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CHAPTER 10

Summary & General discussion
NIPT is already clinically available in many countries, yet many research questions remain unanswered.
In this thesis, important aspects for implementation are addressed. The main conclusions are summarized below, followed by a discussion on future studies, future dreams and remaining research questions.

**SUMMARY**

**PERFORMANCE of NIPT**

As stated in the general introduction (part I), detecting fetal trisomy using a non-invasive test has been challenging, but after decades of research it is now truly feasible by sequencing cell-free DNA in maternal plasma. The diagnostic accuracy appears high. We systematically reviewed the published literature on accuracy of NIPT for the prediction of trisomy 21 using the QUADAS guidelines. This review (chapter 1) concluded that NIPT has great potential with high diagnostic accuracy, however, before implementation, large-scale prospective studies both in high-risk populations as well as in a general population of pregnant women should be performed, verifying the real-life diagnostic accuracy and cost effectiveness of NIPT. After our systematic review various other studies have been published on the performance of NIPT.1

As soon as NIPT became clinically available, first in November 2011 in the USA, Dutch pregnant women were highly interested. Dutch genetic laboratories in several University Medical Centres were already for years involved in research to perform NIPT themselves. However, to be allowed to offer NIPT to Dutch women, the Ministry of Health stated that a specific license was needed, based on the Population Screening Act (Wet op het Bevolkingsonderzoek, WBO). It took the national NIPT consortium almost three years to obtain such a license (granted per April 1st, 2014).

In the mean time, Dutch pregnant women found their way to foreign laboratories to have NIPT performed. First, after the launch of NIPT in clinical care in 2011, pregnant women were flying across the ocean to the United States for NIPT. In 2012, the option of having a blood sample taken in Belgium, and have it shipped to the American laboratories, became available, and this route was increasingly taken by Dutch pregnant women. With the EU-NITE study (chapter 2) we showed that shipping whole blood samples across the Atlantic Ocean is an accurate and feasible option.

Another important finding of the EU-NITE study was one case of a false negative trisomy 21 result, the first described in more than 5000 cases with the DANSR/FORTE method of Ariosa Diagnostics. The percentage fetal DNA in this case was low (4%), just on the threshold for the lab to give a result. The higher the fetal fraction, the percentage of fetal DNA in the total
amount of circulating cell-free DNA is, the more accurate the test result. More and more we understand that the fetal fraction is of great importance for the accuracy of the NIPT, being influenced by high body mass index, singleton or multiple gestation including vanishing twin, mosaicism and gestational age. Using the current techniques (2013), a fetal fraction < 4% results in a test failure, so if the fetal fraction is not measured, a test with a fetal fraction below 4% will be marked as negative, although rarely this is a true false negative result.

A potential disadvantage of NIPT is the lower diagnostic accuracy of NIPT for trisomy 13 (T13) as compared to NIPT for T21, as we discussed in chapter 3. Screening for a lethal disease such as T13, with false positives leading to risky invasive procedures in healthy pregnancies, may do more harm than good. Especially in a general population, T13 is very rare (1.4 per 10,000 live births in the UK). The positive predictive value, which is highly dependent on the prevalence of the disease, is very low, resulting in exactly the problem we aimed to avoid with NIPT, namely procedure-related miscarriages in pregnancies without a trisomy. All cases of T13 are associated with multiple structural anomalies that are hard to miss on detailed ultrasound, at the routine 20 week anomaly scan or often even earlier. With NIPT for T13 there might be earlier detection, thus the option of earlier termination, but also the chance of losing an unaffected child due to the invasive diagnostic test. Given the unfavorable balance between benefit and harm related to using NIPT to test for T13, we suggest not using it at this moment in a general population. The technique of detecting T13 by NIPT can probably be improved, leading to a lower false positive rate in the future. If T13 prediction is done using NIPT, we would strongly advise to first perform a detailed anatomical ultrasound, and only proceed to an amniocentesis and not CVS when abnormalities are seen.

