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The Final thesis

Noninvasive Prenatal Testing in Modern Day Pregnancy Care

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ABSTRACT

BACKGROUND

Literature review of noninvasive prenatal testing in modern-day pregnancy care for collecting information about methods, limitations and benefits of this technique. Noninvasive prenatal testing has been a significant breakthrough in prenatal diagnosis, as it provides important information about the unborn fetus without needing direct access into the uterus and thus reduces the small but significant risk of miscarriages and fetal injury.

METHODS

This Research Paper is a literature review written in English language and in a descriptive manner in which a variety of qualitative scientific sources were utilized as well as secondary sources in addition to some specific quantitative data.

The literature research was concentrated on recent scientific sources in English language, which were not older than ten years.

CONCLUSION

Noninvasive prenatal testing is a promising approach as information about the unborn fetus can be gained by taking a simple blood test from the mother. This test should not just be available in high-risk pregnancies, but for all women who wish to find out more about their child without needing invasive testing, which is associated with minor risks for the fetus and the pregnancy. Even though some limitations currently exist, the benefits outweigh them and pave the way for improvements and further research in this field.

KEYWORDS

Prenatal diagnosis; Prenatal genetic testing; Cell-free DNA screening; Noninvasive prenatal testing; NIPT; Next-generation sequencing; Fetal fraction; Trisomy

ABBREVIATIONS

NIPT – Noninvasive prenatal testing

cffDNA – cell-free fetal DNA

cfDNA – cell-free DNA

FF – Fetal fraction

s-MPS – Shotgun Massively Parallel Sequencing

t-MPS – Target Massively Parallel Sequencing

SNP's – Single nucleotide polymorphisms

DR – Detection rate

FPR – False positive rate

cFTS – Combined first trimester screening

SUMMARY

The detection of cell-free fetal DNA in maternal plasma in 1997 was a groundbreaking discovery in prenatal diagnostics, though it took some more time for it to be introduced into the health care setting (1).

Firstly introduced in 2011, noninvasive prenatal testing was initially introduced by commercial providers (2). In recent years, noninvasive prenatal testing has been implemented into public healthcare systems as either a first-line test or a supplement to existing prenatal screening programs (3,4).

Depending on the country and type of test, it is now possible to detect diseases like trisomy 21, trisomy 18, trisomy 13, sex chromosome aneuploidies, 22q11.2 microdeletion, 1p36 deletion syndrome or fetal sex with a simple blood sample of the mother – without needing direct access into the uterus (5–10). The test can be performed as early as 10 weeks of gestation and is validated for women of any age or risk category (5,6). Indications to perform noninvasive testing vary from high-risk pregnancies in the combined first trimester screening, past history, anxiety, or simply the wish for a "perfect" child (11–13). Even though studies reported excellent performance with overall detection rates for trisomy 21 exceeding 99% with false-positive rates of less than 1% (14), limitations like false-positive or false-negative results, the influences on the tests or incidental findings have to be acknowledged as well (13,15,16).

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1. INTRODUCTION

In 1959, six decades after the death of John Langdon Down, Jérôme Lejeune discovered trisomy 21 as the genetic reason for Down syndrome. Screening for Down syndrome has been ongoing since the 1960s by using maternal age as the main risk parameter (17,18).
Over the past decades, numerous progresses have been made. First trimester screening, combining factors like maternal age, maternal serum parameters and ultrasound findings – such as fetal nuchal translucency -, first appeared in obstetrics in the 1990s (18).
Prenatal screening for fetal anomalies had traditionally focused on chromosome abnormalities as they are the major causes of perinatal morbidity and mortality. Down syndrome, also called trisomy 21, is the most common autosomal aneuploidy leading to live birth and, consequently, the main genetic cause of intellectual disability (1).
Before serum biochemical screening tests were developed in the 1980s, aneuploidy was detected exclusively through invasive technology such as amniocentesis or chorionic villus sampling to provide a sample of fetal genotype for analysis (19).
As invasive testing with chorionic villus sampling or amniocentesis carries a considerable risk of miscarriage, estimated at around 0,5% to 1,0%, it has been a long-standing goal and wish of prenatal diagnosis to develop a noninvasive test for trisomy 21. The detection of cell-free fetal DNA in maternal plasma in 1997 was a defining moment in noninvasive prenatal testing (20). The trophoblast is the primary source of circulating cell-free fetal DNA, which releases DNA into the circulation due to apoptosis. Cell-free DNA of placental origin is detectable as early as 5 weeks of gestation and is quickly cleared after delivery (1). The term noninvasive prenatal diagnosis is generally used to refer to DNA-based tests of fetal wellbeing that do not require direct access into the uterus and that are satisfactorily adequate not to require follow-up confirmation with invasive testing methods. The various terms noninvasive prenatal testing (NIPT), noninvasive prenatal screening (NIPS) and noninvasive DNA-based testing (NIDT) have been coined to differentiate this test from NIPD, as it is not considered a diagnostic test (1). Since 2011, cfDNA testing has been available for screening for trisomies and evidence suggests that analysis of cfDNA in maternal blood can detect a high rate of positive results (21). In recent years, NIPT has been implemented into public healthcare systems as either a first-line test or a supplemental test to already existing prenatal screening programs. The increased use of non-invasive prenatal testing has significantly reduced the number of invasive tests (3). While prenatal testing has historically focused on the termination of

pregnancy, it is nowadays agreed that the goal of prenatal genetic testing should be focused on improving outcomes for women and families (22).
In the following, indications, techniques, different tests and interpretation of test results will be described and discussed, as well as the ethical burdens and future of this method.

