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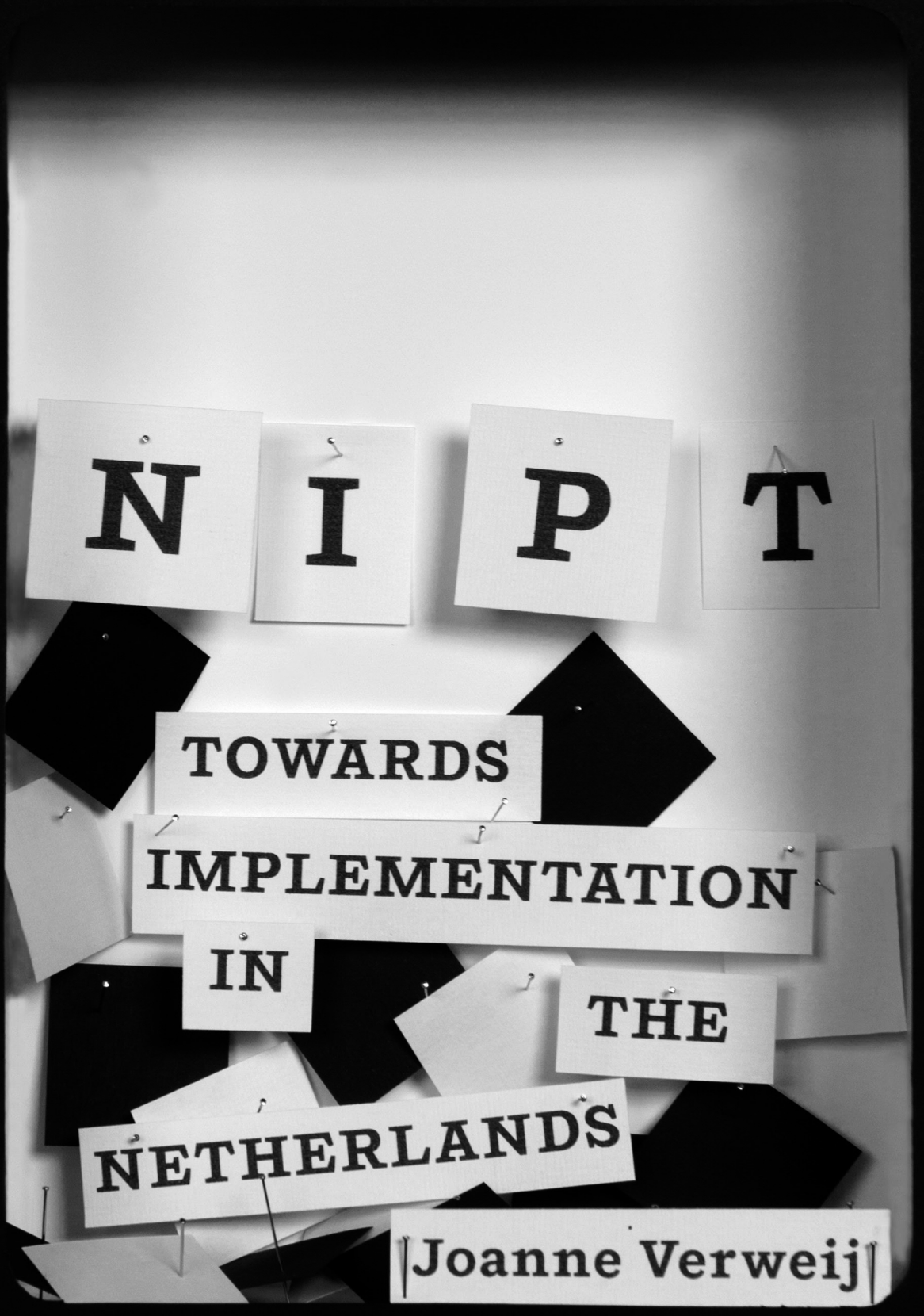
NIPT


NIPT
TOWARDS
IMPLEMENTATION
IN
THE
NETHERLANDS
Joanne Verweij

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NIPT: towards implementation in the Netherlands

The studies described in this thesis were performed at the Department of Obstetrics of the Leiden University Medical Center, the Netherlands.

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NIPT

non-invasive prenatal testing:

towards implementation in the Netherlands

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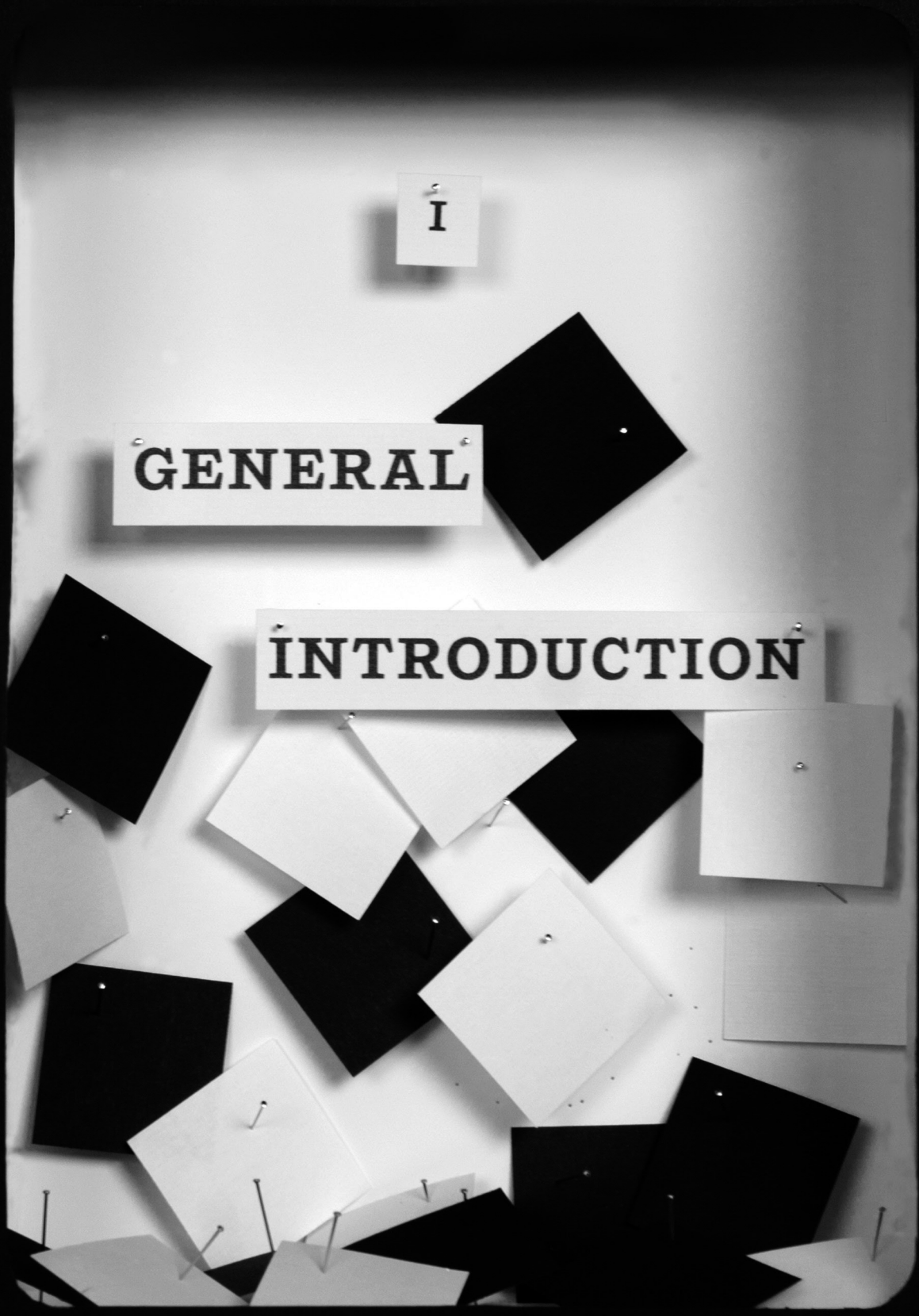
I
GENERAL
INTRODUCTION

The aim of providing prenatal screening for chromosomal conditions is to enable reproductive choice with respect to carrying to term or terminating the pregnancy of a child with a serious disorder or disability.[1] To elect for prenatal screening or diagnosis is a patient's choice.

CURRENT PRENATAL SCREENING PROGRAM FOR FETAL CHROMOSOMAL ANOMALIES

The most common chromosomal abnormality in live born children is Down syndrome (trisomy 21 (T21)). The prevalence of Down syndrome in the Netherlands is estimated to be 1:500. The risk for T21 is age related, the older a women is during pregnancy the higher the risk of an affected child.
In most western countries, pregnant women are offered prenatal screening for T21. In figure 1 the history of prenatal screening in the Netherlands until 2013 is depicted. Before 2007, an invasive procedure was offered to women of 36 years or older, based on the age-related risk. Age alone yet is a poor predicator for T21. Around 1% of the results were positive for T21, but due to the procedure, 0,5-1% of (most often healthy) pregnancies were lost. For this reason in 2007 a prenatal screening program was launched to predict the risk of T21 more precisely. In the first trimester, women are counselled about the option of the so-called first trimester combined test (FCT). The FCT is an individualised risk-calculation to estimate the chance of carrying a fetus with T21. The test algorithm consists of maternal age, maternal serum markers and nuchal translucency measurement and can be performed between 11-14 weeks of gestation (figure 2).[2] The nuchal translucency is a fluid accumulation behind the fetal neck, and is associated with fetal trisomy, and many other anomalies such as heart defects. The accuracy of the measurement depends on the experience and precision of the sonographer. In addition, screening on trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) is offered using the same test, with an adapted algorithm since 2010.
The prevalence of trisomy 13 and 18 is lower, however these syndromes are often lethal. Together with the introduction of the FCT, the 20 week-anomaly scan was introduced to screen for neural tube defects and other structural abnormalities.

The introduction of the FCT resulted in a significant reduction in invasive procedures and was considered a big step forward. The accuracy depends partly on the quality of the ultrasound resulting in a false negative rate of 10–25% in clinical practice.[3-7] In case of a false negative result women are falsely reassured after the FCT, though confronted with a child with T21 after birth. If a woman decides to choose for the screening by FCT in order to have the possibility of terminating an affected pregnancy, a false negative result is clearly an unwanted outcome.
The false positive rate of FCT is a choice that can be made using the test characteristics and the cut-off between a high and a normal risk (in the Netherlands 1 in 200), and is most often set at 5%. Therefore, 1 in 20 women will be referred for an invasive procedure, while >90% of them do not carry an affected child.
Another serious limitation of FCT is its restricted time-window of 11–14 weeks gestation. Women who are late for their first visit, for any reason, are not able to elect for the FCT.

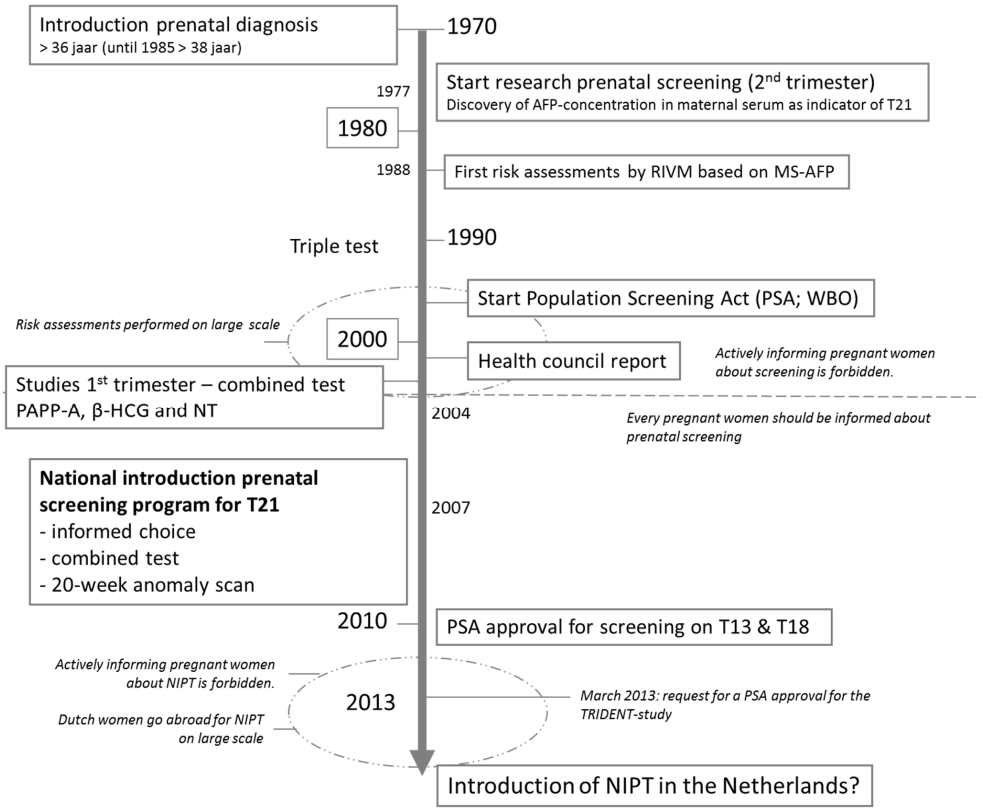

Figure 1. *History of prenatal screening in the Netherlands until 2013*

Invasive procedures

If a woman receives a FCT result with a high risk for trisomy 21,18 and 13, she is offered invasive testing using chorionic villus sampling (CVS) or amniocentesis. CVS is usually performed at 11 to 14 weeks of gestation either transabdominally or transcervically. Amniocentesis is the most commonly used invasive procedure and is usually performed from 15 weeks of gestation onward. Both the chorion villi as the amniotic fluid cells are investigated, mostly by rapid aneuploidy detection (RAD), short –and long term culture or a microarray. RAD using QF-PCR results in a quick result (2-4 days), detecting only the most common chromosomal abnormalities. Microarray is an extensive investigation, mostly applied only in case of an ultrasound abnormality after a normal QF-PCR or short-term culture.

These invasive tests are highly accurate and are associated with an iatrogenic miscarriage rate around 0.5-1%.[8,9] The CVS has a very high accuracy but the amniocentesis performs better. The accuracy of CVS is described to be 99.7% with a very small risk of failure due

to maternal cell contamination, clinical significance of mosaic confined to the placenta or laboratory failure.[10] In these cases resampling by amniocentesis is necessary. The accuracy of an amniocentesis is almost 100%. However, the actual procedure-related miscarriage rate remains a debate, as some obstetricians believe it is lower than 0.5-1%. Tabor et al. studied the fetal loss rate after an invasive procedure during an 11-year period in Denmark describing miscarriage rates of 1.4% (95% CI,1.3–1.5) after amniocentesis and 1.9% (95% CI,1.7–2.0) after CVS.[9] Another result of their study was that the number of procedures a department performed had a significant effect on the risk of miscarriage.[11,12] Wijnberger et al. observed this too in an earlier study where the learning curve for CVS was studied. They concluded that the operator experience influences the safety and success of the procedures.[13]
As described above, the majority of invasive tests (>90%) are carried out in pregnancies with a healthy fetus. However, women fear the invasive procedure, and have an anxious period waiting for the result. Although most of these women are reassured by a favourable result, the situation of strong anxiety is described to influence the pregnancy and the postpartum period negatively.[14] According to unpublished numbers of the National Institute for Public Health and the Environment (RIVM) only half of the women in the Netherlands with a high-risk assessment after FCT elect for an invasive prenatal diagnostic test. The reason for refraining from an invasive procedure after a high-risk assessment is unknown. It could be fear of losing the child after an invasive procedure, or fear for pain or needles, or depending on the actual FCT result, the feeling that the true risk is not really high.

Age related reimbursement

In the Netherlands, for most medical costs a fully covered health care insurance system provides equal health care for every citizen. Insurance companies therefore reimburse the 20-week anomaly scan for everyone. The government decided, however, that FCT for women <36 years was not to be included in the national insurance system. The costs of the FCT (2013: €154) for women ≥36 years are reimbursed. In case of a positive high-risk assessment after FCT further specialist counselling and invasive procedures are reimbursed.
Around 25% of the Dutch pregnant population decides to have a FCT performed. In comparison to other European countries this is a low uptake. Different reasons could account for this, like no desire to know whether the fetus has T21, characteristics of the test, the costs, the counselling, or not willing to take the risk of an invasive procedure following a positive result.

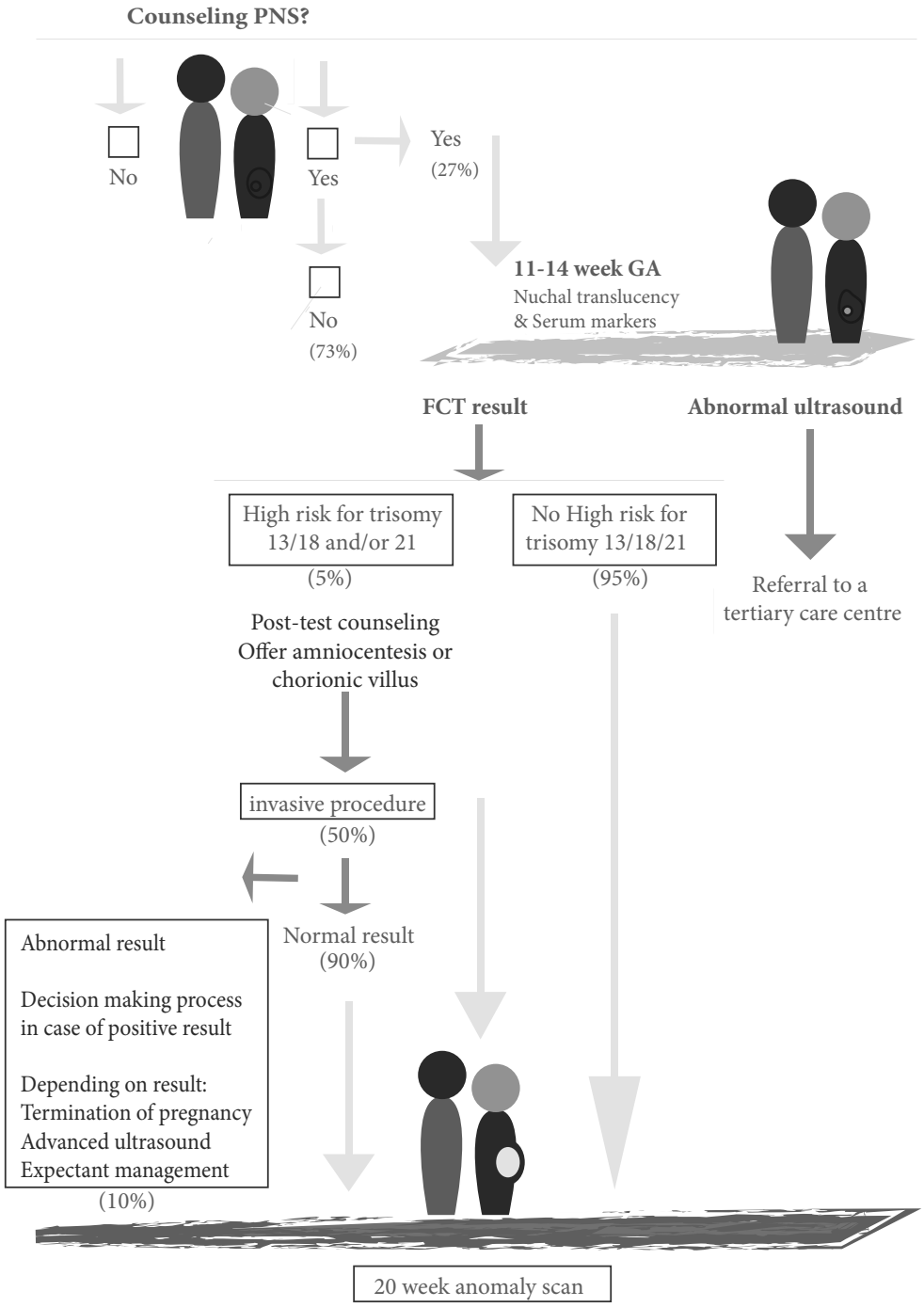

Figure 2. *Current first trimester screening program (estimated percentages)*

NON-INVASIVE PRENATAL TESTING (NIPT)

Background

Although the introduction of the prenatal screening program in 2007 was a big step forward, the current system has many disadvantages. Mainly because of the procedure-related miscarriage, other safer options for prenatal screening and diagnosis have been explored. First, years of investigation were done on fetal cells in the maternal circulation, but this was not successful.[15,16,17] Fetal cells can be detected in the maternal blood years after the pregnancy, even after miscarriages and for this reason are not reliable to evaluate chromosomal abnormalities of a specific pregnancy. In 1997, Dennis Lo et al. developed innovative methods for the analysis of fetal cell-free nucleic acids in maternal plasma and serum.[18] At first NIPT for fetal sex determination and rhesus D, technically easier than testing for fetal trisomy, have been investigated.[19] With the subsequent development of real-time quantitative PCR two proof-of-principle publications were published in 2008 using massively parallel shotgun sequencing (MPSS) to detect trace amounts of extra copies of chromosome 21 in the plasma of pregnant women carrying fetuses with T21.[20,21] The identification of T21 is more complicated because there are no unique fetal gene sequences, in contrast to a male fetus or a fetus with a D-gene in an R-D negative mother.[22]

The arrival of the MPSS or 'next generation' sequencing techniques has opened an era of many new options. A clinically applicable technology was developed for non-invasive testing of fetal chromosomal anomalies, using cell-free DNA (cfDNA) fragments of the placenta in maternal plasma. The development of NIPT for clinical use has been driven by several commercial laboratories in the United States and China. They invested many millions of dollars into this project over the last 5-10 years, because they believed that there would be a great demand, thus a market, for a safe and accurate fetal trisomy test. This seems indeed to be correct; in the first 2 years of the availability of their MaterniT21 test, the first company to launch NIPT (Sequenom Laboratories) already performed more than 200,000 tests worldwide.

