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Chapter 15

Clinical Implementation of Next-generation Sequencing in the Field of Prenatal Diagnostics

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Abstract

The possibility to receive genetic information of the fetus from maternal blood during the course of pregnancy has been one of the main goals of research in prenatal medicine for decades. First, the detection of cell-free fetal DNA in maternal blood and finally, the development of the powerful technique of "next-generation sequencing" (NGS) were required to finally transfer this analysis into clinical practice. Since its introduction in 2011, the clinical demand for the technique of non-invasive prenatal testing (NIPT) has been enormous. NIPT initially was available for the most common aneuploidies (trisomy 21, 13, and 18), but the varieties of diseases that can be detected prenatally by NIPT are increasing rapidly.

In this chapter, we aim to describe the current basic concepts of NIPT, give an overview of the currently available NIPT tests and associated technical aspects. We will present our studies on the clinical uptake of NIPT into clinical care in two different European centers and its impact on prenatal diagnosis.

Keywords: Non-invasive prenatal testing, prenatal diagnosis, prenatal ultrasound, cell-free fetal DNA, fetal aneuploidies

1. Introduction

The analysis of the fetal genome by an indirect approach from maternal blood during pregnancy has been the focus of research in prenatal medicine for decades. The only option to investigate the genetic condition of the fetus so far had been an invasive procedure such as chorionic villous sampling and amniocentesis, which carries a 1% risk of miscarriage.

The basis of the current concepts to this non-invasive approach was the detection of cell-free fetal DNA (cffDNA) in maternal blood in 1997 [1]. It finally was the development of the

technique of next-generation sequencing (NGS) that lead to the transfer of this research into clinical practice. After the clinical availability and introduction of cell-free DNA analysis for the most common fetal aneuploidies (Trisomy 21, 13, and 18) in 2011, there has been an extremely high demand by pregnant women and to date approximately 1.4 million analyses have been performed worldwide assuming that there will be around 1 million/year in 2015 [2]. Most current tests count DNA fragments, map them to the chromosomes, and quantitatively compare the cell-free-DNA in maternal blood with a euploid reference genome. This new screening tool in prenatal diagnostics has marked the beginning of a new era in prenatal care and has significantly reduced the rate of invasive prenatal procedures such as chorionic villous sampling and amniocentesis.

With the broad availability of non-invasive prenatal genetic testing, a number of new issues have emerged concerning its reasonable clinical application, ethical concerns, integration into current public healthcare plans, counseling issues, and the role of prenatal ultrasound screening. In the following, we will discuss the current and future concepts of prenatal cell-free fetal DNA testing and show the current impact on clinical care among different risk groups taking into account medical, social, and ethical aspects.

2. Fetal cells and cell-free DNA

The idea that genetic information of the fetus can be discovered by investigating maternal blood during pregnancy stems from the historic concept of Georg Schmorl, who described cross-placental trafficking of fetal cells into the maternal circulation. Fetal trophoblast cells were first demonstrated in lung tissue in mothers who died from eclampsia [3]. The isolation of fetal cells has remained a challenge due to their very low quantity [4,5], the limited knowledge on the characteristics and suboptimal markers for identification [6]. The focus has moved to the analysis of fetal cell-free DNA fragments which were first described in 1997 [1]. Cell-free DNA in maternal blood is comprised of extracellular DNA fragments that can be found in the maternal plasma and serum. The majority of cell-free DNA in maternal circulation is of maternal origin and around 10% is of fetal origin. Cell-free fetal DNA is released into the maternal circulation from cells of the placenta. It can be detected very early in pregnancy and is cleared a few hours after birth [7].