2. METHODS

This Research Paper is a literature review written in a descriptive manner in which a variety of qualitative scientific sources were utilized. Initially, a broad research about the topics prenatal diagnostics and noninvasive prenatal testing was performed in order to collect an overview and get familiar with the topic. Thereafter, popular medical literature about the topics of prenatal diagnostics of any type, such as “Noninvasive Prenatal Testing: Applied Genomics in Prenatal Screening and Diagnosis” by Lieve Page-Christiaens and Hanns-Georg Klein and “Prenatal Diagnosis” by Mark Evans, as well Williams Obstetrics were used to gain basic knowledge for comprehension and association of contents. The VPN offered by Vilnius University allowed unlimited access and was very helpful in finding material about the topic in other reliable online sources such as ScienceDirect, 5-minute-consult and Access Medicine. I mainly focused my literature research on articles and studies published on ResearchGate and PubMed, which were not older than 10 years and written in English language.
Furthermore, I used the companies' official websites that manufacture noninvasive prenatal tests and are mainly used in Europe to acquire knowledge about workflow, techniques, and recommendations to the patients.

3. DESCRIPTIVE PART

3.1 HISTORICAL PERSPECTIVE OF NIPT

Historically, prenatal diagnosis began to expand drastically with augmented interest in pregnancies carrying a fetus with trisomy 21, also called Down syndrome (19). Down syndrome is a genetic disorder caused by the presence of a portion or all of a third

chromosome 21 and is the most common chromosomal abnormality occurring in humans. Typically, patients present with characteristic facial features, mild to moderate intellectual disability and growth retardation (23). The overall incidence is 1 in 800 birth in the general population, but can be as high as 1 in 35 term births for women over 45 years. Before serum biochemical screening tests were developed in the 1980s, aneuploidy was exclusively detected through invasive testing using chorionic villus sampling or amniocentesis to provide a sample of the fetal genotype for analysis. Invasive techniques began almost 5 decades ago and consist of chorionic villus sampling, which can be done between 9,5 and 12,5 weeks of gestation, or amniocentesis which is usually performed after 14 weeks of gestation (19). Because those techniques require direct access into the uterus (1) and therefore add a small risk of causing fetal injury or miscarriage, it has been a long-standing desire of patients and a goal of physicians to develop a less risky and invasive test for detection of trisomy 21(19,20). The first such screening tests were prior history and advanced maternal age, defined as age older than 35 – which also was the first indication for invasive diagnostic testing. Subsequently, with the discovery that abnormalities in the levels of specific biochemical markers could provide better identification of those women at an increased risk, the introduction of serum markers was soon followed, as well as progress in fetal ultrasound findings indicative of structural anomalies that could also help in the identification of pregnancies at risk (19). A major breakthrough in prenatal diagnostics was the detection of cell-free fetal DNA in maternal plasma in 1997 and was subject to remarkable achievements in the past years (1). By analyzing this source of fetal genetic material, obtained through a simple blood sample from the mother, NIPT has been developed (14). Since then, noninvasive prenatal testing with the use of fetal fraction in maternal blood revolutionized prenatal diagnostics and gave women a new perspective and hope to avoid invasive techniques to get information about their pregnancy.

3.2 cffDNA AND FETAL FRACTION

Cell-free fetal DNA (cffDNA) discovered in maternal plasma originates from placental cell turnover and its main source is the trophoblast, which releases DNA into the maternal circulation as a result of apoptosis (1,14). It consists of short fragments of DNA rather than whole chromosomes and represents approximately 5-10% of the total cffDNA in the maternal

plasma. As early as 4 weeks of gestation the cffDNA can be detected and it disappears quickly from maternal plasma due to its short half-life of 16 minutes and is even undetectable at around 2 hours post delivery. These characteristics make cffDNA and interesting part of NIPT with the potential to eliminate the necessity of invasive genetic procedures (14).
Fetal fraction (FF) is the percentage of total maternal plasma cfDNA that is of fetoplacental origin (24). Therefore, it is a function of both maternal and fetal cfDNA levels in the maternal plasma. The "fetal" DNA is actually placental, and the average FF between the 10th and 20th week of gestation makes up approximately 10-15%. It is a function of both biological factors and bioinformatic algorithms used to interpret DNA sequencing results and is a crucial quality control component of NIPT results. Measuring the FF ensures that cffDNA is detectable in the maternal plasma in adequate amounts to generate a valid result. The minimum amount of FF for sufficient NIPT performance varies by assay, but is typically between 2% and 4% (24 – 26). Low fetal fraction can result in a "No Call" result or test failure (27). Fetal influences on FF can be different fetal aneuploidies: trisomy 21 resulting in a higher FF, whereas trisomies 13 and 18 are associated with a lower median FF. Aneuploidies reported after a failed NIPT test result include: triploidy, trisomy 13, trisomy 18, trisomy 21, trisomy 16 mosaic and monosomy X. Also maternal components could influence fetal fraction, such as increased maternal weight, inflammatory conditions and active autoimmune diseases (25,26,28). The fundamental principle of NIPT for trisomy 21, 18 and 13 is the detection of an excess number of DNA fragments from either chromosome 21, 18 or 13 in the maternal plasma. A fetus affected with either of the named trisomies will release relatively more chromosome DNA fragments into the maternal circulation than other chromosomes. DNA sequencing of maternal plasma cfDNA allows a precise estimate of the fetal dosage of the chromosomes. The number of fragments counted from the chromosomes are then compared to a reference derived from other diploid chromosomes and statistical analysis is performed to determine the chromosomal count (29).

