared to fetal DNA. During pregnancy, placental DNA methylation progressively increases, and

therefore the size of fetal cfDNA fragments in maternal plasma increases with gestational age [18].

Use of cffDNA for prenatal diagnosis

After the discovery of fetal cfDNA circulating in the mother's blood, it was proposed to test it to analyse genetic markers that the fetus may inherit from its father. The first steps were to determine the sex of the fetus by the presence of the SRY gene for the risk of X-linked diseases and the fetal Rh-positive gene in Rh negative pregnant women in 1998. These tests were quickly adopted by a number of laboratories in the UK and the Netherlands [19].

In 2007, the principle of non-invasive prenatal diagnosis of trisomy 21 in the fetus by cfDNA analysis was proposed. In case of trisomy 21, the fetal karyotype contains three copies of the 21st chromosome instead of two, as is normal, so the amount of genetic material of chromosome 21 in the mother's blood will be increased [20]. This principle was successfully implemented in 2011 through the use of massive parallel genomic sequencing (NGS) [17, 21]. The authors conducted a large-scale clinical trial of NIPT and achieved 100% sensitivity and 97.9% specificity for the prenatal diagnosis of trisomy 21.

NIPT has demonstrated high efficiency in detecting chromosomal diseases associated with changes in the number of chromosomes (aneuploidy). When analysing the effectiveness of tests, sensitivity, specificity, and the positive predictive value (PPV) are taken into account [22]. Sensitivity is the probability that a test result will be positive when a disease is present. Specificity – the probability that the test result will be negative when the disease is not present. The PPV indicates the proportion of patients with a positive test result who actually have the disease [22]. The high sensitivity and specificity of NIPT has been proven in numerous clinical trials. According to a 2017 meta-analysis for trisomy 21, the test sensitivity ranged from 94.4 to 100% with a false positive rate of 0 to 0.94% (99.7% and 0.04% on average, respectively). The sensitivity for trisomy 18 ranged from 87.5 to 100% with a false positive rate of 0 to 0.22% (on average 97.9% and 0.04%, respectively). The sensitivity for trisomy 13 ranged from 40.0 to 100% with a false positive rate of 0 to 0.25% (on average 99.0% and 0.04%) [23]. Modern methods of molecular genetic testing of cfDNA provide a sensitivity of 99–100% and a specificity of more than 99% for trisomies 21, 18, 13 [24–26].

For diseases caused by a change in the number of sex chromosomes, the method's efficiency is also high. For sex chromosome trisomies (polysomy X syndromes in women, polysomy Y in men, and Klinefelter syndrome), the average sensitivity and false positive rates were 100% and 0.004%, respectively [23]. When using NGS, the sensitivity of NIPT for monosomy X is 93.9% and the specificity is 99.6% [27].

If the sensitivity and specificity of the test for trisomies 21, 18, 13 are similar, the PPV is significantly different. According to the literature data, the PPV for trisomy 21 ranges from 84 to 98.9%, for trisomy 18 – from 47.6 to 82%, and for trisomy 13 – from 10 to 92.3% [28, 29]. A study conducted in China (Guangzhou) in 2017–2020 showed the PPV for these trisomies of 86.1%, 57.8% and 25.0%, respectively [28]. Unlike sensitivity, the PPV also takes into account the prevalence of the pathology and increases or decreases according to its higher or lower frequency in the group under examination. If the prevalence of the disease in the investigated group is low, even tests with excellent sensitivity and specificity will have a low PPV and generate more false positive than true-positive results [22]. This, in turn, increases the number of unnecessary invasive prenatal diagnostic procedures [30].

For the group of aneuploidies on the sex chromosomes as a whole, the average PPV ranges from 31.39% to 54.54% according to different studies [29, 31, 32]. The lowest PPV is in monosomy 45,X (from 12.5 to 29.4%) [28, 31, 32]. For trisomies on the sex chromosomes, the PPV is higher. Thus, according to Li Y et al., the PPV for the 47,XXX syndrome was 58.82%, for the 47,XXY and 47,XYY syndromes – 85.71% [31].