Initially, it was only feasible to analyze sequences of paternal origin and de novo mutations that were different from the maternal genome due to the high percentage of maternal cell-free DNA. Therefore, early studies focused on fetal Rhesus-status and on the detection of autosomal-dominant disorders of paternal inheritance [8]. Real-time quantitative PCR technology proved to be suitable for the detection of fetal loci that are different from the maternal genome such as the Y chromosome. Fetal gender determination was applied in families with a high risk for X-chromosome-linked disorders in which only male fetuses are affected from the disease and for the detection of fetal Rhesus D in pregnancies at risk for hemolytic disease of the newborn [9–11]. Just recently, non-invasive prenatal testing for routine fetal Rhesus D genotyping in Rhesus-negative women has been proven to be highly accurate over a 2-year

period after its implementation in Denmark and proved to have the ability to direct the use of Anti-D Rhesus prophylaxis in prenatal care [12].

With the technique of next-generation sequencing, it is now possible to also reliably quantify specific DNA sequences and therefore assess sequences that are not only present in the fetus but also present in the maternal genome. This is accomplished by comparing the measured quantity with a reference genome, hence offering the possibility for the widespread analysis for the detection of most common fetal aneuploidies [13].

3. Technical principles of the clinically available Non-Invasive Prenatal Tests (NIPT)

In the following passage, we will focus on the basic principles of the commercially available cell-free DNA test that offers analysis for the three most common aneuploidies today. Basically, there are three different types of approaches of prenatal cell-free DNA testing: whole genome sequencing, targeted genome sequencing, and single-nucleotide polymorphism (SNP)-based sequencing. Another fourth approach, epigenetic testing of fetal DNA methylation, which is not yet clinically available, has shown promising results. It detects fetal-specific epigenetic patterns and unique methylation profiles [14,15].

All techniques use massive parallel genomic sequencing (MPS) or NGS, which refers to the high-throughput DNA sequencing technology that can sequence millions of DNA molecules in parallel [13]. For prenatal testing, both cell-free DNA of maternal and fetal origin present in maternal peripheral blood are sequenced and these fragments are mapped to a reference chromosome. It is important to keep in mind that the majority of sequenced DNA is of maternal origin and that the difference between a normal fetus and fetus with an additional chromosome will only show a slight increase compared to a normal reference chromosome since the aneuploid part forms only about 10% of the sequenced DNA. Quantitative accuracy of the applied method, therefore, is crucial to exclude an aneuploidy. A minimum percentage of fetal DNA is required to reliably perform an analysis and is usually set at a minimum of 4%.

3.1. Whole genome sequencing

For this analysis, the entire cell-free DNA is sequenced in short reads and compared to a reference human genomic database and each sequence is matched to a specific chromosome. The counts observed in the individual probe are then compared to an euploid reference sample. If the fetus carries an additional chromosome (as in trisomy 21, 13, and 18), more fragments are expected for the additional chromosome compared with a normal fetus. However, it is necessary to sequence many millions of DNA fragments (12–15 × 10^6 mapped sequences) to ensure that there are sufficient chromosome fragments (reads) from the specific chromosome to detect statistically significant differences between aneuploid and euploid fetuses. Also, there are several other aspects of sequencing and the fetal fraction as well as the guanine–cytosine content, etc. that need to be taken into account.

3.2. Targeted sequencing

Targeted sequencing sequences only the regions / chromosome of interest and thus can be more time- and cost-efficient compared to whole genome sequencing. The principle is to selectively amplify the regions from chromosome 21, 13, and 18 followed by NGS. This method is also referred to as digital analysis of selected regions (DANSR). The amount of sequencing for a reliable detection is significantly lower around 40,000 and 1 million mapped sequences / sample. Unique to this type, the analysis uses a fetal fraction optimized risk score (FORTE) and takes into account the a priori risk (maternal age and gestational age) and uses an odds ratio approach to calculate the risk for aneuploidy.