Picture 1:

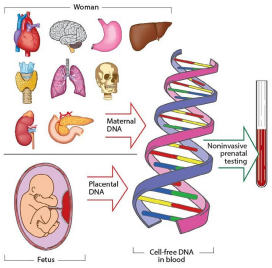


Figure 1: Multiple organ sources of cell-free DNA in maternal plasma (28). Source: Hui L, Bianchi DW. Fetal fraction and noninvasive prenatal testing: What clinicians need to know. Prenatal Diagnosis. Januar 2020;40(2):155–63.

3.3 ADEQUATE INFORMED CONSENT AND PRETEST COUNSELING

As with other screening tests, the decision about whether or not to undergo aneuploidy screening should be made with respect to the patient's needs and values (11). It should be made clear that testing is optional and the decision to proceed should depend on how each individual perceives the benefits of obtaining information when weighed against the potential emotional and physical risks of testing (30). That's where pretest counseling is crucial: it should include discussions about the diagnostic testing as well as the potential limitations and benefits of screening options, review of clinical features and variability of conditions (11,30). Patients should also be informed about sensitivity of NIPT (30). The American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine recommend pretest counseling, which should include information regarding the non-diagnostic nature of the test, as well as a review of the parents family history to review for other chromosome or single-gene disorders (11).

It should be clearly stated and discussed with the parents that there is a possibility that a NIPT result may not correlate with the fetal chromosomes as well as the limitations of follow-up diagnostic testing with invasive techniques, which may not reflect the actual karyotype in cases of confined placental mosaicism. Pretest counseling should also include information about the possibility of discovering maternal chromosomal variations, which have not been previously detected.

Another important consideration that has to be taken into account in the counseling of patients ultimately desiring diagnostic testing is that NIPT cannot yet replace invasive methods as a definite test. Because NIPT is a screening test, so using it as a "secondary screen" following abnormal serum marker screening or ultrasound findings may delay definitive diagnostic testing. Patients with "screen-negative" results should be counseled about the chances of false-negative results. It is recommended to follow-up a screen-positive result with genetic counseling and further diagnostic testing and accurate information about the conditions being tested should be provided to the patients (11,31).

3.4 AVAILABLE TESTS

3.4.1 TESTING METHODS

Thus far, three different techniques for analyzing cfDNA in maternal blood are available. The Shotgun Massively Parallel Sequencing (s-MPS), which is based on the MPS sequencing method, can sequence DNA fragments from the entire genome. It is a technique based on identifying and counting a large number of DNA fragments from maternal plasma samples. Millions of fetal and maternal DNA fragments can be simultaneously sequenced, and, since the complete human genome is known, each sequence mapping a specific locus of that genome can be accredited to the chromosome from which it is originated (32). The second method available is the Target Massively Parallel Sequencing (t-MPS). The t-MPS selects sequences from genomic regions of interest, counts only those sequences and then evaluates when there is a relative excess of one chromosome over another, instead of randomly sequencing genomic fragments of all chromosomes like s-MPS. This results in lower sequencing costs and increased efficiency, since it alternatively counts a bigger number of DNA fragments matching to specific chromosome regions (15,33). The third noninvasive prenatal testing method is based on the analysis of single nucleotide polymorphisms (SNPs) and determines the relative quantitative contribution of maternal and fetal DNA circulating in maternal blood. It is the only method that can distinguish maternal and free fetal DNA. This instrument involves the simultaneous amplification of about 20,000 SNP sequences in a single PCR reaction using maternal plasma DNA followed by sequencing. After considering the SNP position in the chromosomes and the possibility of recombination between them, the probability of an euploid, aneuploid or triploid fetus is calculated. Furthermore, it can identify regions of fetal chromosome homology that indicate consanguinity or uniparenteral disomy. SNP technology can also provide information on the origin of aneuploidy, on recombination events and on inherited mutations (15,34).

Picture 2:

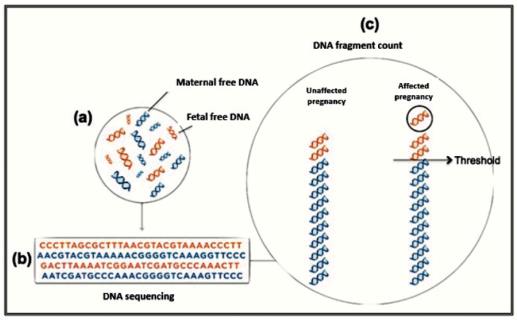


Figure 2 Schematic illustration of MPS sequencing. (a) Mother's blood with free DNA fragments of fetal and maternal origin; (b) all fragments are sequenced simultaneously; (c) each sequence is assigned to the chromosome that originated it and the excess or deficit in the number of fragments, relative to an probable limit for euploid pregnancies, is considered aneuploidy. (15) Source: V.C.M A, L.C.S B, G.D.T T, Borges Peixoto A, DBSP P, Braga A, u. a. Article - Noninvasive prenatal testing 2020. 18. Mai 2020;85.