Differences in the obtained PPV results may be explained with different characteristics of the investigated populations by size, demographics, clinical characteristics, and different NIPT techniques. A higher PPV is observed in the group of pregnant women with an increased risk of fetal chromosomal abnormality and increases with maternal age. Thus, with the 99% sensitivity and specificity of NIPT, the PPV in pregnant women aged 20 years for trisomy 21 is 38–80%, for trisomies on chromosomes 18 and 13, respectively, 11–41% and 5–13%. At the age of 40 years, the PPV for trisomy 21 is as high as 91–99%; for trisomy 18 it is 66–92% and for trisomy 13 it is 43–71% [33]. An increase in the PPV for trisomies 18 and 13 is observed in the group of pregnant women with an increase in the fetal NT [26].

The non-invasive test can be used to detect rare aneuploidies, microduplications and microdeletions, including deletion of 15q (Prader–Willi and Angelman syndromes), 22q11.2 (DiGeorge syndrome), 4p (Wolf–Hirschhorn syndrome), 5p (cat's cry syndrome), 11q (Jacobsen syndrome), etc. However, the sensitivity and PPV of the method for these syndromes are significantly lower. For rare aneuploidies, the PPV was less than 28%, and for deletions and duplications, it ranged from 29% to 50% [28, 34].

The expected positive result of NIPT is higher than the PPV of combined screening in the first trimester of pregnancy. For pregnant women with a trisomy 21 risk of 1:300 based on the results of combined screening in the first trimester, the PPV of combined screening is 7%, and the PPV of non-invasive testing is 76.8%. For 25-year-old pregnant women who have a 1:950 risk for trisomy 21, the PPV of combined screening is 2.3% and that of NIPT is 51.1% [6].

Fetal cfDNA testing can be attractive for many reasons. The test is more sensitive and specific than other prenatal screening methods, does not carry the risk of miscarriage, allows early screening for fetal chromosomal abnormalities, and leaves parents more time to decide whether to terminate or prolong the pregnancy [3]. The fetal cfDNA fraction is low before 10 weeks. At 10 weeks, it is approximately 10%, which is considered sufficient for analysis [12, 35, 36]. Accordingly, NIPT is recommended to perform from the 10th week throughout pregnancy [1, 3, 6, 37]. The test can give false positive results, so in 2016, the American College of Obstetricians and Gynaecologists emphasised that NIPT is a screening test, but not a diagnostic test. Confirmation of positive results by invasive testing is mandatory [33, 38]. In addition to false positives, false negatives and no-call results are also recorded.

Possible causes of false positive, false negative, and no-call NIPT results

False positive results are observed on average in 0.4% of cases [1]. This may be due to limited placental mosaicism [39, 40], the vanishing twin syndrome [41, 42], maternal mosaicism or chimerism, the presence of bone marrow or tissue transplants in pregnant women, or recent blood transfusion [6, 42]. False positive results can also be caused by an undetected malignant or benign maternal tumour [43, 44]. This is explained by the fact that a tumor DNA can contain numerous duplications and deletions. In this case, it is not the fetus that has health problems, but the mother. It is believed that in case of false positive NIPT results, especially when more than one chromosomal aberration is detected, it is necessary to discuss with the pregnant woman the risk of such a pathology. Accordingly, NIPT is not recommended for screening fetal anomalies in pregnant women with a diagnosed neoplasm [44]. It is the possibility of false positive NIPT results that requires mandatory confirmation by invasive diagnostics [45].

False negative NIPT results are less common than false positive results. For example, during examination of 81,601 pregnant women, the false negative rate was 0.01% [46]. According to Hartwig et al., 92% of all analysed false negatives were due to true fetal mosaicism [47]. Other causes may include placental mosaicism [48] and low fetal cfDNA fraction [47, 49].

No-call result occurs in approximately 0.3% of cases [43]. One of the main reasons for this is the low fetal cfDNA fraction (<4%) [50]. Thus, in study of 303 pregnant women with a no-call result, 135 women (44.6%) had a low fetal fraction [51]. The reasons for this include conducting the test earlier than the recommended time, high maternal body mass index, *in vitro* fertilisation, treatment of the pregnant woman with low-molecular-weight heparins and other anticoagulants, autoimmune diseases in pregnant women, and multiple pregnancy [43, 51, 52, 53]. Low fetal fraction is observed in trisomies 18 and 13 due to the small size of the placenta [23], in monosomy X and triploidy [6]. Therefore, pregnant women with a no-call result should be classified as a high-risk group for fetal chromosomal syndromes. According to the recommendations of the American College of Obstetricians and Gynaecologists, a no-call NIPT result can be considered an indication for invasive testing [38].