Technique

In MPSS, the total amount of cell-free DNA fragments, consisting of a 'fetal fraction' thought to be mainly derived from the placenta and the much larger maternal fraction is sequenced. The relative amounts of plasma DNA molecules derived from the various chromosomes are analyzed. Trisomy 21 is caused by the presence of a third copy of chromosome 21. So in case of a trisomy 21 fetus an increased number of sequences are derived from chromosome 21. The maternal genome is mostly euploid, so abnormalities in the proportions originate from the fetal genome. The MPSS method as described above is currently most widely used. A directed cfDNA method, using digital analysis of selected regions (DANSR), combined

with an analysis algorithm, the fetal-fraction optimized risk of trisomy evaluation (FORTE) has been shown to have similar accuracy.[23-26] The third method is the single nucleotide polymorphisms (SNPs) approach, where polymorphic loci are selectively sequenced on the different chromosomes. When measured between 10 and 20 gestational weeks, the average fetal fraction in the maternal plasma is 10% to 15% but can range from under 3% to over 30%. Screening performance is better with increasing fetal fraction. The strongest factor associated with low fetal fraction is high maternal weight.[27]

Performance

Both the sensitivity and specificity of NIPT for fetal T21 exceed 99%.[28] The published studies were generally performed in populations with a known high-risk for trisomies. In most studies archived samples were used, analyzed in batches, with a selected, known to be normal control group.

In 2011, NIPT for fetal trisomy was introduced in clinical practice in the USA, China, and Hong Kong. After introduction of the test, more studies have been performed.[28] The performance of NIPT, for high-risk populations in prospective studies is very accurate for T21 but a higher false positive rate and false negative rate is reported especially for trisomy 13 (T13).
Explanations for false positive NIPT results include technical reasons, the presence of confined placental mosaïcism, or a lost, perhaps unrecognized, co-twin which may provide an increased amount of DNA fragments in maternal plasma. In such cases the test itself can be considered true positive on a cfDNA level, however, the fetus may have a normal chromosome configuration. Some researchers suggest calling such results 'discordant' instead of false positive.

INFORMED DECISION-MAKING

In the light of all these exciting technological opportunities the importance of the social and ethical considerations should not be underestimated.
In the Netherlands the antenatal screening program is designed to provide every pregnant woman the necessary information to make an informed choice about whether or not to request FCT. Women should be able to make an autonomous decision. Multiple factors influence pregnant women in their decision to accept or decline prenatal screening.
Parity, fertility history, family history for chromosomal anomalies, education level, ethnicity and religion are acknowledged to attribute in women's choices for prenatal screening.[30] Until now no studies were performed on the influence of personal costs on the decision-making process. The main reasons to request the test are reassurance and the desire to have knowledge

about the health of the fetus.[31,32] The decision to decline FCT seems to be connected with the woman's view on termination of pregnancy (TOP).[31,32] At present, the vast majority of women confronted with a confirmed diagnosis of fetal trisomy request TOP. In the Netherlands, 93% of women receiving the diagnosis fetal T21 terminate the pregnancy (according to the 2010 annual report on prenatal diagnosis). Some women receive the diagnosis of fetal T21 after 24 weeks of gestation; in this situation it is not legally possible to terminate pregnancy in the Netherlands.

With NIPT, decision-making in prenatal screening is likely to change. Ethical debates revolve around the issue of a possible consequence of this increased testing rate: 'Will the world be without children with Down syndrome in a few years?' There is also concern by some, often quoted in the media, that increased testing with likely reduced numbers of live-born children with T21 may lead to less acceptation of people with T21 in society, or a change towards blaming their parents for their birth.

DUTCH SITUATION

Towards NIPT in the Netherlands

Although other studies were published on NIPT, the Dutch media suddenly broadly covered the subject of NIPT in March 2011, after the publication of Papageorgiou et al.[33] In the same month the first steps towards a national consortium were made. All stakeholders including all Dutch academic centres participated. Several meetings followed to design a national study (the so-called Non-Invasief TRisomie Onderzoek (NITRO)-study) to investigate the feasibility and real time diagnostic accuracy of NIPT, a head-to-head comparison with the FCT in Dutch laboratories. A website was designed as a platform on NIPT for all participating stakeholders and for patients (www.niptconsortium.nl). On the website there is a part secured by a password for participating stakeholders. In 2011 and 2012, meetings with the Ministry of Health, Health Council and Health Insurance Companies were organized to open the dialog about the implementation of NIPT in the Netherlands including a request for Population Screening Act approval. There was, and still is, considerable discussion among professionals as to whether such an approval would be needed, since the proposed application of NIPT would first be only as an alternative for the diagnostic tests in screen-positive women, amniocentesis and CVS.

Population Screening Act

Prenatal screening for untreatable disorders can only be performed if there is a permission of the Minister of Welfare, Health and Sports (VWS) according to the Population Screening Act (PSA; Wet op Bevolkingsonderzoek - 1996).[34,35] The Population Screening Act provides for a permit system for population screening involving the use of ionising radiation, concerning

cancer or concerning serious diseases or abnormalities for which no treatment is possible (www.gr.nl). Ministerial approval is needed for every adjustment on a screening program, so is the case for the implementation of NIPT. The Health Council has an advisory function.

AIM OF THE THESIS

Because of the good test characteristics NIPT will undoubtedly find a place in our healthcare system. The aim of this thesis is to explore and gain more insight regarding the future implementation of NIPT in the Netherlands. Careful preparation for the implementation is essential. Many possible consequences of the implementation of NIPT for pregnant women are unknown.
Research questions were:

- What is the available evidence published about the performance of NIPT for fetal trisomy?
- Is it feasible to send maternal blood samples to laboratories in the United States, and what is the diagnostic accuracy of NIPT using this route?
- Non-invasive prenatal testing for T13; does it do more harm than good?
- Will there be changing attitudes towards termination of pregnancy with the implementation of NIPT?
- What do pregnant women want with the introduction of NIPT?
- What is the influence of personal costs on the decision making process for prenatal screening?
- What is the expected uptake of NIPT?
- What is the attitude of Dutch midwives on the current screening program and on NIPT?
- The Population Screening Act protects the population for potential harm, but also brings a moral dilemma to caregivers. What to do if a test is superior for your patients, but you are not allowed to offer the test?
- And what are the options for Dutch women to have testing by NIPT done outside the Netherlands?
- The next step – an implementation program – what are the most important issues that need to be solved?

OUTLINE OF THE THESIS

Part I. General introduction
Part II. Performance

- Detecting fetal trisomy using cell-free DNA in maternal plasma has been challenging, but after decades of research it is now feasible, and the diagnostic accuracy appears high. We aimed to systematically review the published literature on accuracy of NIPT for the prediction of T21 (1997 – May 2011) using the QUADAS guidelines. This review is described in chapter 1.
- NIPT was not available in the Netherlands at the time of writing the thesis. In 2011/beginning 2012 pregnant women wanting NIPT travelled to the United States. Shipping blood samples across the ocean instead of pregnant women flying to the United States seemed a more feasible way. For this reason the primary aim of the EU-NITE study, discussed in chapter 2 was to evaluate the performance of a directed non-invasive prenatal testing method of cell-free DNA analysis for fetal T21 by shipping whole blood samples from Europe to a laboratory in the United States.
- Chapter 3 discusses some of the potential disadvantages of NIPT. The diagnostic accuracy of NIPT for T13 is reported to be lower. 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.

Part III. Decision Making

- Currently 93% of the women who receive a positive result following an invasive procedure elect for TOP. With the elimination of the risk of an iatrogenic miscarriage, decision-making might change. In chapter 4 we sought to evaluate whether and how the assumed increased rate of detection with the introduction of NIPT would influence the rate of TOP for affected pregnancies.
- In chapter 5 two questions are evaluated. Currently the uptake of the FCT is low, compared to other countries. Earlier studies concluded that the test characteristics of the FCT and the iatrogenic miscarriage risk negatively influence the choice for electing FCT and invasive procedures. If a new test is implemented with better test characteristics the uptake will likely change. We sought to evaluate the attitude of pregnant women towards the future implementation of NIPT. Secondly we sought to evaluate the price that women would be willing to pay for NIPT, which may reflect how women value the risk-free NIPT.
- In Chapter 6 the influence of personal costs is discussed in the decision to undergo FCT. A study was performed comparing the number of women opting for FCT during a period of time where the test was fully reimbursed, with a more recent time-period where women younger than 36 years had to pay for the FCT themselves.

Part IV. Towards NIPT in the Netherlands

- In the Netherlands most pregnant women receive care by independent primary care midwives, including the counseling for the FCT. Until now it was not known what the attitude of primary care midwives is towards the current prenatal screening system and towards NIPT. The aim of the study described in chapter 7 was to investigate the attitude of primary care midwives towards the current system and towards NIPT.
- In 2013, offering NIPT was still forbidden in the Netherlands, since such a change in the national government-approved prenatal screening program requires a new version of the Population Screening Act license. Increasingly, pregnant women became aware of the option to have NIPT performed across the border, in Belgium and Germany. The Dutch NIPT Consortium has requested a license from the Minister of Health, to perform a prospective evaluation project of NIPT in high-risk pregnancies. In chapter 8, we discuss the situation at the time of writing this thesis concerning NIPT, ethical and legal considerations and advise for obstetric care professionals confronted with either requests from patients or their own desires to offer NIPT as an alternative to invasive testing, while awaiting formal permission to incorporate this test into clinical practice.

Part V. Opinion

- In chapter 9 we debated an opinion by Benn et al., published in Ultrasound in Obstetrics & Gynecology with the title 'Non-invasive prenatal diagnosis for Down Syndrome: the paradigm will shift, but slowly'.

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II
PERFORMANCE

E.J.T. Verweij ● J.M.E. van den Oever
M.A. de Boer ● E.M.J. Boon ● D.Oepkes

Fetal Diagnosis & Therapy 2012;31:81-86

CHAPTER 1

Diagnostic accuracy of noninvasive detection of fetal trisomy 21 in maternal blood: a systematic review

ABSTRACT

BACKGROUND

Both pregnant women and providers of obstetric care are aware of the rapid advances in noninvasive prenatal diagnosis (NIPT) of fetal trisomies, and appear to look forward to its clinical introduction.

OBJECTIVES

To review and critically assess the published literature on diagnostic accuracy of NIPT using cell-free fetal DNA or RNA in maternal blood to detect fetal trisomy 21. Method: An electronic search was performed in MEDLINE, EMBASE and the Cochrane library (1997 to April 2011). Of a total of 201 citations, 9 studies were eligible for full-text analysis by 2 independent reviewers, using the QUADAS tool.

RESULTS

Two of the 9 analyzed studies complied with the criteria of the QUADAS tool. Combining the selected 2 studies, with a total of 681 pregnancies included, overall sensitivity was 125/125 (100%, 95% CI 97.5–100%) and specificity 552/556 (99.3%, 95% CI 98.7–99.3%).

CONCLUSIONS

NIPT of fetal trisomy 21, using fetal nucleic acids in maternal plasma, appears to have a high diagnostic accuracy. Large-scale prospective studies are awaited before implementation in clinical practice.

INTRODUCTION

Pregnant women have been offered prenatal diagnosis to detect trisomy 21 (T21, Down syndrome) since the 1970s. T21 is the most common chromosomal abnormality with a birth prevalence of 11–14 per 10,000.[1-3] In most countries, only women considered to be at relatively high risk of a chromosomally abnormal fetus are offered diagnostic testing, since the only available methods are chorionic villus sampling or amniocentesis, invasive procedures with inherent hazards to the pregnancy. Selection of a high-risk group was for many years done based only on maternal age, with low detection rates and hundreds of invasive tests needed to find 1 fetus with T21. Screening has improved by using a combination of maternal serum markers and nuchal translucency measurement, the combination test, in the best programs identifying 90% of T21 cases for 5% false positives.[4] Since the first report in 1997 by Lo et al. of the possibility to use circulating cell-free fetal DNA in maternal plasma for fetal diagnosis, there has been the expectation that someday the complex multi marker, operator-dependent screening test and the invasive testing for karyotyping could be replaced by just taking a maternal blood sample.[5] In the past few years, determination of fetal sex and Rh-D type using cell-free fetal DNA in maternal plasma already has become routine clinical practice.[6-9] Detecting fetal T21 however is technically more challenging, since maternal plasma contains both cell-free fetal and maternal DNA fragments. Various methods to diagnose fetal trisomy using maternal plasma DNA or RNA have been developed. Recently, several relatively small studies have been published reporting on the diagnostic accuracy of these methods. Most authors of these studies agree that there is a need to evaluate the performance of these new tests in large unselected populations. In order to prepare for such studies, we aimed to systematically review and critically appraise the published literature, using the QUADAS guidelines,[10] on the accuracy of non-invasive methods using cfDNA or mRNA from maternal plasma to detect fetal T21.

METHODS

This systematic review was conducted using a protocol with generally accepted methods.[11]

Eligibility criteria

We considered all studies from 1997 until May 2011 in which diagnostic accuracy was determined for non-invasive detection of T21 using nucleic acids, DNA or mRNA, in maternal plasma regardless of the method used. For a study to be included in our review, the non-invasive detection method had to be compared to the gold standard for the determination of trisomies, karyotyping or rapid aneuploidy detection using fluorescent in situ hybridization,

quantitative fluorescent polymerase chain reaction or multiplex ligation-dependent probe amplification on fetal, placental or neonatal cells. Evaluation of the quality of the studies was done using the QUADAS tool.

Information sources and search

Librarians from the Walaeus Library, University of Leiden, searched MEDLINE, EMBASE and the Cochrane Library for relevant papers. The Medical Subject Headings (MeSH) terms 'prenatal (diagnosis)', 'Down syndrome', 'aneuploidy' were used, and combined by Boolean operators ('and' and 'or') with 'non-invasive', 'non-invasive' and 'maternal'. In addition, the reference lists of all primary articles and recent articles, editorials and reviews published on non-invasive prenatal diagnosis (NIPT) were screened to identify articles not found by the initial search. No restrictions were used for publication type or language.

Study selection

Two trained reviewers independently screened titles and abstracts for relevance (E.J.V. and M.A.d.B.). Selected full papers were independently evaluated for inclusion and analysis (E.J.V. and D.O.). Studies were independently assessed by 2 reviewers (E.J.V., M.A.d.B.) for methodological quality against the quality assessment of diagnostic accuracy studies (QUADAS) criteria.[10] Disagreements were resolved by consensus including a third reviewer (D.O.). In case of multiple publications of one dataset we included only the most recently published study.

QUADAS

The QUADAS criteria are a validated evidence-based tool consisting of a 14-item checklist, which encompasses the most sources of bias and variation observed in diagnostic accuracy. The quality assessment items are: representative patient spectrum, description of selection criteria and reference standard, acceptable interval before outcome, partial and differential verification, incorporation bias, adequate test description, blinding of index and reference test, clinical data available and description of uninterpretable test results. All reviewers were trained using the QUADAS tool. Each item was scored 'yes', 'no' or 'unknown' as recommended by the authors of the QUADAS tool.[10]

Data extraction

We included test accuracy studies allowing construction of one or more 2 x 2 contingency tables for each study containing the various methods of non-invasive detection of T21 cross-classifying with the gold standard. We combined results from all selected studies to assess an overall sensitivity and specificity of NIPT to detect T21, with 95% confidence intervals (CI).

RESULTS

Included studies

Figure 1 summarizes the selection process. From the initial 201 publications, 21 full-text articles remained after evaluation of title and abstract. Another 12 studies were excluded after reading the full text focusing on methodology of the laboratory process rather than the performance of the test. The remaining 9 studies were assessed for eligibility and discussed by the expert panel. In case of unknown information we contacted the authors for more information. All studies were scored using the QUADAS instrument.[10] Seven studies [12-18] failed to meet the required criteria for diagnostic test evaluation according to the QUADAS instrument (table 1; fig. 2).

In all these studies sampling was performed only in high-risk pregnancies with an indication for invasive testing and compared with the golden standard of karyotyping. The test description was adequate in all studies. The 9 studies used different methods of non-invasive testing as well as different calculation methods and different cut-offs for standardized fractional genomic presentation (Z score). After scoring all studies following the QUADAS criteria, 2 studies remained for quantitative synthesis. The study with the largest sample size investigated 753 samples of pregnant women with a high risk of fetal T21 with 2 test methods, one 8-plex and one 2-plex procedure.[19] The 2-plex showed the best performance, used in a total of 232 samples of which 86 were from T21 cases. The inclusion criteria were singleton pregnancies with clinical indications for chorionic villus samples or amniocentesis. The investigators used both prospectively recruited samples as well as archived maternal samples. The sensitivity was 86/86 (100%) and the specificity was 143/146 (97.9%). The median gestational age at the time of maternal blood sampling was 13 weeks and 1 day. Insufficient quality of samples was present in 5.6%. Failure to obtain results occurred in 1.5%. The study by Ehrich et al. prospectively tested 480 high-risk pregnancies with 39 women carrying a T21 fetus.[20] They used a multiplexed massively parallel shotgun sequencing assay. High-risk pregnancies were described as pregnancies with clinical indications for chorionic villus sampling or amniocenteses including a positive combination test, maternal age 35 years, family history with T21, a previous T21 pregnancy or ultrasound abnormalities suggestive of T21. The samples were collected prospectively, but were analysed later (all within 10 months after sampling). None of the samples were analysed prospectively or as fresh samples. The sensitivity was 100% and the specificity was 99.7%. One sample was misclassified as T21 (false positive). The median gestational age at blood sampling was 16 (range 8–36) weeks. Ehrich et al. described a sample loss of 2.6% before processing for several reasons (plasma volume 3.5 ml; one sample tube dropped during DNA extraction; samples mixed into each other, and tube broken during centrifugation).[20] In 3.8% the sample was excluded during the process for a variety of reasons including insufficient percentage of fetal DNA, total DNA or library

concentration. Three of the samples excluded from analysis were identified as T21. Overall, combining the results by Ehrich et al. and the 2-plex data from the study by Chiu et al., 681 samples with 125 T21 cases analysed by massively multiplexed parallel sequencing resulted in a sensitivity of 100% (95% CI 97.2–100) and a specificity of 99.3% (95% CI 98.7–99.3; table 2).[19-20]

Summary of evidence

From the recent literature, we can conclude that, after more than a decade of research, NIPT of fetal T21 has become a clinical reality. The 2 studies included meeting all items of the QUADAS criteria aimed to validate the multiplexed massively parallel sequencing. Both studies suggest that T21 can reliably be detected early in the first trimester from maternal plasma with a sensitivity and specificity of nearly 100%. Yet in a number of samples (1.5–3.8%) no results could be obtained. From these excellent results however, we cannot conclude that this new test will have similar performance when implemented into routine obstetric care. The current evidence seems to almost justify such use, however, in the studies performed the samples were mostly stored and then run in a large batch, while for true clinical use, a real-time rapid testing for each patient is needed. This was acknowledged by the authors, who stated that their promising method requires clinical validation in a larger multicenter study. As Chiu et al. concluded, more research is needed to evaluate the use of NIPT as a first-line test for all pregnant women.[19] All studies published thus far were based on high-risk pregnancy samples.

The sensitivity of the current type of screening for the detection of T21 in clinical practice, the combination test, varies from 70 to 91%. The false-positive rate is usually set at 5%, with cut-off values around 1:200 to separate high risk from normal risk. This screening policy, reporting of risks and counselling of pregnant women, is considered by most to be complex and time-consuming. Uptake of screening varies enormously per country, with 30% in the Netherlands to 90% in Denmark. The false-positive rate results in many invasive tests in healthy pregnancies, with one procedure-related miscarriage of a healthy fetus for every 2–3 T21 detected.[21-24]

The method used for non-invasive trisomy detection in the studies by Ehrich et al. and Chiu et al. was massive parallel sequencing of maternal plasma DNA.[19-20] Other studies however used a variety of alternative methods, including tandem single-nucleotide polymorphism array, RNA to single-nucleotide polymorphism allelic ratio approach, reverse transcriptase multiplex ligation-dependent probe amplification, epigenetic-genetic chromosome dosage studies and others. Rapid improvements in these methods, the platforms used and in data analysis, leading to even more reliable, faster and cheaper testing, are expected in the near future. Which method will be preferable for clinical use remains to be elucidated. It is still unclear whether evaluation for fetal trisomy using maternal plasma nucleic acids is feasible

and reliable in the first trimester. Published studies have used blood samples from a wide range of gestational ages, with insufficient numbers to assess accuracy per week gestation. Other important aspects that require further study are failure rate, need for retest rate, and time to reporting to the patient. A next step will be a large prospective study in a low risk population in a real-life setting in which apart from test characteristics throughput capacity, turnaround times, and costs need to be studied. The costs will need to be weighed against the costs of the current practice of performing a combination test and invasive procedures.