Picture 3:

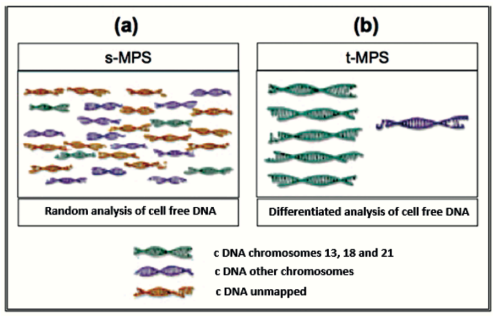


Figure 3 Schematic illustration showing the difference between s-MPS and t-MPS techniques. (a) the s-MPS method analyzes the cfDNA fragments of all chromosomes; (b) the t-MPS method only analyzes the cfDNA fragments of the chromosomes of interest. It incorporates an initial target sequencing step in which specific regions of each chromosome of interest are selectively amplified (15). Source: V.C.M A, L.C.S B, G.D.T T, Borges Peixoto A, DBSP P, Braga A, u. a. Article - Noninvasive prenatal testing 2020. 18. Mai 2020;85.

Picture 4:

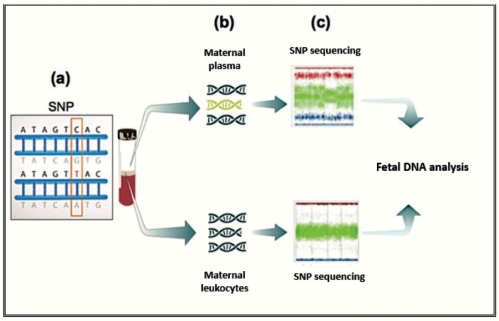


Figure 4 Representative scheme of NIPT for SNP aneuploidies. (a) SNPs are polymorphisms on a single nitrogenous basis; (b) the free DNAs of the maternal plasma are sequenced; separately, only the DNA of the white maternal cells is sequenced; (c) after considering the location of the SNPs in the chromosomes and the option of recombination between them, the relative quantitative contribution of the fetal and maternal DNAs is determined by crossing information between the two sequences. Then the probabilities of the fetuses (euploid, aneuploid or triploid) are calculated (15). Source: V.C.M A, L.C.S B, G.D.T T, Borges Peixoto A, DBSP P, Braga A, u. a. Article - Noninvasive prenatal testing 2020. 18. Mai 2020;85.

3.4.2. HARMONY ® TEST

Roche is one of the companies offering t-MPS, namely "Harmony ®" test, developed together with Ariosa Diagnostics in California. Massive parallel DNA shotgun sequencing (MPSS) is used. In order to address some limitations of MPSS, Ariosa Diagnostics developed a method called digital analysis of selected regions (DANSR), which selectively evaluates specific genomic fragments of cfDNA. By enabling selective analysis of cfDNA, DANSR provides more efficient use of sequencing in the detection of fetal aneuploidy, avoiding the large amounts of unused sequencing data generated my MPSS. Therefore, DANSR enables highly multiplexed sequencing of selected loci from specific chromosomes of interest (35,36).
According to the website of Roche their Harmony ® Test screens for trisomy 21, trisomy 18 and trisomy 13. Additional options to add include monosomy X and other sex chromosome aneuploidies (like Klinefelter syndrome XXY or Turner syndrome XO), 22q11.2 microdeletion (DiGeorge syndrome) and fetal sex. The test can be performed as early as 10 weeks of gestation and is validated for pregnant women of any risk category and any age, and can be used in singleton, twin and self- and non-self egg donor pregnancies. Physicians are advised to order the Harmony ® test as early as 10 weeks of pregnancy and then send the patient's blood sample for analysis using the Harmony ® specimen and transportation box. Results will be received as soon as 3 days, most within 5 days after sample receipt, depending on country. Roche also offers clear reports and support from their provider portal, client services and team of genetic counselors (6).
Detection rates and false-positive rates of Harmony ® test are as follows: For trisomy 21 DR is 99,3% and FPR is < 0,1%, for trisomy 18 DR is 97,4% and FPR is < 0,1% and for trisomy 13 DR is 93,8% and FPR is < 0,1% (5), for microdeletion 22q11.2 DR is 75% and FPR is 0,5%. FPR for monosomy X is ~0,8%. False-positive rates can be influenced by confined placental mosaicism or a twin pregnancy, where the deceased twin suffered from a chromosomal disorder.
In approximately 1,6% of cases, it is impossible to obtain results with the Harmony ® test, most often due to fetal fraction being under the 4% limit. Approximately 50% of tests that cannot be evaluated in the first attempt, the test can be carried out at a later point in time by taking another blood sample and is free for the patients (37).