The no-call NIPT results requires high-quality medical and genetic counselling and identification of possible causes. Further recommendations should take into account the results of fetal ultrasound or combined screening in the first trimester of pregnancy. If there are no additional risk factors for fetal chromosomal abnormalities and the gestational age is early, NIPT should be repeated. When repeated, the test is informative in 50–80% of pregnant women [6, 39]. However, in case of high body mass index, repeated examination is not recommended [6].

Due to the presence of false positive, false negative and no-call results, it is recommended that the non-invasive test be accompanied by an ultrasound for more accurate analysis and to avoid diagnostic errors [42].

Implementation of NIPT in different countries

NIPT technology was first introduced commercially in Hong Kong in 2011 and later in the United States. Nowadays, NIPT is approved by many professional organisations and is used in more than 60 countries, including Ukraine [24].

There are different methods for detecting and evaluating fetal cfDNA in pregnant women's blood. In some countries, NGS is performed, in which all DNA fragments are "read". This provides information about aneuploidy on any chromosome or its part (non-targeted sequencing). Other countries use targeted (chromosome-selective) sequencing methods. In this case, information is read only for chromosomes 21, 18, 13, X and Y [37, 54].

The NIPT can be used as a primary screening test (first-line test) for all pregnant women without prior tests and risk assessment, or as a secondary screening test (second-line test). The first model has been implemented in Belgium and the Netherlands. For example, in Belgium, since 2017, NIPT has been offered as a first-line test and is almost fully reimbursed by the National Institute for Health and Disability Insurance (NIHDI) [55, 56]. Pre-test counselling for pregnant women is mandatory and is provided by obstetricians, family doctors, and midwives. Women are informed about the diseases that can be detected in the fetus, possible test results, and the cost of the test (€8.68). If a positive result is obtained, professional genetic counselling is required.

In other countries (Switzerland, the United Kingdom, Norway, Canada, etc.), NIPT has been introduced as a second-line test. It is suggested in groups of pregnant women with an increased risk of fetal aneuploidy calculated according the results of combined first trimester screening or determined by maternal age, previous pregnancy with trisomy 21, 18, 13. The aim of this approach is to reduce the frequency of invasive procedures. In this case, NIPT serves as an intermediate step between combined first trimester screening and invasive diagnosis [45]. Attention is drawn to the need for mandatory counselling of pregnant women both before testing and in case of positive test results. For example, the Swiss Society for Medical Genetics and the Federal Office of Healthcare recommend NIPT since July 2015 for pregnant women with a risk of trisomies 21, 18, 13 ≥ 1:1000 based on the results of combined first trimester screening [57]. Fetal cfDNA testing for these trisomies is performed in singleton and twin pregnancies and is fully funded by insurance companies [57, 58]. In singleton pregnancies, screening for aneuploidies on the sex chromosomes is also offered without additional charge. If the risk of trisomy 21, 18 or 13 is < 1:1000, the test can be performed at the patient's own expense.

The UK National Screening Committee recommends non-invasive testing for pregnant women with a trisomy risk of 1:150 based on the results of a combined first trimester test or a second trimester quadruple test [59]. In Norway, the government introduced NIPT into the public antenatal care programme in March 2017 for women over 38 years of age who have a risk of trisomy 21 greater than 1:250 or trisomy 18/13 greater than 1:150 based on the results of a combined first trimester screening [60]. Since May 2020, all pregnant women over 35 years of age, as well as all pregnant women with an increased risk of fetal malformation/aneuploidy based on the results of a routine ultrasound in the first and second trimesters, have been authorised to undergo NIPT.

The implementation of NIPT in different countries depends on national healthcare systems, economic resources, the cost of the test and, finally, on the attitude of society towards the problem of pregnancy termination, raising children with chromosomal abnormalities, etc. [23, 54, 55].

In Ukraine, combined screening in the first trimester is mandatory, followed by ultrasound screening and maternal serum markers in the second trimester. NIPT is offered by private laboratories and is carried out at the patient's own expense. It is possible to sequence the entire genome or study the DNA of individual chromosomes. Unfortunately, there are no published data on the number and age of examined pregnant women, the reasons for fetal cfDNA testing, or the test results.

Conclusions. Fetal cfDNA testing (NIPT) is a modern method of prenatal screening characterised by higher specificity, sensitivity, and expected positive result compared to the combined first trimester screening. It is widely used in many countries as a first- or second-line test. The method is available in Ukraine and its use can be expected to increase in the future. This will require the development or adaptation of existing guidelines for the use of NIPT and the interpretation of test results. It is important to inform obstetricians and gynaecologists about the possibilities and limitations of NIPT and to train specialists to counsel pregnant women on the test results.

