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First trimester screening with biochemical markers and ultrasound in relation to NIPT

Introduction

The introduction of NIPT into clinical medicine from 2012 was greeted with great acclaim by physicians and patients. There was talk of breakthrough, NIPT replacing amniocentesis, diagnostic testing procedure and much more. However, clinical research over the past eight years has succeeded in painting a more realistic picture of what the NIPT method can actually do, where its opportunities lie, but also its methodological limitations. This also makes it possible to draw a comparison of the value of NIPT with the standard method "combined NT test", which has been established at this point, that better reflects the real conditions in everyday life.

NIPT is an innovative, sophisticated genetic determination method: it thus stands at the interface between laboratory medicine, genetics, pediatrics and prenatal medicine. The experience of the last eight years in dealing with NIPT has shown that not only the view of unborn life itself, but also the technical language and medical concepts of these disciplines vary slightly from each other at this point. Therefore, in order to understand each other well beyond our medical specialties, it is essential that we use unambiguous terminology across disciplines. We should also have an unambiguous, clear, common terminology about which epidemiological key data characterize the field of prenatal medicine.

Epidemiology of congenital malformations

About 130 million children are born worldwide each year (Fig. 1) ([i]). The UN puts the proportion of children with birth defects at just under 8 million per year ([ii]). This corresponds to 6% of all births. Of these 8 million, 3.2 million are disabled for life ([iii]). Disabled children account for a large proportion of pediatric mortality ([iv]). Over 3.3 million children die annually from congenital defects before reaching the age of five ([v]). Interestingly, the rate of congenital anomalies appears to be lower in developed countries: it is approximately 3% in the United States as well as in Germany ([vi]).

The causes of congenital anomalies can be divided etiologically into three groups: The first group is that of genetic causes. Here, it can be further roughly divided into chromosomal disorders and single gene disorders. The next group concerns exogenous teratogenic factors. These include infections, substance deficiencies, substance abuse- toxic factors, diabetes, and in the broadest sense, maternal age. The third group includes cases of unknown cause ([vii], [viii]). This includes structural malformations in the embryonic phase and thus a large proportion of fetal malformations diagnosed prenatally. Quite a few malformations also result from the combination of the above causes: This is the model of multifactorial disease genesis.

As a result of these acting factors, there may be a disturbance of the structure of the body or its function. Often one causes the other and we observe an overlapping of both forms. As a consequence, these disorders can lead to physical, intellectual and developmental impairments. Due to the mutual dependencies, such impairments are often combined (Fig. 2).

This etiological-taxonomic classification cannot be consistently applied to the prenatal situation: Prenatally, the possibilities are limited to assess the functional impairments that may result from a recognizable structural defect - The view ahead is clouded. Prenatal medicine recognizes three major groupings of fetal disorders: The group of disturbance of fetal structure, the group of genetic defects, and the group of disturbance of growth as an expression of the stability of the maternoplacental axis of supply (Fig. 3). Biologically-practically, these three pathological conditions are closely intertwined. They are mutually dependent. Here is the classic example: A

fetus with trisomy 21 has a structural heart defect in about 50%. At the same time, his risk of developing growth retardation or placental insufficiency is significantly increased.

Conversely, this means for the practical prenatal medical procedure: Whenever the fetus is diagnosed as belonging to one of the three groups of fetal pathologies, simultaneous involvement of the other group of fetal pathologies should be excluded with maximum possible certainty. The more pathology groups the fetus combines, the more probable is the causal presence of a common genetic cause.

Two of these three prenatal pathology groups can be diagnosed authochtonously only by sonography. Even genetic disorders can be detected with high sensitivity by fetal nuchal translucency (NT) measurement and fetal echocardiography.

In a clinical weighting, it should be noted that clinically relevant, prognosis-determining structural defects of the fetus generally occur 10 times more frequently than chromosomal disorders. Therefore, the first commandment of prenatal medicine is: No genetics without fetal ultrasound examination!