DECISION MAKING

Counseling for prenatal screening to facilitate informed reproductive choices should remain the fundamental basis of prenatal screening programs. A non-directive-based counselling approach by healthcare workers will be as important as ever. Caregivers should be aware of the undesirable situation that these prenatal tests may be performed ‘routinely’, in the sense that the possible consequences are not considered careful enough before testing. With the introduction of NIPT, nearly complete elimination of iatrogenic miscarriages due to invasive prenatal diagnosis, and thus absence of fear of women for these risks, will lead to more balanced, autonomous reproductive choices. New questions arise: what will be the effect on the rate of termination of pregnancy? And what will be the effect on uptake of prenatal screening, and on reimbursement of costs?
Termination of pregnancy
Currently 93% of the women who receive a positive result following an invasive procedure elect termination of pregnancy (TOP). With the virtual elimination of the risk of an iatrogenic miscarriage, decision-making might change. We assume that a significant proportion of women who do not want to terminate a pregnancy affected by T21, still want to be informed about the health of the fetus if there is a safe and reliable test-option. The overall percentage of women who opt to terminate their pregnancy upon detecting T21 will likely be reduced if NIPT becomes available for all (chapter 4). We speculate that the main and important difference with the current screening programs will be that, unlike now, most live-born children with T21 will be born in families who made the deliberate choice not to test for fetal trisomy, or to accept and care for a child with T21.
A shift will likely occur following the introduction of NIPT among the selected group of women who mainly have a positive attitude towards TOP, leading to a more diverse group containing a larger proportion of women who will continue their pregnancy of a fetus with Down syndrome. In either situation, professional counsellors must support the woman in whatever decision she might make. Preparing for a life with a child with Down syndrome requires up-to-date information regarding Down syndrome, an explanation of potential often unpredictable mental and physical handicaps, long term prognosis, and—if desired—a referral, for example, to a patient support group.

Expected uptake
Women showed to have a positive attitude towards NIPT. The uptake of prenatal testing will rise as we showed in chapter 5. More than half of the women who rejected prenatal screening in the current program would request NIPT if available. The most important reason for the rise in uptake is the elimination of the risk of iatrogenic miscarriage.

Age-related reimbursement and willingness-to-pay
In the Netherlands, a fully covered health care system provides equal health care for every citizen. The government decided, however, that first trimester combined test (FCT) in women <36 years is not included in the national insurance system. Therefore, women <36 years, have to take the personal costs into account in deciding whether or not to undergo FCT. Published studies have shown that the performance of NIPT is not related to the age of the patient. In chapter 5 we investigated the willingness-to-pay (WTP) for NIPT. We sought to obtain information regarding how women value NIPT for detecting T21 and the test's risk-free diagnostic certainty. The mean amount of money women were willing to pay was slightly higher than the current costs of FCT, and some women were prepared to pay much more. WTP was correlated with both age and income, but not with religion.
In chapter 6 the influence of withdrawal of reimbursement on the uptake of the FCT was studied. We concluded that the financial impact on the uptake of FCT should not be underestimated, as there was a significant reduction in FCT after the period of withdrawal of reimbursement.

**TOWARDS NIPT IN THE NETHERLANDS**

The Netherlands is a special country in the view of prenatal care. Besides the above-mentioned Governmental license need for screening pregnant women, we also have a unique midwifery system. Most pregnant women receive care by independent primary care midwives, including the counselling for the FCT. The attitude of primary care midwives towards the current prenatal screening and towards NIPT was unknown, albeit considered very important when aiming to implement NIPT. In the study described in chapter 7 we found that the majority of Dutch midwives would welcome the implementation of NIPT. We can conclude that primary care midwives prefer NIPT for a general population replacing the FCT. Main concerns described by the interviewed midwives were about well-informed decision-making.