BIBLIOGRAPHY

1. Reinsperger I. Regulation and financing of prenatal screening and diagnostic examinations for fetal anomalies in selected European countries. AIHTA Policy Brief No. 12; 2022. Vienna: HTA Austria. Austrian Institute for Health Technology Assessment GmbH22. Available from: https://eprints.aihta.at/1369/1/Policy%20Brief_012.pdf
2. Order of the Ministry of Health of Ukraine dated August 9, 2022, No. 1437 On approval of the Standards of medical care 'Normal pregnancy' (in Ukrainian). Available from: https://moz.gov.ua/article/ministry-mandates/nakaz-moz-ukraini-vid-09082022--1437-pro-zatverdzhennja-standartiv-medichnoi-dopomogi-normalna-vagitnist.
3. LeFevre NM, Sundermeyer RL. Fetal Aneuploidy: Screening and Diagnostic Testing. *Am Fam Physician.* 2020 Apr 15; 101 (8): 481–8. Available from: https://pubmed.ncbi.nlm.nih.gov/32293844/.
4. Order of the Ministry of Health of Ukraine dated October 20, 2015, No. 685. Unified clinical protocol of primary, secondary (specialized) and tertiary (highly specialized) medical care. Down syndrome (in Ukrainian). Available from: https://zakon.rada.gov.ua/rada/show/v0685282-15#n14.
5. Choe SA, Seol HJ, Kwon JY, et al. Clinical Practice Guidelines for Prenatal Aneuploidy Screening and Diagnostic Testing from Korean Society of Maternal-Fetal Medicine: (1) Prenatal Aneuploidy Screening. *J Korean Med Sci.* 2021 Jan 25; 36 (4): e27. doi: 10.3346/jkms.2021.36.e27.
6. Harraway J. Non-invasive prenatal testing. *Aust Fam Physician.* 2017 Oct; 46 (10): 735–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29036772/.
7. Beenish R, Taqveema A, Divika S, Aatish M, Jyotdeep K. Circulating Cell-Free Nucleic Acids as Epigenetic Biomarkers in Precision Medicine. *Front Genet.* 2020 Aug 11; 11: 844. doi: 10.3389/fgene.2020.00844.
8. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet.* 1997; 350 (9076): 485–7. doi: 10.1016/S0140-6736(97)02174-0.
9. Alberry M, Maddocks D, Jones M, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenat Diagn.* 2007; 27 (5): 415–8. doi: 10.1002/pd.1700.
10. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet.* 1998; 62: 768–75. doi:10.1086/301800.
11. Illanes S, Denbow M, Kailasam C, Finning K, Soothill PW. Early detection of cell-free fetal DNA in maternal plasma. *Early Hum Dev.* 2007; 83 (9): 563–6. doi: 10.1016/j.earlhumdev.2006.11.001.
12. Wang E, Batey A, Struble G, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on cell-free fetal DNA in maternal plasma. *Prenat diagn.* 2013 Jul;33(7):662-6. doi: 10.1002/pd.4119.
13. Lun FM, Chiu RW, Chan KC, Leung TY, Lau TK, Lo YM. Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. *Clin Chem.* 2008 Oct; 54 (10): 1664–72. doi: 10.1373/clinchem.2008.111385.
14. Ariga H, Ohto H, Busch MP, et al. Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. *Transfusion,* 2001; 41: 1524–30. doi: 10.1046/j.1537-2995.2001.41121524.x.
15. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet.* 1999; 64 (1): 218–24. doi: 10.1086/302205.
16. Lichtenstein AV, Melkonyan HS, Tomei LD, Umansky SR. Circulating nucleic acids and apoptosis. *Ann N Y Acad Sci.* 2001; 945: 239–49. doi: 10.1111/j.1749-6632.2001.tb03892.x.
17. Jiang P, Lo YM. The Long and Short of Circulating Cell-Free DNA and the Ins and Outs of Molecular Diagnostics. *Trends in Genetics* 2016; 32 (6): 360–71. doi: 10.1016/j.tig.2016.03.009.
18. Jiang P, Tong YK, Sun K, et al. Gestational age assessment by methylation and size profiling of maternal plasma DNA: A feasibility study. *Clin Chem.* 2017 Feb; 63 (2): 606–8. doi: 10.1373/clinchem.2016.265702.
19. Lo YM. Discovery of Cell-Free Fetal DNA in Maternal Blood and Development of Noninvasive Prenatal Testing2022 Lasker-DeBakey Clinical Medical Research Award. *JAMA.* 2022; 328 (13): 1293–4. doi:10.1001/jama.2022.14982.
20. Lo YM, Lun FM, Chan KC, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. *Proc Natl Acad Sci USA* 2007; 104: 13116–21. doi: 10.1073/pnas.0705765104.
21. Chiu RW, Akolekar R, Zheng YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ.* 2011; 342: c7401. doi: 10.1136/bmj.c7401