3.4.3 PRENATEST ®

The PrenaTest ® is another NIPT using the s-MPS method. According to their website the PrenaTest ® can be done starting from the 10th week of pregnancy, is a safe and reliable noninvasive prenatal test and can determine trisomy 21, trisomy 18, trisomy 13, maldistributions of sex chromosomes X and Y (Klinefelter syndrome, Turner syndrome, Triple X syndrome and XYY syndrome), monosomy 21, monosomy 18, monosomy 13, 22q11.2 microdeletion (DiGeorge syndrome) and trisomies and monosomies of all other chromosomes 1-12, 14, 15, 16, 17, 19, 20 and 22.
The PrenaTest ® can be performed in the case of a twin pregnancy, fertility treatment (IVF or ICSI), after egg donation or simply to find out fetal sex (7).
Physicans are advised to determine the chromosomal disorders to be tested for with their patients, collect the blood sample and place them in the specific box. The box should be stored in the refrigerator until it is shipped. The test is completed within 4-6 business days and the responsible physician will receive the results report by fax and mail (38).
In singleton pregnancies, for trisomy 21, 18 and 13 sensitivity is > 99,9% and specificity is 99,90%.
In twin pregnancies, for trisomy 21 sensitivity is 96,4% and specificity is 99,9%. For trisomy 18 sensitivity is 95,7% and specificity is > 99,9%. For trisomy 13 sensitivity is 93,6% and specificity is > 99,9% (39).
Like every other prenatal test, PrenaTest ® also has some limitations. In general, no statements regarding structural chromosomal changes, mosaics or polyploidy can be made. The company also emphasizes that it is only possible to achieve a level of diagnostic certainty close to that reached with direct chorionic villus sampling. Consequently, mosaics or fetoplacental discrepancies in trisomies 21, 18 or 13 gonosomal aneuploidy are not recognizable. Undisclosed vanishing twins can contribute to the total cffDNA fraction to cause a positive PrenaTest ® result being not representative for the continuing singleton pregnancy. An existing maternal mosaic, maternal gonosomal aneuploidy, or some maternal tumors can lead to a conspicuous PrenaTest ® result that may not represent the unborn child. If the mother is a carrier of 22q11.2 microdeletion test result can be false positive. False-negative test results could be caused by too small size of the microdeletion (40).

3.4.4 PANORAMA ® TEST:

Panorama ® evaluates SNP's – the 1% of the DNA that makes us different from another. On their website, natera states about their Panorama ® test that it is the most rigorously validated NIPT and the only noninvasive prenatal test that distinguishes mother's DNA from the DNA of the fetus. However, they emphasize that this test is a screening test and does not make a final diagnosis. Panorama can be performed as early as nine weeks of gestation in single, twin, egg donor and surrogate pregnancies. This test also detects the fetal sex, trisomy 21, trisomy 18, trisomy 13, monosomy X, triploidy, XXX, XXY, XYY, 22q11.2 deletion syndrome, 1p36 deletion syndrome, Angelman syndrome, Cri-du-chat syndrome and Prader-Willi syndrome (8). The company also states that Panorama ® is able to detect conditions that other tests cannot, including molar pregnancy, triploidy and vanishing twin, as well as assess zygosity, individual fetal sex and individual fetal fraction (for dizygotic twins) in twin pregnancies (9).
With the Panorama ® test sensitivity and specificity of the different conditions are as follows (8):

Table 1:

Condition	**Sensitivity**	**Specificity**
Trisomy 21	> 99%	> 99%
Trisomy 18	98,2%	> 99%
Trisomy 13	> 99%	> 99%
Monosomy X	94,7%	> 99%
Triploidy	> 99%	> 99%
XXX, XXY, XYY	n/a	n/a
22q11.2 deletion syndrome	90,0%	> 99%
1p36 deletion syndrome	> 99%	> 99%
Angelman syndrome	95,5%	> 99%
Cri-du-chat syndrome	> 99%	> 99%
Prader-Willi syndrome	93,8%	> 99%
Fetal sex: female	> 99,9%	> 99,9%
Fetal sex: male	> 99,9%	> 99,9%

Table 1: Sensitivity and specificity of conditions screened with by Panorama ® test

3.5 INDICATIONS

According to the American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine patients with an increased risk for fetal aneuploidy can be offered testing with cfDNA. High-risk women were defined as those of maternal age 35 years or older at delivery, with fetal ultrasonographic findings indicating an increased risk of aneuploidy, with a history of prior pregnancy with trisomy, with a positive maternal serum screen for aneuploidy or with a balanced Robertsonian translocation with an increased risk for fetal trisomy 13 or trisomy 21 (11,12). The International Society for Prenatal Diagnosis recommends more limited use of NIPT, with first-tier prenatal screening recommended using serum marker analyte and ultrasound screening for all women, including those defined as geriatric pregnancy. They also recommend the consideration of NIPT only as a second-tier test for women who have increased risk for aneuploidy determined through serum marker analytes and ultrasound findings, or for women who present to care too late to undergo serum or ultrasound screening that depends on gestational age (41,42). Though those recommendations are 10 years old and should be revised.

A study done by the University Hospital Brugmann in Belgium about the indications of Harmony Prenatal Test were as follows: high risk pregnancy for trisomy 21 or trisomy 18 in 17,2%, a history of chromosomal anomaly in 2,0%, presence of soft markers during the second trimester scan in 2,3%, high-risk according to second-trimester triple test screening in 0,7%, advanced maternal age defined as ≥ 35 years in 49,6% and maternal request in 28,2% of pregnant patients. In conclusion the indications for the NIPT can be classified into two categories: 71,8% of women had a clear indication and 28,2% with anxiety and uncertainty as an indication. The study also focused on maternal requests as an indication to perform noninvasive prenatal testing and found that it was more common in younger women, earlier gestational ages, nulliparous women and those with twin gestations. Surprisingly maternal requests as an indication was not more common in pregnant patients following in vitro fertilization (35).

Other indications or wishes from the mother could be the societal pressure to have a healthy baby or wish for the “perfect” child (13).