From the year 2000: Combined NT screening

The turn of the millennium saw the introduction of NT measurement ([ix], [x]). This was initially done as NT-stand alone, then as a combined NT test with Papp-A and free ßHCG. This test was initially intended to be genetic only: it yielded unprecedentedly high sensitivities of around 90% for the three classical trisomies ([xi]). It soon became apparent, moreover, that the combined NT test could also be predictive for rare chromosomal disorders and occasionally for other genetic diseases ([xii], [xiii], [xiv], [xv]). Thus, NT already exceeded all expectations in this field. What was not foreseeable to this extent in the early 2000s was the increasingly clear realization that increased NT as a screening method in conjunction with early systematic ultrasound examination could also detect up to 80% of relevant structural malformations ([xvi], [xvii], [xviii], [xix]). Thus, NT was a door opener toward systematic early sonographic malformation diagnosis between 11 and 14 gestational weeks. It has now established itself as an integral part of a professional approach to the question of fetal health in prenatal centers worldwide ([xx]).

The core of this concept is the application of early fetal echocardiography ([xxi]). This represents the cornerstone of the diagnosis in a holistic health assessment. This is based on the following biological relationship: the heart anatomy is closely linked to the genetic constitution of the unborn child. Genetically healthy fetuses have structurally normal hearts in the vast majority. Genetically diseased fetuses have a high proportion of structural heart defects.

The combined NT test, in conjunction with early malformation exclusion, detects a large proportion of chromosomal defects and simultaneously a large proportion of structural malformations in fetuses at the biologically earliest possible time (completed embryonic phase) (Fig. 4). Thus, in a holistic approach, this combined sonographic-biochemical screening procedure was and is the best, most applicable reasonable approach to the regularly recurring question of the mother: Is my child healthy? This question, too, is holistic in nature and goes far beyond considerations of fetal genetics alone.

In the years between 2000 and 2012, a systematic, stepwise expansion of NT occurred: by introducing additional sonographic markers, the test sensitivity could be expanded to 95% for trisomy 21 ([xxii], [xxiii], [xxiv]).

From 2012: NIPT and combined NT screening

In 2012 NIPT was introduced into prenatal medicine. It has become firmly established worldwide in non-invasive prenatal genetic diagnostics as a robustly applicable search procedure for trisomy

21 ([xxv], [xxvi], [xxvii]). Studies have shown a slight increase in sensitivity in trisomy 21 to 99% compared to NT at 95% ([xxviii], [xxix]). The detection rates of NIPT in trisomy 18 and 13 range between 90 and 97% and are thus comparable on the level of sensitivity with NT in experienced examiner's hands ([xxx], [xxxi]). Rare chromosomal anomalies are not addressed by NIPT in its current standard configuration. Therefore, in comparison, the total number of genetic disorders considered, and thus the diagnostic breadth, is lower with NIPT than with the combined NT test. In a comparative outcome analysis of the detection (test sensitivity) of all fetal pathologies (structural malformations and genetic disorders), NIPT without simultaneous application of ultrasound is inferior to the combined NT test (sensitivity) when viewed in this way. The better detection of trisomy 21 with NIPT has slightly shifted the focus of consideration toward trisomy 21 compared with NT (Fig. 5).

The actual superiority of NIPT compared to NT ([xxxii]) is found in the significantly higher discriminatory power of the procedure in trisomy 21 due to the impressive reduction of the false positive rate (type 1 error) and thus increase in specificity. But also the false negative rate (type 2 error) decreases by at least one power of ten compared to NT. This significantly increases the positive predictive value (PPV) when considering test performance figures for trisomy 21 (Tables 1 and 2). Thus, NIPT has the potential to replace NT on the genetic-chromosomal side of a combined sonographic-genetic search strategy between 11 and 14 gestational weeks. This does not affect the potential of NT on the morphological side of this holistic screening concept.

NIPT for microdeletions

Around 2015, there was an expansion of the spectrum of genetic disorders addressed by NIPT towards microdeletions in professional investigation kits. For the five most common microdeletions (DiGeorge, Cri-du-Chat, Wolf-Hirschhorn, 1p36, Prader-Willi/Angelman), in high-risk collectives, the sensitivity of NIPT is 75% ([xxxiii]) for DiGeorge and over 90% for the others. All disorders have a steeply decreasing disease prevalence starting from DiGeorge with 1:1000-1:4000. This has a statistical effect in search procedures such that the false positive rate is relatively high and the PPV is relatively low (5-10%). A high false positive rate means a high unnecessary puncture rate. Thus, NIPT for microdeletions is scientifically-statistically effective in detection, but not efficient in practical prenatal life (cost-benefit consideration). Thus, the efforts of the laboratories, which are quite successful on the marketing side, to give NIPT a broader profile by including microdeletions, come to a dead end medically. This is reflected in the current DEGUM recommendations: The use of NIPT in screening is currently not recommended ([xxxiv]).