22. Slabky GO. Methodology for assessing the effectiveness of preventive medicine in the activities of a family doctor: method. recommendations. Kyiv, 2011. 38 p. (in Ukrainian). Available from: https://dspace.uzhnu.edu.ua/jspui/bitstream/lib/24438/1/%2B%2B%20%20%D0%9C%D0%B5%D1%82%D0%BE%D0%B4%D0%BE%D0%BB%D0%BE%D0%B3%D1%96%D1%8F.pdf.
23. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: Updated meta-analysis. *Ultrasound Obstet. Gynecol.* 2017 Sep; 50 (3): 302–14. doi:10.1002/uog.17484.
24. Ravitsky V, Roy MC, Haidar H, at al. The Emergence and Global Spread of Noninvasive Prenatal Testing. *Annu Rev Genomics Hum Genet.* 2021 Aug 31; 22: 309–38. doi: 10.1146/annurev-genom-083118-015053.
25. Wang F, Fu K, Wang Y, at al. Clinical evaluation of non-invasive prenatal screening in 32,394 pregnancies from Changzhi maternal and child health care hospital of Shanxi China. *J Med Biochem.* 2022 Jul 29; 41 (3): 341–6. doi: 10.1016/j.apsb.2023.12.010.
26. Xiang L, Zhu J, Deng K, at al. Non-invasive prenatal testing for the detection of trisomies 21, 18, and 13 in pregnant women with various clinical indications: A multicenter observational study of 1,854,148 women in China. *Prenat Diagn.* 2023 Jul; 43 (8): 1036–43. doi: 10.1002/pd.6312.
27. Soukkhaphone B, Lindsay C, Langlois S, Little J, Rousseau F, Reinharz D. Non-invasive prenatal testing for the prenatal screening of sex chromosome aneuploidies: A systematic review and meta-analysis of diagnostic test accuracy studies. *Mol Genet Genomic Med.* 2021 May; 9 (5): e1654. doi: 10.1002/mgg3.1654.
28. Liu S, Yang F, Chang Q, at al. Positive predictive value estimates for noninvasive prenatal testing from data of a prenatal diagnosis laboratory and literature review. *Mol Cytogenet.* 2022 Jul 6; 15 (1): 29. doi: 10.1186/s13039-022-00607-z.
29. Wei L, Zhang J, Shi N, et al. Association of maternal risk factors with fetal aneuploidy and the accuracy of prenatal aneuploidy screening: a correlation analysis based on 12,186 karyotype reports. *BMC Pregnancy Childbirth.* 2023 Mar 2; 23 (1): 136. doi: 10.1186/s12884-023-05461-4).
30. Moufarrej MN, Bianchi DW, Shaw GM, Stevenson DK, Quake SR. Noninvasive Prenatal Testing Using Circulating DNA and RNA: Advances, Challenges, and Possibilities. *Annu Rev Biomed Data Sci.* 2023 Aug 10; 6: 397–418. doi: 10.1146/annurev-biodatasci-020722-094144.
31. Li Y, Yang X, Zhang Y, Lou H, et al. The detection efficacy of noninvasive prenatal genetic testing (NIPT) for sex chromosome abnormalities and copy number variation and its differentiation in pregnant women of different ages. *Heliyon.* 2024 Jan 7; 10 (2): e24155. doi: 10.1016/j.heliyon.2024.e24155.
32. Lu X, Wang C, Sun Y, Tang J, Tong K, Zhu J. Noninvasive prenatal testing for assessing foetal sex chromosome aneuploidy: a retrospective study of 45,773 cases. *Mol Cytogenet.* 2021 Jan 6; 14 (1): 1. doi: 10.1186/s13039-020-00521-2.
33. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol.* 2013; 122 (6): 1374–7. doi: 10.1097/01.AOG.0000438962.16108.d1.