3.6 WHEN NIPT IS NOT THE IDEAL TEST

Common chromosomal conditions can be excellently screened for with a NIPT, however this test does not provide the detail and range of genomic information as it is gained with invasive testing techniques. An early ultrasonography should be performed before considering NIPT as up to 16% of high-risk women will have ultrasound findings at 10-14 weeks of gestation that alters the prenatal counselling strategy. Those ultrasound findings include correction of gestational age, detection of multiple pregnancy, fetal death or structural abnormality (29,43). Women who have an increased risk of an atypical abnormality should also be counselled about the possibility of missing a clinically significant diagnosis if NIPT is chosen over genome-wide diagnostic testing (29). Additionally, if any woman longs for maximum information about her pregnancy, it may be reasonable to offer a diagnostic test without prior screening – regardless of her background risk (44).

3.7 NIPT RESULTS

As previously mentioned, NIPT is a screening test not a diagnostic test. The results will get divided into low risk, high risk and "no call". A high-risk result does not mean that the fetus has a chromosomal abnormality; rather it indicates a very high probability that the fetus may have that condition. Physicians should advise their patients to follow-up with genetic counseling or a maternal fetal medicine specialist. Furthermore, patients can be offered invasive diagnostic testing such as amniocentesis or chorionic villus sampling.
The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine state: "All women with a positive cell-free DNA test result should have further detailed counseling and testing and should have a diagnostic procedure before any irreversible action is taken". Confirmation prior to birth can also help with pregnancy and neonatal management (45).
If sonographic abnormalities are detected and NIPT result is normal, additional invasive tests are necessary. As a matter of fact, in this situation, time and expenses for NIPT should be saved by clarifying the situation directly with an invasive diagnostic test (13).
A second blood draw should be requested in the case of a "no call" result (10). This result could be caused by a low fetal fraction of placental DNA. If the test is repeated and is still not

feasible, the pregnancy is at higher risk for an adverse outcome and subsequent pregnancy control is recommended (13).

3.8 BENEFITS AND LIMITATIONS

As NIPT is a topic in medicine which is widely discussed, I want to elaborate its advantages and disadvantages in greater detail.

3.8.1 BENEFITS

1. As NIPT can be done with just a blood sample from the mother, it is considered noninvasive, safe and easy. No direct access into the uterus is needed (1). Invasive procedures may induce a miscarriage in 0.3-1.0% (46) – this can be bypassed with the usage of noninvasive prenatal testing.
2. NIPT is highly accurate for detecting the main fetal trisomies: trisomy 21, trisomy 18 and trisomy 13 (16). Also diseases like monosomy X and other sex chromosome aneuploidies (like Klinefelter syndrome XXY or Turner syndrome XO), 22q11.2 microdeletion (DiGeorge syndrome), monosomy 21, monosomy 18, monosomy 13, trisomies and monosomies of all other chromosomes 1-12, 14, 15, 16, 17, 19, 20 and 22, 1p36 deletion syndrome, Angelman syndrome, Cri-du-chat syndrome, Prader-Willi syndrome and fetal sex can be determined – depending on the specific test and extent of "test package" chosen (5–8,10,39).
3. The tests can be performed in the case of a twin pregnancy, fertility treatment (IVF or ICSI), and after egg donation (5–7).
4. Studies reported excellent performance with overall detection rates for trisomy 21 exceeding 99% with false-positive rates of less than 1% (14). Detection rates and false-positive rates of Harmony ® test are as follows: For trisomy 21 DR is 99,3% and FPR is < 0,1%, for trisomy 18 DR is 97,4% and FPR is < 0,1% and for trisomy 13 DR is 93,8% and FPR is < 0,1% (5), for microdeletion 22q11.2 DR is 75% and FPR is 0,5%. FPR for monosomy X is ~0,8% (5,6). In singleton pregnancies with the PrenaTest ®, for trisomy 21, 18 and 13 sensitivity is > 99,9% and specificity is 99,90% (7,39).

5. The test can be performed as early as 10 weeks of gestation and is validated for pregnant women of any risk category and age (5,6).
6. Results will be received as soon as 3 days and most within 5 days after sample receipt, depending on country. Companies like Roche also offer clear reports and support from their provider portal, client services and team of genetic counselors (5,6). The PrenaTest ® is completed within 4-6 business days (38).

3.8.2 LIMITATIONS

Some limitations appear for the use of NIPT technologies for fetal chromosomal abnormality testing. Even though studies reported excellent performance with overall detection rates for trisomy 21 exceeding 99% with false-positive rates of less than 1%, other factors need to be taken into consideration (14).

1. False-positive and false-negative NIPT results may occur. False-positive results have been reported because of confined placental mosaicism, vanishing twin and maternal mosaicism (14,16). Because of the false-positive results immanent in any screening test most guidelines recommend that a “screen positive” NIPT result should be confirmed by invasive diagnostics (3). Malignant tumors may have chromosomal abnormalities, therefore, if a pregnant woman has a malignant tumor, the NIPT result may be false positive or non-reportable. A recent review suggests, that neuroendocrine cancer, angiosarcoma and small-cell carcinoma may cause false-positive results. Malignant neoplasia in pregnant women is relatively rare, but occurs in approximately 1 in 1000 cases and accounts for about 15% of NIPT false-positive results. False-negative results could also occur due to confined placental mosaicism. As with all laboratory tests, confusion with rare samples and other technical errors may lead to false-positive or false-negative results (16). Furthermore the website or Prenatest ® explicitly states: “In general, no statements regarding structural chromosomal changes, mosaics or polyploidy can be made. The company also emphasizes that it is only possible to achieve a level of diagnostic certainty close to that attained via direct chorionic villus sampling. Consequently, mosaics or fetoplacental discrepancies in trisomies 21, 18 or 13 gonosomal aneuploidy are not identifiable. Undisclosed vanishing twins can contribute a sufficient proportion to the total cffDNA fraction to cause a positive PrenaTest ® result being not

representative for the continuing singleton pregnancy. An existing maternal mosaic, maternal gonosomal aneuploidy, or some maternal tumors can lead to a conspicuous PrenaTest ® result that may not represent the unborn child. If the mother is a carrier of 22q11.2 microdeletion test result can be false positive. False-negative test results could be caused by too small size of the microdeletion" (7,39,40).