Limitations

The main limitation of using the QUADAS tool to evaluate selected studies is that it relies on published data. Some studies may receive a negative score on certain items based on unclear reporting, while the study itself may have met the criteria. We have tried to overcome this by contacting the investigators for more detailed information. Not all authors responded to these requests. Received responses were imported into our results.

CONCLUSION

Both pregnant women and providers of obstetric care are aware of the rapid advances in NIPT, and appear to look forward to its clinical introduction. Therefore, there is some urgency to perform large-scale properly conducted clinical evaluation studies while we still can. Consumer-driven genetic testing and commercial parties offering tests to anyone who pays may interfere with scientific and diagnostic evaluation. We believe now is the time, preferably in multicenter and if needed international collaboration, to design and carry out large-scale studies to rigorously analyse the diagnostic accuracy and cost effectiveness of NIPT. In parallel, we should also thoroughly evaluate all ethical and social implications of the revolutionary changes in prenatal diagnosis that await us.

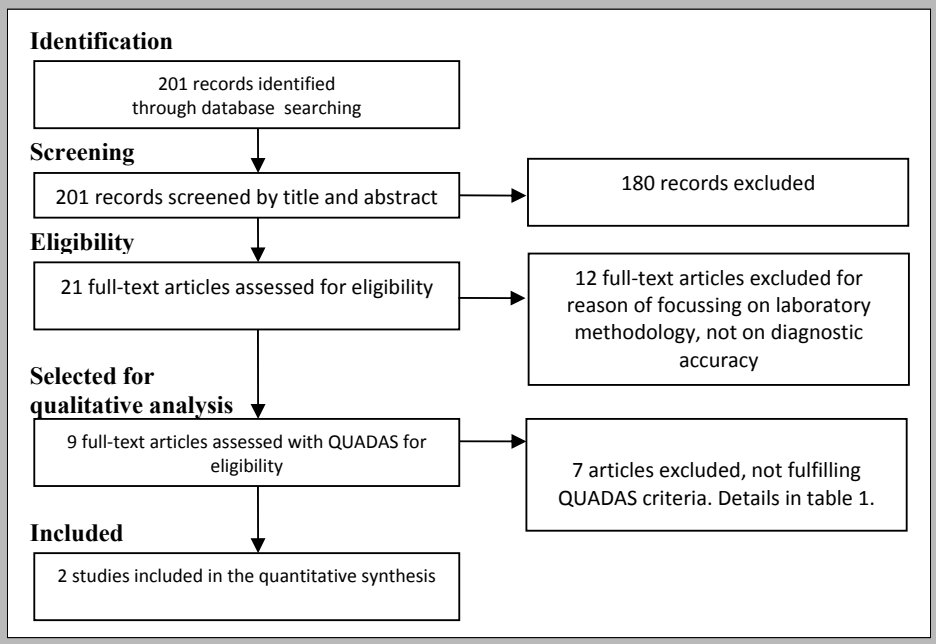

Figure 1. *Flow through the different phases of the systematic review*

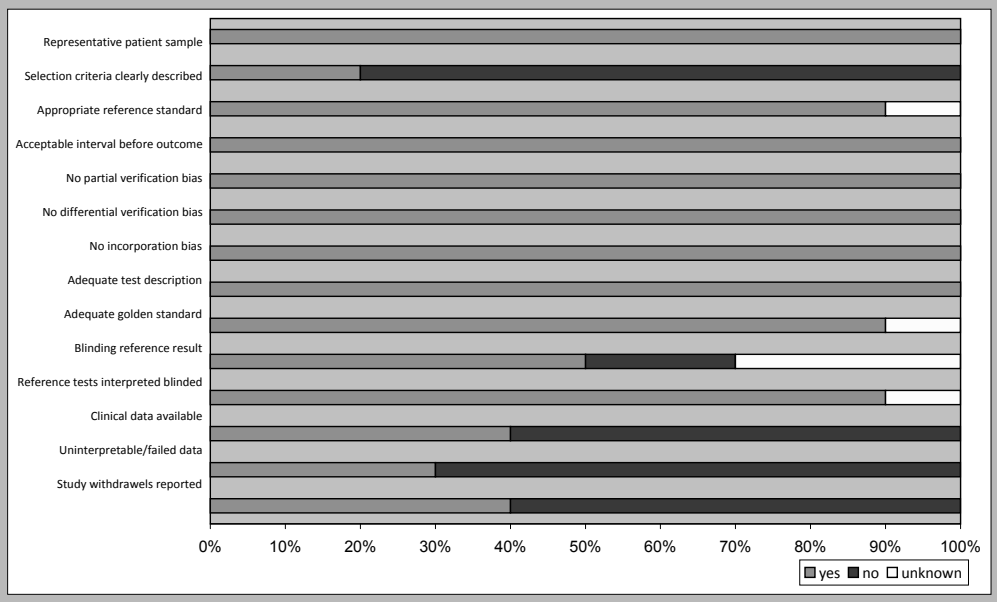

Figure 2. *Summary of quality of the reviewed studies using the QUADAS-instrument*

First author, year	*n*	*n T21*	*Sensitivity (%)*	*Specificity (%)*	*Method*	*Sampling*	*Comments according to QUADAS criteria*
Fan, 2008 [18]	18	9	100	100	Massive parallel genomic sequencing	after invasive procedure	Selection criteria not clearly described, selected samples used failed or retested samples not mentioned
Chiu, 2008 [19]	28	14	100	100	Massive parallel genomic sequencing	euploid samples before invasive procedures and T21 samples before and after invasive procedure before TOP	Selected samples used, failed or retested samples not mentioned
Tong, 2010 [20]	24	5	100	95.8	Epigenetic–Genetic Chromosome-Dosage Approach	not mentioned	Selection criteria not clearly described, no blinded samples, failed samples not mentioned
Tsui, 2010 [21]	153	16	100	89.7	PLAC 4 SNP (RNA) by mass spectrometric and digital PCR methods	before invasive procedure	Failed samples not mentioned, clinical data not clearly described
Ghanta, 2010 [22]	40	7	100	100	Tandem SNP	before and after invasive procedure	Selection criteria not clearly described, selected samples used
Deng, 2011 [23]	121	23	92	100	PLAC 4 SNP (RNA) RT-MLPA	before invasive procedure	Selection criteria not clearly described, failed samples not mentioned
Papageorgiou, 2011 [24]	40	14	100	100	methylated DNA immunoprecipitation	Archived samples, time of sampling not mentioned	Selection criteria not clearly described, archived samples used, failed samples not mentioned.

Table 1. *Overview of excluded studies that were assessed using the QUADAS instrument. N T21: number of women in study carrying a foetus with trisomy 21, TOP: termination of pregnancy, SNP: Single Nucleotide Polymorphisms*

First author, year	*n*	*n T21*	*Sensitivity (%)*	*Specificity (%)*	*Method*
Chui, 2011 [12]					
8-plex	753	86	79.1	98.9	Massive parallel genomic sequencing
2-plex	232	86	100	97.9	
Ehrich, 2011 [13]	480	39	100	99.7	Massive parallel genomic sequencing
Combined data Ehrich and Chiu 2-plex protocol	681	125	100	99.3	

Table 2. *Overview of the included studies*

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E.J.T. Verweij ● B. Jacobsson ● P.A. van Scheltema
M.A. de Boer ● M.J.V. Hoffer ● D. Hollemon
M. Westgren ● K. Song ● D. Oepkes

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CHAPTER 2

European Non-Invasive Trisomy Evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing

ABSTRACT

OBJECTIVE

To evaluate the performance of a directed non-invasive prenatal testing method of cell-free DNA analysis for fetal trisomy 21 (T21) by shipping the whole blood samples from Europe to a laboratory in the USA.

METHODS

A European multicenter prospective, consecutive cohort study was performed enrolling pregnant women from Sweden and the Netherlands. Blood samples were drawn just prior to a planned of invasive diagnostic procedure in a population at increased risk for fetal T21 and then shipped to the USA without any blood processing.

Chromosome-selective sequencing was carried out on chromosome 21 with reporting high risk or low risk of T21. Karyotyping or rapid aneuploidy detection was used as the clinical reference standard.

RESULTS

Of the 520 eligible study subjects, a T21 test result was obtained in 504/520 (96.9%). Risk assessment was accurate in 503/504 subjects (99.8%). There was one false negative result for T21 (sensitivity 17/18, 94.4%, and specificity 100%).

CONCLUSION

This is the first prospective European multicenter study showing that non-invasive prenatal testing using directed sequencing of cell-free DNA applied to blood samples shipped across the Atlantic Ocean, is highly accurate for assessing risk of fetal T21.

INTRODUCTION

In most European countries, pregnant women are offered screening for trisomy 21(T21), regardless of age, using the first trimester combined screening (FCT), consisting of maternal serum markers and nuchal translucency measurement.[1] Screen-positive women are offered invasive testing using chorionic villus sampling (CVS) or amniocentesis. These invasive tests are highly accurate. However, they are associated with an iatrogenic miscarriage rate up to 1%.[2] The majority of invasive tests (>90%) are carried out in pregnancies with a healthy fetus. The prevalence rate of T21 in the group requesting screening is around one in 500, whereas one in 20 women undergoing FCT are categorised as high risk. Many women fear the risks of invasive testing, and a significant proportion therefore refrains from testing or even from screening.[3] In addition, FCT in 'real world' clinical practice has a false negative rate of 10–25%.[4–8] Lastly, a serious limitation of FCT is its restricted time-window of 11–13 completed weeks, in particular for populations reporting late for their first clinic visit. After a decade of research, a clinically applicable technology was developed for non-invasive testing of fetal chromosomal anomalies, using sequencing of cell-free DNA (cfDNA) fragments in maternal plasma.[9] This method promises to eliminate iatrogenic miscarriages caused by invasive diagnostic procedures, and the fear of many women have for invasive testing. Recently, non-invasive prenatal testing (NIPT) for fetal trisomy was introduced in clinical practice in the USA, China, and Hong Kong. The first test used in these countries was based on massively parallel DNA shotgun sequencing (MPSS)[10,11] an apparently accurate but complex and expensive method. An alternative and more efficient approach using more directed evaluation of cfDNA fragments has been developed and shown to have similar accuracy as compared with MPSS.[12–14] A recent large cohort study showed a sensitivity of 100% and a false positive rate of 0.03% for the prediction of T21 using digital analysis of selected regions (DANSR), combined with an analysis algorithm, the fetal-fraction optimized risk of trisomy evaluation (FORTE).[15] This directed cfDNA method has also been recently evaluated in a general screening population and shown to be highly accurate.[16]

In several European countries, NIPT for fetal sex determination and Rh type are now standard practice. However, until recently, no laboratories were able to clinically provide NIPT for fetal trisomy testing. In August 2012, a laboratory in Germany (Lifecodexx AG, Konstanz, Germany) started to offered testing for T21 using MPSS for women from German-speaking countries, at relatively high cost as expected with this method. Several European clinical research sites participated in the large non-invasive chromosomal evaluation study, by Norton et al., on the accuracy of the DANSR and FORTE methods.[15] From this experience, it appeared that the logistics of shipping the whole blood samples for rapid analysis from Europe to the laboratory of Ariosa Diagnostics in San Jose, California, USA, was feasible enough to consider applying this route for introduction of NIPT in European countries without the need, for now,

to perform the testing itself in Europe. In this European multicenter prospective cohort study, our primary aim was to evaluate the performance of a directed method of a non-invasive prenatal test for fetal T21 by shipping the whole blood samples from Europe to a laboratory in the USA.

METHODS

Study population

In this prospective, consecutive cohort study, pregnant women scheduled to undergo an invasive diagnostic test (CVS or amniocentesis) for fetal genotyping were asked to participate by donating a blood sample just prior to the invasive procedure. Women could be included in the study, after informed consent, when ≥18 years old and carrying a singleton pregnancy with a gestational age of at least 10 weeks. Two groups of indications for the invasive test were identified: I: Women with an increased risk for T21 based on first trimester screening (serum screening, nuchal translucency measurement, and/or maternal age) and II: Women choosing to undergo invasive testing after the detection of fetal anomalies on ultrasound examination. Women requesting invasive testing for psychosocial or anxiety reasons, without abnormalities on ultrasound, were included in group I. Exclusion criteria were pregnant women with >1 fetus, an invasive procedure performed prior to the blood sampling, history or active significant malignancy requiring major surgery or systemic chemotherapy, or language restriction with failure to understand the study information. Women were prospectively enrolled at different sites in the Netherlands (Leiden University Medical Center, Leiden and satellite hospitals) and Sweden (Karolinska University Hospital, Stockholm and Sahlgrenska University Hospital, Gothenburg). The study protocol was approved by the respective Institutional Review Boards of the participating centers.

Sample collection

Eligible subjects were asked to participate in the study after the counselling session for the invasive diagnostic test. Approximately 20mL of whole blood was collected in two Cell-Free DNA BCTTM tubes (Streck, Omaha, and NE) from each subject just prior to the invasive procedure. After blood collection, samples were placed into ambient shipping polystyrene containers with two room temperature gel bricks inside the container. Samples were sent the same day to the laboratory of Ariosa Diagnostics, (San Jose, California, USA) without processing. Upon receipt, blood was processed immediately into plasma, and then plasma was stored at 20 °C until all subjects had been enrolled. At time of sample analysis, only those samples received within 5 days of blood collection were deemed eligible. All samples from the invasive diagnostic tests were analysed at the respective certified genetic laboratories

of the participating university medical centers, using either full karyotyping or quantitative fluorescent polymerase chain reaction.

Test method

Each subject's of cfDNA sample was isolated and quantified using the DANSR assay as described previously.[14] Briefly, this method uses ligation of locus-specific oligonucleotides to produce a sequencing template only from selected genomic loci. The FORTE algorithm, also previously described, was used to estimate the risk of T21 in each sample.[13] The FORTE risk score is determined by calculating the odds ratio for T21 based on chromosome 21 cfDNA counts, and fraction of fetal cfDNA in the sample, then applying this as a likelihood ratio to the a priori T21 risk based on the maternal age and gestational age. A predefined cut-off value of one in 100 (1%) was designated as the threshold for classifying a sample as high risk versus low risk. Samples that did not generate a result were classified as low (<4%) fraction of fetal cfDNA, inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, and failed sequencing. The laboratory personnel who performed the analyses were blinded to the clinical information.

Data analysis

On the basis of national screening program data from the Netherlands, we estimated the prevalence rate of T21 in our cohort to be around 5%. The anticipated target performance of the NIPT using DANSR and FORTE was set at 98%. During the course of the European non-invasive trisomy evaluation (EU-NITE) study, we expected to see the publication of the large NICE study, which was using the same testing methodology.[15] Because showing the efficacy of the directed cfDNA approach using the DANSR platform, and the FORTE algorithm was the main aim of the NICE study, we set out to analyse our data to evaluate whether collecting blood samples from European pregnant women and shipping it across the Atlantic Ocean for analysis in the same laboratory would lead to comparable accuracy. Because the results of the NICE study were unknown at the start of our study, we planned to include 1000 samples, which would enable assessment of a sensitivity and specificity for the European cohort with a lower 95% confidence interval (CI) of 93 and 98%, respectively. Using the results of the NICE study and the findings of the interim analysis, the sample size was adjusted after the first 500 samples.

Statistical analysis

Categorical variables were summarised by the number and percentage of subjects. Continuous variables were described as total number and the mean with standard deviation (SD) or range. Correlation between continuous variables (e.g. percent fetal fraction and gestational age at blood sampling)was analysed using linear regression analysis. Sensitivity and specificity were

calculated by standard formulas for a binominal proportion. The accuracy of the test was determined by dividing the sum of the true positive and true negative results by the total number of subjects. Wilson's interval method was used to calculate 95% CI. The comparison of the accuracy of the current study with the NICE study was based on the comparison of unpaired proportions using the Poisson test.[17] Analysis of samples using DANSR and FORTE included all evaluable subjects who had undergone invasive testing with fetal genotype analysis by karyotype or quantitative fluorescent polymerase chain reaction. Prior to study unblinding, all abnormal karyotypes were reviewed by a clinical geneticist and categorized as T21 or other chromosomal abnormality. Other chromosomal abnormalities included sex aneuploidy, trisomy 13 and 18, triploidy, and balanced Robertsonian translocation involving chromosomes 13 and 14. Subjects with chromosomal abnormalities other than T21 were not included in the primary analysis. Results from the DANSR assay and FORTE algorithm were provided as a T21 risk score, with the upper and lower risk value capped at 99% (99 in 100) and 0.01% (one in 10 000), respectively. Calculations for sensitivity and specificity were based on a 1% (one in 100) cut-off to designate results as high risk or low risk for T21.

RESULTS

A total of 595 subjects were enrolled in the Netherlands and Sweden between May 2011 and March 2012. A total of 188 (31.6%) subjects were enrolled in the Netherlands, 283 (47.5%) subjects were enrolled in Stockholm, and 124 (20.8%) subjects were enrolled in Gothenburg, Sweden. There were 75 subjects ineligible for the primary study analysis because of the failure to meet the inclusion criteria (n=21, e.g. non- invasive procedure was performed, twin pregnancy, or blood draw was not successful), insufficient plasma volume (n=19), logistic problems (n=11, e.g. shipping time >5 days or incorrect labelling), or other chromosome abnormalities besides T21 (n=24). Samples were sent to the laboratory using FedEx International Priority Service, with door-to-door times of less than 36h. Of the 520 eligible subjects and corresponding blood samples, a T21 test result was obtained in 504 subjects (96.9% test result rate). Of the two blood collection tubes drawn from each subject, one tube was processed at a time. The second tube was used in 51 cases. Low (<4%) fraction fetal DNA was present in seven/520 (1.3%) samples and nine/520 (1.7%) of samples were excluded because of laboratory processing or specimen issues. Graphical representation of the subject sample flow is given in Figure 1.

Patient characteristics

The mean maternal age of the eligible study cohort was 36.4 years (range 20–47 years). The mean gestational age at the time of blood sampling was 14.0 weeks (range 10–28 weeks).

As showed in Table 1, the vast majority of women (n=441/520, 84.8%) were of Caucasian origin, the remaining were Asian (n=17/520, 3.3%), Mediterranean (n = 31/520, 6.0%), Black (n=7/520, 1.3%), and other (n=24/520, 4.6%). In the group, 64 (12%) subjects underwent an invasive procedure because of an earlier child with a chromosomal abnormality, one of the parents with a chromosomal abnormality or close family with a chromosomal abnormality.
In the group of 520 subjects analysed for T21, there was an even distribution of CVS (n=280, 54%) and amniocentesis (n=240, 46%). Cytogenetic results of these invasive tests confirmed T21 in 18 cases.

Fetal fraction

In the cohort of 504 subjects with samples analysable for T21, the overall fraction of fetal cfDNA was 11.1% (SD 4.1, range 4–30), with seven samples containing less than the pre-specified cut-off of 4%. The fraction of fetal cfDNA did not vary with ethnicity or maternal age. The fraction of fetal cfDNA by gestational age showed no statistically significant difference for gestational ages between 10 and 22weeks.

Test performance

A T21 test result was obtained in 504/520 (96.9%). Risk assessment was accurate in 503/504 cases (99.8%). In Figure 2, the T21 risk probability results are shown. Applying the predefined 1% cut-off to the 18 cases of T21, 17 of the T21 were classified correctly as high risk (sensitivity 94.4%, 95%; CI 72.7–99.9%). One T21 case, determined by CVS, was classified as low risk with cfDNA testing. This false negative case was a real miss with a risk for T21 calculated with NIPT of 0.01%. This individual was a 39-year-old-Caucasian woman with a gestational age of 13weeks and 5 days. The percentage fraction of fetal cfDNA was 4%. Of the euploid cases, all 485/485 were identified as low risk (specificity 100%, 95%; CI 99.4–100%). There were no false-positive cases. In comparison with the results from the larger NICE study, accuracy was 99.8% in the EU-NITE versus 99.9% (p=0.2790).

