UNIVERSIDADE D COIMBRA FACULDADE DE MEDICINA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

CATARINA ISABEL TORRÃO PATO DE MACEDO

Noninvasive prenatal screening: the future of obstetric care

ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE OBSTETRICIA

Trabalho realizado sob a orientação de:

JOSÉ JOAQUIM SOUSA BARROS

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Abstract

Noninvasive prenatal testing (NIPT) brought into obstetrics the unprecedented ability to access the fetal genome with a noninvasive technique. Currently, this technique's most reliable application is in prenatal screening for trisomy 21. Several studies comparing its performance with traditional maternal serum screening have been published in the last decade, showing its potential in improving overall aneuploidy detection rate, reducing the need for invasive diagnostic procedures (IDP) and broadening our ability to explore fetal genetic features. Its reliable performance and noninvasive nature have resulted in its inclusion in many different prenatal screening models worldwide. However, the use of NIPT in prenatal care does not obviate the need for a confirmatory IDP after a positive test result. The existence of a significant number of discordant and "no call" test results justifies the need for a confirmatory IDP. Pregnancy, maternal and fetal-related conditions may be the reason for these discordant results. In addition, it is important to highlight its limitations regarding congenital conditions other than the most common trisomies, where ultrasound, traditional serum screening and IDP still play an irreplaceable role. Health care providers are thus responsible for recognizing the test's limitations and interpreting its results, as well as defining situations where NIPT may not be the most adequate screening test.

Keywords

Aneuploidy, cell-free fetal DNA, noninvasive prenatal testing, prenatal diagnosis

Introduction

A fundamental aspect of prenatal care is to educate parents on screening, diagnosis and management of pregnancy-related complications. Congenital malformations are among the most common conditions responsible for poor obstetric outcomes (1). In Europe, about 2% of all pregnancies are complicated by congenital malformations and syndromes (2).

Aneuploidies, defined by the existence of an extra or missing chromosome in the fetal karyotype, are among the most prevalent congenital conditions. Their incidence is about 1 in 150 live births. Autosomal trisomies are the most frequently encountered, with trisomy 21 (T21), known as Down syndrome, being the most common, followed by trisomy 18 (T18) and trisomy 13 (T13). Sex chromosome aneuploidies (SCA) occur in 1 in 1000 births. The most common are Klinefelter's syndrome and monosomy X (MX). The latter is the only viable monosomy. The prevalence of the most common aneuploidies is described in Table 1 (1).

Table 1 – Prevalence of the most common aneuploidies

Aneuploidy	Prevalence
T21 (Down syndrome)	1/800
T18 (Edward syndrome)	1/6,000
T13 (Patau syndrome)	1/10,000
47,XXY (Klinefelter syndrome)	1/500 males
45, X (Turner syndrome)	1/10,000

T (trisomy). ***Adapted from Casanova et al. (1)***

Clinically, changes in the quantity of genomic material can lead to a wide range of consequences. T21 clinical manifestations, in particular, may vary from mild to severe. Around 43% of affected pregnancies end in miscarriage or stillbirth. Individuals with T21 may present congenital heart defects, distinctive facial features and intestinal atresia, and have increased risk of seizures, childhood leukemia, learning disabilities, infertility and early onset Alzheimer disease. The average lifespan of people affected by Down syndrome is 60 years in developed countries (3,4).

Obstetricians are responsible for determining whether a woman presents an increased risk of fetal abnormalities and for offering appropriate prenatal screening and/or diagnostic tests. Risk stratification begins with identification of factors associated with a higher likelihood of fetal chromosomal anomalies. Advanced maternal age, history of a previous pregnancy affected by chromosomal abnormality and past early pregnancy loss (at least half of first trimester

miscarriages are estimated to result from fetal chromosomal anomalies) are examples of such risk factors (1).

Traditional prenatal screening tests, such as ultrasound (US) and maternal serum screening, are an essential tool in risk assessment for neural tube defects (NTD) and the most common trisomies. They can be performed during the first and second trimesters, and are ideally applied to all pregnant women regardless of their past history and age – however, they are not able to confirm or exclude disease. In contrast, invasive diagnostic procedures (IDP) are able to establish the presence or absence of a specific disease through karyotype analysis, and should be offered when a screening test is positive. The most commonly performed IDP are amniocentesis, chorionic villus sampling (CVS) and cordocentesis. Less frequently used IDP include fetal skin sampling, fetal tissue (mostly liver or muscle) biopsy and fetoscopy, performed only in the suspicion of rarer diseases (1). The more accurate screening tests are, the less IDP are necessary, resulting in a safer and more economical approach for both mother and fetus.

Noninvasive prenatal testing (NIPT) is the most recent element added to prenatal screening. It was introduced in 2012 and consists in the analysis of cell-free fetal DNA (cffDNA), derived from placental cytotrophoblast apoptosis, present in a sample of maternal blood (5). Different aneuploidy screening models which include NIPT have been rapidly approved and widely accepted around the world and its implementation has significantly reduced the number of invasive procedures performed. However, a positive result in NIPT does not bypass the need for a diagnostic test confirmation. cffDNA quantification is also not advised to all pregnant women, especially those with higher risk for discordant test results. Given its promising results in aneuploidy screening, new applications for NIPT have been tested, such as fetal sex determination, subchromosomic anomaly detection and fetal rhesus-D genotyping.

This work aims to review NIPT technical aspects and compare its performance with traditional aneuploidy screening models.

Methods

Research was performed between June and December 2018, in the online databases Embase and Pubmed, using the term ‘cell free fetal DNA’ and the following MeSH terms: ‘aneuploidy’ and ‘prenatal diagnosis’. Research was restricted to English and Portuguese language publications, published between ‘2008/11/21’ and ‘2018/11/18’. Results were reviewed and selected for relevant articles, and supplemented with manual search of citations included in pertinent reviews. Some American College of Obstetricians and Gynecologists (ACOG) clinical guidelines and opinions, articles from Acta Obstétrica e Ginecológica Portuguesa and

documents from Registo Nacional de Anomalias Congénitas (RENAC) were also selected and included.

Results

1. Traditional aneuploidy screening

1.1. First trimester combined screening (FTCS) and Quadruple (quad) test

In the past, the most important risk factor for fetal aneuploidy was maternal age greater than 35 years. Although aneuploidy risk increases with age, fetal aneuploidy can affect any pregnancy regardless of maternal age. In fact, the majority of cases happen in younger mothers, since the majority of pregnancies occur at those ages. An observational study including 38,000 women demonstrated that if only women aged more than 35 years had been submitted to IDP, the detection rate of T21 would only have been 21.6% (4). For this reason, prenatal screening of aneuploidies is recommended in all pregnancies. Positive ultrasound findings, a positive screening test, a positive history for a previous child with aneuploidy or a parental translocation involving chromosomes of interest are others factors which define high risk of aneuploidy (6).

Traditional first trimester screening (usually performed between 10 and 13 weeks of gestation) includes US evaluation and the quantification of specific maternal serum markers: pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotropin (beta-hCG). PAPP-A is secreted by the placenta, is responsible for cell differentiation and proliferation and increases in maternal serum throughout pregnancy. Beta-hCG is produced exclusively by the outer layer cells of the trophoblast, reaching its peak in maternal serum at week 8 and then maintaining stable levels until term (6). Screening results are influenced by prior history of aneuploidy, maternal weight, race and parity. A typical positive FTCS for T21 occurs when maternal age is higher than 35, nuchal translucency (NT) is increased (above 3.0 mm or above the 95th percentile), beta-hCG is increased (at least 1.90 multiples of the median [MoM]) and PAPP-A is decreased (less than 0.40 MoM) (4,7).