3.3. SNP-based sequencing

This third approach was the most recent method introduced to the variety of clinically available NIPT options. This technique involves targeted amplification and sequencing of single-nucleotide polymorphisms (SNPs). SNPs are single base pairs that occur approximately once / 300 base pairs on the human genome and can be used to distinguish individuals. In addition to the above mentioned applications, maternal and fetal DNA also can be distinguished by SNP analysis. For this analysis, both maternal DNA from white cells from the buffy coat and maternal plasma which includes fetal and maternal DNA are used. In the SNP-technology originally introduced by Zimmermann et al. [16], 19,488 SNPs on the chromosomes 21, 13, 18, X, and Y are analyzed simultaneously. Taking into account the parental genotype, the fetal fraction, and the fetal chromosome copy number, billions of possible genotypes at a specific locus are considered by a complex algorithm and the observed allele distributions are compared to the expected allele distributions. By this method, the most likely fetal genotype can be calculated and a specific risk score for the analyzed aneuploidies is reported [16–19].

4. Evidence on the quality of NIPT from published literature

The initial studies on test quality for the most common aneuploidies were performed in high-risk collectives and focused on the sensitivities and specificities of the different cell-free DNA tests [20–26]. After the rapid clinical application of NIPT including many women at low risk, there was a demand for information on the positive predictive value of each individual test. The positive predictive value then was found to vary widely depending on the investigated cohort and could be as low as 45.4% for trisomy 21 [27], meaning that when a NIPT-test was positive only 45.4% of the fetuses were affected. This underlines the fact that although cell-free DNA testing performs better than the previous screening algorithms for aneuploidy, a positive test result requires confirmation with an invasive procedure such as amniocentesis or chorionic villous sampling.

4.1. Trisomy 21, 13, and 18

The data for the three most common aneuploidies now stem from a number of large-scale studies from mainly high-risk collectives. The detection rate for trisomy 21 ranged from 97.5%

to 100%, with most of the studies showing sensitivities above 99%. For trisomy 18, the outcome is similar ranging from 92.8% to 100%. The sensitivities for trisomy 13 are slightly lower ranging from 78.6% to 100% [18–32]. All of the reported screening methods have significantly lower false positive rates below 1% compared to conventional first trimester screening, which typically is set at a 5% false positive rate.

4.2. Sex chromosome aneuploidies

While reporting of fetal gender is feasible with cell-free DNA testing with high sensitivities of more than 95%, the reporting of sex chromosomal aneuploidies is more challenging. The most common sex chromosomal aneuploidies are 45X0 (Turner syndrome), XXX (Triple X syndrome), XXY (Klinefelter syndrome), and XYY (Jacob syndrome).

While Turner syndrome can be detected on prenatal ultrasound, the others typically do not show sonographic signs but have been detected incidentally if an invasive procedure was performed for another reason. Compared to the most common aneuploidies, the detection rates of sex chromosomal aneuploidies have lower specificities leading to higher false positive rates [23,33]. This is most likely due to the guanine–cytosine content of the X chromosome, which affects the reliability and accuracy of the sequencing data, the small size of the Y chromosome, and the sequence similarity between the X and the Y chromosome. Furthermore, an unknown maternal or fetal mosaicism can interfere with the quantifications of the chromosomal representations. The reported numbers on detected sex chromosome aneuploidies other than Turner syndrome are very low with less than seven cases of each aneuploidy per study [23,34–36] so that reliable data are not present to date. The data on Turner syndrome need to be interpreted with caution since there may be a bias toward the non-viable cases and those detected with sonography. Furthermore, the follow-up data on test negative cases might be incomplete due to the fact that children with Turner syndrome might not show a noticeable phenotype at birth. Also, the rate of tests that do not receive a result due to difficulties with the interpretation of the sequencing data (non-reportables) seems to be higher compared to the autosomal aneuploidies. Taking into account some of these limitations, the detection rate for Turner syndrome ranges between 75% and 92% at a false positive rate of up to 0.3% [23,34–36].