DISCUSSION

This is the first prospective European multicenter study showing that non-invasive prenatal testing using directed sequencing of cfDNA, applied to blood samples shipped across the Atlantic Ocean, accurately assesses risk of fetal T21. A T21 test result was obtained in 504/520 (96.9%), with an accuracy of 99.8%. This is comparable to the recent large predominantly US study by Norton et al. using the same technology.[15] These results are of great importance in current debates in many countries on how to best incorporate this new, safe, and non-invasive trisomy test in health care programs. The possibility of choosing NIPT for European women

becomes more realistic as it appears feasible to ship the whole blood samples to the USA for processing and analysis by experienced laboratories, who have proven in large studies to master this new technology. In addition, pregnant women in German-speaking countries now have access to NIPT, through shipping of blood samples to the Lifecodexx laboratory in Konstanz, Germany. This laboratory accepts also blood samples from women from other countries, however, they currently need to travel to Germany for counselling and blood draw. Until now, no scientific evaluation of tests performed by this laboratory has been published in peer reviewed scientific literature. In the NICE study by Norton et al., 1.8% of analysed samples had to be excluded because of insufficient fetal fraction of cfDNA versus 1.3% in our study, suggesting that transatlantic shipping does not negatively influence this important parameter.[15] Similarly, assay failure in the NICE study was 2.8% versus 1.7% in the current study.

In the NICE study, all 81 T21 cases were correctly predicted by the DANSR and FORTE methods, and one in 2888 normal cases was classified incorrectly as T21, whereas in our study there were no false positives and one false negative result. This is the first false negative result reported with the DANSR/FORTE approach. In this subject, the percentage of fetal DNA was low (4%). Combining our results with published studies using DANSR/FORTE, a total of 5421 analysed samples of which 175 were T21, the overall sensitivity for T21 is 99.4% (95% CI 97.5–99.9%) and specificity 99.98% (95% CI 99.9–100%).[12,13,15,16]

Although NIPT has a higher accuracy than currently used screening methods, this discordant case 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 actually undergoing any type of testing, including the likelihood of test failure, incorrect results, and findings of unclear significance. For this reason, the introduction of NIPT should be designed carefully and addressed thoroughly by healthcare workers and policy makers. In the current screening and diagnosis programs, women may be falsely reassured by the first line screening test, or may be put through a time of stress and anxiety, fearing both the adverse outcome of either losing their fetus because of the invasive test, or being told that their child has a chromosomal anomaly. Recently, Hill et al. published women's strong preference for tests with no risk of miscarriage, even if such a test would not be entirely accurate.[18] They found that consideration for safety of the fetus is paramount in decision making. As clinicians, we should facilitate in all information so a women can make an informed choice.

The strength of this study is that this is an international multicenter study with one of the largest European cohorts published until now. In contrast with earlier nested-case control studies, this study analysed a prospective consecutive cohort, which is more representative of actual clinical practice. Similar to all studies on NIPT thus far, our study showed that not all

subject samples were provided a test result. When introducing NIPT in clinical practice, an option not possible in published studies arises, namely a rapid redraw of a blood sample in case of test failure. In addition, careful analysis of sampling and logistics may further reduce test failure to a proportion comparable to current invasive testing or even better.

CONCLUSION

The EU-NITE study shows that shipping the whole blood samples across the Atlantic Ocean, using a directed cfDNA approach for analysis is an accurate and feasible option. NIPT rapidly becomes a more realistic option for European women. Detailed prenatal counselling is needed to ensure that women understand the possible implications of a result. Further investigations are needed to determine the accuracy in a general population and to evaluate the causes of assay failures and how best to address them.

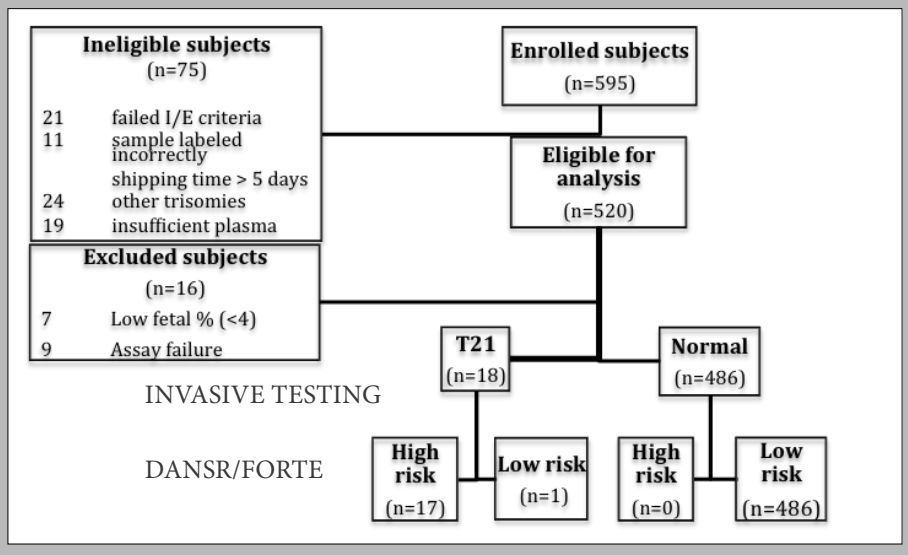

Figure 1. *Graphical representation of the subject sample flow. Eligible subjects for analysis were classified into trisomy 21 and normal based on invasive testing results. DANSR, Digital Analysis of Selected Regions; FORTE, Fetal-fraction Optimized Risk of Trisomy Evaluation; I/E, inclusion/exclusion.*

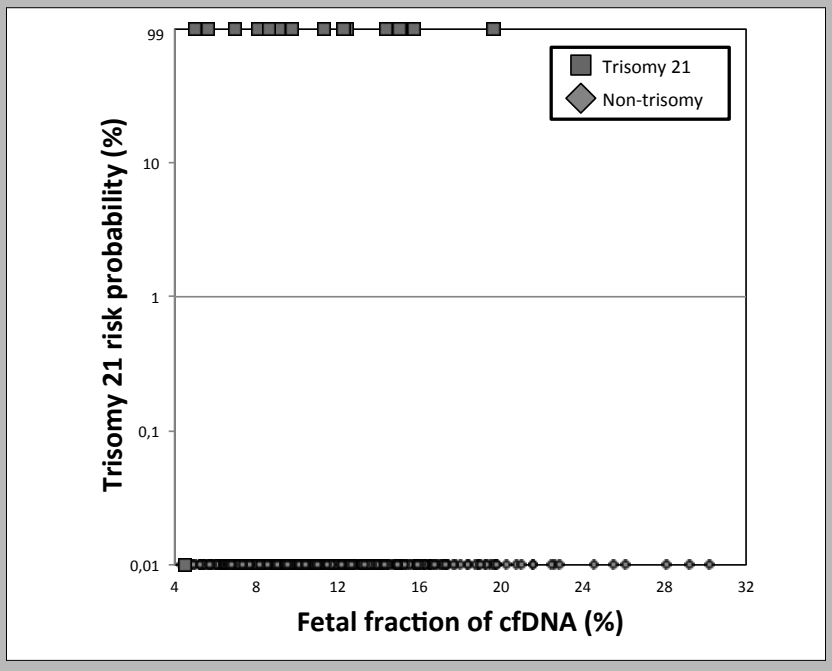

Figure 2. *Trisomy 21 (T21) detection with Digital Analysis of Selected Regions and Fetal-fraction Optimized Risk of Trisomy evaluation, with cut-off line between high risk and low risk at 1%. T21 cases are squares, normal cases are diamonds.*

Demographic	*Euploid (n=502)*	*T21 (n=18)*	*Total (n=520)*
Maternal age y, mean±SD (range)	36.4±4.6 (20-47)	36.7±4.0 (28-43)	36.4±4.6 (20-47)
≥ 35 years (%)	243 (48.4)	5 (27.8)	248 (47.7)
Gestational age, wk, mean±SD (range)	14.0±2.1 (10-28)	13.3±1.6 (11-18)	14.0±2.1 (10-28)
Maternal Ethnicity, n (%)			
Caucasian	425 (84.7)	16 (88.9)	441(84.8)
Mediterranean	31 (6.2)	-	31 (6.0)
Asian	17 (3.4)	-	17 (3.3)
Black	6 (1.2)	1 (5.6)	7 (1.3)
Other	23 (4.6)	1 (5.6)	24 (4.6)
Fetal DNA in sample,%, mean±SD (range)	11.2± 4.1(4-30)	10.2±4,1(4-20)	11.1±4.1(4-30)
Screening for trisomies, n (%)			
First trimester	174 (34.7)	12 (66.7)	186 (35.8)
Second trimester	2 (0.4)	0	2 (0.4)
Other risk factor (e.g. previous affected pregnancy), n (%)	63 (12.5)	0	63 (12.1)
Other (e.g. maternal anxiety), n (%)	45 (9.0)	1 (8.3)	46 (8.8)

Table 1. *Demographic characteristics of eligible subjects*

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E.J.T. Verweij ● M.A. de Boer ● D. Oepkes

Submitted

CHAPTER 3

Non-invasive prenatal testing for trisomy 13; more harm than good?

INTRODUCTION

Trisomy 13 (T13; Patau syndrome) is a rare and lethal disease. Fetuses with T13 often die in utero, and neonates rarely survive beyond the first few months following birth.[1] The associated structural anomalies can be almost always reliably detected by ultrasound examination. In this paper, we discuss whether early testing for T13 using cell-free DNA (cfDNA) testing in maternal serum has benefits that outweigh the potential harm associated with false positive results, which may lead to unnecessary invasive procedures and anxiety.

Non-invasive prenatal testing (NIPT) using chromosome selective sequencing of cfDNA has been shown to be an excellent test to predict presence or absence of fetal trisomy 21 (T21; Down syndrome) from as early as 10 weeks gestation. The major reason for many pregnant women to request testing for T21 is, apart from being a relatively common genetic condition, the fact that T21 is *not* a lethal disease, but a non-treatable serious handicap associated with a life expectancy of over 50 years. NIPT can also be used to evaluate the risk for other chromosomal anomalies, including T13. Studies published thus far however reported a lower detection rate and, more importantly, higher false positive rate (FPR) and higher false negative rate than reported for T21 and T18.[2-7] Explanations for false positive NIPT results include technical reasons such a relatively high Guanidine-Cytosine (GC) content of chromosome negatively influence sequencing reliability. In addition, the presence of confined placental mosaicism, or a lost, perhaps unrecognized, co-twin may provide an increased amount of DNA fragments in maternal plasma from chromosome 13. Strictly speaking, in such cases the test can be considered true positive on a cfDNA level, however, the fetus itself may have a normal chromosome configuration. Consequently, physicians, professional organizations like ACOG, and companies offering NIPT advocate confirmation of positive NIPT results by amniocentesis, rather than confirmation by chorion villus sampling.

One of the most important advantages of cfDNA testing is the expected significant reduction of invasive diagnostic procedures and the associated iatrogenic miscarriages in healthy pregnancies. For T21, this is undoubtedly true. For a more rare condition such as T13, combined with a higher FPR, there may not be a reduction in invasive procedures. We would like to illustrate this with a case report, followed by a mathematic assessment of the potential harm caused by large scale application of NIPT for T13.

Case

In the fall of 2012, a German 35 year old pregnant women, primigravid, pregnant after IVF, contacted us because of a T13/T18 risk of 1:55 based on the first trimester combined test. After searching the internet she found that the Leiden University Medical Center just started a pilot project sending maternal blood samples for NIPT to the United States (MaterniT21

Plus test, Sequenom, San Diego, CA). At that time, the German Praena NIPT test (Lifecodexx, Konstanz) was only available for T21 testing. She and her partner decided to have blood drawn in Leiden at 14+5 weeks' gestation. Ultrasound to verify the gestational age revealed a CRL on the 2.3 percentile, with no obvious structural anomalies, and no evidence for a second gestational sac. After 13 days we received an email by the head of the laboratory, and a fax the next day, with the result. The test was positive for T13. The fetal fraction was 7%.

We advised her to contact her obstetrician in Germany, and we stressed the fact that before considering any irreversible action, confirmation by amniocentesis was warranted. Alternatively, she could have an advanced ultrasound and refrain from invasive testing if no abnormalities were seen. Obviously, the next days were particularly stressful for the couple. Detailed ultrasound examination revealed no obvious anomalies. After counselling, the patient elected to undergo an amniocentesis 5 days after receiving the NIPT result. The QF-PCR result was available 3 days thereafter revealing no signs of a trisomy, and full karyotyping confirmed a normal 46XY result. The couple still expressed being grateful for the opportunity of NIPT, despite the false positive result and the associated 8 days of significant anxiety.

DISCUSSION

In table 1, the available literature on the diagnostic accuracy of NIPT for T13 is summarised. A total of only 71 T13 cases have been reported thus far. Although we realize that several different laboratory techniques were used in the different studies, and that techniques have been improved over time, we elected to include all published studies in our primary analysis, which gave an overall detection rate of 91.6%, with a 0.097% false positive rate. All of the tested samples thus far were selected from stored samples obtained from high-risk pregnancies; consequently the prevalence of T13 in this cohort was high. Ashoor et al acknowledged that the total number of cases of T13 examined in their series was too small for accurate assessment of the detection rate.[7] Walsh et al evaluated the published evidence for NIPT as either a 'primary' or an 'advanced' screening test for the different trisomies. They concluded that future studies must address its use as a primary screening test for T13 in high-risk women.[10]

We conclude that the performance, thus the benefit of NIPT for the prediction of T13 is not clear yet, even in high-risk populations. Yet benefits in a low risk population are more doubtful.

Our concerns about the use of NIPT for T13 are clarified with a hypothetical calculation. When we extrapolate the test characteristics to a general average-risk pregnant population of 1.000.000 women, with an expected prevalence in the first trimester, of 1 case of T13 per10.000, and all women would be tested using NIPT, 1059 women would receive a positive

test result for T13 (92% detection rate of 100 T13 cases = 92 true positives, 0.097% false positive rate of 1,000,000 women = 967 false positives.) The positive predictive value will be 92/1059 = 8.7%. If all these women would elect further testing by amniocentesis, with a one percent additional risk for miscarriage, 10 (0.913x0.01x1059) healthy fetuses would be lost for 92 T13 cases detected. In addition, the other 957 women not losing the pregnancy but experiencing serious stress for at least some days to even a few weeks, represent another disadvantage.
In contrast, we can extrapolate the test characteristics to a high-risk pregnant population of 1,000,000 women, with an expected prevalence in the first trimester, of T13 of 1:200, or a 5000 fetuses with T13. If all women would be tested using NIPT, 5540 women would receive a positive test result for T13 (91.6% detection rate of 5000 T13 cases = 4578 true positives, 0.097% false positive rate of 1,000,000 women = 962 false positives.) The positive predictive value will be 4578/5540 = 82.6%. If all these women would elect further testing by amniocentesis, with a one percent additional risk for miscarriage, 10 healthy fetuses would be lost for 4578 T13 cases detected.

These hypothetical calculations show that although the test performance is similar, the positive predictive value is highly depending on the prevalence of the disease. Before introducing NIPT in a screening and diagnosis program we should balance harm and benefits.
Especially in a low risk population the only benefit for the few women that have the misfortune to carry a fetus with T13 is earlier detection, thus the option for earlier termination, when NIPT is offered at 10 weeks' gestation. The end result, losing the fetus, is the same for all. Given the issue of confined placental mosaïcism, a chorionic villus sampling is not recommended. Amniocentesis can be done from 15 weeks' gestation onwards, providing a rapid aneuploidy detection results close to 16 weeks. Since all T13 cases are associated with multiple anomalies that are hard to miss on detailed ultrasound examination, T13 will be detected at the routine 20-week scan, and often even earlier. Papageorghiou et al described that >90% of T13 cases are identified at the 11 to 14-week scan.[11] Given the unfavourable balance between benefit and harm related to using NIPT to test for T13, we suggest reconsidering its use especially in a general population. We lose health children for a few week earlier detection.
One option, likely the most practical, would be to discuss these issues in the pre-test counselling for both high and general risk women, and to arrange a detailed ultrasound as soon as possible after a positive T13 result. Refraining from invasive testing in the absence of any ultrasound abnormality would seem justifiable, however, there may be residual anxiety experienced by the parents until birth. A second option would be to design the NIPT in such a way that only those abnormalities are tested that, after careful consideration by professional bodies and policy makers, are regarded clinically relevant and for which the test has acceptable false positive rates. When introducing NIPT for low risk populations we suggest to consider

not including T13 testing or rather not reporting this result, although we understand the difficulties of this concept.

CONCLUSION

NIPT is a revolutionary improvement of prenatal care, undoubtedly soon finding its way into routine practice. Importantly however, irrespective of what policy will eventually be implemented, all obstetricians and genetic counsellors should be fully aware of their responsibility to properly counsel women both before and after NIPT, including the consequences of the less than perfect test characteristics, in particular for T13. Screening for diseases that are lethal in the fetal or early neonatal period, at the expense of serious anxiety and iatrogenic miscarriages of healthy fetuses may do more harm than good.

Finally, clinicians in our view could reassure the patient and refrain from invasive testing in case of a positive NIPT result for T13 and a completely normal detailed ultrasound examination.

	Method	*Detection rate*	*False pos. rate*	*PPV**	*NPV**
Chen et al (2011)[2]	MPS	100% (25/25)	1.1% (3/264)	89.3% (25/28)	100% (261/261)
Lau et al (2012)[3]	MPS	100 % (2/2)	0%	100% (2/2)	100% (106/106)
Bianchi et al (2012)[4]**	MPS	78.6 % (11/14)	0%	100% (11/11)	99.4% (485/488)
Palomaki et al (2012)[5]	MPS	91.7 % (11/12)	0.1% (1/1959)	91.7% (11/12)	99.9% (1958/1959)
Jiang et al (2012)[6]	MPS	100% (2/2)	0%	100% (2/2)	100% (901/901)
Zimmerman et al (2012)[7]	Selective	100% (2/2)	0%	100% (2/2)	100% (143/143)
Liang et al (2013)[8]	MPS	100 % (4/4)	0.3% (1/408)	80.0% (4/5)	100% (407/407)
Ashoor et al (2013)[9]	Selective	80 % (8/10)	(0.1%) (1/1938)	88.9% (8/9)	99.9% (1937/1939)
Total sample		**91.6% (65/71)**	**0.097% (6/6204)**	**91.6% (65/71)**	**99.9% (6198/6204)**

Table 1. *literature published until April 1, 2013 on the diagnostic accuracy of NIPT for T13*
**PPV: positive predictive value, NPV: negative predictive value*
*** 2/2 unclassified samples were both positive for T13 by karyotyping*

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III
DECISION
MAKING

E.J.T. Verweij ● D.Oepkes ● M.A. de Boer

Prenatal Diagnosis 2013;33:996-1001

CHAPTER 4

Changing attitudes towards termination of pregnancy for trisomy 21 with non-invasive prenatal trisomy testing: a population-based study in Dutch pregnant women

The aim of providing testing for chromosomal conditions is enabling reproductive choice with respect to carrying to term, or terminating the pregnancy of a child with a serious disorder or disability. Except for a few countries such as Denmark and Hong Kong, the uptake of screening for fetal trisomy is relatively low, ranging from 25% (The Netherlands) to around 50% in many other Western countries. Reasons for refraining from screening include a number of perceived disadvantages of current screening programs, of which the risk of iatrogenic miscarriage associated with follow-up testing with invasive diagnostic procedures (0.5 to 1%) is an often reported one.

At present, the vast majority of women confronted with a confirmed diagnosis of fetal trisomy request termination of pregnancy (TOP). In the Netherlands, 93% of women receiving the diagnosis fetal T21 terminate the pregnancy (according to the 2010 annual report on prenatal diagnosis), which is similar to published European data.[1] A recently published systematic review presented evidence of decreasing termination rates in the USA (67%), which was speculated to be associated with progress in the medical management of Down syndrome children.[2] Another study underlines women's strong preference for tests with no risk of miscarriage demonstrating that consideration for safety of the fetus is paramount in decision making.[3] With the newly developed non-invasive prenatal testing (NIPT) approach using cell-free fetal DNA obtained from maternal plasma, decision-making in prenatal screening is likely to change. Both the sensitivity and specificity of NIPT exceed 99%.[4] However, ethical debates revolve around the issue of a possible consequence of this increased testing rate: 'Will the world be without children with Down syndrome in a few years?' There is also concern that increased testing with likely reduced numbers of live-born children with T21 may lead to a reduction in scientific progress, and funding, aiming for treatment of children with Down syndrome. We sought to evaluate whether and how the assumed increased rate of detection with the introduction of NIPT would influence the rate of TOP for affected pregnancies. This information may aid in the planning of new screening strategies.

In two hospitals and nine community midwife practices, self complete questionnaires were administered to pregnant women shortly after women received counseling for first trimester combined test (FCT) by their own midwife or doctor between 1 August 2011 and 31 December 2011. All women received information about prenatal screening for trisomies following the current guidelines. Questionnaires were given to all women, independent from their expressed interest in prenatal screening. All questionnaires were handled anonymously. The questionnaire addressed questions regarding prenatal screening in the current pregnancy and regarding NIPT if available. Background information about NIPT was included prior to questions to determine the attitude of women towards NIPT. Participating women were asked to indicate the likelihood that they would choose the option of terminating their pregnancy should their fetus be diagnosed with Down syndrome based on a visual analog scale (VAS).

