Restrictions and Advantages of Non-Invasive Prenatal Testing (NIPT)

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There are two major principle modalities to perform prenatal genetic diagnostics: by invasive or non-invasive means. Invasive prenatal genetic diagnostics depends on fetal or placental tissues, which can only be acquired by approaching the unborn with a needle to gain this material for further in vitro studies. Conversely, non-invasive tests do not disturb the fetal or placental tissues at all. The newest non-invasive prenatal diagnostic test approach is the so-called non-invasive prenatal testing (NIPT). Here an overview on real advantages and still critical issues of this approach are put together.

Keywords: Non-Invasive Prenatal Testing (NIPT) ; teratogen effects ; multigenetic diseases ; pregnant woman perspective ; first trimester-screening (FTS)

1. Introduction

During the last five decades, a dream of humankind has come true; what was previously purely science fiction is now reality: owing to the technical progress over this period it became routine and commonplace that pregnant women could obtain sound “prenatal information”, regarding the health of their unborn child [1]. At first, there is the opportunity to visualize the unborn child by ultrasound imaging [2]. While progress in sonographic approaches were already striking [2], the fast developments of (cyto-) genetics and genomics opened new and previously unexpected ways to even obtain information, concerning the genetic status of a fetus [1]. There are two major principle modalities to perform prenatal genetic diagnostics: either by (i) invasive or by (ii) non-invasive means [3][4].

Invasive prenatal genetic diagnostics depends on fetal or placental tissues, which can only be acquired by approaching the unborn with a needle to gain this material for further in vitro studies. Conversely, non-invasive tests do not disturb the fetal or placental tissues at all; they depend on sonography, on proteins or free placental DNA accessible from the maternal blood. While all, so-called “non-invasive prenatal diagnostic approaches,” are in reality screening tests, it is only invasive tests, based on fetal tissue that can provide clear answers on whether the unborn has a specific genetic condition or not [1][2][3][4].

All invasive prenatal tests involve a certain risk for the fetus. In the 1980s to 1990s, chorionic villi sampling (CVS) from placenta, amniocentesis (AC) or umbilical cord blood (UCB) acquisition had an abortion risk between >1% and 3% [5], which dropped to 0.5% to 1% by the 2010s [6]. Since then, the application of needles with smaller diameters has reduced these rates down to between 0.1% and 0.3% [3][5][7].

The aforementioned initially relatively high-risk figures of invasive approaches [5][6] stimulated substantial medical research, which developed many tools for non-invasive prenatal diagnostics (for the currently available invasive and non-invasive prenatal screening tests by week of gestation (w.o.g.) see **Table 1** [1][5][6][9][10]).

Table 1. Overview on the presently available invasive and non-invasive prenatal diagnostic tests.

Type of Test	Invasive	
	Can be carried out from w.o.g.:	Is the fetus studied?
(cyto)genetics from CVS	~11	No
(cyto)genetics from AC	~14	Yes
(cyto)genetics from UCB	~20	Yes

Type of Test	Invasive Can be carried out from w.o.g.:	Is the fetus studied?
Type of test	**Non-invasive** **Can be carried out from w.o.g.:**	**Is the fetus studied?**
Early sonography	**~6**	**(Yes) [1]**
Late fine sonography	**~19**	**(Yes) [1]**
First trimester-screening (FTS)	**11–14**	**(No) [2]**
Tests for protein markers (e.g., alpha fetoprotein, etc.) from maternal blood serum	**~11**	**No**
Non-invasive prenatal testing (NIPT) from free placenta-derived DNA in maternal blood serum	**~10**	**No**
Molecular genetic/molecular cytogenetic tests on fetal nucleated erythrocytes from maternal blood	**before 10**	**Yes**

[1] In sonographic studies, the fetus is observed visually, but no genetic material is studied—thus here 'Yes' is given in brackets. [2] FTS includes sonography—thus here 'No' is given in brackets.

The newest non-invasive prenatal diagnostic test approach is the so-called non-invasive prenatal testing (NIPT), also referred to as non-invasive prenatal screening (NIPS) or non-invasive prenatal diagnostics (NIPD). However, the wording NIPD should not be used, as NIPT is clearly a screening test [1][2][3]. As highlighted in **Table 1**, the only non-invasive approach assessing fetal tissues is the study of fetal nucleated erythrocytes from the maternal blood [10]. The latter is a promising idea, still hampered at present, mainly by the fact that reliable and efficient isolation tools for fetal nucleated erythrocytes are not yet available [11]. However, single reports prove that molecular [12] and even molecular cytogenetic analyses can be completed, based on these fetal blood cells [13].

2. Technical Bases of NIPT

NIPT is based on free placental DNA, intentionally and misleadingly referred to as 'cell free fetal DNA = cffDNA' in NIPT-related literature, from the very beginning [14]. This cffDNA can be reliably detected in the blood of a pregnant woman at approximately 10 w.o.g.; the cffDNA concentration rises during pregnancy and disappears completely from the maternal blood serum only hours after birth [9]. The latter is in contrast to fetal lymphocytes, which can circulate for decades in the maternal body, while nucleated erythrocytes normally die within the range of weeks [10].

