

## The implementation of NIPT in The Netherlands

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### Abstract

In the Netherlands Prenatal Screening is offered as a mean to increase reproductive choices of couples. All women are counseled on the existing options by trained midwives. The government puts a great emphasis on informed choice and on the women opinion and reactions to screenings options. Since 2017 NIPT is offered as first tier screening for aneuploidies in the genome-wide variant at the cost of 175 Euro's. Uptake is around 45%. This rather advanced screenings offer is perceived as unusual for the traditionally cautious Dutch system. This has raised some criticism and concerns in the international arena.

In the Netherlands (NL) a unique law, the Population Screening Act, regulates screening for untreatable diseases<sup>1</sup>. The law aims at protecting citizens against the potential negative effects of screening. As a consequence, every time a new screening for these conditions is proposed a governmental license is required before the screening can be implemented<sup>2</sup>. This background explains why implementation of prenatal screening (PS) in the Netherlands has always been careful and thoughtfully weighed, with special attention for its potential side-effects<sup>2,3</sup>. The foundation on which Prenatal Screening has been endorsed in the NL is that the offer should enhance reproductive choices and it is essential that it rests on informed choice of women/couples<sup>4</sup>. This cautious policy explains why the NL was the last country in Europe to embrace PS for congenital conditions. The national screening program was implemented in 2007.<sup>5,6</sup> All pregnant women were from the outset counseled regarding the following screening options: 1) the combined testing (CT) as screening for trisomy 21 (Downsyndrome) and a structural ultrasound examination at around 20 weeks as screening for structural anomalies. In 2011, screening for trisomies was extended to trisomies 13 (Patau syndrome) and 18 (Edwards syndrome). Whereas mid-trimester US screening is free, women have to pay 165 Euro's for aneuploidies screening. This fact may have contributed to the low uptake of screening for trisomies<sup>7</sup>. Uptake increased from 25% in 2013 to 34% in 2016. In 2014, the Dutch NIPT Consortium, a collaborative partnership including all sort of stakeholders involved in PS, was granted a governmental license to introduce NIPT in the Netherlands by means of the implementation study Trial by Dutch Laboratories for Evaluation of Non-Invasive Prenatal Testing (TRIDENT).<sup>2</sup> In TRIDENT-1, women at increased risk for common trisomies (risk 1/200) based on the CT or medical history and not advanced maternal age alone, could choose between NIPT or invasive diagnostic testing. This study confirmed a remarkable reduction in the number of invasive procedures performed to confirm a high-risk result.<sup>8</sup> In April 2017, a second governmental license was granted to evaluate the implementation of NIPT as a first tier screening test for trisomies 21, 18, and 13 within the government-supported national prenatal screening program (TRIDENT-2 study)<sup>6</sup>. All women in the general obstetric population are counseled by midwives and can elect either NIPT (TRIDENT-2) or FCT as a first-tier test at the cost of 175 euro.<sup>9</sup> Tridents-2 is a study focusing on test performance and characteristics, as well as on women's perspectives. Women with an increased *a-priori* risk, based on the criteria described above, are excluded from TRIDENT-2 but are still eligible to have NIPT under the TRIDENT-1 study, or choose directly for invasive testing. A unique feature of TRIDENT-2 is that women who elect NIPT can choose to be tested for trisomy 21, 18, and 13 either with or without extra chromosomal aberrations of the other autosomes (size resolution of 10–20Mb). Sex chromosomes are excluded from the analysis.

Together with Belgium, The Netherlands is the only other country in Europe where NIPT is offered as first tier screening test and in the genome-wide option.<sup>10</sup> Three Dutch laboratories analyze the samples and provide the results by using Illumina platforms with the method WISECONDOR<sup>11</sup> first and more recently by using the VeriSeq 2.0 solution.