Second trimester biochemical screening (from 15 to 22 weeks of gestation), also called quadruple or quad screen, consists in measuring alpha-fetoprotein (AFP), beta-hCG, inhibin-A and unconjugated estriol in the maternal serum. In addition to aneuploidy risk information, this test also quantifies the risk of NTD (4). Fetal AFP is a plasma protein similar to albumin, produced by the yolk sac, liver and gastrointestinal tract. In fetal plasma, AFP reaches its peak at week 10-13 and then decreases until the end of gestation. On the other hand, maternal serum levels achieve their peak in the third trimester. While amniotic and maternal levels of AFP are increased in several malformations such as neural tube and abdominal wall defects,

lower values have been associated with T21. Unconjugated estriol results from precursors secreted in fetal adrenal and liver cells, which are metabolized and released by the placenta. Typically, unconjugated estriol increases until term; however, it is known to be decreased in T21 and T18. Finally, inhibin-A is produced by the corpus luteum and fetoplacental unit and decreased levels in maternal serum are considered a positive screening result for Down syndrome. A different modality of the second trimester screening, called triple screening, is possible, by measuring onlyserum AFP, beta-hCG and unconjugated estriol (4,6).

FTCS can be used independently or combined with second trimester biochemical screening, as an integrated, sequential or contingent model. An integrated screening model consists in performing FTCS followed by second trimester quadruple test, resulting in a single test result, only available in the second trimester. In a sequential model, first trimester results are immediately reported to parents. This allows early identification of high risk pregnancies. The high risk group is referred to IDP while the remaining population is proposed the second trimester quad test. This model has a detection rate of 91–93%. Finally, the contingent model stratifies women as low, intermediate and high risk based on FTCS results. High risk women are offered IDP, intermediate risk pregnancies are proposed the quad test and the low risk population does not undergo any further testing (4).

In terms of accuracy, concerning T21, FTCS alone provides a positive predictive value (PPV) of only 2-3% (8), a detection rate (DR) of 82-87% and a false positive (FP) rate of 5% (6). Second trimester biochemical screening alone provides a PPV of 2%, a FP rate of 7% and a detection rate of less than 80% (6,8). Triple screening provides a lower sensitivity (69%) than FTCS and the quad test, and a PPV of 5% (4). Sequential screening offers a slightly higher PPV of 4% (8), but the fact that results are only revealed in the second trimester leaves parents less time to decide on a potential termination of pregnancy. In addition, high false-positive rates for FTCS can result in many unnecessary invasive procedures.

1.2. Ultrasound

US plays a fundamental role in Obstetrics, allowing the identification of syndromes which result in abnormal fetal morphology. T13 and T18 usually show major structural anomalies on US; T21 features are sometimes more subtle (4). First trimester ultrasound, performed between week 11 and week 13, is essential for the detection of major malformations and early aneuploidy markers. Second trimester US screening is also used to identify morphological anomalies, major and minor aneuploidy markers.

Regarding first trimester US, the isolated marker most associated with aneuploidy is nuchal translucency (NT). The risk of an adverse outcome is proportional to the degree of enlargement of NT (4). The NT test has a sensitivity of 77% for trisomy 21 and a false-positive rate of 6%

(6). It is important to note that NT increase is not specific for aneuploidy, since it may also be present in congenital heart defects, abdominal wall defects and diaphragmatic hernia in euploid fetuses.

Second trimester US identifies major and soft aneuploidy markers, being only capable of detecting 50-60% of Down syndrome affected fetuses. The more US markers are identified, the higher is the risk of aneuploidy. However, a single US marker is more commonly found among affected (22,6%) than non-affectes fetuses (11,3%), highlighting the importance of keeping single findings in mind, especially among women who received a high risk FTCS result (9). Increased nuchal skinfold thickness confers the highest risk of aneuploidy, contrasting with an isolated echogenic intracardiac focus, which carries one of the lowest risks of fetal aneuploidy (1,4).

Overall, the screening value of US is so high, that after the identification of a major fetal structural abnormality, an IDP is the most adequate next step, rather than performing other screening options (8,10).

However, like every procedure, it also possesses some disadvantages, such as its dependence on professional expertise, some maternal and gestational features (e.g. maternal body mass index and parity), software and hardware quality and the lack of standardization of some cut-offs (4).

1.3. Invasive procedures

Fetal nucleated cells are needed for fetal DNA analysis and definitive diagnosis, and these can only be obtained by IDP.

CVS can be performed via transvaginal or transabdominal approach from week 11 of gestation, being the only IDP available in the first trimester (11). Its performance before 11 weeks is not recommended, since it is associated with risk of limb reduction and oromandibular defects. Apart from the possibility of being performed in earlier pregnancy stages than amniocentesis, CVS may also have a shorter sample processing time. However, it may present complications such as vaginal bleeding, which occurs in 32% of patients and is more frequent with the transvaginal approach. This approach also carries a higher risk of complications (1).

Amniocentesis consists on a sampling of 20 to 30 mL of amniotic fluid by transabdominal approach, under US guidance. It is usually performed between weeks 15 and 20 of gestation. Several studies have shown its safety and diagnostic accuracy (more than 99%). The most frequent complications are vaginal spotting, amniotic fluid leakage and chorioamnionitis (1).

Amniocentesis and CVS are considered relatively safe tests, although there is a risk of complications (such as premature rupture of membranes, early delivery, chorioamnionitis or miscarriage) of 0.13% and 0.22%, respectively (5).

2. NIPT role in aneuploidy screening

2.1. Sample collection, sequencing and quantification of cffDNA

Cell free DNA fragments from mother and fetus are both present in maternal blood. cffDNA is originated from programmed cell death of placental trophoblastic cells and can be detected since day 18 of gestational age until day 2 postpartum (12).

Currently, different laboratories have different techniques to sequence and quantify cffDNA. One possible approach is to analyze the whole fetal genome, for which massive parallel sequencing is the most frequently used method. Targeted or chromosome-selective sequencing only quantifies and sequences chromosomes of interest (21, 18, 13, X…), resulting in a more cost-effective performance. Additionally, some laboratories base sequencing on single nucleotide polymorphism (SNP) detection. By comparing SNPs present in cffDNA with those in DNA present in maternal leukocytes, it is possible to differentiate one from the other, to quantify fetal fraction (FF) and infer about fetal karyotype (13). This method also allows detection of information like uniparental disomy, parental source of aneuploidies and other mutations, nonpaternity and consanguinity (5). SNP technology is not appropriate in cases of multiple pregnancy, pregnancy resulting from a donor egg and maternal bone marrow or solid organ transplant (8,13).

FF is the amount of fetal DNA found in the total free DNA present in maternal plasma. It varies between 7-19% and increases throughout pregnancy. Apart from gestational age, other factors contribute to its variation: maternal ethnicity, body mass index (BMI), smoking status, multiple pregnancy, fetal ploidy and conditions such as preeclampsia (13). A low FF, defined as values under 4%, originates inconclusive results (8).