34. Van Den Bogaert K, Lannoo L, Brison N, et al. Outcome of publicly funded nationwide first-tier noninvasive prenatal screening. *Genet Med.* 2021 Jun; 23 (6): 1137–42. doi: 10.1038/s41436-021-01101-4.
35. Kinnings SL, Geis JA, Almasri E, et al. Factors affecting levels of circulating cell-free fetal DNA in maternal plasma and their implications for noninvasive prenatal testing. *Prenat Diagn.* 2015 Aug; 35 (8): 816–22. doi: 10.1002/pd.4625.
36. Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on cell-free fetal DNA in maternal plasma. *Prenat Diagn.* 2013 Jul; 33 (7): 662–6. doi: 10.1002/pd.4119.
37. Burka OA, Cherevashko VS, Martynova DA, Ishchenko GI, Maksian OI, Knyhnytska SO. Performance capabilities of prenatal diagnosis of chromosomal anomalies: what changed with the introduction of non-invasive prenatal test (NIPT)? *Reproductive endocrinology.* 2021; 60: 21–30 (in Ukrainian). doi.org/10.18370/2309-4117.2021.60.21-30. Available from: https://reproduct-endo.com/article/view/242991/240934.
38. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins – Obstetrics; Committee on Genetics; Society for Maternal-Fetal Medicine. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. *Obstet Gynecol.* 2020 Oct; 136 (4): e48–e69. doi: 10.1097/AOG.0000000000004084.
39. Palomaki GE, Kloza EM. Prenatal cell-free DNA screening test failures: a systematic review of failure rates, risks of Down syndrome, and impact of repeat testing. *Genet Med.* 2018 Nov; 20 (11): 1312–23. doi: 10.1038/gim.2018.22.
40. Kang KM, Kim SH, Park JE, et al. Inconsistency between non-invasive prenatal testing (NIPT) and conventional prenatal diagnosis due to confined placental and fetal mosaicism: Two case reports. *Front Med (Lausanne).* 2022 Dec 15; 9: 1063480. doi: 10.3389/fmed.2022.1063480. Available from: https://pubmed.ncbi.nlm.nih.gov/36590946/.
41. Kamath MS, Antonisamy B, Selliah HY, Sunkara SK. Perinatal outcomes of singleton live births with and without vanishing twin following transfer of multiple embryos: analysis of 113 784 singleton live births. *Hum Reprod.* 2018 Nov 1; 33 (11): 2018–22. doi: 10.1093/humrep/dey284.
42. Jayashankar SS, Nasaruddin ML, Hassan MF, et al. Non-Invasive Prenatal Testing (NIPT): Reliability, Challenges, and Future Directions. *Diagnostics (Basel).* 2023 Aug 2; 13 (15): 2570. doi: 10.3390/diagnostics13152570. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10417786/.
43. Jay AM, Mason B, Lebovic D, Chuba P. More than an Incidentaloma: The Nonreportable NIPT. *Case Rep Obstet Gynecol.* 2022 Apr 30; 2022: 2496057. doi: 10.1155/2022/2496057.
44. Lenaerts L, Van Calsteren K, Che H, Vermeesch JR, Amant F. Pregnant women with confirmed neoplasms should not have noninvasive prenatal testing. *Prenat Diagn.* 2019 Nov; 39 (12): 1162–5. doi: 10.1002/pd.5544.
45. Baldus M. "Overestimated technology – underestimated consequences" – reflections on risks, ethical conflicts, and social disparities in the handling of non-invasive prenatal tests (NIPTs). *Med Health Care Philos.* 2023 Jun; 26 (2): 271–82. doi: 10.1007/s11019-023-10143-1.