2. Heparin administration can influence NIPT testing. Heparin, which is for example used to prevent abortion in antiphospholipid antibody syndrome can influence the analysis of NIPT. In pregnant women on LMWH medication the cfDNA contains a higher proportion of small DNA fragments featuring an unusually high guanosin-cytosin (GC) content than in unaffected women. This apparently influences NIPT results so that they cannot be interpreted correctly and even may provide false results. Thus, not assessing the GC content of a sample as a quality criterion of NIPT leads to false-positive results for trisomy 18 or false-negative results for trisomy 13 or trisomy 21 (47). Because Heparin has a short half-life, blood collection when the concentration of heparin is low can minimize the effects (16).

3. Due to low amount of fetal fraction and placental DNA "no call" results are observed in around 3-5% of cases and thus could lead to invasive testing (13). This could happen due to too early testing, maternal obesity or vanishing twins (16).

4. Autoimmune disease can also affect NIPT. Several cases of repeated non-reportable results in pregnant women with autoimmune thrombocytopenic purpura (ITP) have been reported. A low fetal cfDNA fraction can occur in mothers with autoimmune diseases as inflammatory reactions can increase maternal cfDNA in the maternal blood. Also, a different pattern is seen in NIPT analyses using next-generation sequencing in patients with Systemic Lupus Erythematosus (16). Affected woman should be counseled about that.

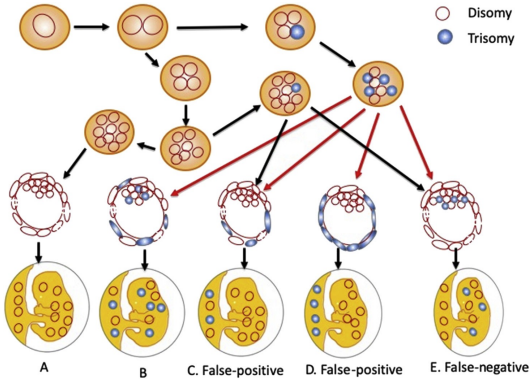
5. If a pregnant woman suffers from obesity, the percentage of cffDNA is in general lower than in normal weight women and unsuccessful tests become more frequent (~6%) (13). A study showed that from obesity class I and above, the incidence of "no call" results increased considerably. The "no call" rate for women in obesity class III was highly variable, ranging from 5,4% to 70,1%, but was lowest in more recent and larger studies (48).

6. Since NIPT does not screen for open neural tube defects, maternal serum alpha-fetoprotein testing and/or fetal anatomic ultrasound would still be needed during the second trimester (14).

7. Unfortunately one could argue that, even though the main abnormalities can be tested for with the NIPT, not as many possible genetic changes are detectable as with invasive prenatal testing (13).

8. In an abnormal or positive NIPT result further invasive testing may be needed nevertheless (13).
9. To date, while a reasonable amount of evidence supports the use of noninvasive prenatal testing in high-risk women to detect fetal trisomy 21 and 18, the cost effectiveness of NIPT implementation in a routine pregnancy health care workflow setting is controversial (14).
10. In many countries the NIPT is not yet included into the obstetric screening program and thus has to be self-financed by patients, many of which may not have the financial means even though they might wish for the test (3)

Picture 5:



Mechanisms of false-positive and false-negative results in NIPT (16). Source: Samura O, Okamoto A. Causes of aberrant noninvasive prenatal testing for aneuploidy: A systematic review. Taiwanese Journal of Obstetrics and Gynecology. Januar 2020;59(1):16–20.

3.9 NIPT AROUND THE WORLD

Noninvasive prenatal testing was introduced in 2011, initially launched by commercial providers (2). In recent years, NIPT has been implemented into public healthcare systems as either a first line test or a supplement to existing prenatal screening programs (3,4). Nevertheless, not in all countries NIPT is freely available. A study about the current use of noninvasive prenatal testing in Europe, Australia and the USA by Kasper Gadsbøll was completed in 2019 and showed interesting results (3).