Technically, NIPT is predominantly based on whole genomic sequencing (WGS), either shotgun massively parallel sequencing (s-MPS), targeting massively parallel sequencing (t-MPS) or single nucleotide polymorphism (SNP) based WGS. In MPS based approaches, the maternal and placental DNA cannot be distinguished; aneuploidies are recognized as changes in copy numbers for the studied chromosomal regions. Only the SNP-based approaches allow the separation of cffDNA from the maternal, serum derived DNA, and also allow for the detection of triploidy [15]. In addition, the combination of WGS with the real-time polymerase chain reaction [16] or target enrichment [17], and NIPT based on microarray-technology or rolling circle amplification [18] are reported in the literature, but are not widely used as of yet.

A major problem of the publications reporting on NIPT, is that these technical differences between platforms are not discussed extensively [15][19]. NIPT results are reported for in-house developed or the application of commercially available platforms, and the comparison of the results is achieved interchangeably, as if differences in platforms and their technical restrictions do not matter.

Apart from these problems, NIPT-studies can include testing for

(a)trisomy 13, 18 and 21, only;

(b)aforementioned trisomies and for changes in the copy numbers of sex chromosomes;

(c)all mentioned in b) plus only DiGeorge syndrome [20];

(d)all mentioned in b) plus other selected microdeletion or microduplication syndromes [21];

(e)all mentioned in (b), (c) or (d) plus all other copy number changes of all other autosomes;

(f) whole genome, for any kind of copy number alteration.

(g)Finally, some NIPT providers have started to also offer screening for point mutations of specific genes, such as the Rhesus factor and blood groups.

Overall, there are dozens of variants of NIPT available, but they are not really distinguished in the literature [19][22]. This combination issue, if applied to all studies using molecular cytogenetics/fluorescence in situ hybridization (FISH) in prenatal samples [23], would be as if they were all reported as similar results e.g., “invasive prenatal FISH testing” (IPFT), a misnomer, which would fail to distinguish whether the results were based on interphase or metaphase FISH, and/or which and how many different probes were applied. This exaggerated comparison is merely intended to illustrate what is being lumped together in the literature within the NIPT field.

3. NIPT and Its Advantages

The idea to perform NIPT, based on WGS is indisputably brilliant. The concept to study cells from the placenta without bothering or endangering the developing individual is laudable. Screening the literature for bolstering-points for NIPT include conclusions that the platform:

I. can be applied earlier than other tests during pregnancy;

II. has the potential to reach populations with no access to centers offering invasive diagnostics [24];

III. can exclude and detect a trisomy 21 with the highest probability of all non-invasive approaches—the positive predictive value (PPV) in cases of a NIPT suggesting trisomy 21 is >99%;

IV. can bring a psychological relief to an anxious pregnant woman in the case of a normal NIPT result; and

V. can lead to an early pregnancy termination in cases of an early detection of an adverse genetic condition via NIPT [9][15][19].

Interestingly, these are the only five points to identify in the literature as advantages of NIPT. Moreover, as outlined in the next chapter, the interpretations of NIPT results are either not really understood in detail and/or at the least not well communicated [15][19]. Further compounding the issue, the advantages, and restrictions of NIPT may be unknown to both the MDs ordering the test, and the pregnant women taking the test [25].

4. NIPT and Its Practical Restrictions and Shortcuts

Some of the problems of NIPT have already been reviewed and were recently summarized by [15][19]; thus, herein, only the main keywords are repeated and summarized in one to two sentences. The supporting evidence can be found in references [15][19].

(a)The term NIPT suggests to the uninformed public that the platform studies cell free fetal DNA (cffDNA); however, all cell free DNA in the pregnant individual’s blood (not being of maternal origin) derives from the placenta (and not the fetus); thus, in 1–2% of the cases studied by NIPT, a placenta confined mosaic condition must be expected. They constitute a part of the false positive and negative NIPT results [15][19].

(b)Each abnormal NIPT result has to be checked by an invasive prenatal test, optimally an AC, as indicated by each commercial NIPT provider in the package leaflet [9]. However, one major motivation to do NIPT is to avoid any invasive procedure, due to the previously suggested high abortion risk [3][5][6][7].

(c)A normal NIPT result can maximally exclude (to a certain extent) genetic conditions, which are covered by the NIPT platform used. A normal NIPT is never synonymous with the statement: ‘a healthy child will be born’ [26]. As recently shown, NIPT can at best detect 5–10% of all cases potentially born with birth defects [19] (**Figure 1**).