4.3. Triploidy

The presence of a third additional copy of each chromosome is called triploidy. The third copy stems from either the mother (digynic triploidy) or the father (diandric triploidy) and is a challenge for NIPT. Since whole genome sequencing and targeted sequencing rely on the proportions of chromosomes in relation to each other, it is impossible to detect this condition. Only very few cases have been investigated in SNP-based arrays [37] and have shown that the detection of diandric triploidy is feasible but digynic triploidy is difficult, most likely due to the severe growth restriction and a very small placenta which is the typical phenotype associated with this condition that will lead to non-reporting of NIPT due to the low fetal fraction.

4.4. Mosaicism

In mosaic autosomal trisomies, the detection with NIPT is less effective compared to complete fetal trisomies. The major reason is that the representation of the fetal chromosome is only partial. The detection of a fetal mosaicism is dependent on the fetal fraction and on the percentage of abnormal cells in the mosaic. There have been two relevant studies investigating the ability of detecting mosaicisms showing far less sensitive results for mosaic aneuploidies with NGS. Since cell-free "fetal" DNA stems from the trophoblast, a confined placental mosaicism can be a reason for a false positive result. Also, maternal mosaicism can lead to false positive results. On the other hand, mosaicisms can be missed since it is more difficult to detect due to the lower percentage of abnormal cells [38]. However, mosaicism is found in approximately 0.25% of pregnancies in women undergoing amniocentesis and conventional karyotyping [39]. Finally, if NIPT is positive for a trisomy, the distinction of mosaic versus complete trisomy can only be made after karyotyping. This shows the importance of confirmation of the findings detected by NIPT through an invasive procedure as recommended by the professional societies.

4.5. Twins

Most of the approaches using whole genome NGS and targeted NGS offer an analysis for twin pregnancies. The analysis, however, is more complex since maternal blood then carries the cell-free DNA from three individuals. For monozygotic twins that usually carry the same genetic information, the analysis can be made analogue to singletons. In dizygotic twins it is likely that only one fetus is affected from an aneuploidy. NGS relies on a small increase of reads identified for the trisomic chromosome. The total cell-free fetal DNA fraction is larger compared to singleton pregnancies most likely due to a larger placental volume [40] and this would be an advantage for NGS compared to singletons. However, this advantage is reduced by the fact that in most cases only half of the fetal DNA fraction stems from the aneuploid fetus. Furthermore, it is possible that the cell-free-DNA, which is found in the maternal circulation, is not equally released half by half from each of the two fetuses. So the aneuploid fetal fraction could be lower compared to the euploid fetus [41]. To circumvent the mistakes of the total fetal fraction, the lower fetal fraction is used for the risk assessment. A consequence of this policy is that the rate of non-reporting will be higher for twin pregnancies.

The published data from twin pregnancies now count almost one thousand analyzed twin pregnancies [40,42–47]. The SNP-targeted approach does not yet offer twin analysis. The most recent analysis on 515 twin pregnancies showed a test failure rate of 5.6% compared to 1.7% in singletons. The median lower individual fetal fraction was lower than in singletons (8.7% versus 11.7%). Among the 351 pregnancies with complete follow-up and with a test result, there were no false positives among 334 euploid fetuses. All 5 cases of trisomy 18 were detected, but there was 1 false negative case of trisomy 21 among the 12 pregnancies discordant for trisomy 21 [43].

The analysis for twins, however, will not reach a diagnostic level with NGS from maternal blood since it will never be able to tell which one of the fetuses is affected until this information is acquired via separate analysis of each twin through an invasive procedure.

4.6. Factors explaining false positive and false negative results

Even though NIPT is the best available screening test for the detection of the three most common aneuploidies trisomy 21,13, and 18, the method of analyzing cell-free DNA in maternal blood by NGS, false negative, as well as false positive results are possible. To understand the technology, one has to keep in mind two essential things: first, cell-free "fetal" DNA stems from the trophoblast rather than from the fetus itself [7], and second, the cell-free DNA analysis of maternal and fetal cell-free DNA in NIPT uses maternal blood as the DNA source for the analysis. As known from chorionic villous sampling for many years, there is the phenomenon of feto-placental mosaicism in which only the cytotrophoblast but not the fetus is affected by the aneuploid cell line or vice versa [48]. If only the cytotrophoblast is affected, this would lead to a false positive result while a false negative NIPT result is expected if only the fetus but not the trophoblast is affected from the aneuploid cell line.