FF determination is not performed by all laboratories, but it is essential when clinicians are faced with maternal conditions that can result in lower FF, increasing the risk of false-negative (FN) results (5,14). In a study published in 2015 by Kinnings et al. (15), including 140,377 samples of cffDNA in maternal blood, the influence of gestational age, fetal ploidy, maternal BMI and redrawing in FF was assessed. In a normal gestation, FF was proven to increase throughout pregnancy, although at different rates. Between week 10 and 13 it seems to increase 0.44% per week, reducing to a rate of 0.08% per week until week 20. After week 20, FF may increase around 0.82% per week, maintaining this evolution until the end of gestation. Fetal ploidy is also an important variable affecting FF. The influence of the most common aneuploidies is discriminated in Figure 1.

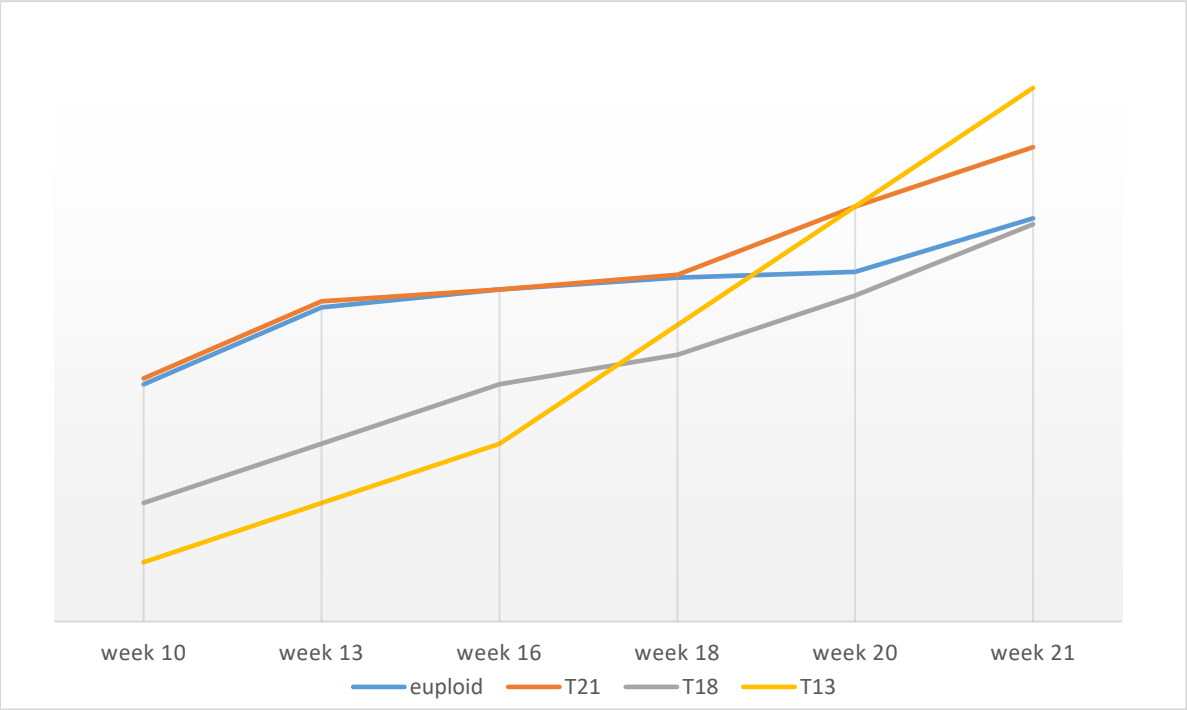

Figure 1 – Fetal fraction variation according to fetal ploidy. *Adapted from Kinnings et al.* (15)

Similarly to other published studies, BMI was shown to be inversely correlated with FF values. More precisely, Kinnings et al. showed that within the range of 20-40 Kg/m2 of BMI, FF decreased 1.17% per every increase in BMI of 5 Kg/m2. Above 40 Kg/m2, FF remained stable. Observations that 75% of samples are suitable for analysis when considering BMI of 60 kg/m2 and above allowed the conclusion that overweight and obese women are still eligible for NIPT, bearing in mind that the accuracy of the test is inferior compared to the normal BMI population. Redrawing blood after insufficient FF on a first test resulted in greater and eligible values of cffDNA when repeated with an interval as little as 1-10 days after the first draw, with 71.4% of the samples presenting meaningful results at the second draw. Broader redrawing intervals did not present further improvement in FF. The increase in FF in redraw samples was attributed to advancing gestational age (15).

2.2. NIPT vs. traditional aneuploidy screening

The best timing to perform NIPT is after week 10 of gestation and it can be offered at any time until birth (10).

Since its introduction in clinical practice, several studies have established NIPT performance in prenatal screening for the most common aneuploidies (T21, T18, T13 and SCA). Table 2 shows the DR and false positive rate (FPR) for each condition, based on a meta-analysis including mostly high risk pregnancies. Studies performed in the general population have shown similar performances (16).

Table 2 – NIPT performance in T21, T18, T13 and Monosomy X screening

Aneuploidy	DR	FPR
T21	99.7%	0.04%
T18	97.9%	0.04%
T13	99.0%	0.04%
MX	95.8%	0.14%

Noninvasive prenatal testing (NIPT), detection rate (DR), false positive rate (FPR), trisomy (T), monosomy X (MX). ***Adapted from Gil et al.(16) .***

Compared to traditional serum biomarkers used in aneuploidy screening, NIPT offers significant improvements in T21 DR (99% vs. 80-95%) and higher PPV (80-99% vs. 2-3%). Additionally, NIPT needs only one simple blood sample to be executed, while some other screening modalities are more complex. Nevertheless, NIPT has the highest screening failure rate (Table 3). Maternal obesity, for example, can originate a significant rate of FN or "no-call" results when NIPT is performed due to reduced FF. Traditional serum screening remains unaffected in these situations, since risk calculation is adjusted for maternal BMI (8).

Table 3 – Comparative analysis of Down syndrome prenatal screening tests

	FTCS	2nd trimester quad test	Integrated screen	NIPT
DR	90%	80%	95%	>98%
PPV	2-3%	2%	4%	80-99%
FR	<1%	<<1%	<1%	0.3-3%
Procedures	US + 1 BS	US + 1 BS	US + 2 BS	1 BS

Detection rate (DR), positive predictive value (PPV), failure rate (FR), first trimester combined screening (FTCS), noninvasive prenatal testing (NIPT), ultrasound (US), BS (blood sample). ***Adapted from Gray et al. (8)***

Given the conventional screening tests and NIPT's FPR (4.5% and 0.04-0.14%, respectively), an IDP (CVS or amniocentesis) must always be performed after a positive result, to confirm the diagnosis and calculate the risk of recurrence (10,17). The same happens after an US showing morphological anomalies.

Some scientific societies, such as the Society for Maternal-Fetal Medicine, have recently updated US guidelines concerning women who underwent NIPT. For instance, this society does not recommend the determination of NT after a negative cffDNA result. In addition, women with negative results that present one single aneuploidy soft marker on second trimester US (such as choroid plexus cyst or echogenic intracardiac focus) have no need for a diagnostic procedure and such marker should be considered a normal variant (8).

Vora *et al*. performed a retrospective cohort study (18) including 2,337 high risk women (based on advanced maternal age) to define how often a first trimester US in the context of NIPT would influence the prenatal screening/diagnostic strategy and parental counseling. US evaluation was offered at the time of NIPT (between weeks 10-14 of gestation), to look for nonviable pregnancies, structural anomalies, multiple gestations and incorrect pregnancy dating, where NIPT would not be recommended. About 16% of women presented one or more of such criteria (Table 4). US evaluation earlier than 10 weeks of gestation was shown to be inconclusive, since 22% of those evaluations subsequently showed fetal anomalies or demise at the 10-14 week US. Based on these findings, it may be more adequate to perform NIPT only after or at the time of first trimester US.