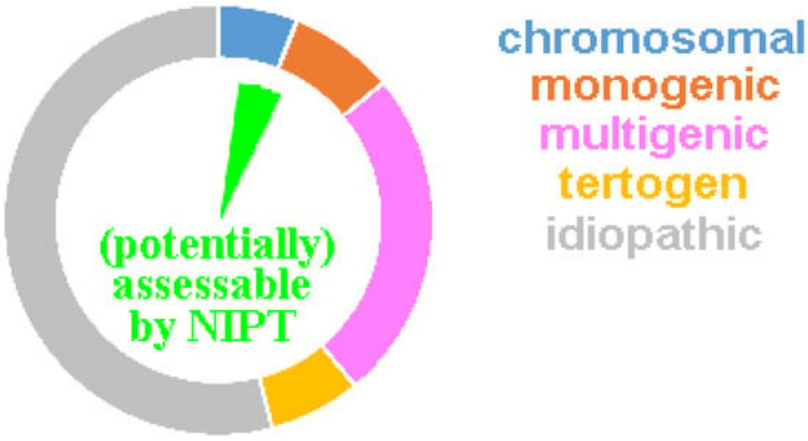

Figure 1. The ring diagram includes the 3–6% of newborns with major inborn abnormalities. A chromosomal disorder is present in ~6%, teratogenic damage in ~7%, and a monogenetic or multigenetic disease in ~8% or ~25%, respectively. For the remaining ~54%, the diagnosis usually remains a lifelong suffering attributed to an "idiopathic disorder", i.e., the cause remains unclear. The green inner pie diagram shows the ~5 to max 10% of cases potentially assessable by NIPT.

(d)90% of aneuploid fetuses detected in NIPT would be aborted naturally and do not survive to birth [19]. Thus, 90% of pregnant women with a true abnormal NIPT result would not have to undergo an induced abortion.

(e)5–10% of NIPTs provide no result at the first attempt—this is most often due to the low fraction of cell-free placenta derived DNA. The greater the obesity severity in pregnant women, the more likely a 'no-call' result will occur [15][19]. The number of cases repeated is not extensively reported in the literature, which could yield data on how many of these cases ultimately yield an informative result. However, it should be noted that within this population, there is overrepresentation of cases ending in an early abortion, largely attributed to a placenta that is too small for the gestational age and preeclampsia.

(f) False positive NIPT results can be due to confined placental mosaicism, a vanishing twin, a maternal sex chromosome mosaic, or a maternal undetected tumor [9][15][19].

(g)There can be unexpected findings, even for NIPTs evaluating only trisomies 13, 18 and 21. Partial trisomies or even tetrasomies, e.g., supernumerary isochromsome 18p syndrome can be picked up: several publications report this surprise detection in the literature (e.g., [27][28][29]).

(h)As aforementioned, the PPV for trisomy 21 is >99% in all available NIPTs; however, it is neither known nor studied why screening for trisomies 13 and 18, is less reliable. PPVs for all other tested copy number changes (including sex chromosomes, other autosomes, and microdeletion/-duplications) remain substantially lower between 5 and 60—which means 40 to 95% of women receiving an abnormal NIPT result have indeed received a false positive result [19].

(i) In a worldwide perspective, there are hints in the literature that in ~20% of cases, there is a strong tendency to trust an abnormal NIPT result so much that a second test is skipped and termination of the pregnancy is simply based on a single screening test [19][30]. Such policies are also reinforced by the cost-effectiveness of NIPT analyses, which inappropriately encourage NIPT use as a diagnostic versus a screening tool [31].

As recently summarized, all of these nine points must be considered and should be covered in pre-test counselling for everyone considering a NIPT. Therefore medical specialists working with and offering NIPT need to be aware of these peculiarities [15][19][32]. It is surprising that most NIPT related publications do not discuss any of these points and instead provide an overall astonishingly positive view on NIPT. Examples similar to the two following excerpts can be easily found in dozens of NIPT papers:

> *(1) "The high specificity, efficiency and safety (non-invasiveness) of NIPT can effectively improve the detection rate of common chromosomal aneuploidy, thereby reducing the occurrence of birth defects"* [33]*; or (2) "NIPT has revolutionized the approach to prenatal diagnosis and, to date, it is the most superior screening method for the common autosomal aneuploidies".* [34]

5. Conclusions

While the theoretical possibilities of NIPT are promising problems and limitations must also be considered. Overall, NIPT results are not well understood and the reliability of the obtained data is, at the least, also not communicated well to the MDs ordering the test nor to the pregnant women taking the test. As stated by Hessel and Henn, recently: "*Fact is, that the NIPT is perceived by many pregnant as a standard measure for pregnant women and is carried out unquestioningly*" (translated from [35]). This attitude may lead to a rude awakening [26]. Thus, appropriate training and continuing education of the physicians providing NIPT counseling [14] are urgently necessary. As outlined elsewhere [15] this can only be achieved by unbiased offers of ongoing education—not from NIPT providers, but from medical associations or independent researchers.

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