Another potential cause for a false positive result could stem from cell-free DNA from an unrecognized vanishing twin [42,49]. Fetal aneuploidy is a common reason for early fetal loss and has been described as a reason for a false positive NIPT result [42]. In fact, an additional fetal haplotype was identified in 0.42% of over 30,000 routine NIPT samples from a SNP-based assay [49].

If an abnormal karyotype is present in the mother, this might lead to a false positive result. False positive findings have been reported associated with maternal malignancies [50] or with maternal X-chromosome abnormalities in otherwise healthy women [51]. As mentioned before, the depth of sequencing and a low fetal fraction can be the causes of false negative results due to the counting technology.

5. Integration of NIPT into current prenatal care

Although NIPT has just reached clinical application, the broad use of NIPT in high-risk and low-risk pregnancies is remarkable. Most professional societies have given recommendations to limit the application to women at higher risk [52–54], but the number of studies emerging from low risk and general populations are increasing and models for integration into health care plans are emerging.

A growing number of trials have now shown that NIPT can also be used in women at low risk for aneuploidy [19,27,31,33,55,56]. Although the positive predictive value is assumed to be lower in low-risk patients, test performance is still superior to conventional first trimester screening [27]. With a broad acceptance among specialist societies that a positive NIPT result requires confirmation by invasive testing, there seems to be no reason to withhold NIPT from low-risk women.

Basically, there are two discussed options: one is to use NIPT as a primary screening test that is offered to every pregnant woman and the second is to use NIPT as a secondary (contingent) screening test used only in certain risk groups. This could be either women of increased maternal age or women that screen positive in conventional screening. All discussed options

refer to NIPT for trisomy 21,13, and 18 in singleton pregnancies as in traditional first trimester screening. All the other available NIPT options are not considered in a form of general clinical screening at this point.

A primary screening would lead to the highest detection rates of aneuploidies by lowering the false positive rates and also the need for invasive procedures [32]. However, the benefit of the first trimester ultrasound screening apart from aneuploidy detection needs to be remembered carefully since correct pregnancy dating by measuring crown-rump length is crucial for lowering perinatal mortality. Furthermore, the determination of twin chorionicity and an evaluation of maternal adnexae are part of the routine workup in the first trimester. Also, the majority of major fetal malformations that are not necessarily associated with genetic changes can be assessed by ultrasound. Further, primary screening also would be an expensive option by neglecting other benefits of first trimester ultrasound.

Considering contingent screening makes more sense from a healthcare point of view.

Since first trimester screening is widely used in many countries, it would make sense to offer NIPT to a selected population which is screen positive after first trimester screening. Such an approach was modeled with a test positive cut-off of 1:2,500 by first trimester screening and showed an increase of the detection rate of Down Syndrome with a decrease of invasive testing [57] at considerably lower costs compared to first-line screening.

In cases of a positive result, there is consensus among the specialist societies such as the American College of Obstetricians and Gynecologists (ACOG), the Society of Maternal-Fetal Medicine (SMFM), the International Society of Prenatal Diagnosis and the National Society of Genetic counselors that they need to be confirmed with an invasive procedure and fetal karyotyping. This seems especially important when a termination of pregnancy is considered following a positive NIPT result. As discussed previously, this is mandatory due to the occasional false-positive results, especially in low-risk patients.

Switzerland is the first country in Europe to have introduced a national policy on obligatory health care coverage for NIPT for women with singleton pregnancies that have a risk of > 1:1,000 for trisomy 21, 13, or 18 after conventional first trimester screening.