The VAS is a graphic tool with a 100mm horizontal line with the left end marked as 'very uncertain' and the right end marked as 'very certain'. The subject is asked to mark the point that is corresponding most with their feeling about the subject questioned. The last part of the questionnaire included sociodemographic questions (age, educational level, religion, and income). The Dutch legislation does not require informed consent for a prospective study using questionnaires when results are treated anonymously. Data were analyzed using SPSS version 17. Completed questionnaires were received from 147 (43%) of the 340 women who were sent a questionnaire. In this group of responders, 79/147 (54%) opted for FCT in their current pregnancy; 82% (121/147) of the women answered they would elect to undergo NIPT if it were available. There were no women opting for FCT in the current pregnancy and declining NIPT, if available. The data of the women who preferred (82%) or declined (18%) NIPT were analyzed separately. Figure 1 shows the frequency distribution of the likelihood of TOP among the 121 women with a positive attitude towards NIPT. Among women electing to receive NIPT if available, those who elected to undergo FCT in their current pregnancy were more likely to request TOP (median likelihood score of 70, range: 0–100) than those not performing FCT in their current pregnancy (median score of 34, range: 0–100). Women who chose not to perform either FCT or NIPT were extremely unlikely to terminate a pregnancy of a T21 fetus, with a median score of 0 (range: 0–95). Women currently electing FCT were more likely to terminate a T21 pregnancy than those who currently rejected FCT but elected for NIPT screening for T21 (p=0.03). In both groups, the attitude towards TOP was not related to age, education level, income, or religion.

Our study suggests that implementing NIPT may result in a higher uptake of prenatal screening. The percentage of women who opt to terminate their pregnancy upon detecting T21 will likely be reduced if NIPT becomes available for all. With the introduction of NIPT, nearly complete elimination of iatrogenic miscarriages due to invasive prenatal diagnosis, and in particular the fear of women for these risks, will lead to more balanced, autonomous reproductive choices. 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. Most women wish to be reassured regarding the health of their baby, as reflected by the high number of women who choose to undergo a mid-trimester structural anomaly scan.

This statement also holds true for women who choose not to terminate after receiving an antenatal diagnosis of T21 because they value the certainty of the diagnosis during pregnancy and trust their ability to prepare themselves adequately. Although we acknowledge that because of cultural differences, our results cannot be extrapolated to all countries, the introduction of NIPT must be designed carefully and its implications addressed thoroughly

by healthcare workers and policy makers. Counseling for prenatal screening to facilitate informed reproduction choices should maintain the fundamental basis of prenatal screening programs. Specifically, women should retain their 'right not to know'. 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 before testing. In our experience, in the current situation of offering FCT, many pregnant women are poorly informed regarding the implications of Down syndrome itself. The counseling is focused on explaining the test rather than on the condition itself. With the introduction of NIPT, counseling about the test will be easier, 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 cannot be predicted antenatally.

Korenromp et al. reported that when Down syndrome is diagnosed, medical caregivers are among the most important individuals to the woman in guiding her decision whether to terminate the pregnancy.[5] 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, the woman must be accompanied by supportive counselors. Preparing for a life with a child with Down syndrome requires up-to-date information regarding Down syndrome, an explanation of potential ultrasound abnormalities, and - if desired - a referral, for example, to a patient support group. On the other hand, for many women, the choice to terminate the pregnancy is associated with long-lasting psychological issues. As we have described, NIPT has many advances compared with current testing; therefore, it can even be perceived as unethical to withhold it from pregnant women. However, NIPT needs to be carefully incorporated into a well-designed screening program that is based on informed decision-making. A non-directive-based counseling approach by healthcare workers will be as important as ever. Limitations in our study are the relatively small sample size and the limited response rate. The difference between the national uptake of FCT (around 30%)[6] and the uptake in our study population (54%) may be explained by the fact that women who perform FCT are more willing to complete a questionnaire about FCT than women who reject FCT. A study with a larger sample size or with choice experiments should be undertaken to obtain more information about this important topic.

In conclusion, the reproductive choices of pregnant women will likely change following the introduction of NIPT. The uptake of prenatal screening will likely increase. However, this does not necessarily mean that the abortion rate of T21 fetuses will rise similarly. Our

study shows that the introduction of NIPT is likely to cause a shift in decision-making in which more women will choose prenatal screening to gain knowledge without the intention to terminate the pregnancy. NIPT needs to be carefully incorporated into a well-designed screening program that is based on informed decision-making.

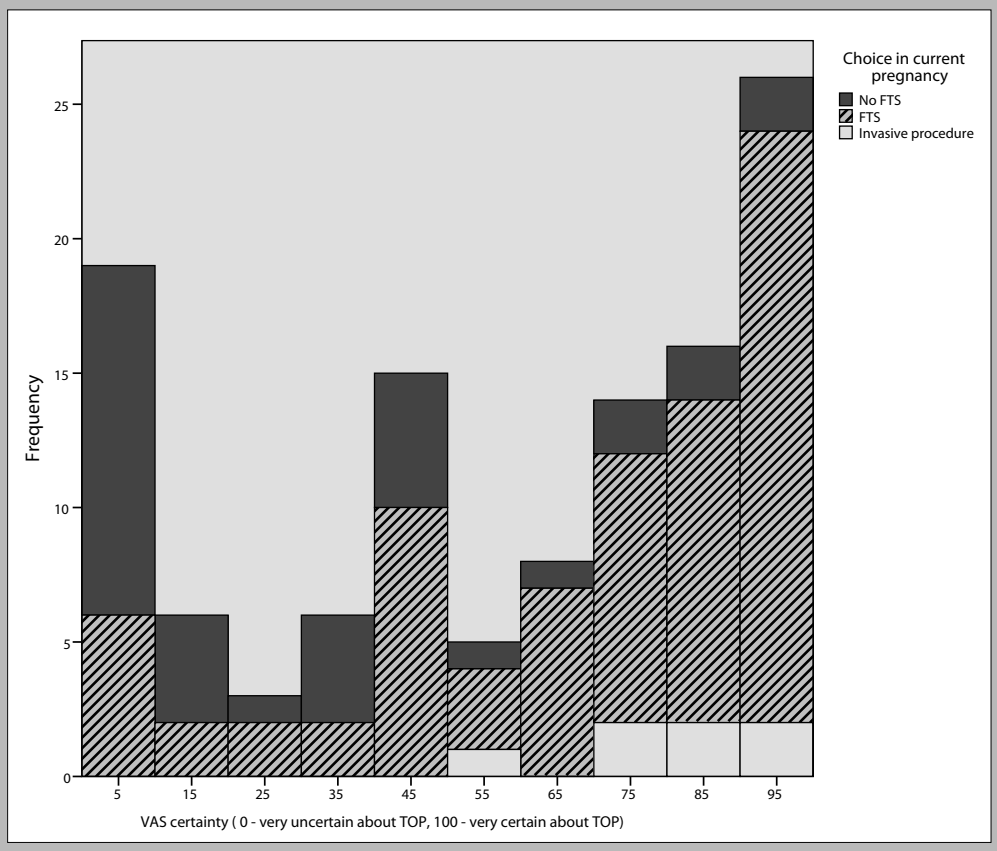

Figure 1. *Likelihood of termination of pregnancy (TOP) for T21 in pregnant women who would opt for NIPT if available. The number of women for the 3 different groups (frequency; y-axis) is plotted against VAS certainty (x-axis). A VAS score of 0 indicates high uncertainty regarding TOP and a VAS score of 100 indicates high certainty regarding TOP.*

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E.J.T. Verweij ● D. Oepkes ● M. de Vries
M.E. van den Akker ● E.S. van den Akker ● M.A. de Boer

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CHAPTER 5

Non-invasive prenatal screening for trisomy 21: what women want and are willing to pay

ABSTRACT

OBJECTIVE

To investigate the attitude among pregnant women regarding non-invasive prenatal testing (NIPT) for detecting trisomy 21 (T21) and to quantify their willingness to pay for NIPT.

METHODS

A questionnaire was administered to pregnant women who received counselling for first-trimester combined test (FCT) in two hospitals and nine midwife practices in the Netherlands.

RESULTS

A total of 147 women completed the questionnaire, yielding a response rate of 43%. If NIPT for detecting T21 were available, 81% stated they would choose to have this test, and 57% of women who elected not to undergo FCT in their current pregnancy would perform NIPT if available. Willingness to pay for NIPT was correlated with age and income, but not education level. The price that participants were willing to pay for NIPT was similar to the current price for FCT.

CONCLUSION

The pregnant women in our study had a positive attitude regarding NIPT for T21, and more than half of the women who rejected prenatal screening would receive NIPT if available.

PRACTICE IMPLICATIONS

Due to the elimination of iatrogenic miscarriage, caregivers should be aware that informed decision-making can change with respect to prenatal screening with the introduction of NIPT.

INTRODUCTION

Non-invasive prenatal testing (NIPT) can use cell-free foetal DNA circulating in Maternal blood to detect chromosomal trisomy, and NIPT was recently introduced into clinical practice. NIPT has both high sensitivity and high specificity.[1] In the Netherlands, first-trimester combined test (FCT) is currently offered to all pregnant women as part of a national antenatal screening programme that is based on the "informed choice" principle, meaning that the individual's decision is voluntary and made with full understanding of the circumstances, including all expected benefits, burdens, risks and available alternatives. Invasive testing using chorion villus sampling (CVS) or amniocentesis is offered when the risk of trisomy is ≥1:200. In the Netherlands, approximately 25% of women elect to receive FCT, which is low compared to other countries, and women over the age of 36 have the right to request CVS and/or amniocentesis. Decision-making regarding prenatal screening includes pre-paring for the next step, which is an invasive procedure in the event of increased risk of trisomy 21 (T21, or Down syndrome). At this stage, the decision requires balancing the probability of having a child with T21 against the risk of a procedure-related (iatrogenic) miscarriage. The most frequently cited reason for screening is to gain both knowledge regarding the health of the foetus and reassurance.[2] The principal reasons for declining screening include unfavourable characteristics of the screening test, ethical and/or religious objections, post-testing anxiety or uncertainty, and risks associated with invasive testing.[2] These arguments suggest that if a near 100% accurate, non-invasive test for foetal trisomy were available, women may make different choices regarding prenatal screening. Depending on cost and/or availability, NIPT may eventually replace current screening methods. Although nearly everyone in the Netherlands has medical insurance, the cost of FCT (approximately €150) is only reimbursed for women ≥36 years of age. We therefore asked whether – and how much – women would be willing to pay for NIPT for T21 with risk-free diagnostic certainty. The price that women are willing to pay might also reflect how women value the test's risk-free diagnostic certainty.

MATERIALS AND METHODS

Data were obtained from questionnaires that were completed by pregnant women. Information regarding prenatal screening for T21 was provided in accordance with current guidelines. The questionnaires were distributed by midwives and doctors following patient counselling for prenatal screening within the patient's first trimester. Questionnaires were distributed to all women in their first trimester, independent of their expressed interest regarding prenatal screening. The women were recruited from August 2011 through December 2011 from two hospitals and seven midwife practices in two regions (Leiden and Amsterdam) in the Netherlands. All questionnaires were treated anonymously (no name or address was listed

on either the questionnaire or the envelope). The questionnaires were returned to one central hospital in pre-paid envelopes. In total, 340 women were invited to participate. Background information regarding NIPT was provided, followed by questions designed to determine the participant's attitude towards NIPT. NIPT for T21 was described as a safe test with high (nearly 100%) diagnostic accuracy. The first part of the questionnaire addressed women's attitudes towards receiving information regarding prenatal screening and the reason(s) they might accept or decline prenatal screening in their current pregnancy. The participants were asked to indicate whether they would prefer NIPT replacing screening and/or invasive testing. Content analysis was used. The visual analogue scale (VAS) was used; the VAS is a graphic tool with a 100-mm horizontal line; the left end is labelled "very uncertain", and the right end is labelled "very certain". The participants were instructed to indicate the point on the scale that corresponds best with their feelings regarding the question.[3] Willingness to pay (WTP) was assessed using a payment card, consisting of a list of nine costs ranging from €50 to €500. For each amount, the women were asked to indicate whether they would be willing to pay this amount for non-invasive screening for trisomy 21. If they indicated a willingness to pay more than €500, they were asked to indicate the maximum amount they would be willing to pay.[4-6] The last part of the questionnaire included sociodemographic questions regarding age, education level, religious preference and household income. Education level was determined by asking respondents to indicate their highest completed level of education. Religious preference was determined by asking respondents to describe themselves as belonging to one of the following eight categories: no religion, Catholic, Protestant, other Christian, Islamic, Hindu, Humanist, or Other (specify). Income was determined by asking the respondents to indicate the range corresponding with their monthly net household income. The following hypotheses regarding the relationship between the aforementioned sociodemographic factors and WTP were tested:

- Higher-income respondents have a higher WTP.
- Highly educated respondents have a higher WTP.
- Older participants have a higher WTP.
- Religious participants have a lower WTP.

The questionnaires were developed and pre-tested in both healthcare workers and pregnant women (n=10/group) to determine the clarity of information, and several questions and answers were then optimised based on this pre-test. The Dutch legislation does not require informed consent for a prospective study using questionnaires if the results are handled anonymously. Data were analysed using SPSS 17.0. 2.1.

Participants

Table 1 shows the demographics of the participants. The mean age of the participants was 32.9 years, which is older than the average age of pregnant women in the Netherlands (31 years).[7]

The percentage of women <36 (68.7%) and ≥36 years of age (31.3%) was consistent with the age distribution of pregnant women in the Netherlands.[8] Relatively few participants had a low level of education and/or low income.

RESULTS

In total, 340 women were given a questionnaire and invited to participate in the study, and 147 women (43%) completed and returned the questionnaire. In total, 79 respondents (54%) opted for FCT in their current pregnancy, 7 respondents (5%) opted for an invasive procedure (all of whom were ≥36 years of age), and 61 respondents (42%) rejected prenatal screening, including 5 respondents who also declined information regarding the availability of prenatal screening. Forty-eight respondents (33%) were recruited by the two hospitals, and the remaining 99 participants (67%) were recruited by their midwife. The reasons stated (via an open-text field) for choosing screening were "we want to obtain knowledge regarding the baby's health" (41%); "'I have a higher risk for having a T21 baby because of my age" (24%); "we want reassurance" (5%); "if we receive a diagnosis of T21, we will terminate the pregnancy" (8%); "preparing for a possible child with Down syndrome" (4%); "if the child has T21, I do not want to burden my other children with the care of this child" (4%); and "I received screening during a previous pregnancy" (1%); 13% did not provide a reason. The reasons for declining prenatal screening (indicating more than one reason was possible) included (n = 61) "not wanting to gain knowledge regarding T21 (15%); "I do not want to perform an invasive follow-up test" (23%); "I am opposed to terminating a pregnancy" (33%); "women felt that their risk of having a T21 child was too low to warrant testing" (41%); unfavourable features of the test (46%); and "I cannot or do not want to pay for FCT" (10%). All 86 participants who opted for FCT in their current pregnancy expressed a positive attitude towards NIPT. Among the respondents who did not receive prenatal screening, 57% (n=35) said that they would choose NIPT if available. Finally, 26 participants who did not opt for FCT in their current pregnancy would also not opt for NIPT if available. As noted above, 121 of the 147 participants (82%) expressed an interest in performing NIPT, and 89 participants (61%) were interested in NIPT only as a replacement for invasive procedures or screening. A few respondents (6.4%) preferred to receive FCT before NIPT in order to have an additional ultrasound in their first trimester. Thirty-two women (22%) specifically stated in an open-text field that they would prefer NIPT as a replacement screen. The following arguments were stated: "easier and more efficient"; "why do you need a risk assessment when you get certainty with NIPT?"; "the result will be available earlier in the pregnancy"; and "less time living with uncertainty". Of the women who were recruited in a hospital, 94% had a positive attitude towards NIPT, compared with 77% of women recruited by their midwife (p=0.011).

Participants were asked to indicate on a VAS scale their certainty with respect to accepting or declining NIPT if it were available. Among the participants with a positive attitude regarding NIPT, the median score was 95.0 (range: 10–100), indicating high certainty for accepting NIPT. Among the participants with a negative attitude towards NIPT, the median score was 97.5 (range: 0–100), indicating that this group was also highly certain about rejecting NIPT. The mean price that participants were willing to pay for NIPT was €169 (median: €150; range: €0–1000). Three of the 121 participants (2%) were willing to pay €1000 for the NIPT test for T21. Table 2 shows the relationship between willingness to pay and age, income, education level and religious preference for the 121 participants with a positive attitude towards NIPT. We found no significant correlation between willingness to pay and either education or religious preference. However, as we hypothesised, willingness to pay correlated significantly with both income (p<0.001) and age (p=0.049). Interestingly, women age 36 and older were willing to pay more for NIPT (mean WTP: €218) than women <36 years of age (mean WTP: €185).

DISCUSSION AND CONCLUSIONS

Discussion

This study is the first in which pregnant women were asked in the first trimester whether they would opt for NIPT if it were available. The timing of the questioning regarding a sensitive topic such as FCT is extremely important, as confronting a pregnant woman regarding the uncertainty of her baby's health could change her opinion of FCT. If available, the vast majority (81%) of women in our study indicated that they would choose to undergo NIPT. This positive attitude towards NIPT is consistent with a study published by Kooij et al., who studied both pregnant women (past the first trimester) and not-pregnant students (82 and 79%, respectively, had a positive attitude towards NIPT)[9], and with a study published by Tischler et al., who studied women in their third trimester (72% of whom had a positive attitude towards NIPT).[10] An intriguing and novel finding of our study was that more than half (57%) of the women who rejected prenatal screening in the current system would elect to have NIPT if it was available. This suggests that more than half of the women rejected FCT because of unfavourable test characteristics and/or to avoid undergoing an invasive test. The question of whether to accept or decline NIPT became more relevant with the recent introduction of commercially available NIPT in the United States.[11] Moreover, in several European countries, NIPT is offered as a commercially available alternative to invasive procedures in high-risk pregnancies. Interestingly, the women surveyed in our study were positive regarding the introduction of NIPT in general, but were even more positive regarding the introduction of NIPT as a screening tool. The reasons cited included reduced anxiety and uncertainty because

of the one-step method and higher diagnostic accuracy compared with current FCT methods. The willingness to pay for NIPT revealed information regarding how women value NIPT for detecting trisomy 21 and the test's risk-free diagnostic certainty. The mean amount that women were willing to pay was slightly higher than the current average cost of FCT (which is €150), and some women were prepared to pay much more. The range of willingness to pay was wide (€0–1000), and this could be a reflection of the study population's diversity and/or personal preferences. Interestingly, consistent with our hypotheses, willingness to pay was correlated with both age and income, but it was not related to the level of education. However, in contrast with our hypothesis, WTP was not associated with religion. Analysing willingness to pay is often subject to criticism. For example, the method used to estimate WTP is a common source of criticism. The advantages and disadvantages of the payment card as used in this study compared with other methods to estimate WTP have been widely discussed; however, it is a commonly accepted tool. Another criticism of the WTP method is that hypothetical answers are obtained based on a hypothetical survey situation, and these answers may differ from answers given in a real-life situation, causing a so-called "hypothetical bias". In general, hypothetical willingness to pay overestimates one's actual willingness to pay.[12] Therefore, the current WTP estimates might be an overestimation of actual WTP. The strength of this study is that the participants were asked at the same time in their pregnancy as they would have to decide about NIPT, if available. In addition, this study included both women who declined prenatal screening and women who opted for prenatal screening. Although several previous studies examined the attitudes of pregnant women, they included high-risk women only or failed to obtain sufficient information from participants who refused prenatal screening.[10-13] Although Kooij et al. studied a low-risk group, they did not mention whether the women received prenatal screening or an invasive procedure.[9] Our study was limited by the relatively small sample size, a low response rate, and an underrepresentation of women with lower education and lower income levels. The difference in opinion between the participants and the non-responders are not known. The patients might have been influenced by the attitude of the healthcare provider while handling the questionnaire. The difference between the national FCT compliance rate and the response rate in our study population may be explained by the circumstance that women who receive FCT are more willing to complete a questionnaire regarding FCT than women who refuse FCT. Furthermore, the women in this study were slightly older than the average age of pregnant women in the Netherlands; older pregnant women are generally more aware of the risks and are more interested in prenatal testing. This study may have also included a selection bias. First, participating midwives and doctors may have felt that less-educated women would be less interested in completing the questionnaire; in their first trimester, these women are particularly sensitive to receiving an overload of information regarding pregnancy and prenatal screening in a single visit.[14] Second, only women who could read Dutch were invited to participate in the study. Several publications

have addressed the roles of ethnic and socio-economic differences in the decision to receive screening.[15-18] Although the reasons why certain groups do not opt for screening are unclear, Dormandy et al. suggested that ethnic inequalities regarding access to prenatal testing might play a role.[14] This so-called "health gap" could arise from the relative complexity of the multi marker FCT. Importantly, this "health gap" might be reduced when the NIPT – which is relatively easy to explain to patients – is introduced. Our study did not investigate these issues, and future studies should examine the difference in attitude towards NIPT between lower and higher education levels and various ethnic groups. Finally, our study was performed in the Netherlands, and similar studies conducted in other countries could yield different results. Although many other countries appear to have higher screening uptake, most countries (particularly Denmark) lack reliable nationwide data.