3.9.1 EUROPE

NIPT is still used by less than 25% of women in most European countries and is more widely used in Italy, Spain, Austria and Belgium. All Nordic European Countries offer combined first trimester screening (cFTS) either to all women or selected groups. At the cFTS, a risk estimate is calculated based on maternal age, ultrasound-determined nuchal translucency and maternal blood tests. If the risk is found to be high, then women are offered either invasive testing or NIPT. The Nordic European national healthcare systems fully cover all prenatal screening costs. In Iceland, high-risk women are offered invasive testing, but the costs will be covered publicly if NIPT is specifically requested. In the UK, Wales is the only country that has integrated NIPT in public prenatal screening, offering NIPT as a financially covered alternative to invasive testing in high-risk women after cFTS. In Slovakia, Russia and the Czech Republic, there are no publicly funded offers for NIPT, thus this test is self-financed through private clinics. Poland and Romania offer only invasive testing after cFTS to women at very high risk. In Spain, there is no national policy on the use of NIPT. Some regions have decided to offer NIPT to high-risk patients, in other regions hospitals have integrated NIPT according to their needs and budget and in these cases NIPT is publicly financed. The proportion of women receiving NIPT in Spain is approximately 25-50%. In Italy, there are official guidelines supporting the use of NIPT in high-risk women, but only the regions Toscana and Bolzano currently compensate the test. Italy has a high use of NIPT (25-50%) through private clinics. There are no national guidelines for NIPT in Greece and Cyprus, but NIPT is available as a self-financed service. In Slovenia, there is only an offer of NIPT if invasive testing is contraindicated due to maternal factors. In that case NIPT is publicly funded. France offers NIPT to high-risk women after cFTS free of charge. Though, women only receive results on trisomy 21. In Germany, a national decision on the use of NIPT is still pending. In the Netherlands NIPT is available for all pregnant women since 2017 as a first-line screening test, unfortunately less than 42% of women currently select NIPT. There, NIPT is partially reimbursed and partially self-financed. In Belgium, NIPT is offered to all pregnant women in addition to ultrasound and is reimbursed by insurance, thus the proportion of women opting for NIPT is over 75%. The Netherland, Belgium, Lithuania, Italy, Cyprus and Greece primarily offer NIPT for trisomies 21, 18, 13, sex chromosome aneuploidies, microdeletions and/or whole-genome coverage (3).

3.9.2 AUSTRALIA

The Royal Australian and New Zealand College of Obstetricians and Gynecologists (RANZCOG) states that either cFTS or NIPT are acceptable primary prenatal screening tests. cFTS in Australia is government funded, whereas NIPT is self-financed. There is a different uptake of NIPT according of model of care, with over 50% to 75% of women in private obstetric care using NIPT as a primary screen, compared to less than 25% of public patients. NIPT genome coverage in Australia generally varies by provider more than by state (3).

3.9.3 USA

In the USA, nearly all commercial insurance companies and Medicaid programs cover NIPT for high-risk women, such as women aged 35 years or older or a positive cFTS. In six states, Medicaid programs cover NIPT for average risk women, whereas in nine states Medicaid programs do not cover NIPT at all. Approximately 25-50% of pregnant women receive NIPT. Most frequently used is the "package" for trisomy 21, 18 and 13 and sex chromosome aneuploidies. Screening for rare aneuploidies, triploidy and some microdeletions is available but generally not recommended (3).

4. DISCUSSION

As described in the previous chapters, prenatal testing and especially noninvasive prenatal testing is currently on the up and up. Having the opportunity to gain complicated genetic information with a simple blood test of the mother and without needing invasive procedures and direct access into the uterus is what makes noninvasive prenatal testing so attractive for many women all around the globe. Even though NIPT includes many advantages, such as a high sensitivity and specificity for many genetic diseases, low false-positive and false-negative rates and the possibility to perform this test in twin pregnancies, fertility treatments or after egg donation, the limitations of this technique should not be overlooked. Even though the chances are low, false-positive or false-negative NIPT results can occur. Both these cases could have a major impact on the mental health of the parents and family, leaving the parents

non-prepared in case of a false-negative result for example. Furthermore, it is possible that the fetal fraction in the mother's blood is very low and may lead to "no call" results, which will further lead to invasive prenatal testing, which should have been prevented with NIPT. Also, until today, not as many genetic changes can be detected with the NIPT as can be detected with invasive prenatal testing, such as chorionic villus sampling or amniotic fluid testing. However, one of the most arguable points – which is also present in invasive prenatal testing – are the ethical points of view. Ethicists are worried about the normalization and trivialization of early selective abortion and the demands of modern society for the "healthy, perfect child". The ethics of termination of a pregnancy with a fetus with a congenital disability still remains controversial.

Another problem is the financing, equality and possibilities for women in different countries to have access to invasive and noninvasive prenatal testing – prenatal diagnosis should not be withheld for social or financial reasons.

Diagnostic tests are generally performed to benefit the person affected by the disease while avoiding any harm that might be greater than the expected benefits. Nonetheless, one could argue that prenatal diagnostic methods may harm the fetus while the benefits are not always clear. Thus, the pregnant woman should understand that not all detected disorders are expected to affect the child's future quality of life. Because of the above mentioned ethical issues, it is critical how gynecologists and genetic counsellors give information and advice for mothers and couples, as their decisions are based on the information and advice given by professionals in the light of the couple's own individual circumstances and attitudes (49). However, the development of NIPT is simply an amazing opportunity to gain information about the unborn fetus without needing invasive techniques. Even though it is still a way to go to perfect the technique, availability and financial aspects, it is a great start and way into future medicine.

5. CONCLUSION

The inclusion of noninvasive prenatal testing into routine prenatal care is a significant breakthrough in prenatal diagnostics, notably because this technology has potential to offer earlier results without multiple blood samples and substantially reduce the number of invasive procedures (14,50).

It is a very promising approach and might encourage more pregnant women to undergo prenatal testing, without the risk of needing direct access into the uterus and thus having a small but significant risk for miscarriage.

Nevertheless, there is still a long way to go to perfect the techniques, decrease the limitations, and make it available for every woman.

However, the benefits outweigh the limitations and pave the way for further improvements and research in this field.

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