6. Influence of NIPT on diagnostic procedures and changes in prenatal care

With the introduction of clinical available NIPT for the most common aneuploidies, a risk-free additional option of prenatal testing has become available. So far, most pregnant women in the western world had access to a detailed sonographic examination of the fetal anatomy (Figures 1 and 2), correct pregnancy dating based on Crown rump length at 11–14 weeks, and were offered the "combined first trimester test", which is a risk assessment for the trisomy 21, 13, and 18. The first trimester screening combines the statistical background risk of the mother incorporating her age, fetal anatomical markers, nuchal translucency measurements, and biochemical markers in maternal blood (pregnancy associated plasma–protein–A (PAPP-A) and free beta human chorionic gonadotropin (HCG). With this, aneuploidy screening for

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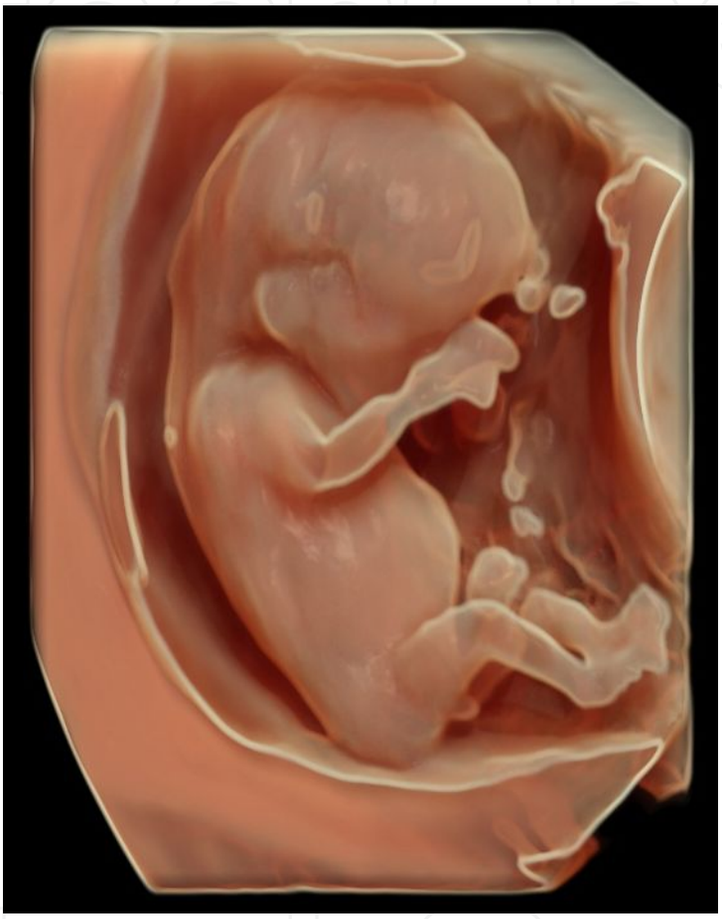
trisomy 21 can be achieved with a sensitivity of 90% at a false positive rate of 5% [58]. Women at increased risk would usually undergo an invasive procedure such as amniocentesis or chorionic villous sampling for karyotyping. Although this type of screening was better than any previous serum marker tests or using the maternal age-risk alone, it still lead to a large number of invasive tests and only few positive results. Putting mothers through an invasive procedure exposes them to a risk of fetal loss of 0.5–1% [59,60].



(Archive Dr. G. Manegold-Brauer, University of Basel, Department of Prenatal Medicine and Gynecologic Ultrasound)

Figure 1. 4D-ultrasound image of a fetus in the first trimester

With NIPT a new technology was introduced, which has lead to changes in algorithms previously used to guide patients. Since NIPT only requires a fetal blood sample, patients report that the greatest benefit is the decreased rate of miscarriage as compared to amniocentesis or chorionic villous sampling [61,62].