In 2013, offering NIPT was still forbidden in the Netherlands, although the Minster of Health was considering providing a Population Screening Act license. In chapter 8, the situation for obstetric care professionals is described while awaiting formal permission to incorporate the test into clinical practice. If women ask for information about NIPT, the caregivers are allowed and obliged to tell women what they know, thus to counsel women about NIPT. However, unsolicited offering of NIPT was still considered illegal. This situation was often a dilemma for many obstetricians, especially in case of a complicated obstetric or fertility history. Our article in the Dutch Journal for Obstetricians and Gynecologists summarized the medical-legal issues concerning this dilemma, and concluded that although it may seem morally difficult, doctors should not feel forced to break the law.

**OPINION**

In 2012, Benn et al. published an Editorial in Ultrasound in Obstetrics & Gynecology with the title ‘Non-invasive prenatal diagnosis for Down Syndrome: the paradigm will shift, but slowly.’

In chapter 9 we debate several key issues in their opinion and we point to their in our view unjustified or exaggerated negative remarks on NIPT. We predicted, in contrast to Benn et al. that the paradigm would shift rather fast, and already history appears to prove us right.
One of the important, and still repeatedly returning issues was - NIPT: screening or diagnosis? Whether a test is diagnostic or for screening is not related to its accuracy. Screening involves offering a test to a population without a known increased risk, while diagnostic testing is done in patients with symptoms or a known high risk. In our opinion NIPT is both a (very good) diagnostic as well as a (extremely good) screening test, this only depends on where in the sequence of testing the test is placed.

**TRIDENT-STUDY – THE FIRST STEP**

The NIPT consortium received a population screening act approval for the TRIDENT-study, starting in April 2014. The goal of the TRIDENT-study is to evaluate all aspects of a trial of implementation of NIPT as part of standard obstetric care. The evaluation will include feasibility and logistic analysis, uptake, patient preferences, technical performance, costs and suitability for high-throughput analysis. The power of the project does not allow reliable determination of the accuracy of the NIPT, but we assume this will be similar to published results using the same methods. Obviously, the dataset will be used to calculate test characteristics, with the aim to compare this to published work, but undoubtedly there will be wide confidence intervals. Many questions need to be answered during the TRIDENT study such as, what is the turn-around time, failure and redraw rates. The evaluation of women's perspective focused on the decision-making process, experiences, attitudes and opinions about NIPT will be studied in the parallel ESPRIT-study. This information is essential for the design of the future logistics and counselling.

The TRIDENT-study will be a first step towards implementation of NIPT for a general population.
Counselling during the TRIDENT-study

The initial design of the TRIDENT-study was to sustain the current screening program. Women ≥36 years of age would be allowed in the initial design to perform NIPT without performing a FCT first. The Health Council however, advised to eliminate 'maternal age' as a screening criterium. Only after a positive FCT women will be offered NIPT.

All pregnant women, electing to be informed about the FCT, should be informed about the new possibility of NIPT following the FCT (figure 1). Patients should be aware that it is a study, and not all information is available yet.

It is important to mention all advantages and disadvantages of NIPT and both invasive procedures. The informed choice principle is the basis of the counselling.

The pre-test counselling of NIPT should include the (estimated) performance, positive –and negative predictive value, the failure rate (with factors mentioned to influence the failure rate like BMI) and the turnaround time. The limitations of only detecting T21, T18 and T13 should be mentioned, including the not perfect performance of NIPT of especially for T13. Fetal sex or sex chromosome abnormalities will not be reported. The fetal fraction will not be determined in the TRIDENT-study, and for this reason not reported.

Figure 1. The flowchart of the TRIDENT-study
All women with a positive NIPT result are strongly advised to perform an amniocentesis or CVS. The post-test counselling is very important and should be individualised. No selective abortion should take place before confirmation of the trisomy by QF-PCR/karyotyping. The disadvantage of an iatrogenic miscarriage should be mentioned, but also the advantage of a very rapid result (STC, QF-PCR in 3 days) and the extensive detection opportunity of a microarray. In case of an ultrasound abnormality (including NT >3.5 mm) it is advised to perform an invasive procedure followed by a microarray.