Conclusions

The majority of pregnant women in our study expressed a positive attitude towards NIPT and indicated that they would request NIPT if available. Importantly, more than half of the women who rejected prenatal screening in the current system would opt for NIPT if available. A prospective study to determine the practicality of NIPT in the general population is needed. The price that women <36 years of age are willing to pay for NIPT is similar to what they currently pay for prenatal screening. Healthcare workers should be aware that if prenatal costs are not reimbursed, the uptake rate will be dependent on income. Clearly, the answers given in a questionnaire are a stated preference, and although they can approximate the preferences of pregnant women, they might not predict their actual preference in real-life situations.

Practical implications

The reasons stated by pregnant women for receiving or declining prenatal screening were quite similar to those described by Van den Berg et al.[19] Not unexpectedly, the lack of a risk of procedure-related miscarriage was the most important reason for a woman to opt for NIPT. As noted by several groups regarding the current prenatal screening programme, because of insufficient knowledge, women do not generally make an informed choice, despite receiving counselling.[19,20] We agree with other groups that although NIPT differs from other tests, patients should still receive counselling after NIPT becomes available in order to discuss potential decision-making and the consequences of knowing whether the foetus has trisomy 21.[10,21] The finding that approximately half of pregnant women reject FCT in the current system because of unfavourable test features must be taken into account by policy makers, laboratories and insurance companies when preparing to introduce NIPT into existing screening programmes.

Variables	*Participants (n=147)*	*%*
Age		
Mean age (range)	32.9 (21 - 44)	
SD	4.6	
Low (<36 years)	101	68.7
High(≥36 years)	46	31.3
Level of education		
Low	13	8.8
Medium	15	10.2
High	57	38.8
Academic	62	42.2
Religious affiliation		
Religous	46	31.3
Not religious	100	68.0
Missing	1	0.7
Income household per month (euro)		
<1500	2	1.4
1500-3000	28	19.0
>3000	101	68.7
Missing	16	10.9

Table 1. *Sociodemographic characteristics*

Variables	*N (total)*	*Mean (Euro)*	*p*
Age (years)			
<36	77	185	0.049
≥36	44	218	
Income euro/month			
<1500	2	no information	<0.001
1500-3000	21	145	
3000-5000	64	190	
>5000	23	304	
Education			
Low	6	183	NS
Medium	12	125	
High	49	196	
Academic	54	217	
Religion			
Religious	31	229	NS
Not religious	89	187	
Unknown	1	150	

Table 2. *Variations of the maximum willingness-to-pay for NIPT in women with a positive attitude*

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E.J.T. Verweij ● D. Veersema
E. Pajkrt ● M.C. Haak

Submitted

CHAPTER 6

Decision making in prenatal screening: money matters

ABSTRACT

OBJECTIVE

Multiple factors influence pregnant women in their decision to accept or decline prenatal screening. This study aimed at determining the influence of withdrawal of reimbursement on the uptake of the first trimester combined test.

MATERIAL AND METHODS

Between 2004 and January 2007 the combined test was offered to all pregnant women in a designated geographical area as a pilot study prior to the introduction of the National screening program to test the logistics. All tests were performed in one ultrasound centre and were reimbursed by the insurance companies. After the introduction of the screening program the insurance companies stopped paying for the combined test in women <36 years by decision of the government. The influence of reimbursement was studied by examining the difference in the number of women opting for a combined test 12 months before and 12 months after the introduction of the national screening program in January 2007.

RESULTS

In the year 2006 the combined test was performed in 4616 pregnant women. With the introduction of the national screening program and withdrawal of the reimbursement 3459 combined tests were performed (reduction of 25%). In January 2007 a significant decline was observed in the uptake of the combined test in women <36 year (p<0.001) as opposed to a significant increase in the uptake in women ≥36 year (p<0.001).

DISCUSSION

Withdrawal of reimbursement of the combined test has led to a significant reduction in the uptake of the combined test in this selected area. The financial impact on the uptake of the combined test should not be underestimated.

INTRODUCTION

Prenatal screening aims to detect women at high risk for fetal trisomies in a population of normal pregnancies. One of the available screening methods is the first-trimester combined test (CT), consisting of maternal serum screening and nuchal translucency (NT) measurement. This test identifies women at risk for trisomy 21 (T21, Down syndrome), 18 or 13. The serum test is normally performed between 9+0 weeks and 13+6 weeks of gestation and the nuchal translucency is performed between 11+0 and 13+6 weeks of gestation. In the Netherlands the antenatal screening program was designed to provide every pregnant woman with the information necessary to make an informed choice. Multiple factors influence pregnant women in their decision to accept or decline prenatal screening. If the risk assessment shows a high risk for fetal trisomies or if fetal anomalies are detected, invasive testing is offered. Invasive testing caries a risk of iatrogenic pregnancy loss. Parity, fertility history, family history for chromosomal anomalies, education level, ethnicity and religion are acknowledged to attribute in women's choices for prenatal screening.[1] The main reasons to undergo the test are reassurance and the desire to have knowledge about the health of the fetus.[2,3] The decision to decline the first-trimester combined test may be related to personal views on pregnancy termination.[2,3]

In the Netherlands a fully covered health care insurance system provides equal health care for every citizen. With the introduction of the National prenatal screening program in 2007, the government decided that the 20-week anomaly scan should be reimbursed by the insurance companies to all pregnant women. In contrast, the combined test (€154) would be reimbursed only to women of 36 years or older. Although younger women <36 years have to be informed about the combined test, they have to take the personal costs into account when deciding whether or not to undergo first trimester screening. Invasive prenatal diagnosis, such as amniocentesis and chorionic villous sampling, is subsequently reimbursed to all women with an increased risk, either based on maternal age or on the combined test.

Very little is published in literature about the influence of personal costs in the decision to undergo first trimester screening. The aim of this study was to determine the influence of personal costs on the uptake of the combined test.

METHODS

In the period 2004 – January 2007 the combined test and 20 week anomaly scan were performed without personal costs in a regional ultrasound centre (Diagnostic centre Diagnostiek voor U, Eindhoven, the Netherlands), designated to service a specific geographical area. This was done as a pilot before the start of the national screening program, to test the logistic procedures

and quality aspects. Counselling concerning the combined test and 20 weeks'scan was done at about 9-12 weeks of gestation at the booking visits in regional midwifery practices or at regional hospitals.
With the introduction of the program in 2007, the insurance companies suddenly stopped paying for the combined test in women <36 years in this region as decided by the Ministry of Health Department. Counselling did not change, except for the fact that women <36 years were informed about the costs of the test and the fact they had to pay for the test themselves.

We assessed the influence of the stop of reimbursement by studying the difference in uptake of the combined test 12 months before and 12 months after January 2007. The monthly number of 20-week anomaly scans performed in the same ultrasound centre, free of costs independent of age, was used as a reference to rule out demographic changes.

RESULTS

In the year 2006 the combined test was performed in 4616 pregnant women. With the introduction of the national screening program and withdrawal of the reimbursement 3459 combined tests were performed, a reduction of 25%. Figure 1 shows a significant decline of first trimester screening for the group women <36 year in January 2007 (p<0.001) and a significant rise in the uptake of the combined test in women ≥36 year (p<0.001). As a comparison the numbers of the 20-week anomaly scans are shown. Before and after January 2007 the mean number of combined tests performed per month in women <36 year was 327 (range 277–390) and 126 (range 73–353), respectively, as opposed to 58 (range 46–86) and 161 (range 86–241), respectively in women ≥36 year. During the study period, the total number of 20-week anomaly scans in the same ultrasound centre remained stable (p=0.74), indicating that no demographic changes occurred in this period.

DISCUSSION

These data illustrate that personal costs may play a significant role in the decisions of pregnant women whether or not to undergo certain tests. We have observed a significant reduction in the uptake of the combined test for women <36 year with the introduction of the national screening program.
This study is the first to show the influence of reimbursement in prenatal screening. Decision making in prenatal screening depends on many factors like obstetric history, individual experience regarding previous pregnancies, family history, education and religion. Probably

there are many potential confounders, but we believe the personal costs can be an important incentive. As this is a population based cohort study we are not able to rule out other confounders.

Up to date, no reports have been published about the influence of reimbursement on the uptake of prenatal screening. In a high-risk population deciding whether or not to undergo an amniocentesis, the effect of reimbursement has recently been published.[4] This information supports our finding that the absence of costs has a largely positive effect on the probability of choosing for a prenatal test. The fact that women <36 years have to pay personally in a fully insurance-covered health care system might act as a sign from the government that first-trimester screening is not important for younger women. Although Dormandy et al concluded that healthcare professionals' attitudes are unrelated to the uptake of screening, it is imaginable that personal costs for the patient could influence their counselling.[5] Prenatal care providers are often aware of the financial situation of their clients, thus a certain bias towards the potential benefits of the combined test could be present because of the costs. Although all women were counselled about prenatal screening in the pilot area in 2006, women ≥36 were not likely to choose first trimester screening. The rise in uptake in January 2007 for women ≥36 year remains unexplained, but may be partly because women were suddenly made more aware that the combined test was indeed a good alternative for invasive testing based on maternal age. Possibly information in magazines and on television about the introduction of the national screening program in the first months of 2007 influenced the uptake with a reduction in invasive procedures. Currently, like in many countries prenatal screening is changing with the introduction of the non-invasive prenatal test (NIPT) using chromosome selective sequencing of cfDNA. NIPT has been shown to be a very good test to predict presence or absence of fetal trisomy 21 from as early as 10 weeks gestation.
For the implementation of NIPT these study results should be taken into account, as age related reimbursement for prenatal screening tests produces unequal access to prenatal care.

CONCLUSION

The influence of personal costs on the uptake of prenatal screening tests should not be underestimated. Policy makers and health insurance companies should reconsider if the introduction of personal costs for a selected group in a national screening program is ethical as we believe this regulation is against the principle of non-discrimination, the principle of equally accessable health care in relation to the ethical principle of fairness. Future implementation studies for example for NIPT should be carefully designed based on this knowledge. Caregivers should take into account the important financial incentive in the decision making process.

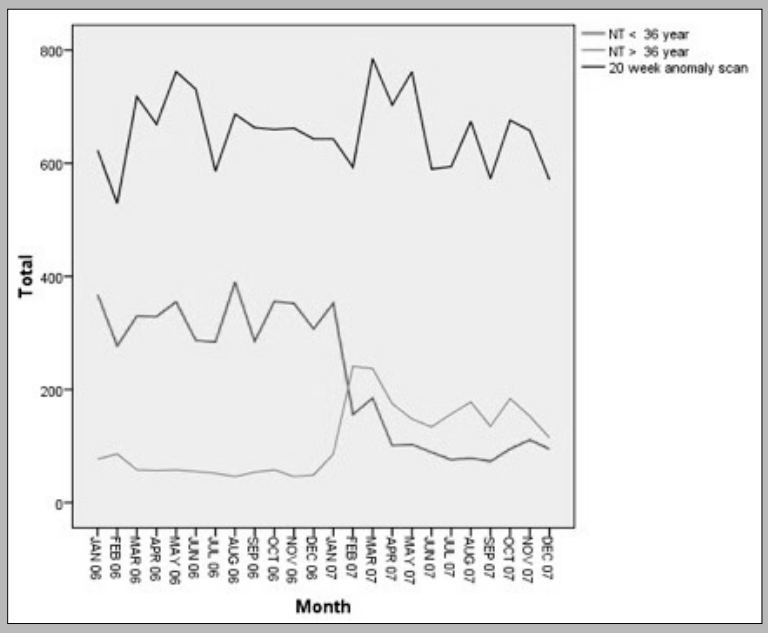

Figure 1. *shows a significant decline of FCT (serummarkers and NT) in January 2007 for women <36 year. The 20-week anomaly scan is used as a reference*

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IV
TOWARDS
NIPT
IN
THE
NETHERLANDS

E.J.T. Verweij ● E.J. Romeijn ● J.M. Koelewijn
K.M. Heetkamp ● M.A. de Boer ● D.Oepkes

Submitted

CHAPTER 7

Attitudes of Dutch primary care midwives towards the current prenatal screening and non-invasive prenatal testing

ABSTRACT

BACKGROUND

With the likely introduction of non-invasive prenatal test (NIPT) in the near future, we wanted to gain knowledge about the attitude of primary care midwives concerning current and future prenatal screening programs.

METHODS

In a cross-sectional study a digital semi-structured questionnaire was used to explore the attitudes of Dutch primary care midwives.

RESULTS

The response rate was 24% (489/2072). The background characteristics of the responders match those of the entire population of Dutch primary care midwives. The attitude of the respondents towards NIPT was positive in 72%, whereas 47% were positive towards the current FCT program. The attitudes towards NIPT were significantly influenced by religion. Advantages of NIPT mentioned were mainly the absence of iatrogenic miscarriage risk (97%), easier to understand counseling (82%), fewer referrals for invasive diagnostics (91%) and pregnancy termination in earlier gestation (77%). Disadvantages of NIPT mentioned were concern about less informed decision-making by pregnant women (70%), the test becoming a routine test (41 %), increased uptake rates (30%) and increased abortion rates (24%).

CONCLUSION

The majority of Dutch midwives responding to the questionnaire would welcome the implementation of NIPT. Specific counseling courses are recommended for all who will be counseling pregnant women on NIPT.

INTRODUCTION

In the Netherlands, the first trimester combined test (FCT) is offered to all pregnant women since 2007 as part of a national prenatal screening program. Prenatal screening aims to offer an individual risk estimate for fetal trisomies in the first trimester or early second trimester of pregnancy. FCT consists of maternal serum screening and nuchal translucency (NT) measurement, which identifies women at elevated risk for trisomy 21, 18 or 13 with a sensitivity of 80-85% for a false positive rate of 5%. The serum test is performed between 9+0 weeks and 13+6 weeks' gestation. The nuchal translucency measurement is performed between 11+0 and 13+6 weeks gestation. If the FCT shows an elevated risk (above 1:200) for trisomy invasive testing using chorion villus sampling (CVS) or amniocentesis is offered. In the Netherlands the uptake of the FCT is low (around 25%) compared to other countries.[1]

Changes in the prenatal screening program are expected with the availability of the non-invasive prenatal test (NIPT) in the Netherlands. With NIPT trisomies can be detected using cell-free fetal (cff) DNA circulating in maternal blood with a reported sensitivity and specificity of >99%, and has recently been introduced in clinical practice in many countries.[2] NIPT can be performed from 10 weeks gestation onwards and is completely safe for the fetus. With the introduction of NIPT the uptake of prenatal screening might increase because of better test characteristics and less need for invasive tests.[3] Whether NIPT should be used as primary care screening, replacing FCT, or only in case of an elevated risk as a replacement for an invasive test is subject of debate. Ethical, financial and logistic issues play a main role in this discussion, but these issues may change over time. Because NIPT is a safe test that requires only a maternal blood sample, some caregivers worry that the decision to perform NIPT might be taken to easily and good counseling will be omitted.[4,5]

In the Netherlands most pregnant women receive prenatal care by independent primary care midwives, who also do the counseling for the FCT. Until now it is not known what the attitude of primary care midwives is towards the current prenatal screening system and towards NIPT.

The aim of this study was to investigate the attitudes of primary care midwives towards the current Dutch prenatal screening program and towards the future introduction of NIPT.

METHODS

Design

In a cross-sectional design a digital semi-structured questionnaire was used. The time the respondents needed to complete the questionnaire was around 20 minutes.

Study population

The study population consisted of all primary care midwives in the Netherlands, who were members of the Royal Dutch Organization of Midwives (KNOV) in January 2012 (n=2093). In total, 95 % of all primary care midwives are KNOV members. The midwives were invited to complete an online questionnaire between February 16 and March 20, 2012. The invitation was placed both on the KNOV-website and stated in their online newsletter. After a week a postal reminder with a link to the questionnaire was sent to all primary care midwives.

Setting

At the time of the study NIPT was not yet available in the Netherlands. We provided uniform information about NIPT at the beginning of the questionnaire, including the purpose of this study, the sensitivity and specificity of the FCT, the miscarriage risk of invasive diagnostics, the characteristics of the NIPT test, the reported sensitivity and specificity of NIPT and the possible consequences of the introduction of NIPT.

Two possible scenarios for the introduction of NIPT were described, either NIPT for the elevated risk population, replacing invasive testing, or NIPT offered to all pregnant women, replacing the FCT. In the current situation primary care midwives receive financial compensation for prenatal counseling.

Outcome measures

The main outcome measures were the attitudes of midwives towards the current screening program and towards NIPT.

Data collection

The questionnaire included 35 closed and 4 open questions and consisted of three parts: firstly, the background variables of the midwives (*age, place of graduation, religion, urbanization of working area and function in the primary care center*); secondly the attitude towards the national screening program and thirdly the attitude towards NIPT.

The questions concerning the attitude towards the current FCT based national screening program focused on counseling and training. They addressed the capability of counseling, the difficulties in counseling (based on the following aspects: the seriousness of the disease, the

false positive result, the false negative result and the iatrogenic miscarriage risk when electing for an invasive procedure) the time required for counseling and counseling difficulties. participants were asked to indicate their attitude towards the current screening program and the influence of this attitude on their counseling. Most of the questions concerning the aspects of attitudes were measured on a 5 point Likert scale. The assumed influence of the personal attitude on counseling was measured on a "forced" 4 points scale (without neutral).

In the last part of the questionnaire, participants were asked questions about NIPT. The first question was whether they were aware of the existence of this new test. The attitude towards NIPT was addressed twice on two different moments in the questionnaire. The participants were asked to make a choice on the way of implementation of NIPT, only for high risk women or for all pregnant women. Several questions addressed the possible positive and negative influences of the introduction of NIPT for both the respondent as society.

Statistical analysis

All analyses were performed using SPSS 20.0 for windows (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The answers on the open questions for the perceived needs by implementation were coded into categories, labeled and entered in SPSS.

Ethical considerations

All data were processed anonymously. Ethical review for this form of research is not required in the Netherlands.

RESULTS

A total of 489 midwives completed the questionnaires, of a total of 2086 primary care midwives, which gives a response rate of 24%. The background characteristics are listed in table 1.

Training and counseling

A total of 463 (95%) respondents were counseling patients about the FCT, and 480 (98%) answered that they had attended courses on prenatal screening, either during their initial midwifery training or during post registration courses. A majority of the respondents (98%) felt adequately trained to counsel pregnant women about the FCT and 402 (82%) of the respondents felt adequately trained to present the result of the FCT. The respondents spent a median of 10 minutes (range 3-50 min) on counseling. In total, 33/463 (7%) midwives needed more than 20 minutes per counseling. The respondents estimated that the percentage of

pregnant women electing for FCT was 25% (median, range 1-99). A minority (75/463, 17%) answered to have uptake rates of over 50%. A statistically significantly positive correlation was found between time needed for counseling and uptake rate (p=<0.001).

The respondents noted that counseling on false positive and false negative results was most difficult (respectively 59% and 52% of the respondents noted this to be hard or very hard). In contrast, 80% of the respondents considered counseling about the risk of an iatrogenic miscarriage to be easy. The severity of Down syndrome was valued easy to explain by 54% of the respondents, and 20% found it hard to explain. Most respondents commented on the complexity of the risk calculations, the difficulty of explaining the FCT in particular to people with insufficient knowledge of the Dutch language and the costs.

The attitude towards the current screening program

Of the 483 respondents, 230 (47%) reported to have a positive attitude, 84 (17%) respondents a negative attitude, and 169 respondents (35%) a neutral attitude towards the current screening program (table 2). Most midwives (89 %) answered that their personal attitude towards FCT did not affect their way of counseling. Yet, 50 respondents (10%) answered that personal attitude was certainly playing a role in their counseling. In this subgroup, no particular attitude (positive, negative or neutral) was overrepresented.

Attitudes towards NIPT

The majority, 75%, of respondents reported to be informed about the existence of NIPT, mostly by the KNOV, newspapers and training or conferences. Both the first measurement on attitude towards NIPT, in the beginning of the questionnaire, as the second measurement, after more detailed information and questions, are noted in table 2. Being religious (OR=5.30; 95%CI 2.30-12.02) was a significant determinant for having a negative attitude towards NIPT. However, religious midwives had a more positive attitude towards NIPT compared to FCT (56 % versus 40%). Working in non-urban areas was also a significant predictor for a negative attitude (OR= 2.06, 95%CI 1.10-3.85).

Uptake and consequences of NIPT

A rise in uptake of NIPT if it would replace the FCT was expected by 75% of the respondents. A rise in uptake if NIPT would replace invasive procedures was expected by 57% of the respondents. Midwives had a preference for the option that NIPT would replace the FCT in the prenatal screening program (strong preference 25%, preference 39%) as compared to the option that NIPT would replace invasive diagnostics (strong preference 9%, preference 16%). Fifty-three (11%) of the respondents noted to be neutral.
In table 3 the respondents' attitudes towards the possible consequences of NIPT are listed.