The medical profession rapidly had to face and solve many challenges on offering and counseling patients about NIPT. It is especially challenging to distinguish scientific information on the different NIPT tests from commercial announcements due to the many different laboratories that offer these tests and the flood of published studies that emerged in only a few years. Adequate counseling has become very complex and should incorporate all the options,

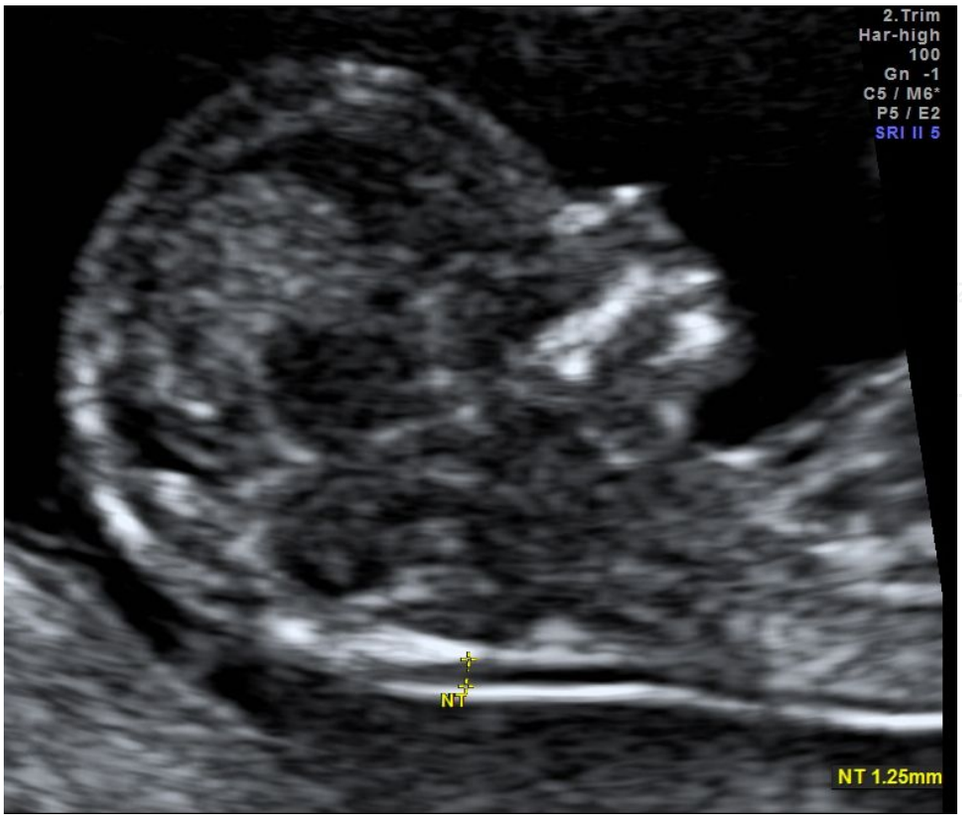


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Figure 2. 2D-ultrasound image of a fetal profile at 11–14 gestational weeks

limitations, and risks for each type of prenatal testing (ultrasound screening, biochemical screening, invasive procedures, NIPT, conventional karyotyping, and microarray analysis) in a non-directive manner and in the end should allow pregnant women to make an informed decision. For NIPT, it seems important to also counsel on non-reporting due to low fetal fraction in correlation to maternal weight and gestational age and fetal karyotype [63]. Further patients need to be informed on the need for an invasive procedure for confirmation in cases of positive findings.

However, in clinical practice the changes in prenatal care were incorporated differently in different health care systems and were highly dependent on the cohort that was investigated. The high costs associated with NIPT might also have played a role in the uptake in different societies. The introduction of NIPT has lead to an increased rate of prenatal testing in general. Many women that might have relied on first trimester screening in the past would now choose NIPT even if the results of first trimester screening were normal (Table 1). Not surprisingly, the increase of additional testing in the intermediate-risk group was most significant [64,65]. While the total number of invasive testing decreased by 70% in some studies [65], the reduction of invasive procedures was not significant in high-risk cohorts, especially when there is a high percentage of patients that present with anomalies seen on prenatal ultrasound. This management, however, is comprehensible since there is a high risk of chromosomal anomalies other than trisomy 21, 13, and 18 when ultrasound anomalies are present (about one third) that

would not necessarily be picked up by NIPT but which can be detected by conventional karyotyping or microarray analysis.