Table 4 – US findings which can change prenatal counseling at the time of NIPT

US findings	Subsequent approach
Nonviable pregnancy →	No more tests
Structural anomalies →	Diagnostic procedure
Multiple gestations →	FTCS instead of NIPT
Lower gestational age →	Delay screening tests

Ultrasound (US), first trimester combined screening (FTCS), noninvasive prenatal testing (NIPT). ***Adapted from Vora et al. (17)***

2.3. NIPT implementation models

There are three main possible models of implementation of NIPT in prenatal care: Advanced, Universal and Contingent.

Universal screening models (Figure 2A) offer NIPT to all pregnant women around week 12, along with US. This provides the best rates of aneuploidy detection, but is the most expensive

model. Besides, giving all women only NIPT and abandoning traditional serum screening leaves some rarer chromosomic/subchromosomic abnormalities, such as microdeletions, microduplications, rarer aneuploidies, mosaicism and triploidy/tetraploidy undiagnosed,, since trisomies 21, 18, 13 and SCA only constitute 80% of all karyotype anomalies (8). FTCS also allows the detection of a higher risk of other pregnancy complications (such as fetal growth restriction (FGR), preterm delivery and preeclampsia), based on reduced values of PAPP-A. Despite its less important role in prenatal screening, second trimester serum screening can also alert for complications that are not screened for by NIPT. For example, an increased AFP is considered a good risk marker for NTD; low estradiol may indicate Smith Lemli-Opitz syndrome; higher levels of beta-hCG and inhibin-A may indicate some pregnancy-related complications, such as preeclampsia (4,8). This results in a 2% risk of having a FN result which could have been avoided by traditional serum screening (7,8). For this reason, if all chromosome abnormalities are considered, serum screening has a higher overall detection rate than NIPT in the general population (8).

When NIPT is applied to a low risk population (e.g., Universal models), its PPV is lower, since it depends on prior risk of a certain condition on a specific population. For women under 35 years of age, the PPV is around 50% (vs. 80-99% in a high risk population). However, this is still higher than the PPV of traditional serum screening. Also, though the main points of interest of NIPT are trisomies 21, 18 and 13, SCA are already a target for some laboratories. For these conditions, risk is not as dependent on maternal age, which means the PPV will be less variable (8).

In an Advanced screening model (Figure 2B), also known as second-tier screening, all women are submitted to FTCS. Then, NIPT can be offered to all high risk women as an advanced screening test, before performing an IDP. If parents choose NIPT, only those with a positive result are counseled to perform an IDP. This model reduces the need for invasive procedures but does not improve the overall DR, mostly because of the lower detection rate of serum screening (80-90%) (8). Consequently, around 20% of affected fetuses are not detected by serum traditional screening and will not receive NIPT. Since in this model only about 5% of pregnancies receive NIPT, it is reasonably cost-effective.

In a Contingent model (Figure 2C), pregnancies are stratified in low/intermediate/high risk based on FTCS. IDP are recommended to high risk women and NIPT to the intermediate risk population (e.g., FTCS risk between 1/100 and 1/1000). NIPT results dictate whether it is recommended to perform an IDP or no further testing. This model tries to balance the advantages and disadvantages of the other two, in terms of detection rate and costs. This strategy is less expensive than the Universal model and offers better detection rates than the Advanced model (19).

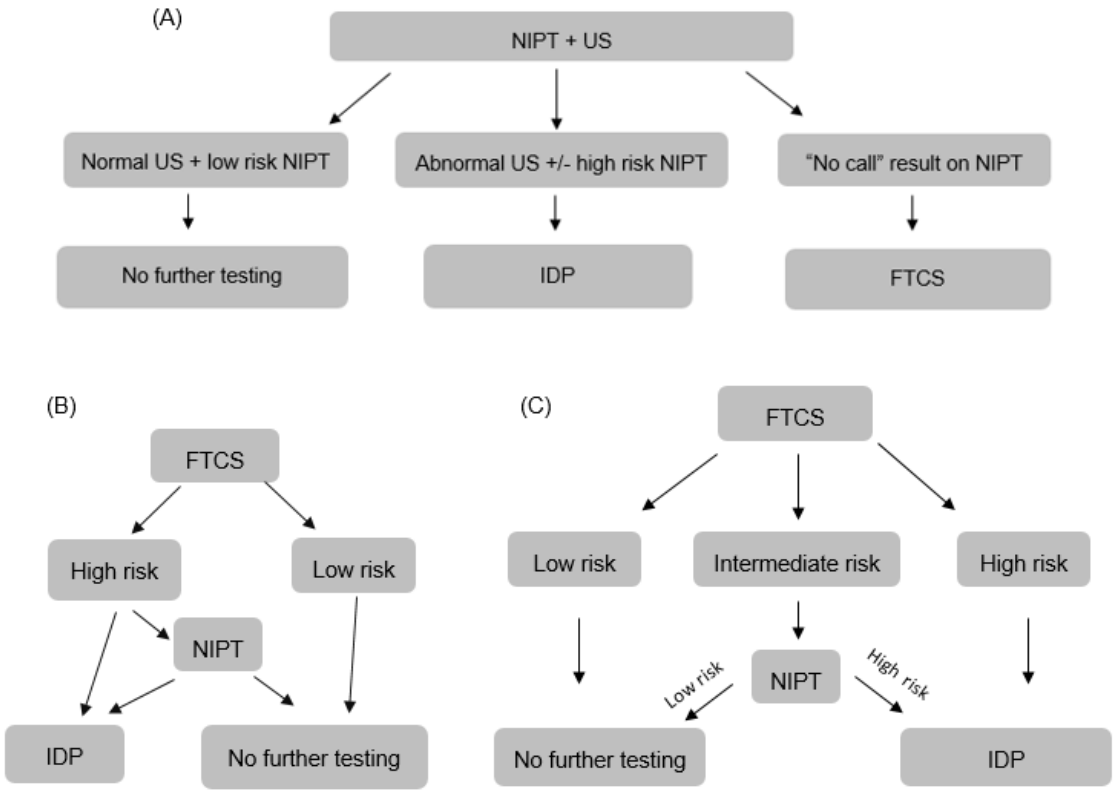

Figure 2 – Implementation models of NIPT, incorporating conventional screening/diagnostic procedures: (A) - Universal model, (B) – Advanced model, (C) – Contingent model. Noninvasive prenatal testing (NIPT), ultrasound (US), invasive diagnostic procedures (IDP), first trimester combined screening (FTCS). ***Adapted from Hui et al.*** (19)

Additionally, some implementation models may not correspond exactly to any of the previously described. ACOG recommendations, for instance, advise NIPT performance in high risk women defined by the following criteria: women who are 35 years of age or older, who have fetuses with US anomalies suggestive of an increased aneuploidy risk, women with previous trisomy affected offspring, couples with increased risk of trisomy due to a parental balanced Robertsonian translocation, and women with increased risk based on first or second trimester screening tests (10).

3. Discordant test results and NIPT limitations

When a NIPT positive result is not confirmed by IDP, it is labeled a FP result. FN results happen when a negative result of NIPT is not confirmed by IDP or karyotyping after birth. Some authors refer to both these situations as discordant results (20).

3.1. FP results

The main causes of FP results are associated with fetal or maternal characteristics, synthesized in Table 5.