When asked about the consequences for their own practice respondents with a positive attitude reported significantly more often possible advantages of NIPT for their own practice than respondents with a negative attitude, like easier counseling (87% resp. 58%) and reduction in referrals for invasive diagnostics (90% resp. 76%). Respondents with a negative attitude valued an increase in the uptake rate of NIPT, an increase in detection T21 and an increase in selective abortion rate as disadvantages. Many midwives noted in a blank field that they believe that although NIPT would replace the FCT the first trimester ultrasound is believed to be maintained. For the society, possible advantage of NIPT noted by the respondents, respectively with a positive or a negative attitude, was the reduction of referrals for invasive diagnostics (95% resp. 87%). Disadvantages mentioned were the possible increased uptake rates (30% resp. 79%) and performing the test as a routine (43% resp. 87%) with less counseling and worse informed decision-making. Many respondents (n=45) commented on a possible increase of discrimination of people with Down syndrome, resulting in social pressure to elect for NIPT and to elect for termination of pregnancy in affected pregnancies. Also the fear of a society not accepting people with handicaps anymore is a comment made by the respondents. Some respondents (n=10) worried about the possible high costs for society with the implementation of NIPT, but are also worried about an unequal access to prenatal care if reimbursement is not available for every pregnant woman. Respondents with a negative attitude were less likely willing to participate in studies concerning prenatal screening.

DISCUSSION

This study is the first evaluation of attitudes of Dutch primary care midwives concerning NIPT. We found that 71% of the respondents were positive about NIPT, considerably more than the 47% who were positive about the current screening program. Respondents with a positive attitude towards screening reported a higher than average uptake of testing. A negative attitude towards both FCT and NIPT was significantly associated with religion.

Up to now, there is little information about the experience and the attitudes of midwives towards the current Dutch screening program and the extent to which the attitude of midwives is influencing the uptake rates of FCT. In a Dutch study in 2007, neither uptake rates, nor attitude of the pregnant women towards prenatal screening could be predicted by the counselors attitude towards prenatal screening.[6] Another study did not detect a correlation between positive attitudes of healthcare professionals towards screening and uptake rates of prenatal screening.[7] The main advantages of NIPT for pregnant women mentioned by midwives were the absence of iatrogenic miscarriage risk, easier-to-understand counseling, fewer referrals for invasive diagnostics and pregnancy termination in earlier gestation.

Reasons for concern towards NIPT were less well-informed decision making, increase in uptake rates and increase in abortion in the case of T21.

Hill et al published a study about the preferences of both women and health professionals in the United Kingdom showing that women preferred the absent risk of miscarriage whereas health professionals preferred a high accuracy.[8] Implementation of NIPT for Down syndrome into routine antenatal care will depend on many factors, including test accuracy, costs, and care pathways. In addition, preferences of the many stakeholders in prenatal care is important. In our study, the respondent midwives prefer NIPT replacing the FCT rather than NIPT replacing invasive procedures. However, policy makers or other stakeholders may prefer offering NIPT only for high-risk women. We believe successful implementation of NIPT into routine health care depends on close collaboration between all stakeholders from the earliest time of planning the changes. The majority of pregnant women (84.2% in 2010) in the Netherlands start their prenatal care with an independently working midwife. Understanding the views of the midwives is essential for planning changes in our national prenatal screening program and appears highly valuable in the interdisciplinary discussions.

The main aim of counseling and prenatal screening is to enable pregnant women to make an informed choice. Counseling of FCT has its difficulties because the FCT only provides a risk assessment. In our study more than 40% of the primary-care midwives found false negative and positive results with risk calculations such as 1 in 250 or 1 in 400 difficult to explain. With NIPT, this will be less difficult, since the result is either highly likely abnormal (>99%), or extremely unlikely abnormal (i.e. less than 1 in 10,000). The positive and negative predictive value will be important to address in the counseling as well as the need to verify the positive result with an invasive procedure before termination of pregnancy NIPT has a potential risk for less adequate informed consent and becoming a routine diagnostic test. On the other hand, with the test becoming safer, more women will have this test performed to get informed about important aspects of the health of their baby. Similar to anomalies detected by ultrasound, also parents who would never decide to terminate the pregnancy for this condition,[9] could very well want to be informed. We need to design a robust screening program together with all stakeholders when for the implementation of NIPT, acknowledging our study outcomes. Our study data should prepare us for an increased uptake of testing once NIPT becomes available. Caregivers should be prepared so they will be able to offer good counseling.

Strengths and limitations

This study provides the first evidence that Dutch primary care midwives hold significantly different attitudes towards the current prenatal screening compared to NIPT. An important limitation is the low response rate of 24%. Despite a postal reminder to the home address of

the KNOV-members we did not receive permission of the KNOV to use email addresses of the KNOV-members. It is possible that this resulted in a lower response rate as the respondents received a paper letter and had to complete the questionnaire online. The response rate might have been higher if a personal email was send with a direct link to the online questionnaire. However, background characteristics were representable for the entire population of primary care midwives. Furthermore, in our study midwives with positive or negative attitudes towards FCT and NIPT are both represented. Obviously, the results of a survey with less than half of the targeted group of midwives actually responding need to be interpreted with caution. Our study does not claim to provide definite answers on the view of all midwives, it is however a useful starting point for discussions and collaboration between obstetric care professionals and other stakeholders in this field.

A second constraint has to do with the nature of opinion-survey. We have not only asked midwives about their attitudes but also asked for estimations of uptake rates and expectations of NIPT-consequences. Estimated numbers may very well differ from actual numbers and none of the respondents has had any real life experience with the execution of NIPT yet.

Furthermore, NIPT has not been yet implemented and attitudes are based on what is expected. If NIPT is actually implemented it would be interesting to repeat our survey and compare the data.

CONCLUSION

Dutch midwives appear to welcome the implementation of NIPT. Their attitudes towards NIPT are more positive than towards the current FCT. Main concerns for implementation are about counseling and well informed decision-making. Counseling courses specifically for NIPT are recommended for all who counsel pregnant women on NIPT.

ACKNOWLEDGEMENTS

Special thanks to A. Stiggelbout, professor in Decision Making, Department of decision making, Leiden University Medical Centre, Leiden, The Netherlands

BIJLAGE - VRAGENLIJST

Geachte collega,

Welkom bij de digitale vragenlijst over prenatale screening op Downsyndroom in het algemeen en in het bijzonder over de nieuwste ontwikkeling: Non-Invasieve Prenatale Test (NIPT).

- **Waar gaat deze vragenlijst over?**
  Het in kaart brengen van de mening van eerstelijns verloskundigen over de huidige prenatale screening op Down syndroom in het algemeen en in het bijzonder over de nieuwe ontwikkeling NIPT.
  De vragenlijst bestaat uit 3 delen:
  - Algemene vragen
  - Huidige prenatale screening
  - Non-invasieve prenatale testen (NIPT)
- **Hoe lang duurt het om de vragenlijst in te vullen?**
  Het invullen van de vragenlijst duurt ongeveer 10 minuten.
- **Tot slot**
  Heel hartelijk dank voor uw deelname.
  Heeft u vragen tijdens het invullen van deze vragenlijst? Belt u dan met
  Enja Romeijn of mail naar verloskundigenoverscreening@kpnmail.nl

Wilt u niet deelnemen, klink dan hier op 'nee'. Wij stellen het op prijs als u wel de algemene gegevens wilt invullen.

❍ Ja, ik wil deelnemen. Question Wilt u niet deelnemen, klink dan hier op 'nee'. Wij stellen het op prijs als u wel de algemene gegevens wilt invullen.

❍ Nee, ik wil niet deelnemen (door naar de algemene gegevens en alleen naar pagina 14) Wilt u niet deelnemen, klink dan hier op 'nee'. Wij stellen het op prijs als u wel de algemene gegevens wilt invullen.

Deel 1: Algemene gegevens

1 Wat is uw leeftijd?
☐☐ jaar

2 Wat is uw geslacht?
M☐V☐

3 In welke postcodegebied bent u werkzaam?
□□□□

4 In welk jaar bent u als verloskundige afgestudeerd?
□□□□

5 Waar bent u afgestudeerd als verloskundige?
- ❑ Amsterdam
- ❑ Groningen
- ❑ Maastricht/Kerkrade/Heerlen
- ❑ Rotterdam
- ❑ Overig namelijk ……

6 Wat is uw huidige functie?
- ❑ eigen praktijk/maatschap
- ❑ in loondienst van een gezondheidscentrum, STBN, etc.
- ❑ klinisch werkzaam
- ❑ in loondienst van een zelfstandig gevestigde verloskundige
- ❑ waarneemster
- ❑ anders, nl. ……..

7 In welk gebied bent u werkzaam:
- ❑ Zeer sterk stedelijk
- ❑ Sterk stedelijk
- ❑ Matig stedelijk
- ❑ Weinig stedelijk
- ❑ Niet-stedelijk

8 Leeft u vanuit een bepaalde geloofs-/levensovertuiging. En zo ja, vanuit welke geloofs/levensovertuiging?
- ❑ Nee
- ❑ (Rooms) Katholiek
- ❑ Protestants
- ❑ Anders christelijk nl…….
- ❑ Hindoeïstisch
- ❑ Humanistisch
- ❑ Anders, nl……..

9 Was counselen voor prenatale screening (PNS) een onderdeel van uw opleiding tot verloskundige?

- ❑ Ja
- ❑ Nee

10 Heeft u (al) de nascholingsmodule voor counselors prenatale screening (PNS) gevolgd?

- ❑ Ja
- ❑ Nee
- ❑ Ik ben het van plan

Deel 2: huidige prenatale screening

Sinds 1 januari 2007 wordt elke zwangere in de gelegenheid gesteld om gebruik te maken van de eerste trimester-combinatietest ter opsporing van voor onderzoek naar trisomie 21, 13 en 18. Afhankelijk van de maternale leeftijd varieert de sensitiviteit van de combinatietest van 70% -96 % en de specificiteit van 75 -97 %.
Wanneer er sprake is van een verhoogde kans op Downsyndroom trisomie 21 (of 13 of 18) is er de mogelijkheid tot invasieve diagnostiek ter vaststelling van trisomie 21. Invasieve ingrepen hebben een iatrogeen miskraamrisico tussen de 0.5 en 1%.
Het geven van voorlichting over prenatale screening en het begeleiden van zwangere vrouwen bij het keuzeproces wordt counseling genoemd.

Op iedere pagina opnieuw: prenatale screening (PNS) en vervolgens alleen PNS

11 Wat is uw houding tegenover de huidige Nederlandse Prenatale Screening (PNS)?

- ❑ Zeer positief
- ❑ Positief
- ❑ Neutraal
- ❑ Negatief
- ❑ Zeer negatief

12 In hoeverre vindt u zichzelf geschoold voor counseling PNS?

- ❑ Meer dan voldoende
- ❑ Voldoende
- ❑ Neutraal
- ❑ Onvoldoende
- ❑ Ruim onvoldoende

13 In hoeverre vindt u zichzelf geschoold voor het geven van de uitslag van de combinatietest?

- ❑ Meer dan voldoende
- ❑ Voldoende
- ❑ Neutraal
- ❑ Onvoldoende
- ❑ Ruim onvoldoende

14 Wat is u mening over de geadviseerde bedenktijd van de zwangere tussen counseling en bloedafname voor de serumtest (als onderdeel van de combinatietest)?

Zeer negatief	negatief	Neutraal	positief	zeer positief
☐	☐	☐	☐	☐

15 Doet u zelf aan counseling prenatale screening (PNS)?

- ❑ Ja
- ❑ Nee, mijn collega's in de praktijk (u kunt doorgaan naar vraag 19)
- ❑ Nee, momenteel ben ik niet werkzaam in de eerstelijns praktijk (u kunt doorgaan naar vraag 19)

16 Hoeveel tijd besteedt u gemiddeld aan counselen PNS per zwangere?

☐☐ Minuten

17 Welk percentage (schatting) van de zwangeren in uw praktijk maakt gebruik van uw aanbod voor counseling PNS?

☐☐ %

18 Welk percentage (schatting) van degenen die zich door u laten counselen maakt gebruik van prenatale screening?

☐☐ %

19 Kunt u de volgende onderdelen van de counseling rangschikken op moeilijkheid om goed uit te leggen aan de cliënt?
1= meest moeilijk, 5= minst moeilijk (gelijke scores mogen ook, dan komt u niet aan 5)

- ☐ Ernst van de afwijking(en)
- ☐ Foutpositieve uitslag
- ☐ Miskraamrisico bij invasief vervolgonderzoek
- ☐ Foutnegatieve uitslag
- ☐ Anders nl…….

20 In hoeverre heeft uw persoonlijke attitude invloed op de wijze van counselen PNS?

- ❑ Heel veel
- ❑ Veel
- ❑ Weinig
- ❑ Heel weinig

Deel 3: Nieuwe ontwikkelingen: Non-Invasieve Prenatale Test (NIPT) Met de nieuw ontwikkelde test, NIPT kan op een betrouwbare, niet-invasieve wijze het foetaal DNA in maternaal serum worden onderzocht op trisomie 21. In de toekomst zal met NIPT de bepaling van andere trisomiëen ook mogelijk zijn. Uit studies, verricht onder vrouwen met een indicatie voor een invasieve ingreep, blijkt dat NIPT een sensitiviteit heeft van bijna 100% en specificiteit van 99,3% voor het bepalen van trisomie 21. Met deze nieuwe test kan trisomie 21 vanaf ongeveer 10 weken bepaald worden waarbij de zwangere een eenduidige uitslag krijgt na ongeveer 2 weken. Hiervoor zijn 2 buisjes maternaal bloed nodig.

21a Was u voordat u deze vragenlijst ging invullen, al bekend met (Non-Invasieve Prenatale Test) NIPT?

- ❑ Ja
- ❑ Nee

21b Zo ja, op welke manier? (Meerdere opties mogelijk)

- ❑ Collega
- ❑ KNOV
- ❑ Krant
- ❑ Tijdschrift voor Verloskundigen
- ❑ Ander tijdschrift
- ❑ TV
- ❑ Radio
- ❑ Overig……..

22 Wat is uw houding ten aanzien van NIPT met de kennis die u nu hebt?

- ❑ Zeer positief
- ❑ Positief
- ❑ Neutraal
- ❑ Negatief
- ❑ Zeer negatief

Internationaal wordt er discussie gevoerd over de manieren van invoeren van NIPT binnen het prenatale screeningsprogramma.

Er zijn twee mogelijke opties:

A. NIPT vervangt invasieve diagnostiek
De combinatietest aanbieden als screeningstest en bij een verhoogde risico op trisomie 21 NIPD aanbieden ter vervanging van invasieve diagnostiek.

B. NIPT vervangt de combinatietest.
NIPT aanbieden als screeningstest aan alle zwangeren en bij een positieve testuitslag invasieve diagnostiek aanbieden ter vaststelling van trisomie 21.

23 Welke van deze 2 opties heeft uw voorkeur:

Sterke voorkeur **A**	voorkeur **A**	Neutraal	voorkeur **B**	Sterke voorkeur **B**
☐	☐	☐	☐	☐

24 Wat verwacht u dat de invoering van NIPT (als vervanging van invasieve diagnostiek, zoals bij optie A) zal betekenen voor de deelname van zwangeren aan prenatale screening?

- ❑ deelname neemt sterk toe
- ❑ deelname neemt toe
- ❑ deelname blijft gelijk
- ❑ deelname neemt af
- ❑ deelname neemt sterk af

25 Wat verwacht u dat de invoering van NIPT (als vervanging van de combinatietest, zoals bij optie B) zal betekenen voor de deelname van zwangeren aan prenatale screening?

- ❑ deelname neemt sterk toe
- ❑ deelname neemt toe
- ❑ deelname blijft gelijk
- ❑ deelname neemt af
- ❑ deelname neemt sterk af

Enerzijds zal NIPT naar verwachting de counseling makkelijker maken. De zwangere krijgt een betrouwbare uitslag in plaats van een kansbepaling. Bij een positieve uitslag op trisomie 21 zal dit in eerste instantie nog worden bevestigd met invasieve diagnostiek. Uitsluiting van het miskraamrisico zal ook een belangrijke factor zijn in het maken van een keuze. De verwachting is dat meer vrouwen zullen kiezen voor NIPT dan voor de CT.
Anderzijds is een veelgenoemde stelling dat invoering van NIPT zal leiden tot een minder weloverwogen keuze van de zwangere over mogelijke deelname aan prenatale screening en diagnostiek.

26 Hieronder volgen mogelijke consequenties van invoering van NIPT voor de zwangere. Beschouwt u, als verloskundige, ieder van deze consequenties voor de zwangere overwegend als een voordeel, nadeel of neutraal?

Mogelijke consequenties	groot voordeel	voordeel	neutraal	nadeel	groot nadeel
Geen miskraamrisico	☐	☐	☐	☐	☐
Begrijpelijkere counseling voor de zwangere door eigen zorgverlener	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor counseling naar de 2e lijn	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor invasieve ingrepen	☐	☐	☐	☐	☐
Toename deelname test(en) op trisomie 21	☐	☐	☐	☐	☐
Toename opsporing trisomie 21	☐	☐	☐	☐	☐
Weloverwogen keuze neemt af	☐	☐	☐	☐	☐
Zwangerschapsafbreking bij kortere amennorroeduur	☐	☐	☐	☐	☐
Totaal aantal zwangerschapsafbrekingen neemt toe na vaststelling trisomie 21	☐	☐	☐	☐	☐
Er vindt een echo (nekplooimeting) minder plaats	☐	☐	☐	☐	☐

Overige opmerkingen .....

27 Hieronder volgen mogelijke consequenties van invoering van NIPT voor uzelf. Beschouwt u, als verloskundige, ieder van deze consequenties voor uzelf overwegend als een voordeel, nadeel of neutraal?

Mogelijke consequenties	groot voordeel	voordeel	neutraal	nadeel	groot nadeel
Eenvoudigere counseling	☐	☐	☐	☐	☐
Minder onzekere cliënt	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor counseling naar de 2e lijn	☐	☐	☐	☐	☐
Toename deelname test(en) op trisomie 21	☐	☐	☐	☐	☐
Toename opsporing trisomie 21	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor invasieve ingrepen	☐	☐	☐	☐	☐
Totaal aantal zwangerschapsafbrekingen neemt toe na vaststelling tisomie 21	☐	☐	☐	☐	☐
Er vindt een echo (nekplooimeting) minder plaats	☐	☐	☐	☐	☐

Overige opmerkingen .....

28 Hieronder volgen mogelijke consequenties van invoering van NIPT voor de maatschappij. Beschouwt u, als verloskundige, ieder van deze consequenties voor de maatschappij overwegend als een voordeel, nadeel of neutraal?

Mogelijke consequenties	groot voordeel	voordeel	neutraal	nadeel	groot nadeel
Testen op trisomie 21 wordt vanzelfsprekender	☐	☐	☐	☐	☐
Toename deelname test(en) op trisomie 21	☐	☐	☐	☐	☐
Toename opsporing trisomie 21	☐	☐	☐	☐	☐
Totaal aantal zwangerschapsafbrekingen neemt toe na vaststelling trisomie 21	☐	☐	☐	☐	☐
Counseling door eigen zorgverlener is goedkoper	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor counseling naar de 2e lijn	☐	☐	☐	☐	☐
Daling aantal verwijzingen voor invasieve ingrepen	☐	☐	☐	☐	☐

Overige opmerkingen .....

29 Stel dat NIPT wordt geïmplementeerd in het Nederlands screeningsprogramma, wat is volgens u het belangrijkste aandachtspunt?

.....

.....

30 Wat is uw mening over NIPT na de informatie die u hebt gelezen in deze vragenlijst:

- ❑ Zeer positief
- ❑ Positief
- ❑ Neutraal
- ❑ Negatief
- ❑ Zeer negatief

Het Nederlandse NIPT consortium en de NITRO-studie

In het voorjaar van 2011 is het NIPT-consortium opgericht waarin alle academische ziekenhuizen, het RIVM, de KNOV, de NVOG, de VSOP en Sanquin deelnemen met als doel een grootschalige studie op te zetten. De voorbereidingen worden getroffen voor de zogenaamde **Non-Invasief Trisomie Onderzoek (NITRO) studie** waarbij in het huidige studiedesign de combinatietest zal worden vergeleken met de nieuwe NIPT. De counseling voor de NITRO-studie zal voornamelijk bij de verloskundigen plaatsvinden.

31 Bent u bereid in de toekomst vrouwen te counselen voor de NITRO-studie?

- ❑ Ja
- ❑ Nee, om de volgende reden(en)

.....

Hartelijk dank voor het invullen van de vragenlijst!