Tissue after a miscarriage or an intrauterine fetal demise should be collected to test for fetal trisomy.

**Position paper by the Netherlands Society of Obstetricians and Gynaecologists (NVOG)**

Besides preparing implementation processes for the TRIDENT-study, other preparations towards implementation of NIPT were made. An important example is the position paper published in May 2013 by the Netherlands Society of Obstetricians and Gynaecologists (NVOG) underlining the importance of NIPT.³

A summary of the position paper:

1. As soon as the Dutch laboratories are able to offer Non Invasive Prenatal Testing (NIPT) for use in clinical practice, the NVOG recommends offering NIPT as a third option, next to the current standard tests (chorionic villus biopsy and amniocentesis) to women with an increased risk of fetal T21.

2. The definition of increased risk for T21 includes currently accepted indications for invasive testing, either a risk estimate of 1:200 or above based on the first trimester combined test, a previous child with a chromosomal abnormality, or one of the parents carrying a translocation of chromosome 21.

3. In case of a nuchal translucency measurement >3.5 mm other tests directed at a wider range of anomalies are considered indicated. Pregnant women who receive an increased risk result following the first trimester combined test screening for T18 or T13 are advised to have a detailed ultrasound in an academic hospital, followed by individualised counselling including discussing the option of NIPT. The less accurate test performance of NIPT for T13 and T18 needs to be discussed.

4. The NVOG states that until the Dutch laboratories are able and allowed to perform NIPT for fetal trisomy, women with an increased risk for fetal trisomy based on the above mentioned criteria should be informed about the option to have NIPT performed by foreign laboratories.

5. The NVOG believes it is her responsibility to optimise and to ensure uniform patient information, to prevent unequal access in care.
6. In the Netherlands, for (invasive) prenatal diagnosis of fetal chromosomal abnormalities it is mandatory to have a license based on the Act on Exceptional Medical Procedures (WBMV). The opinion of the NVOG is that in the first phase of implementation of NIPT, pre-and post-test counselling, blood draw, reporting and follow-up of results should be performed in Prenatal Diagnostic Centres with this license and expertise. After an implementation and evaluation period of two years it might be clearer whether (a part of) the chain of care could possibly take place in other medical centres (without this license).

7. The NVOG believes that NIPT should be reimbursed by the pregnant woman’s health insurance under the same conditions as the invasive tests in the current system. The NVOG advocates equal access to care for all pregnant women.
DISCUSSION ON SEVERAL TOPICS FOR FUTURE IMPLEMENTATION OF NIPT IN THE NETHERLANDS

Following our studies, but also based on the current literature and personal opinion, a few important topics should be addressed on the implementation in the Netherlands. These topics are: the uptake and logistics, costs, counselling, collaboration between obstetricians and primary care midwives, the value of the first trimester ultrasound and the reduction of invasive procedures.

Uptake and logistics
In 2014 we expect the publication of several large studies in general pregnant populations. We expect the results to be similar to the published high-risk studies. If this is the case, the logic of electing a FCT before NIPT will be hard to explain, although pregnant women possibly value an extra ultrasound. (In the Netherlands, all pregnant women undergo an early first trimester dating ultrasound, which also enables detection of twins and anencephaly). We expect the uptake to rise fast. A rough estimation of an expected uptake would result in an uptake of more than 100,000 samples per year in the Netherlands. The question is, what laboratory in the Netherlands is willing and more important, able to perform NIPT in these large numbers? Worldwide there are only a few NIPT-laboratories, although increasing in number. After speaking to several commercial laboratories it should not be underestimated that the expenses of a fully automatic high throughput, top-quality laboratory, with a back-up system for all machines in case of error, are very high. In the Netherlands we may not need the capability of performing NIPT in larger numbers in every academic centre. It could be cheaper and more efficient to centralise the performance of NIPT to one or two centres or maybe to an external laboratory like Sanquin, Amsterdam. Another option would be to send samples to one of the foreign laboratories. The Health Council stated against this option in case of insufficient capacity because of loss of quality control.