Table 5 – Main causes for a false positive test result in NIPT

Fetal/pregnancy characteristics	Maternal characteristics
Feto-placental mosaicism	Chromosomal/subchromosomal abnormalities
Vanishing twin	Malignancy, transplant

Noninvasive prenatal testing (NIPT). ***Adapted from Hui et al.*** (19)

3.1.1. Feto-placental mosaicism

Mosaicism is the existence of, at least, two different cell lines in gestational tissues, resulting from errors during cell division. If the error occurs in an early pregnancy stage, it may affect both fetus and placenta. If the error occurs later, mosaicism can be confined to a smaller number of cells, existing only in the fetus or placenta. In the placenta, there are two different cell layers – cytotrophoblasts and mesenchymal cells - which can be distinctly affected. When mosaicism occurs exclusively in placental cells, it is termed confined placental mosaicism (CPM). When the fetus is affected it is referred to as true fetal mosaicism (TFM) (21). CPM is thought to occur in 1-2% of viable pregnancies at 10-12 weeks. Since cffDNA is originated from the multiplication of placental originated cells, in a case of CPM where the cytotrophoblast is affected by mosaicism, FP results in NIPT are possible (19). On the other hand, when the fetus is affected but the trophoblast cell line is spared, NIPT can result in FN results. A study including 67,030 cytogenetic diagnoses collected by CVS revealed that autosomal aneuploidies are the most common in placental tissue (21). Another study including 52,673 karyotype samples, obtained by initial CVS of both placental cell layers and subsequently by confirmatory amniocentesis (22), aimed to determine the best confirmatory IDP after a high risk result in NIPT. This is particularly relevant since NIPT can be performed from week 9 and the only IDP available at this time is CVS. Among the most frequent aneuploidies, T13 and Monosomy X showed higher rates of mosaicism among pregnancies undergoing invasive testing with CVS (22% and 59%, respectively). Lower frequencies were found for T21 and T18 (2% and 4%, respectively). Figures 3 and 4 illustrate the relative frequency of CPM and TFM, respectively, for the most common aneuploidies. These findings support that it is adequate to perform CVS after a positive screening for T21 and T18, allowing for earlier counseling and management of the pregnancy, keeping in mind the small risk of a FP result. For suspected

monosomy X and trisomy 13 amniocentesis is the most adequate diagnostic option, avoiding the termination of a normal pregnancy but contributing to a longer period of uncertainty until diagnosis is confirmed. These principles are not applicable when US findings suggest fetal involvement – in these cases, CVS can be immediately performed (19,22).

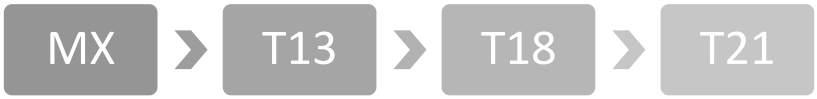

Figure 3 – Relative frequency of confined placental mosaicism in the most common aneuploidies. Monosomy X (MX), trisomy (T). ***Adapted from Grati et al.*** (22)

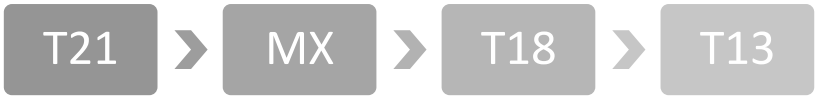

Figure 4 – Relative frequency of true fetal mosaicism in the most common aneuploidies. Monosomy X (MX), trisomy (T). ***Adapted from Grati et al.*** (22)

3.1.2. Vanishing twin

The placenta of a vanishing twin can be the cause of a FP result. It is believed that 1-3% of singleton pregnancies begin as multiple gestations (8), and that the demise of a co-twin is usually associated with chromosomal anomalies. CffDNA belonging to the demised twin can be detected in maternal blood until 8 weeks after demise. This situation is easier to identify when there is a female fetus and Y chromosomes are detected in maternal blood (19). SNP-based quantification can also be useful in this situation, allowing for distinction between cffDNA of each of the fetuses (23).

3.1.3. Maternal chromosomal and subchromosomal abnormalities

Maternal conditions are also a frequent etiology of FP results. Among them, we can find SCA, microdelections, copy number variations (CNV), maternal malignancies and history of maternal transplant. NIPT can be positive for SCA in situations of maternal mosaicism, such as 45,X/46,XX, or maternal 47, XXX karyotype. Another common reason for a FP result is the age-related somatic loss of an X chromosome in the peripheral blood, resulting in low-level somatic mosaicism in some women (19). In fact, a total of 8,6% of positive results for SCA are due to maternal mosaic and loss of X chromosomes, consisting in the most common cause of FP results in NIPT (8,13,18,22). CNV, especially when considerably large and occurring in chromosomes of interest, can also lead to inadequate positive results (8).

3.1.4. Maternal malignancy

Maternal malignancies lead to higher and imbalanced amounts of circulating cell-free DNA, because of increased tumor cell turnover. In these conditions, maternal cell-free DNA can reflect multiple chromosomal abnormalities. This occurs in 0.03% of pregnancies submitted to NIPT and accounts for 18% of maternal conditions that affect NIPT (8). The most documented maternal malignancies detected by NIPT since its application in prenatal care are breast, colorectal and endocrine neoplasms, as well as leukemia. For this reason, when results reveal multiple chromosomal abnormalities, oncologist referral may be indicated.

3.1.5. Maternal transplant

Maternal transplants, such as bone narrow transplant, solid organ transplant or even a simple blood transfusion, introduce foreign biological material to maternal circulation. If a detailed medical history is not performed, NIPT can detect abnormalities reflecting donor conditions, resulting in a FP result (4,18).

3.2. FN results

The probability of an aneuploid fetus with Down syndrome being screened as an euploid fetus is very low. However, there are some reported cases. The main reasons are low FF and CPM (8). Low FF is defined as values under 4% (8). The main etiologies of a low FF are: early gestational age, maternal obesity, fetal aneuploidy, low sample quality and preexisting maternal hypertension. At 10 weeks, average FF is 13% and increases throughout pregnancy (8). Before 10 weeks FF is considerably lower, meaning that early testing or overestimated

gestational age can result in negative results in affected fetuses. Maternal BMI inversely affects the amount of FF. This is thought to result from dilutional effects, since maternal plasma is increased in obesity and there is a rise in maternal cell-free DNA caused by a secondary inflammatory state. Overall, obese women have a 3 to 4-fold increased risk of receiving a FN or failed test result (8). Fetal aneuploidy can also be a reason for FN results, especially due to CPM. This has been shown to be especially frequent in fetuses with trisomy 18 and trisomy 13, since up to 40% of their placentas may have an euploid karyotype (8). Another reason is the fact that aneuploidy itself can result in lower FF, affecting test results. This is true for trisomy 18, 13, monosomy X and triploid pregnancies. Inversely, Down syndrome affected pregnancies have higher values of FF (8). Additionally, quality aspects of blood draw can be associated with maternal cell cytolysis, increasing maternal cell-free DNA, diluting cffDNA and lowering FF(8). Preexisting maternal hypertension is also a cause of low FF. Other pre-pregnancy maternal conditions such as thyroid diseases and preexisting diabetes have also been assessed, showing no significant relationship with FF (24).

3.3. 'No call' results

'No call' results are defined by situations where NIPT fails to return a result. NIPT failure rate ranges from 0% to 12,7% (25), and about 20% of samples with 'no call' results have an abnormal karyotype (8). Consequently, after a test failure result it is reasonable to proceed to an IDP with or without re-testing, based on US findings and prior known risks (14). However, managing women with 'no call' results as screen positive will decrease specificity and increase the FP rate, lowering NIPT's impact on the number of IDP performed. (4). Finally, repeating NIPT is also an option; however, the chances of a second inconclusive result are high (13.9%) and it can delay a more appropriate clinical approach. Thus, some societies such as the American College of Medical Genetics and Genomics advise against this option (13,25). Different situations can induce a 'no call' result.