Risk category after first trimester screening		*n*	No further tests (%)	IPT (%)	NIPT (%)	IPT special indication / termination (%)
Low risk	Group 1	431	95.36	2.09	0	2.55
	Group 2	391	92.58	1.02	5.88	0.51
	p		0.997	0.372	<0.001*	
Intermediate risk	Group 1	37	64.86	35.14	0	0
	Group 2	35	54.29	5.71	40.00	0
	p		0.835	0.018*	<0.001*	
High risk	Group 1	37	40.54	56.75	0	2.71
	Group 2	20	40.00	40.00	15.00	5.00
	p		0.333	0.054	0.103	

Table 1. Differences in prenatal testing according to risk category before and after the introduction of NIPT. Group 1: before the introduction of NIPT, group 2: after the introduction of NIPT (adapted from [63]) IPT: invasive prenatal testing; *p*: *p*-value comparison before and after the introduction of NIPT, significant differences are marked with *

	Structural abnormality (n = 69)	NT >95th percentile (n = 38)	Multiple softmarker (n = 43)	Normal scan (n = 32)
IPT	48 (69.6)	21 (55.3)	12 (27.9)	16 (50.0)
NIPT	0 (0.0)	1 (2.6)	3 (7.0)	8 (25.0)
No further tests	21 (30.4)	16 (42.1)	28 (65.1)	8 (25.0)

IPT: invasive prenatal testing; NIPT: non-invasive prenatal testing.

Data shows number (%).

Table 2. Management choices among high-risk patients after the introduction of NIPT. This table shows the presence or absence of sonographic findings (normal scan) in the high-risk group (*n* = 182) and management choices in the individual subgroups (adapted from [62]).

7. Ethical and social aspects

The introduction of NIPT by the technique of NGS used in prenatal diagnosis has raised some ethical and social concerns. NIPT can theoretically provide information on the entire genome of the mother and the fetus with relative ease. In fact, NIPT has already revealed a small number of occult malignancies [66]. The sequenced DNA, however, could also reveal a BRCA mutation

or mutations on genes encoding for neurodegenerative diseases such as Chorea Huntington that would have major consequences for the mother and the unborn child [67]. It becomes obvious that the professional societies and national guidelines need to carefully regulate which data will be analyzed, stored, and reported. Clearly, the mother needs to give written informed consent to each specific analysis that is performed and needs to approve any individuals or institutions that receive this type of information. Although most of today's available NIPT tests directly report to the physician who indicated the test there remains a concern that NIPT could be offered directly to the pregnant woman without a medical request or indication. It seems of highest importance that the expectant mother is appropriately counseled by a trained health care professional who can offer and discuss all implications for testing, provide for and interpret all options, discuss prognosis and can assist with the management of the pregnancy and the subsequent prenatal care [68,69]. An important further aspect is that adequate educational material is offered to health care professionals and to the public, as it will assist in avoiding misunderstandings about the technology and possible misuse, thereby ease public anxieties [70].

8. Conclusion

With the technology of NGS, prenatal care has reached a new era. It has changed prenatal algorithms and has led to a reduction of invasive procedures which was one of the main goals of this technology [65,71]. At present, the main domain of NIPT is the detection of the three most common aneuploidies trisomy 21, 13, and 18, in singletons. However, further aneuploidies like sex chromosomal aneuploidies and some microdeletions are offered today in a clinical setting and research is aiming on sequencing the whole genome by a non-invasive approach with the ultimate dream of thereby opening an early "window of opportunity" for fetal therapy.

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