Costs
Costs of health care are a hot topic as health care expenditure is rising each year. Will NIPT lead to higher costs for society? Should women pay for NIPT themselves? These are important questions to answer before implementation of NIPT. Eventually we expect NIPT will replace the FCT, but at first NIPT will be implemented as an additional test besides the invasive procedures. For women who receive a positive NIPT result, and who elect to confirm this by an invasive test, costs compared to the current system will increase. This group however is small, at most a few hundred women per year. Women receiving an increased risk for trisomy from the FCT in the current system in about 50% declined further testing. With
NIPT available, we assume that the vast majority, at least if the test is reimbursed, will choose to have NIPT done. Therefore, although the costs of NIPT are considerably lower than for invasive testing, the overall reduction in costs may be limited. If all women at increased risk for trisomy after the FCT choose to have NIPT, then NIPT needs to be at least 50% cheaper than invasive testing to be financially beneficial.

In addition, the uptake of the FCT may increase when women become aware that in case of being screen-positive, there is a safe next testing step available. If the uptake would double, from 45,000 to 90,000, this would mean an increase in costs of almost 7 million euros. Part (roughly estimated 50%) of this amount will be paid by women themselves, if they are under 36 years old.

What else do we take into account when we calculate the costs of the implementation of NIPT? The costs of the test itself are lower than the costs for an invasive procedure, but higher than the current FCT. The costs of NIPT have dropped in the past years (from around 2,000 dollar to around 400 US dollar) and might drop further. Secondary costs are hard to calculate. A few examples. What are the costs of one or two days off work because a woman has to go for an invasive procedure? Or requires bed rest for bleeding or continuous amniotic fluid loss post-procedure? What are the costs of a woman who loses her child because of an invasive procedure? One could argue that the loss of a healthy child in term of economic value translates in the loss of 80 Quality-Adjusted Life Years. The costs for life-long care for children born with a mental handicap is a very sensitive issue, but we know these costs are among the highest in Dutch health care. It does not feel ethically correct to calculate these costs.

What if policy makers decide there will be no or only partial reimbursement of NIPT? Apparently women who go abroad now for NIPT are willing to pay for the test or borrow money for it. We don’t know the characteristics of these women exactly, but we believe this is a selected, often highly educated, therefore financially privileged group of women. The access to health care is not equal and will influence decision-making.

One of the options to reduce costs with the implementation of NIPT is drawing blood >12 weeks of gestation, since this will largely prevent testing pregnancies that end in spontaneous miscarriage. In addition, the chance of test failure is lower, as the fetal fraction increases with gestational age.

Counselling

Although NIPT has a higher accuracy than currently used screening methods, the discordant case in the EU-NITE study underlines the importance, as is with every medical test used in screening and diagnostic settings, of appropriate pre-test and post-test counselling. Women should understand the implications of the test results before undergoing any type of testing, including the likelihood of test failure, incorrect results, and findings of unclear significance. If there is a discordance between NIPT and the karyotyping, there often is an underlying biological reason, such as confined placental mosaicism, maternal mosaicism, co-twin
demise or malignancy. The NIPT itself provides the correct result, i.e. an excess of DNA of a certain chromosome, however the fetus may not have the phenotype of the predicted trisomy. Therefore, one could argue that discordance is a preferable term over false negative or false positive.

A positive result should always be confirmed by an invasive procedure, or at least for women considering termination of pregnancy based on the test result. Of course information about T21, T18 and T13 should be complete.