Previous studies have demonstrated the high sensitivity and specificity of NIPT for the common trisomies in both high- and general-risk populations.<sup>12</sup> However, studies dealing with the nature and clinical relevance of additional findings from whole-genome sequencing (WGS)-based NIPT are limited. The same applies to preferences of patients for this form of NIPT. Another characteristic of the Trident study is that a large unselected population is tested and a lot of effort is put on follow-up in order to clarify the clinical implications of the so called 'additional findings' and on women experience with these results. It is known that counseling over NIPT is challenging. In the Dutch system counseling is performed by the primary care-givers, i.e. midwives or gynaecologists who had to follow a dedicated training in order to be qualified for it.<sup>13,14</sup> Post-test counseling in case of abnormal results involving additional findings is carried out by geneticists. The TRIDENT-1 study, conducted in a high-risk obstetric population, highlighted the potential clinical utility of genome-wide NIPT by showing that 80% of the additional findings originated from the fetus or the placenta (the rest being maternal or unresolved, due to lack of follow-up material), and the majority were of clinical relevance for pregnancy management.<sup>8</sup> Other research groups have reported similar results.<sup>12</sup> In contrast, expanding NIPT beyond trisomies 21, 18, and 13 by including rare autosomal trisomies (RAT's) or segmental chromosomal aberrations (SA) is a new area of investigation, almost exclusive to the TRIDENT-2 study.<sup>15</sup> Extension of NIPT to sex chromosomes and microdeletion syndromes has been shown to be far less sensitive and specific than NIPT for trisomy 21 only and lead to unnecessary invasive diagnostic testing and potentially to pregnancy terminations for relatively mild or uncertain phenotypic conditions.<sup>16</sup>

Exclusion criteria for NIPT are a vanishing twin and the finding of a structural anomaly in the fetus, including a thickened nuchal translucency of 3,5 mm or more. In the latter cases a more detailed genetic investigation is needed, such as arrays CGH or whole exome sequencing (WES), capable of detecting sub-microscopic genetic aberrations.<sup>17</sup> One of the limitation of the current NIPT offer is that a scan is offered for dating of pregnancy and confirmation of viability and exclusion of twins at about 10 weeks 'gestation. The scan is performed by midwife not trained to specifically look for anomalies and at a stage where anomalies or a NT >3,5mm may not yet be visible. This means that the exclusion criteria are in practice poorly applicable. This results in double costs whenever anomalies are detected at a later stage and women are still offered the option of an advanced genetic investigation fater a normal NIPT result.

The results of TRIDENT-1 have been promising and have shown a good DR for trisomies with a high positive predictive value (PPV) of a positive test and a dramatic reduction in the invasive procedures (IP) in comparison with the CT.<sup>8</sup> The cut-off for offering NIPT was set at a CT risk of 1:200. The sensitivity of NIPT for Down syndrome was 97%, 90% for Edwards syndrome, and 90% for Patau syndrome, while the CT had in the NL sensitivities of 85%, 77% and 65% (RIVM, 2019), respectively. Following TRIDENT -1, in spite of the advice of the Dutch Health council to offer NIPT as a first- tier test to all women only as test for trisomies, the Trident consortium decided to offer NIPT in the genome-wide variant (TRIDENT-2).

The study will continue until the end of 2023, after which the Health Council will deliberate on the future standard offer of NIPT in the NL.

In the mean time preliminary results of TRIDENT-2 have been published, reporting the result on 73.239 women who underwent NIPT.<sup>10</sup> Uptake of NIPT was 42%. Sensitivity and PPV for Down, Edwards and Patau syndromes were 98 %, 91%, 100% and 96%, 98%, 53%, respectively. For the additional findings (AF) the overall PPV is 36%, with great variation among the type of AF: from 6% for RATs to 32% for SAs. The PPV of a positive test for abnormal maternal profiles suggestive of an increased risk of malignancy was 64%, although this concerned 11 cases of positive result, of which were uterine fibroids. AF accounted for 35% of the screen-positive results, meaning that more than 1/3 of the invasive procedures were done for this reason. The extremely low PPV for RATs, is mainly

attributable to false positive results due to confined placental mosaicisms (CPM). Especially in case of trisomy 7, 8 and 20 was invariably attributable to a CPM<sup>10</sup>. Trisomy 16 seems to be the only RAT with possible clinical consequences, in view of an increased risk of growth restriction and/or premature birth. It is known that in true fetal mosaicisms for trisomy 16 structural anomalies can occur, but infrequently and intellectual development can vary, but is mostly favorable. The counseling of women after a positive NIPT for AF is particularly challenging. In fact clinical advices and actions are taken in the setting of a research study whose aim is exactly to clarify the clinical outcome of the fetuses affected by these rare aberrations.<sup>18</sup> The question has also arisen as to how is it possible to obtain an informed consent in a study set-up where to obtain the informations necessary for a good counseling is the exact aim of the study. Clinical outcomes for the majority of the additional findings are in fact still largely unknown.<sup>19</sup>