46. Xue Y, Zhao G, Qiao L, Lu J, Yu B, Wang T. Sequencing Shorter cfDNA Fragments Decreases the False Negative Rate of Non-invasive Prenatal Testing. *Front Genet.* 2020 Mar 26; 11: 280. doi: 10.3389/fgene.2020.00280. Available from: https://pubmed.ncbi.nlm.nih.gov/32273885/.
47. Hartwig TS, Ambye L, Sørensen S, Jørgensen FS. Discordant non-invasive prenatal testing (NIPT) – a systematic review. *Prenat Diagn.* 2017 Jun; 37 (6): 527–39. doi: 10.1002/pd.5049.
48. Zhao Q, Chen J, Ren L et al. Two cases of placental trisomy 21 mosaicism causing false negative NIPT results. *Mol Cytogenet.* 2023 Jul 14; 16 (1): 16. doi: 10.1186/s13039-023-00643-3. Available from: https://pubmed.ncbi.nlm.nih.gov/37452352/.
49. Samura O, Okamoto A. Causes of aberrant non-invasive prenatal testing for aneuploidy: A systematic review. *Taiwan J Obstet Gynecol.* 2020 Jan; 59 (1): 16–20. doi: 10.1016/j.tjog.2019.11.003.
50. Suzumori N, Sekizawa A, Takeda E, et al. Classification of factors involved in nonreportable results of noninvasive prenatal testing (NIPT) and prediction of success rate of second NIPT. *Prenat Diagn.* 2019 Jan; 39 (2): 100–6. doi: 10.1002/pd.5408.
51. Kong X, Zhang L, Yang R, et al. Reasons for failure of noninvasive prenatal test for cell-free fetal DNA in maternal peripheral blood. *Mol Genet Genomic Med.* 2024 Jan; 12 (1): e2351. doi: 10.1002/mgg3.2351.
52. Shree R, MacKinnon HJ, Hannan J, Kolarova TR, Reichel J, Lockwood CM. Anticoagulation use is associated with lower fetal fraction and more indeterminate results. *Am J Obstet Gynecol.* 2024 Jan; 230 (1): 95.e1–95.e10. doi: 10.1016/j.ajog.2023.07.005.
53. Palomaki GE, Chiu RWK, Pertile MD, et al. International Society for Prenatal Diagnosis Position Statement: cell-free (cf) DNA screening for Down syndrome in multiple pregnancies. *Prenat Diagn.* 2021 Sep; 41 (10):1222–32. doi: 10.1002/pd.5832.
54. Carbone L, Cariati F, Sarno L, et al. Non-Invasive Prenatal Testing: Current Perspectives and Future Challenges. *Genes (Basel).* 2020 Dec 24; 12 (1): 15. doi: 10.3390/genes12010015. Available from: https://pubmed.ncbi.nlm.nih.gov/33374411/.
55. Lannoo L, van der Meij KRM, Bekker MN, et al. A cross-country comparison of pregnant women's decision-making and perspectives when opting for non-invasive prenatal testing in the Netherlands and Belgium. *Prenat Diagn.* 2023 Mar; 43 (3): 294–303. doi: 10.1002/pd.6329. https://pubmed.ncbi.nlm.nih.gov/36740754/.
56. Van Den Bogaert K, Lannoo L, Brison N, et al. Outcome of publicly funded nationwide first-tier noninvasive prenatal screening. *Genet Med.* 2021 Jun; 23 (6): 1137–42. doi: 10.1038/s41436-021-01101-4.
57. Ochsenbein N, Burkhardt T, Raio L, et al. Expertenbrief No 52: Pränatale nicht-invasive Risikoabschätzung fetaler Aneuploidien. SGGG 2018 Accessed September 01, 2022 (in German). Available from: https://www.sggg.ch/fileadmin/user_upload/Formulardaten/52_def_Praenatale_nicht-invasive_Risikoabschaetzung_fetaler_Aneuploidien.pdf.
58. Proto A, Trottmann F, Schneider S, et al. First Trimester Contingent Screening for Aneuploidies with Cell-Free Fetal DNA in Singleton Pregnancies – a Swiss Single Centre Experience. *Geburtshilfe Frauenheilkd.* 2024 Jan 3; 84 (1): 68–76. doi: 10.1055/a-2202-5282.
59. Okoror CEM, Arora S. Prenatal diagnosis after high chance non-invasive prenatal testing for trisomies 21, 18 and 13, chorionic villus sampling or amniocentesis? – Experience at a district general hospital in the United Kingdom. *Eur J Obstet Gynecol Reprod Biol X.* 2023 Jul 1; 19: 100211. doi: 10.1016/j.eurox.2023.100211. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10345242/.
60. Salvesen KÅB, Glad R, Sitras V. Controversies in implementing non-invasive prenatal testing in a public antenatal care program. *Acta Obstet Gynecol Scand.* 2022 Jun; 101 (6): 577–80. doi: 10.1111/aogs.14351.

Надійшла до редакції 04.04.2024.
Прийнята до друку 30.08.2024.
Електронна адреса для листування marchesn2005@gmail.com