But should we disclose all possible rare (0.5-1%) ‘accidental’ trisomic or non-trisomic abnormal outcomes that could be obtained by examining the sequencing data from NIPT? Or should the laboratory use a blinding method to prevent observations of such findings? Or should a geneticist in the laboratory evaluate such findings to differentiate between clinically relevant and innocent variations? This is still open for debate, however, we have for decades similar experience with the use of the routine use of the 20-week anomaly scan. If we counsel women for the 20-week anomaly scan we tell her that we screen the fetus from head to toe and we inform her, broadly, about the roughly 5% chance of finding a fetal structural abnormality. We give some examples such as neural tube defect, obstructed kidneys and heart defects, we obviously answer any specific question the patient may have, but we don’t tell them details about the thousands of rare possibilities outlined in our 500-page ultrasound textbooks.

In case of an ultrasound abnormality, during post-test counselling, patients are counselled in detail by experts with all information they need to make decisions. We believe it should be the same for NIPT. The post-counselling should be done thoroughly by an experienced obstetrician, if needed supported by a clinical geneticist, like every other abnormal prenatal diagnosis result.

In the current prenatal screening system the counselling is generally focused on explaining the test (the FCT) rather than the condition Down Syndrome itself. With the introduction of NIPT as a first-line test, counselling about the test will be less time consuming, and more time will be available to inform the expectant parents regarding Down syndrome. Health issues common among children with Down syndrome and variability in the degree of intellectual disability are essential elements of this information. In addition, parents should be informed that individual medical and neurodevelopmental outcomes can not be predicted antenatally.

**Collaboration between primary care midwives and hospital care**

In the first step of implementation, NIPT will be offered as a test for a high-risk population, as a third option next to the amniocentesis and chorion villus biopsy. The counselling for the primary care midwife will not change much, only some brief, general information about NIPT will be added. If NIPT is implemented as a test for the general population, it could be an opportunity to strengthen collaboration between primary-care midwives and hospital care. Several options for this collaboration are possible. A joint effort could be done for group
counselling sessions for a low-risk pregnant population. Not all women who receive care in the hospital are at increased risk for trisomies and could easily join group-counselling sessions by midwives. In fact, far fewer women than in the current screening program will have a high risk for trisomies. Women with a positive NIPT result or any other reason for prenatal diagnosis, or for instance women with twin pregnancies will receive counselling by obstetricians in centres for prenatal diagnosis.

Before implementation of NIPT, counselling courses should be organised for both midwives, obstetrician-gynaecologists (in training) and prenatal doctors.

The value of the first trimester ultrasound

The first trimester ultrasound measurement of the NT was originally designed to screen for trisomy 21. However, its use has broadened in the past years. In the Netherlands, NT measurement as part of the FCT is still only directed at calculation of the risk for T21, T18 and T13. As a 'chance finding' additional fetal structural abnormalities can be detected in increasing numbers, with more advanced machines and more experienced operators. A large NT is not only associated with fetal trisomy but with many other, often severe, diseases. It is debated what to do with the first trimester ultrasound when NIPT is implemented. In particular, with implementation of NIPT in the general population, the use of the first trimester ultrasound needs to be re-evaluated. The value of the first trimester ultrasound in a group of pregnancies where fetal trisomy is already excluded should be studied. What can be detected, and how reliable is such a diagnosis? What useful management steps can be taken based on finding an ultrasound abnormality at 13 weeks? In a few selected cases, the diagnosis will be sufficiently clear to consider termination of pregnancy. However, in others, repeating the scan at 16 and again at 20 weeks could be needed, giving rise to many weeks of anxiety. Should an invasive test for microarray be offered for all anomalies? How many false positives would that cause, including, again, iatrogenic miscarriages? And would such a program be cost-effective? Large scale, prospective studies to address these issues should be carried out with some urgency.