3.3.1. Low FF

The majority of laboratories require a minimum of 2-4% of FF to offer a result. Low FF is the most frequent cause of test failure, accounting for about 75% of 'no call' results (26). However, half of these women will have a definitive result if they repeat the test. Apart from the risk of a 'no call' result, women with low FF can be at high risk of complications, such as trisomy 18 or triploidy, deserving special attention and care (19). Clinicians must be aware of situations that increase the risk of low FF and refrain from prescribing NIPT in those cases (14).

3.3.2. Twin pregnancies

The ACOG does not recommend cffDNA screening in women with multiple gestation, because data regarding its application in this type of pregnancy are still very limited (10). Thus, pre-NIPT US surveillance plays an important role in determining whether it is indicated or not, by defining the number of fetuses and chorionicity. Apart from the fact that a normal twin can mask the abnormal karyotype of an aneuploid co-twin, some studies have shown that each fetus can contribute differently for the total amount of cffDNA present in maternal blood. Additionally, the greater BMI of these women contributes to a higher dilutional effect. This is less relevant in the case of monozygous pregnancies, where fetuses share an identical karyotype, but these represent only 34% of the total of multiple pregnancies (23).

In fact, no screening and diagnostic method is as accurate in multiple gestation as it is in singleton pregnancies. For instance, the second trimester quad test can only detect 50% of T21 affected fetuses (vs 80% in singleton pregnancies). When an US anomaly is detected in one of the fetuses or in case of fetal demise, maternal serum screening is discouraged. In those situations an IDP must be considered. In fact, the most accurate method to perform aneuploidy screening in these pregnancies is still measurement of NT during the first trimester US – since it allows the evaluation of both fetuses separately and the standardized reference values and cut-offs do not differ significantly from the ones established for singleton pregnancies (4,8).

The lower accuracy of screening tests in multiple pregnancies deserves special concern, since the rate of aneuploidy is higher than in singleton pregnancies and IDP also present higher complication rates (20).

3.3.3. Maternal BMI

Maternal BMI induces test failure by reducing FF. Almost 7% of women weighing 100kg have a FF lower than 4%. When maternal weight reaches 160kg, ‘no call’ results increase to almost 50% (13).

3.3.4. Specific chromosomal abnormalities

Some specific chromosomal abnormalities, such as T13, T18 and triploidy, present higher rates of test failure. This means that a ‘no call’ result can be indicative of an increased risk of aneuploidy, requiring additional follow-up and counseling (13,27).

4. Performance-studies around the world

One of the main limitations to the more widespread use of NIPT has been the test's cost. Some performance and cost-effectiveness studies have been performed, but more are still needed.

4.1. RAPID trial

In the United Kingdom, the Reliable Accurate Prenatal non-Invasive Diagnosis (RAPID) project (28) was performed between November 2013 and February 2015, to test a contingent model of implementation of NIPT in Down Syndrome screening. NIPT was offered to all women with a risk of T21 greater than 1/1000 after FTCS or quadruple screening. Those with a risk equal to or greater than 1/150 were offered NIPT or invasive prenatal diagnosis. Assessment of the implementation model was based on the DR of Down syndrome affected fetuses, number of IDP performed and cost analysis.

A risk cut-off of 1/150 allowed an increased detection rate of T21, lower estimated costs, decreased number of IDP and, consequently, less procedure-related miscarriages (Table 6). Based on these results, the UK National Screening Program now recommends the performance of NIPT in all women with a risk of aneuploidy of 1/150 or greater after FTCS or quadruple screening.

Table 6 – Advantages and disadvantages of each risk cut-off in the RAPID program, compared to the traditional screening pathway

Risk cut-off	Advantages	Disadvantages
1/1000	176 more cases detected 4,805 less IDP performed 24 less miscarriages	7,809,000 £ more
1/500	152 more cases detected 4,826 less IDP performed 25 less miscarriages	3,365,000 £ more
1/150	102 more cases detected 4,870 less IDP performed 25 less miscarriages 337,000 £ less	

Invasive diagnostic procedures (IDP*). Adapted from Lyn et al* (28)

4.2. TRIDENT trial

TRIDENT-1 is a trial performed by Dutch laboratories for evaluation of NIPT implementation, and supported by The Netherlands Organization for Health Research and Development. Human resources for this project comprised obstetric healthcare professionals, clinical geneticists and laboratory specialists, the national prenatal screening organization, Dutch Genetic Alliance representatives, ethicists, insurance company employees and policy makers. It took place between 1st April and 1st September 2014 and included 23,232 women. Among these, 1,413 (6%) were classified as high risk, based on FTCS aneuploidy risk greater than 1/200. The majority (85.7%) of high risk women chose to perform NIPT. Another 179 additional women were proposed and accepted performing NIPT, based only on their medical history and regardless of their FTCS result. Exclusion criteria for this trial were: history of maternal malignancy or known parental chromosomic anomaly, women under 18 years of age, multiple gestation, vanishing twins, fetal NT>3.5 mm or other structural anomalies, gestational age under 10 weeks and inability to give informed consent. Almost all women (99.7%) received a NIPT result, 0.4% of them only after a redraw. Among the 0.3% of test failures, 75% were due to low FF and the other 25% presented high BMI; all of these women ultimately gave birth to unaffected children. Overall, DR of NIPT considering the most common trisomies (T21, T18, T13) was 97.4% (all the 4 cases of T18 and 4 cases of T13 were correctly detected, while 1 out of 30 cases of T21 was not detected). The only FN result for T21 had a 46,XX,i(21)(q10) karyotype, and was diagnosed by amniocentesis carried out after US anomalies were detected at routine scanning. Parents chose to proceed with the pregnancy. Placental analysis after birth showed no presence of the isochromosome in the cytotrophoblast. Ultimate FPR was 0.4% (including 2 cases of T21, 1 of T18 and 2 of T13). SCA were not screened for because of political constraints. Ultimately, the number of IDP was reduced in 62% (29). The success of this project gave place to the TRIDENT-2 trial which has already been approved and is currently in progress (2017-2020). This second version of the project will offer NIPT to all pregnant women, regardless of their risk for aneuploidies. Women will be able to choose between NIPT and FTCS as their first screening method, and even to perform NIPT in a contingent manner after FTCS. It is relevant to note that, in the Netherlands, NIPT and FTCS already present similar costs, 175 and 168€, respectively (26).

4.3. PEGASUS trial

PEGASUS (PErsonalized Genomics for prenatal Aneuploidy Screening USing maternal blood) is a Canadian project aimed at analysing NIPT implementation in the Canadian health care system. PEGASUS-1 took place between 2013 and 2017. NIPT application in aneuploidy screening proved to be cost-neutral when applied as a second-tier screening, since the

increased cost brought about by NIPT would be offset by the reduction of IDP performed. Additionally, educational tools for health care professionals and decision-making tools to assist couples in making informed decisions were also developed. Given the results of the first part of this project, since 22nd April 2018 NIPT is already offered by the Canadian public health care system as a second tier screening test, after a positive result in FTCS is obtained.