A Dutch journalist has expressed in an article the unpleasant emotional roller-coaster his wife went through after a positive NIPT for trisomy 7.<sup>20</sup>

The introduction of NIPT has given rise to ethical and societal concerns about informed consent, felt pressure to undergo screening, and possible inequality in access due to the financial burden of NIPT.<sup>14</sup> The fee women have to pay may create a threshold and discrimination in access. Moreover, another voiced concern is that offering NIPT as a first-tier screening test may lead to routinization.<sup>21</sup> This concept refers to the fact that women may embark on prenatal screening more superficially as there is less risk of having to undergo an invasive procedure implying a (small) risk of miscarriage.<sup>22</sup> This may also lead to societal pressures to participate in prenatal screening and to stigmatization of women, families who do not do it.<sup>23</sup> Moreover, it has also been suggested that it may be easier to decide for termination of pregnancy of an affected fetus at an earlier stage in pregnancy, as compared to after an amniocentesis performed at 16 weeks.<sup>24</sup> However, all these concerns still lack empirical evidence and may not be founded.<sup>22</sup>

Expanding the scope of NIPT to the genome wide option would seem to benefit pregnant couples, as more subtle genetic aberrations can be detected in the fetus.<sup>25</sup> However, concerns on expansion of NIPT to include microdeletions and other genetic aberrations have been voiced.<sup>18,19</sup> It has been suggested that expanding the scope of NIPT may undermine informed decision-making because of the increased quantity and complexity of pre-test information counselors have to give.<sup>26</sup> Moreover, this may be the beginning of a 'slippery-slope' that may lead in the future to screening for minor abnormalities and even cosmetic variants. Different studies have shown that both professionals and pregnant women find it difficult to grasp the concept of an expanded NIPT.<sup>25</sup> In the mean time, the offer of an expanded NIPT has already started in many developed countries, including the United States besides Belgium and the Netherlands.<sup>27</sup> More care providers offer their patients commercially available expanded NIPT including besides the three trisomies also submicroscopical chromosomal aberrations and microdeletions.

A comment on the first TRIDENT-2 article was recently published in *Ultrasound in Obstetrics and Gynecology*. The authors express several concerns arising from GW-cfDNA testing.<sup>19</sup>

- 1) Increase in the screen-positive rate of a test that was initially meant to reduce it, and increase in the rate of invasive testing for conditions of unknown clinical significance that remain of unknown significance even after an invasive procedure.
- 2) There is uncertainty as to the clinical significance of a heterogeneous set of chromosomal abnormalities and how best to manage a positive result. Consequently, no professional society currently recommends this test.
- 3) There is heterogeneity of home-brew massively parallel shotgun sequencing protocols.
- 4) There are ethical and legal challenges to overcome regarding how best to counsel parents before they give their informed consent, since accurate information is lacking. In fact, women are already undergoing GW-cfDNA screening without clear information about its limitations and drawbacks, and clinical decisions are already being made based on results of uncertain clinical significance.

There are also ethical concerns regarding increased voluntary termination of pregnancy due to positive RAT results even after a normal karyotype and normal ultrasound scan.

5) The test violates World Health Organization screening principles.

The conclusion of the opinion is that, although research should always be encouraged, the benefits vs harms of implementation of GW cfDNA screening must be weighed carefully. Healthcare providers and grant-awarding bodies have a responsibility to ensure that more robust data and management strategies are available before endorsing studies or strategies incorporating GW-cfDNA testing into nationally reimbursed screening programs. “

In spite of these concerns the Dutch Health Council, traditionally extremely thoughtful and sensitive to ethical considerations related to PS, has recently released a new report, allowing the TRIDENT-2 study to continue until completion, with the only exception of not communicating anymore results of RATs almost invariably related to CPM.<sup>28</sup>

To conclude, there is an urgent need for global ethical guidance to determine an appropriate scope of NIPT. In this context, knowledge of women's preferences with regard to the scope of NIPT is of pivotal importance.

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