The reduction of invasive procedures

As Tabor et al studied, experienced operators have a higher success rate and a lower complication rate performing invasive procedures. If NIPT is implemented, and even at this moment (because women go abroad for NIPT) the numbers of invasive procedures are decreasing rapidly. Similar to many other procedures the caregiver should perform sufficient numbers to be and stay capable. It seems obvious and inevitable that (further) centralization of invasive procedures and obligatory annual reports to the Health Care Inspection including follow-up is the only way to be able to retain a quality assurance and monitoring.
The committee of the Health Council published table 2 in the Population Screening Act – manuscript (in Dutch). The table shows the reduction of invasive tests with the implementation of NIPT.

**Table 2**  Detectie van trisomie 21 vergeleken met de kans op een invasieve test in verschillende scenario’s met een combinatietest (CT) en/of NIPT.

<table>
<thead>
<tr>
<th></th>
<th>CT 1 op 200</th>
<th>CT 1 op 200</th>
<th>CT 1 op 300</th>
<th>CT 1 op 500</th>
<th>Geen CT, direct NIPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT: Trisomie 21 (sensitiviteit)</td>
<td>CT: foutpositief (percentage)</td>
<td>CT: foutpositief (percentage)</td>
<td>CT: foutpositief (percentage)</td>
<td>Geen CT, direct NIPT</td>
</tr>
<tr>
<td>CT: Trisomie 21 (sensitiviteit)</td>
<td>170 (85%)</td>
<td>3.393 (3.4%)</td>
<td>170 (85%)</td>
<td>3.393 (3.4%)</td>
<td>194 (97%)</td>
</tr>
<tr>
<td>CT: foutpositief (percentage)</td>
<td>3.393 (3.4%)</td>
<td>170 (85%)</td>
<td>3.489 (5.5%)</td>
<td>194 (97%)</td>
<td>n.v.t.</td>
</tr>
<tr>
<td>NIPT (na CT): Trisomie 21</td>
<td>n.v.t.</td>
<td>169</td>
<td>179</td>
<td>193</td>
<td>199</td>
</tr>
<tr>
<td>NIPT: aantal foutpositief</td>
<td>n.v.t.</td>
<td>10</td>
<td>16</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Aantal invasieve tests</td>
<td>n.v.t.</td>
<td>3.563</td>
<td>179</td>
<td>195</td>
<td>223</td>
</tr>
<tr>
<td>PVW</td>
<td>n.v.t.</td>
<td>94%</td>
<td>97%</td>
<td>87%</td>
<td>40%</td>
</tr>
<tr>
<td>Detectie: miskraam</td>
<td>10:1</td>
<td>189:1</td>
<td>184:1</td>
<td>173:1</td>
<td>80:1</td>
</tr>
<tr>
<td>Invasieve tests l.o.v. nu</td>
<td>0:1</td>
<td>-31:1</td>
<td>-319:1</td>
<td>-3170:1</td>
<td>-2895:1</td>
</tr>
<tr>
<td>Trisomie 21 l.o.v. nu</td>
<td>0:1</td>
<td>-1</td>
<td>-9</td>
<td>-423</td>
<td>-429</td>
</tr>
</tbody>
</table>

* Detectie-miskraamverhouding: het aantal gedetecteerde gevallen trisomie 21 op één miskraam, als de kans op fetogene miskraam 0,5 procent is.

As we showed in chapter 5 and 8 patients and caregivers favour NIPT as a first-line test. The uptake will probably rise. More and more women will ask for NIPT, as a first line test, as they already do – as many women don’t want to perform the FCT, with the chance of a false negative result. As we discussed before the age-related policy in the current screening should be eliminated.

**AFTER THE TRIDENT-STUDY**

With the results and experience of the TRIDENT-study we expect that we can move forward to the next phase in the improvement of the screening program in pregnancy, an exploration of the implementation of NIPT as a screenings test for a (more) general population.