PEGASUS-2 aims to evaluate NIPT as a first-tier screening tool in prenatal care. This second phase of the trial started in 2018 and will last until 2022. This project proposes that a first-tier application of NIPT for Down syndrome screening will benefit prenatal care by providing a higher DR of affected fetuses and a lower rate of FP results than traditional aneuploidy screening.. Additionally, due to this test's increasing applications, other chromosomal abnormalities could also be screened. Cost-effectiveness of expanding screening to other conditions will also be analysed, along with the ethical, social and legal implications of doing so. Finally, another goal is to develop NIPT technology in order to lower its costs and to expand its applications to other anomalies. Web-based tools to aid parental decision making and health care professional training in prenatal screening and counselling will also be reinforced (30).

5. Other applications of NIPT

NIPT allows access to the entire fetal genome, offering the opportunity to screen for other genetic conditions in a noninvasive way. Consequently, less common chromosomal or subchromosomal anomalies, such as microdelections or duplications, may be subject to detection. Some have already been explored, with less promising results than for the most common trisomies.

5.1. Sex chromosome aneuploidies

NIPT was first applied to SCA in 2012, more specifically for monosomy X, which occurs in 1-1.5% of pregnancies (13). Identification of other SCA like 47, XXX; 47, XXY; 47, XYY has since been tested as well. A meta-analysis about the performance of NIPT for monosomy X revealed a sensitivity of 95.8% and a FP rate of 0.14%, compared with 99.7% and 0.04% for T21 (16). FP results for SCA may be associated with confined placental mosaicism, demise of a co-twin with monosomy or trisomy X, or maternal somatic X chromosome numerical abnormalities. Wang et al. (31) concluded that 8.6% of high risk results involving the X chromosome were due to maternal chromosome abnormalities, which as previously noted, may be explained by age-related somatic loss of an X chromosome (19).

Aneuploidies involving the Y chromosome are usually easier to screen for due to the absence of this chromosome in the maternal genome (29).

5.2. Microdelections and microduplications

Subchromosomal abnormalities are present in 1-17% of all pregnancies. Among women younger than 40 years of age, these conditions are much more frequent than the most common trisomies (32). NIPT testing for microdelections and microduplications began in 2013. Although subchromosomal anomalies are rarer than T13, T18 and T21, they represent 16.9% of all cytogenetic anomalies found in the general population. Di-George syndrome (22q11.2 deletion) is the most common (8), followed by 5p deletion (Cri du chat syndrome), 15q deletion (Prader-Willi/Angelman syndrome), 4p deletion (Wolf-Hirschhorn syndrome), 11q deletion (Jacobsen syndrome), 8q deletion (Langer-Giedion syndrome) and monosomy 1p36. All these mutations are already available for testing in some United States screening kits (19).

NIPT including screening for microdeletions can be performed in two different models. It can be applied whenever NIPT is performed or to high risk populations such as pregnancies presenting US anomalies suggesting a congenital syndrome. The latter group would not be representative, since few microdeletion syndromes (with the exception of Di-George syndrome) characteristically present with US anomalies (33). Apart from the detection of US anomalies, a high risk population for these syndromes is very hard to define, because there are no known risk factors. This is very distinct from what happens with common trisomies, where advanced maternal age, biochemical and US findings play an important role in risk definition (34).

PPV depends on NIPT detection rate and prevalence of the disease. The prevalence of DiGeorge syndrome, the most common of these syndromes, is between 1:4000 and 1:2000, resulting in a PPV of 3.2-6.2%. Rarer syndromes have lower PPVs (33). A study performed by Petersen et al. (20) in a population of 712 pregnant women with a positive screening test for any condition detectable by NIPT, found a PPV of 0-21% and a FP rate of 79-100% for the most common microdelection syndromes. Studies including only high risk populations defined by clinical findings (such as US anomalies) or higher prevalence of these conditions showed higher PPV (60-100%).

Based on this evidence, ACOG recommendations state that NIPT is not yet sufficiently understood to be applied in routine screening for microdeletion syndromes (10). Parents who wish to know if their fetus is affected by one of these syndromes must be informed that the best option is to undergo IDP with subsequent microarray analysis (4).

5.3. Fetal sex determination

The detection of the Y chromosome in maternal blood was the first method used to differentiate maternal cell-free DNA from cffDNA. This method has since been abandoned, as it could only be applied to male fetuses. However, recent studies have shown its applicability in fetal sex determination. Most studies used real-time polymerase chain reaction to analyze Y chromosome sequences present in maternal blood, such as SRY and DYS14. Overall sensitivity and specificity for detection of a male fetus were 95.4% and 98.6%, respectively (12). A recent Italian study including 132 pregnant women at week 12 of gestation used Y chromosome sequences to detect its presence in maternal blood. Fetal sex determination revealed 100% concordance with IDP results (35).

Fetal sex can be accurately assessed by US from the 13th week of gestation. NIPT can be performed as early as week 9, presenting an opportunity to act earlier in some conditions such as congenital adrenal hyperplasia in a female fetus. This would allow the administration of dexamethasone in an early gestational stage to prevent virilization (5,12). In addition, early identification of fetal sex can be useful to anticipate the need for special prenatal investigations, therapeutic measures and counseling in couples at risk of an X-linked condition (such as haemophilia, fragile X disorder and Duchenne's muscular dystrophy) (35).

A case-report by Iruretagoyena et al. illustrated some of these situations: in the first case, second trimester US showed a female fetus. Later in pregnancy, FGR ensued, but parents rejected any further testing. At birth, the newborn presented dysmorphic facial features, ambiguous genitalia with labial enlargement, bilateral inguinal hernias, hypertrophic clitoris, hypoglycemia and hypotension. Those findings motivated newborn karyotyping, found to be 46,XY. This child died despite hormone therapy administration, and unilateral adrenal agenesis and contralateral adrenal hypoplasia were detected by autopsy. A prenatal genotyping of this fetus could have allowed a prompter management of the situation. The second case reported a female genital ambiguity detected by second trimester US, after Y chromosome detection by NIPT at week 10. IDP allowed diagnosis of the autosomal recessive Smith-Lemli-Opitz syndrome and parents were tested for the same genetic anomaly. NIPT ultimately allowed timely pregnancy termination, and the added knowledge of a low risk of recurrence for those parents, since they were not carriers (36).

5.4. Fetal rhesus-D genotyping

Hemolytic disease of the fetus and newborn (HDFN) happens mainly in rhesus-D (RhD) negative pregnant women carrying RhD positive fetuses, caused by placental migration of maternal anti-RhD antigen immunoglobulin. Even though the most frequent moment for immunization is during delivery, it can happen in earlier pregnancy (with a peak in the third

trimester), leading to fetomaternal hemorrhage. Clinical consequences of this immunological response vary from fetal hyperbilirubinemia, anemia or, in the worst scenario, *erythroblastosis fetalis* with consequent fetal loss (37). Since the 1990s, isoimmunization has been avoided by antenatal and postnatal anti-RhD immunoglobulin prophylaxis in RhD negative women. Postnatal prophylaxis is only performed after a positive RhD cordocentesis, performed at birth (38). In Portugal (39), all RhD negative women receive anti-RhD immunoglobulin at week 28, and again at postpartum if the newborn is RhD positive.

The introduction of NIPT in clinical practice allows fetal RhD genotyping before birth and the anticipation of prophylactic anti-RhD immunoglobulin administration when needed. It is important to note that this specific application of NIPT is considered a diagnostic tool, not requiring further confirmatory tests, given its high accuracy in the general population (27). The most recent studies report a test sensitivity of 99.5–99.8% and a specificity of 94.0– 99.5% (12,37).