NIPT - Effect of test failure rate on real test performance in primary screening

One aspect that is often left out of the scientific analyses and marketing strategies of laboratory providers is the everyday reality of NIPT screening in a normal or low-risk population. This is envisaged as a model of care by statutory health insurers in Germany from 2021. Here, a factor comes into play that is usually primarily excluded in study collectives and thus leads to significantly better test performance figures than in prenatal everyday reality. This is the test failure (no-call) rate. Taken into account, this leads to a significant increase in the false positive rate of NIPT in trisomy 21, because a test that is not interpretable even when repeated often results in a puncture with good medical and psychological reasons ([xxxv]). Conversely, the increased false positive rate means a significant decrease in the positive predictive value of NIPT for trisomy 21. According to meta-analyses, these effects decrease the test sensitivity of NIPT for trisomy 21 in a normal population to 96%, for trisomy 18 to 87%, and for trisomy 13 to 77% ([xxxvi]). Thus, NIPT screening for trisomy 13 and 18 in the normal population no longer meets the quality criteria that are internationally applied to a screening procedure.

Summary - Conclusion

NIPT is a high-performing procedure at the trisomy 21 level for indicated screening in high-risk populations and is superior to the combined NT test, particularly at the level of specificity at this

point. Due to the high NPV, an inconspicuous test result at T21 predicts a non-T21 fetus with an extremely high probability. However, non-T21 does not mean genetically healthy or even generally healthy. In this respect, the wording "NIPT replaces amniocentesis" is philosophically-epistemologically and medically-practically incorrect and misleading.

NIPT is thus, from a practical point of view, a highly selective search procedure for trisomy 21. In all other respects, it is at best equivalent to the combined NT test with systematic ultrasound examination in genetic terms. NIPT without systematic ultrasound examination of the fetus means a diagnostic relapse into the 1980s for practical prenatal medicine.

NIPT has thus expanded the spectrum of available diagnostic methods in the practical day-to-day work of prenatal medicine, but has not completely displaced NT: Depending on the individual counseling situation, the expectations of the pregnant women seeking advice, and their economic possibilities, NT and NIPT are currently two valuable search methods in prenatal medicine that differ slightly in their breadth and depth of information (Fig. 6).

Figure 1:

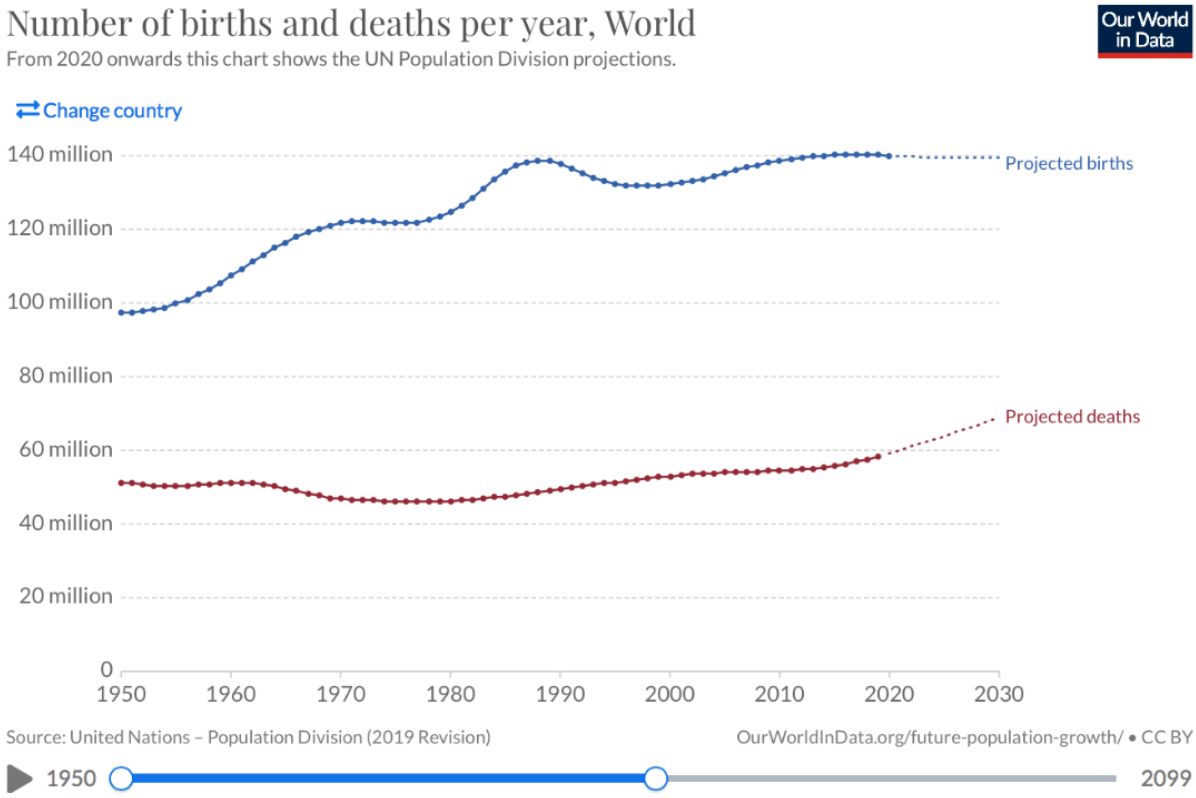
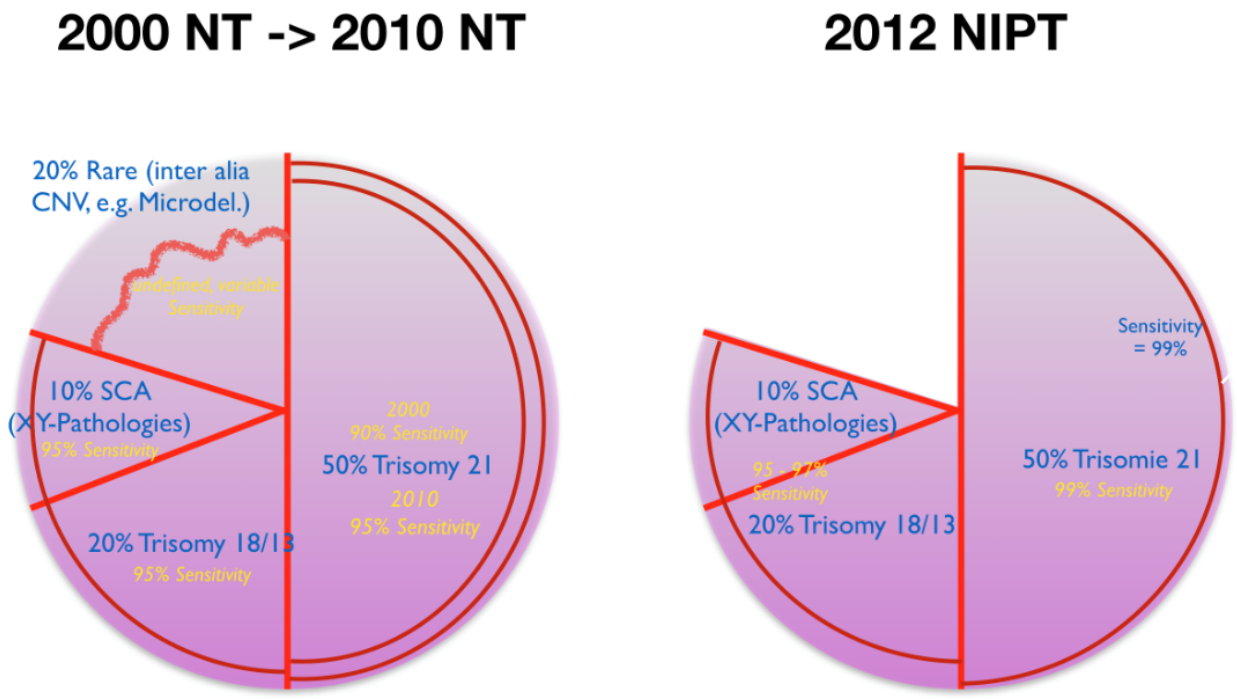
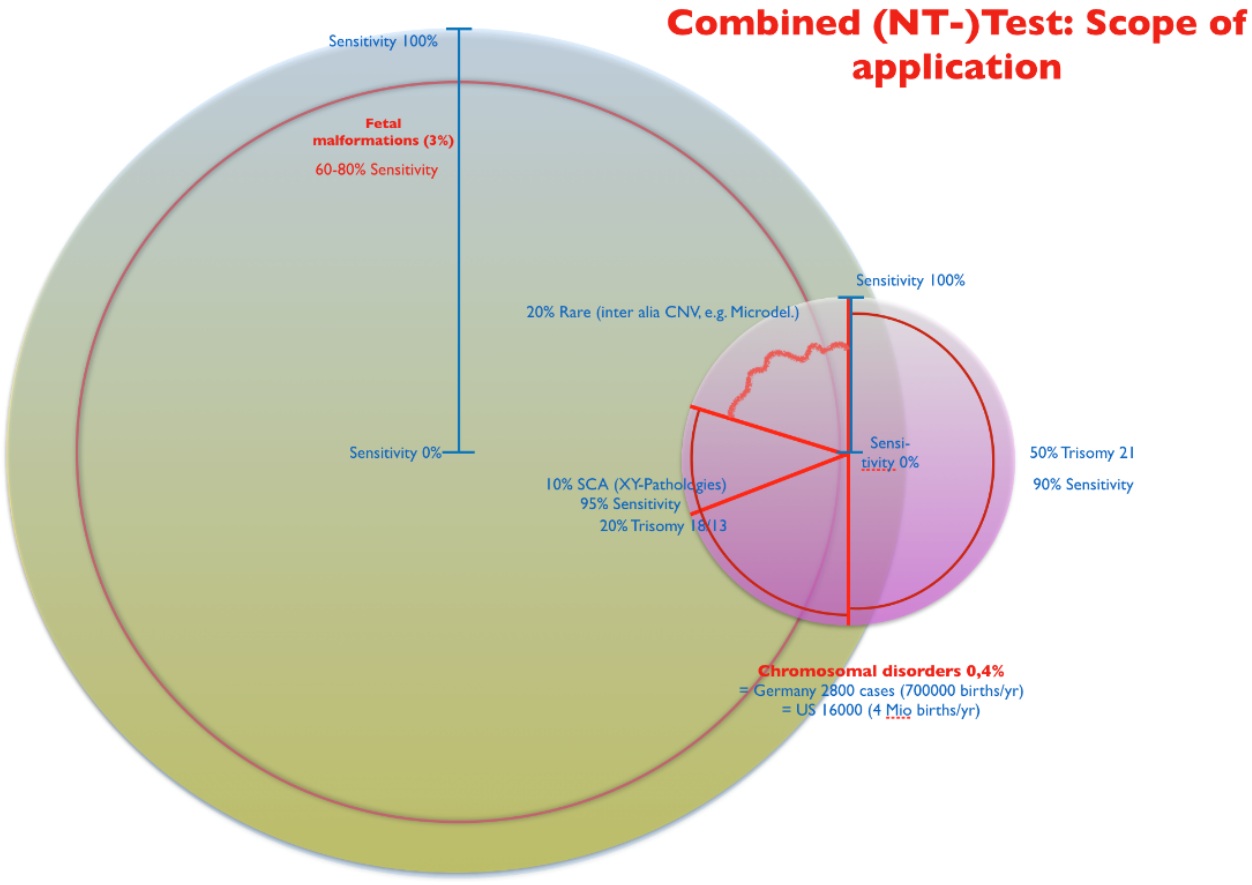


Figure 4:

Figure 5:


2000 NT -> 2010 NT
20% Rare (inter alia CNV, e.g. Microdel.)
undefined, variable Sensitivity
10% SCA (XY-Pathologies)
95% Sensitivity
20% Trisomy 18/13
95% Sensitivity
2000
90% Sensitivity
50% Trisomy 21
2010
95% Sensitivity
2012 NIPT
10% SCA (XY-Pathologies)
95 – 97% Sensitivity
20% Trisomy 18/13
Sensitivity = 99%
50% Trisomie 21
99% Sensitivity


Combined (NT-)Test: Scope of application
Sensitivity 100%
Fetal malformations (3%)
60-80% Sensitivity
Sensitivity 0%
Sensitivity 100%
20% Rare (inter alia CNV, e.g. Microdel.)
Sensi-tivity 0%
10% SCA (XY-Pathologies)
95% Sensitivity
20% Trisomy 18/13
50% Trisomy 21
90% Sensitivity
Chromosomal disorders 0,4%
= Germany 2800 cases (700000 births/yr)
= US 16000 (4 Mio births/yr)

[i] https://ourworldindata.org/grapher/births-and-deaths-projected-to-2100?tab=table&stackMode=absolute&time=1950..2030&region=World

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