It is still too early to draw conclusions, but several implementation options could be envisaged:

1. **No FCT but NIPT as a first-line screening test for all women. In case of a positive NIPT result a 16 week amniocentesis for targeted or whole-genome microarray**
2. **FCT first and NIPT in case of a positive risk assessment ≥1:200**
3. **FCT first and NIPT in case of a positive risk assessment ≥1:1000 or 2500**
4. **NIPT first, followed by a detailed 13-week anomaly scan, and serum screening for several pregnancy complications such as preeclampsia, preterm birth and fetal growth restriction.**

As we showed in chapter 5 and 8 patients and caregivers favour NIPT as a first-line test. The uptake will probably rise. More and more women will ask for NIPT, as a first line test, as they already do – as many women don’t want to perform the FCT, with the chance of a false negative result. As we discussed before the age-related policy in the current screening should be eliminated.
Another question is, especially for the logistics, but also for the costs, what is the best gestational age to draw blood for NIPT? A major advantage of NIPT is that NIPT can be performed from 10 weeks gestation onward, without an upper limit with its' inherent problem for women reporting late for their first visit.

1. Blood draw at 10 weeks of gestation
   Advantages:
   a. early reassurance for most women
   b. in case of a failure of NIPT, women are able to perform FCT
   c. early termination of pregnancy is possible
   Disadvantages:
   a. the fetal fraction is lower, resulting in more failure
   b. spontaneous miscarriages are not uncommon until 12 weeks of gestation
   c. due to the arguments noted above (at a and b) the costs of NIPT will be higher
   d. Blood draw at 12 weeks of gestation
   Advantages:
   a. the fetal fraction is higher, resulting in less failure
   b. Less testing in pregnancies with a spontaneous miscarriages
   Disadvantages:
   a. longer uncertainty for women requesting testing
   b. after NIPT, in case of failure, FCT might not be possible anymore.
   c. termination of pregnancy at more advanced gestation. A curettage is a more invasive procedure beyond 14 weeks, and many obstetricians prefer termination using prostaglandin induction.

LIMITATIONS OF THE THESIS

In the Netherlands no studies have been performed, in which investigators were able to give an NIPT result to the patient because no Ministerial approval was given. For this reason all questionnaire studies done until implementation are hypothetical. A limitation of studies with questionnaires is the limited response rate and the fact that the questionnaires are self-reporting. Studies in a real-time setting after implementation of NIPT with a larger sample size or with choice experiments should be undertaken to obtain more information about this important topics.
FUTURE POSSIBILITIES?

If possibilities broaden using NIPT we might be able to detect syndromes or diseases we can treat during pregnancy resulting in better outcomes. In the future, many possibilities are expected, like the detection of microdeletions or duplications in maternal blood and the detection of monogenic diseases like Huntington. Although the first results in a research setting are hopeful, the feasibility for clinical use should be investigated. Obviously, with rare diseases, evaluation of the reliability of testing becomes more difficult.

We might be able to perform the so-called 'heel prick test', now performed in the first week after birth detecting a range of metabolic disorders during pregnancy. For instance a diet or medication administered to the mother might result in a better postnatal outcome for the fetus.

FUTURE RESEARCH QUESTIONS

Besides the many research questions we will hopefully answer with the TRIDENT-study, other questions should be addressed. What is the percentage of women opting for termination of pregnancy, in case of confirmed Down syndrome? What are the real changes in the decision making process? What will happen with the first trimester ultrasound? We have to re-evaluate the clinical value, and cost-effectiveness of the first trimester ultrasound as a test for other diseases than fetal trisomy. What are the true costs of NIPT in a real-time, real-life setting?

The diagnostic accuracy for multiple pregnancies is not yet optimal. Is there a difference in accuracy between dichorionic and monochorionic twin pregnancies?

The most likely advance in the near future, apart from becoming cheaper and faster, is NIPT on sub-chromosome abnormalities. What to detect and what to ignore needs a thorough clinical and ethical debate, because in the future the options might be endless. In a research setting, the whole fetal genome has been sequenced already.
REFERENCES