RhD genotyping at week 28 of gestation is well accepted, allowing great accuracy of results since cffDNA concentration in maternal blood increases throughout gestation. However, it can be performed since week 10, which provides the opportunity to administrate RhD immunoglobulin in cases of pregnancy termination, miscarriages and IDP (38).

Cost-benefit studies on NIPT use for RhD genotyping have had conflicting results. NIPT technology is still very expensive. However, facts such as the decreased need for postnatal cordocentesis and reduction of the administration of prophylactic immunoglobulin contribute to less unnecessary costs and allow earlier clinical discharge (38).

In the United Kingdom, the National Institute for Health and Care Excellence (NICE) recommends fetal RhD genotyping implementation (38). In Canada, Denmark, the Netherlands, Sweden, Finland, France, Germany and regionally in Belgium, this application has already been implemented as a routine antenatal screening program. In the United States, the associated costs, low risk of immunization and the fact that insurance providers do not consider the test sufficiently relevant are the main reasons for its reduced acceptance (37).

5.5. Single gene disorders

Screening for single gene disorders can be useful after an abnormal US suggests a condition known to be associated with a specific gene mutation (e.g. suspected skeletal abnormalities in achondroplasia with FGFR3 mutation) (13). At present, ACOG does not recommend the use of NIPT in this setting (10).

5.6. Rarer trisomies

Apart from trisomy 21, all other chromosomopathies present a bad prognosis, leading to fetal death in early stages of pregnancy or US anomalies unlikely to be missed. Besides, most of these syndromes are quite rare, making it difficult to gather significant clinical data on its inclusion in screening models and to establish reliable screening accuracy values. Hence, experts and societies do not recommend inclusion of rarer trisomies (other than T21, T18 and T13) in NIPT (33).

5.7. Pregnancy-related complications

The application of NIPT in screening for pregnancy-related complications is based on the fact that cffDNA concentration is altered in some of these situations. Preeclampsia, FGR and preterm labor are the most studied conditions, followed by placenta praevia and hyperemesis gravidarum. In the majority of these studies, cffDNA was shown to increase before the onset of symptoms. However, some studies have also shown no change in cffDNA levels with these pathological situations (40).

A systematic review comparing preeclampsia cases with control pregnancies revealed an increase in cffDNA concentration in 84.6% of 13 studies. Early-onset preeclampsia, defined as preeclampsia before 34 weeks, revealed a stronger association with higher concentrations of cffDNA compared to late-onset preeclampsia. Early-onset preeclampsia is thought to be related with maladaptation of uterine spiral arteries during the early stages of placentation, resulting in placental hypoxia, oxidative stress and increasing levels of placental apoptosis and necrosis. This fact may justify the increasing levels of cffDNA in maternal plasma, since necrosis may cause cffDNA release to maternal circulation (41).

6. Clinical consequences of NIPT in prenatal care

6.1. Reduction of invasive procedures

Due to its improved screening ability, one of the biggest advantages of NIPT implementation is a decrease in the number of IDP. A study published by the Eastern Virginia Medical School Department of Obstetrics and Gynecology in 2016 describes a reduction of 76% and 83%, respectively, of amniocentesis and CVS, following the introduction of NIPT in their clinical practice (20). A more recent study showed how implementation of a cffDNA test could reduce the need for invasive procedures after a positive FTCS (contingent model). CffDNA acceptance in this study was 77.5% and resulted in a 60.5% reduction in IDP. IDP efficiency increased from 15% to 50% in high risk populations. However, the authors postulate that, with

the decrease in the number of IDP, skill and training of health care professionals in its performance may be affected (5).

6.2. Incidental findings

A Canadian study reported a positive NIPT result caused by a partial chromosome 21 duplication. This anomaly was present in both maternal and fetal genome and did not include the critical region for Down syndrome. However, it included the amyloid beta precursor protein gene, resulting in a possible increased risk for early onset Alzheimer disease. Incidental findings such as this, may raise ethical issues, since its consequences are impossible to predict and treatment is not warranted. Consequently, this information should not have been reported to the obstetrician or the patient (42).

Maternal sex chromosome-related findings, such as 45,X/46,XX mosaic, or maternal 47, XXX karyotype are also a relatively common finding. Usually, these situations do not require any subsequent measure. On the other hand, maternal malignancies can also be suspected, especially when several chromosomal abnormalities are detected, and these women should be properly addressed (19).

6.3. Targeted RhD prophylaxis

After the introduction of NIPT in the Canadian health care system, most negative RhD pregnant women carrying RhD negative fetuses were spared unnecessary immunoglobulin administration, avoiding the unnecessary treatment of 40% of all RhD negative women. The Society of Obstetricians and Gynaecologists of Canada currently claims that it is ethically unacceptable to administer RhD immunoglobulin to all RhD negative pregnancies, given the accuracy and safety of RhD genotyping by NIPT, the minimum but existing risk of complications of RhD immunoglobulin and its shortage in supply. Given the FN rate of 0.2% and considering the number of births in Canada per year, the likelihood of prophylaxis-related complications expected is 1 per 5 million administrations of immunoglobulin. FN could be due to variants in the RhD gene not currently detected. New technology is needed in order to identify these variants and reduce FN. In the United States on the other hand, there are still concerns about the costs of NIPT and insurance providers do not cover its performance (37,38).

7. Prenatal counseling

Counseling and informed consent before and after any kind of screening or diagnostic procedure is crucial, and NIPT is no different. The understanding that cffDNA testing is only well established in screening for the most frequent aneuploidies must be clearly conveyed, as well as their lower screening value for most other rarer conditions. In case of a true positive result, parents should be referred to genetic counseling in order to determine their risk of recurrence (e.g. the determination of a parental translocation).

It is very important to educate parents about the meaning of a diagnosis of aneuploidy. Aneuploidies, especially T21, induce a kaleidoscope of possible clinical findings which can vary from mild to severe disability. Screening and diagnostic tools are only responsible for detecting an anomaly with lower or higher probability and are not able to predict the severity of the syndrome. When facing a positive test result, this knowledge is of the utmost importance for informed parental decisions regarding the pregnancy (4).

Conclusion

It is important to keep in mind that no single modality of screening is superior to the others, when considering the whole spectrum of prenatal screening tools. Each screening test presents specific advantages and disadvantages. The most important is that health care providers, particularly the ones in obstetric care, are capable of selecting the best candidates for each of them, in order to achieve the most reliable results. This selection must be based on the type of information desired by parents, gestational age, and the cost and accuracy of screening. The choice must also be adapted to parental needs, concerns and values. Regardless of which test is chosen, parental counseling about its limitations, accuracy and meaning of altered results must always be performed.

At present, NIPT is the most accurate method in screening for the most common aneuploidies. However, NIPT technology is still expensive, affecting its implementation in health care systems around the world. The emergent evolution of technology involving NIPT may, in the future, decrease cffDNA detection costs. PEGASUS-2 and TRIDENT-2 projects will likely bring new perspectives about NIPT cost-effectiveness and implementation in prenatal care.

NIPT application to subchromosomal abnormalities, fetal sex determination, multiple gestation prenatal screening, RhD genotyping, some pregnancy-related complications and other conditions still need to be tested further, in order to better advise parents and define efficient and ethically acceptable models of implementation.

Because of its safety and many different potentialities, NIPT is, at the present, certainly the most promising tool to the future of prenatal screening. For this reason, professionals must be

up to date regarding its possibilities and limitations, in order to provide parents the most appropriate and individualized care.

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