Variables	Median(Range)	N (%)*	National data **
Gender			
Female		473 (97)	98%
Male		9 (2)	2%
Age	33 (21-63)		
<25		55 (11)	7%
25-34		208 (42)	41%
35-44		106 (22)	22%
45-54		83 (17)	15%
>54		36 (7)	7%
Years since graduation	9 (1-41)		
Urbanization of working area			
no		165 (34)	7%
little		65 (13)	22%
moderate		70 (14)	22%
strong		42 (9)	29%
very strong		141 (29)	20%
Religion			
religious/belief		199 (40.7)	58%
non-religious/belief		283 (57.9)	42%
Estimation by midwife of clients:			
wish to receive information FCT	90% (2-100)		91% (84-94)
elect FCT	25% (1-99)		27% (14-49)
Time required for counseling interview	10min (3-50)		23min

Table 1. *Background characteristics of primary care midwives*
**Responses to some questions were missing, thus total values may not add to 100%.*
***National data 2011(primary care midwives)*

Variables	*N (%)**
Attitude towards current FCT	
positive	230 (47)
neutral	169 (35)
negative	84 (17)
Assumed influence personal attitude on counseling FCT	
very little	138(28)
little	280 (57)
strong	49 (10)
very strong	1 (0.2)
Attitude towards NIPT (first measurement)	
positive	322(68)
neutral	119 (25)
negative	31(7)
Attitude towards NIPT (second measurement)	
positive	347 (71)
neutral	95 (19)
negative	38 (8)

Table 2. *Attitudes of primary care midwives towards FCT and NIPT*

** Responses of some questions were missing, thus total values may not add to 100%*

	Advantage (%)	*Neutral (%)*	*Disadvantage (%)*
Absence of iatrogenic miscarriage risk	97	1	0.2
More understandable counseling	82	17	0.4
Reduction counseling consultations in hospital	42	57	0.2
Reduction referrals invasive diagnostics	91	7	0.4
Increased uptake rate NIPT	11	58	30
Increased detection DS	47	40	11
Less well-informed decision	2	27	70
Abortion at earlier gestation	77	17	4
Increase of abortion in case of DS	7	67	24
One ultrasound investigation less (NT)	23	60	16
Routinisation of testing	13	35	41

Table 3. *Attitudes of primary care midwives towards the possible consequences of NIPT NIPT: non-invasive prenatal test; DS: Down syndrome; NT: nuchal translucency*

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E.J.T. Verweij ● D. Oepkes ● E.S.A.van den Akker
M.A. de Boer ● R.E. Hellemondt ● D.P. Engberts

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CHAPTER 8

Déjà vu?
Discussie over de non-invasieve prenatale test (NIPT) voor Nederlandse zwangeren

SAMENVATTING

Al vele jaren wordt gezocht naar een niet-invasief, veilig alternatief voor de vruchtwaterpunctie en vlokkentest en dit alternatief is gevonden in de niet invasieve prenatale test (NIPT). Volgens inmiddels tientallen gepubliceerde studies is NIPT zeer accuraat (>99%) in het voorspellen van de aan- of afwezigheid van trisomie 21. Om deze reden zal NIPT ongetwijfeld een weg vinden in de dagelijkse praktijk van prenatale screening, echter op dit moment is het nog verboden NIPT in Nederland aan te bieden en/of uit te voeren. Wel zijn steeds meer Nederlandse zwangeren op de hoogte van de optie voor NIPT uit te wijken naar het buitenland. De invoering van NIPT, ook in onderzoeksetting of proeffase, in Nederland is WBO-vergunningplichtig, en daarmee een politiek vraagstuk. De hierbij behorende administratieve en juridische processen maken het nog even kan duren voordat de test in Nederland kan en mag worden uitgevoerd. In dit artikel wordt de huidige stand van zaken besproken, worden enkele medisch-ethische en juridische aspecten toegelicht en wordt stilgestaan bij de verloskundige praktijkvoering in afwachting van een meer definitieve regeling.

INLEIDING

De non-invasieve prenatale test (NIPT) wordt beschouwd als een revolutie in de prenatale screening. In dit artikel wordt de huidige stand van zaken besproken, worden enkele medisch-ethische en juridische aspecten toegelicht en wordt stilgestaan bij de verloskundige praktijkvoering in afwachting van een meer definitieve regeling.

Al tientallen jaren wordt gezocht naar een niet-invasief, veilig alternatief voor de vruchtwaterpunctie en vlokkentest. In de richtlijn Prenatale Diagnostiek wordt het risico op een miskraam als gevolg van deze tests 0,3-0,5% genoemd. Een recente review van Tabor en Alfirevic komt op een risico van 0,5-1%.[1] Een ander nadeel is dat het huidige screeningsprogramma niet in staat is om de groep zwangeren die voor een invasieve test in aanmerking komt, goed te selecteren. In de twee belangrijkste indicatiegroepen – verhoogd risico op basis van de combinatietest en maternale leeftijd van 36 jaar of ouder – wordt bij de invasieve tests slechts in respectievelijk 7% en 1% een trisomie 21 vastgesteld. In meer dan 95% van de gevallen wordt de risicovolle invasieve test eigenlijk onnodig gedaan.

In 2010 verschenen de eerste publicaties over de niet-invasieve prenatale test (NIPT) naar trisomie 21 die gebruik maakt van vrij foetaal DNA in maternaal plasma. Deze techniek is al langer in gebruik voor het bepalen van de foetale bloedgroep en het geslacht. Volgens inmiddels tientallen studies is NIPT zeer accuraat (>99%) in het voorspellen van de aan of afwezigheid van trisomie 21.[2] NIPT kan vanaf negen à tien weken zwangerschap worden uitgevoerd, zonder bovengrens. De uitslag is na 10-14 dagen bekend. Amerikaanse en Duitse laboratoria die de test de afgelopen jaren hebben uitgevoerd, rapporteren inmiddels ook of er een normale of afwijkende hoeveelheid foetaal DNA van chromosoom 18, 13, X en Y in het plasma aanwezig is. De meeste studies die momenteel zijn gepubliceerd, zijn verricht met plasmamonsters van zwangeren met een al bekend verhoogd risico op trisomie 21.

In toenemende mate wordt door zwangeren met een indicatie voor een invasieve test, en door gynaecologen die deze tests uitvoeren, de wens geuit om naast de optie van de vlokkentest of vruchtwaterpunctie, NIPT als keuze te krijgen. In veel landen is dit al mogelijk en wordt dit ook door de wetenschappelijke verenigingen van de verloskundig hulpverleners in die landen gesteund (SOGC, ACOG).[3,4] Ook de NVOG heeft recent (mei 2013) een standpunt gepubliceerd waarin wordt gesteld dat NIPT zo spoedig mogelijk voor deze groep vrouwen beschikbaar zou moeten komen.[5] Het primaire doel van invoering van NIPT op korte termijn is het aantal onnodige miskramen door invasieve tests (enkele tientallen per jaar), en de angst hiervoor bij duizenden zwangeren, tot vrijwel nul te reduceren. Aangezien de betrouwbaarheid van NIPT weliswaar zeer hoog is maar geen 100%, zal een deel van de

zwangeren waarschijnlijk toch kiezen voor de invasieve test. Ook zijn de beroepsgroepen unaniem van mening dat een positief resultaat van de NIPT altijd bevestigd dient te worden door een invasieve test, alvorens onomkeerbare keuzes (het afbreken van de zwangerschap) te maken. In de groep die op basis van NIPT een verhoogd risico heeft, zal in tegenstelling tot de huidige praktijk, verreweg het grootste deel van de puncties een trisomie 21 bevestigen. Er gaan ook stemmen op voor het aanbieden van NIPT aan elke zwangere in plaats van de combinatietest. De testeigenschappen in deze groep zijn echter nog onvoldoende duidelijk en ook als die gelijk zouden zijn aan die in de hoog risicogroep, betekent dit dat veel minder van de positieve NIPT-uitslagen inderdaad corresponderen met een afwijking van de foetus. De positief voorspellende waarde van NIPT daalt bij een lagere prevalentie (in Nederland komt trisomie 21 bij ongeveer 1 op 500 zwangerschappen voor) al snel richting 50%. Ook zaken als logistieke grenzen (lab-capaciteit) en de additionele waarde van de combinatietest als voorspeller van andere zwangerschapsafwijkingen betekenen dat discussie over invoering van NIPT voor de algemene populatie voorlopig nog ver weg is.

In Nederland is de test nu nog niet beschikbaar. Een aantal universitaire laboratoria is inmiddels technisch wel in staat de test uit te voeren.[6,7] Voor daadwerkelijke invoering als screeningsinstrument is echter toestemming nodig van de minister van Volksgezondheid, Welzijn & Sport (VWS). Er geldt volgens de minister voor de gehele keten van prenatale screening, inclusief de diagnostiek aan het eind van die keten, een vergunningplicht op basis van de Wet Bevolkingsonderzoek (WBO). Bij een wezenlijke verandering in deze keten, zoals aanbieden van NIPT als alternatief voor de invasieve tests, is aanpassing van de huidige WBO-vergunning, die aan de acht regionale centra is verstrekt, noodzakelijk. Eind maart 2013 is een WBO-vergunning aangevraagd voor de TRIDENT-studie, een Nederlandse proefimplementatie van NIPT in de groep zwangeren met een verhoogd risico op trisomie 21. Na goedkeuring door de minister van VWS zal NIPT in samenwerking met de centra voor klinische genetica na zorgvuldige counseling worden aangeboden als keuze door de gynaecologen die nu de invasieve tests uitvoeren. Het bloed zal worden onderzocht in minstens vier en mogelijk meer laboratoria van de universitaire medische centra. Sociale en ethische aspecten zullen uitgebreid worden onderzocht in het kader van de parallel lopende ESPRIT-studie (meer informatie is te vinden op www.niptconsortium.nl). Het is nog onzeker of en wanneer deze proefimplementatie, waarin bloedmonsters van ongeveer 1000 zwangeren onderzocht zullen worden, van start kan gaan. De Gezondheidsraad zal de minister zeer binnenkort een advies sturen en over de financiering van het project wordt ook nog met meerdere partijen gesproken. Hoewel de NIPT goedkoper is dan de invasieve tests, moet voor formele invoering van een tarief voor een nieuwe test door het College van Zorgverzekeraars een langdurig administratief traject nodig. De NIPT is sinds de zomer van 2012 al wel voor Nederlandse zwangeren toegankelijk als zij hun bloed laten onderzoeken in

een laboratorium in Duitsland of in een van de vier Amerikaanse laboratoria. In meerdere Europese landen, waaronder Duitsland, België en Engeland, zijn gynaecologen bereid om bloed van Nederlandse zwangeren af te nemen en naar deze labs te sturen. De kosten komen voor rekening van de zwangere en bedragen tussen de 600 en 1000 euro, inclusief het consult bij de buitenlandse gynaecoloog. De test zelf kost tussen de 400 en 825 euro, waarbij de verwachting is dat dit bedrag bij toenemende aantallen en automatisering nog zal dalen. De invasieve test kost ongeveer het dubbele.

In de huidige situatie, in afwachting van de formele goedkeuring in de vorm van een WBO-vergunning van uitvoering van NIPT, is in de spreekkamer van verloskundig hulpverleners sprake van een dilemma, ook wel aangeduid als spagaat. NIPT maakt geen deel uit van het officiële aanbod van tests voor zwangeren en de hulpverlener kan NIPT dan ook niet aanbieden. Als zwangeren zelf naar informatie over NIPT vragen, hoort de zorgverlener de patiënt juist en volledig te informeren. Voor degenen die al wat langer in het vak werkzaam zijn, is dit geen onbekend fenomeen. Jarenlang is op deze basis eerst de tripletest en later de combinatietest uitgevoerd bij het beter geïnformeerde deel van de zwangere bevolking, tot uiteindelijk in 2007 de WBO-vergunning werd afgegeven en de tests aan elke zwangere mochten worden aangeboden.
Opnieuw, en voor velen wellicht een déjà vu, treffen gynaecologen en verloskundigen zichzelf aan in deze spagaat. Is het niet-verstrekken van informatie over NIPT tenzij de zwangere patiënte hierom zelf actief vraagt, in strijd met goed hulpverlenerschap, waarbij het geven van de best mogelijke zorg, het optimaliseren van de keuzevrijheid van patiënten en het waarborgen van gelijke toegang tot zorg een centrale plaats hebben? Is het niet gewoon 'achterhouden van informatie' en in strijd met de informatieplicht van de hulpverlener volgens de WGBO? Valt de gynaecoloog iets te verwijten als een zwangere een miskraam krijgt na een invasieve test? Of als een kind met het downsyndroom wordt geboren omdat de zwangere uit angst geen punctie liet doen? Is het feit dat de verzekeraar de in het buitenland uitgevoerde test (nog) niet vergoedt een inbreuk op het recht van patiënten op gelijke toegang tot zorg?

CASUS

Patiënte A, 42 jaar, gravida 3 para 0, heeft in de voorgeschiedenis twee miskramen, vijf en zes jaar geleden. Het daaropvolgend fertiliteitstraject heeft zij uiteindelijk na vier IVF-pogingen gestaakt. Nu is sprake van een zeer onverwachte, maar zeer gewenste spontane zwangerschap. Zij kiest na counseling voor een combinatietest, die een kans op trisomie 21 van 1:166 geeft.

Patiënte B, 38 jaar, gravida 3 para 0, tweemaal een spontane miskraam, is op het moment van consultatie elf weken zwanger. Zij is op de hoogte van de kans op een kind met een trisomie passend bij haar leeftijd en zij zou de geboorte van een kind met een trisomie willen vermijden. Zij weet dat zij recht heeft op een invasieve test, maar ook dat zo'n 99% van die tests in haar leeftijdsgroep een normale uitslag geeft en dus in haar ogen 'voor niks' wordt gedaan. Zij ziet erg op tegen het risico van de invasieve ingreep, vanwege haar twee miskramen en omdat een vriendin een kindje heeft verloren na een vruchtwaterpunctie.

Patiënte C, 33 jaar, gravida 2 para 1 en zeven weken zwanger. Zij is anderhalf jaar geleden bevallen van een kind met onverwacht downsyndroom. De combinatietest gaf destijds een kans van 1 op 500 aan op trisomie 21. Er was geen sprake van een erfelijke vorm, de herhalingskans wordt door de klinisch geneticus als hooguit enkele procenten ingeschat. Zij heeft daar inmiddels vrede mee en heeft weinig problemen met het feit dat haar eerste kind downsyndroom heeft. Zij wil echter wel heel graag voorkomen dat zij een tweede kind met downsyndroom krijgt. Ze vertrouwt de combinatietest niet meer, maar ze ziet op tegen de risico's van een invasieve test.

Patiënte D, 34 jaar, gravida 2 para 0, heeft een combinatietestuitslag met een kans op downsyndroom van 1 op 80. Zij kiest voor een vlokkentest. De dag na de ingreep is er sprake van vaginaal bloedverlies dat in wisselende mate aanhoudt. Bij 18 weken zwangerschap is er sprake van evident gebroken vliezen, waarna bij 22 weken een partus immaturus volgt.
Bij nacontrole vraagt patiënte of zij in een volgende zwangerschap NIPT mag en of zij dan eerst weer de combitest moet ondergaan of ook direct voor NIPT mag kiezen.

BESCHOUWING

De casus die hierboven zijn beschreven, behoren tot de praktijk van een verloskundige hulpverlener. In alle casus lijkt NIPT een aantrekkelijke optie, maar NIPT mag niet worden aangeboden. Mogelijke uitzondering is patiënte C, aangezien haar indicatie voor prenataal onderzoek een zogenaamde 'medische indicatie' betreft en daarmee buiten het screeningsaanbod en de WBO-vergunningplicht valt. De redenering is in dit geval: bij patiënte C is gebleken dat zij een verhoogde individuele kans heeft op het krijgen van een kind met downsyndroom. Daarom is prenataal onderzoek tijdens een volgende zwangerschap, ook middels NIPT, individueel geïndiceerd in het kader van de hulpverleningsrelatie. Bij de andere patiënten uit de casus zijn weliswaar op basis van epidemiologische gegevens verhoogde risico's te berekenen, maar NIPT zou in hun geval een voortzetting zijn van de eerder ingezette theoretische risicoschatting en niet plaatsvinden op basis van een individuele indicatie.

Het is niet moeilijk om de overtuigingskracht van deze redenering te relativeren en het lijkt ondoenlijk om de redenering in de spreekkamer overtuigend te presenteren, maar dat is niet waarop het aankomt. Waarop het aankomt is, dat het de geldende redenering is en dat die niet kan worden genegeerd. De ontstane situatie hangt samen met het op zichzelf juiste onderscheid tussen hulpverlening en screening. Bij hulpverlening is het uitgangspunt dat de patiënt zich met een klacht of probleem tot de hulpverlener wendt, die zich vervolgens inspant om dat probleem op te lossen. Bij screening gaat het initiatief uit van de professional die zich ongevraagd tot mensen zonder klachten richt met het aanbod om nog niet ontdekte ziekten of afwijkingen op te sporen. Dat heet bevolkingsonderzoek. Bevolkingsonderzoek is in een aantal omstandigheden alleen toegestaan als de minister van VWS daarvoor, na advies van de Gezondheidsraad, een vergunning heeft gegeven: bij onderzoek naar kanker, bij onderzoek met behulp van ioniserende straling en bij onderzoek naar ernstige ziekten waarvoor geen preventie of behandeling mogelijk is.

Prenataal onderzoek is daarom vergunningplichtig als wordt gezocht naar aandoeningen die niet kunnen worden behandeld of voorkomen. Dat is dikwijls het geval. Als prenataal wordt gescreend op chromosomale afwijkingen, bijvoorbeeld downsyndroom, dan zijn er geen mogelijkheden voor behandeling of preventie. Weliswaar kan dan worden besloten tot afbreking van de zwangerschap, maar dat is geen preventie van ziekte maar preventie van de patiënt. En dat is niet het soort preventie dat de WBO bedoelt. Daarom is prenatale screening op downsyndroom door onderzoek van foetaal DNA in maternaal plasma vergunningplichtig bevolkingsonderzoek in de zin van de WBO. Dat er geen mogelijkheden zijn tot preventie van ziekte betekent niet dat de vergunning niet zou mogen worden gegeven. Dat mag wel, maar die beslissing is aan de minister van VWS, die voor een dergelijke beslissing ook politiek verantwoordelijk is. Dat betekent dat de beslissing moet worden gesteund – althans: niet wordt bestreden – door een meerderheid in de Tweede Kamer. Die meerderheid is niet vanzelfsprekend. In het verleden waren het vooral de christelijke partijen die daartegen uitgesproken bezwaren hadden; tegenwoordig is verzet tegen prenatale screening die mogelijk leidt tot afbreking van zwangerschappen ook in niet-religieus georiënteerde partijen aan te treffen.

Wat te doen als zwangere zelf vraagt naar de mogelijkheid van NIPT?

Als zwangeren zelf verzoeken om NIPT, welke informatieve mag en wellicht moet de hulpverlener dan geven? Als de patiënte er zelf om vraagt, is de arts volgens de WGBO verplicht de patiënte juist en volledig te informeren. Die informatie omvat ook dat NIPT in Nederland niet als screeningsinstrument is toegestaan en alleen op individuele medische indicatie zou mogen worden uitgevoerd. Een individueel verzoek van een zwangere om NIPT

is niet hetzelfde als een individuele medische indicatie en dat betekent dat aan een dergelijk verzoek geen gehoor mag worden gegeven. De hulpverlener behoort dus op de hoogte te zijn van de mogelijkheden. Door het bijhouden van de wetenschappelijke literatuur, het zoeken van informatie op internet, congresbezoek en nascholing kan elke gynaecoloog voldoende op de hoogte zijn om de bestaande opties te bespreken. In 2012 is het landelijk multidisciplinair NIPT-consortium opgericht, en is aan alle gynaecologen per e-mail het adres van de website met up-to-date informatieve over NIPT gestuurd (www.niptconsortium.nl).
Onder FAQ staat op die website een lijst met mogelijkheden om NIPT in het buitenland te laten uitvoeren.

Het aanbieden van NIPT mag niet, wat als de hulpverlener dit wel doet?

Tot op heden is het ministerie van VWS er duidelijk over geweest; het aanbieden van NIPT is verboden. Indien NIPT als screeningsinstrument wordt aangeboden is dit in strijd met de WBO. Het versturen van bloed vanuit Nederland naar een buitenlands laboratorium wordt door het ministerie van VWS eveneens als ongewenst beschouwd. Het ministerie van VWS heeft aangegeven dat, als bekend wordt dat hulpverleners NIPT als screeningsinstrument aanbieden of bloedsamples versturen naar het buitenland, dit zal worden gemeld aan de Inspectie voor de Gezondheidszorg (IGZ).[8] De te verwachten actie kan dan zijn dat de IGZ gelast dat deze activiteiten worden gestaakt. Gelet op de dwangmiddelen die de IGZ ter beschikking staan, zal een ziekenhuisbestuur niet lang aarzelen om conform de aanwijzingen van de IGZ te handelen.

Valt de gynaecoloog iets te verwijten als een zwangere, onkundig van NIPT een miskraam krijgt na een invasieve test? Of als een kind met downsyndroom geboren wordt omdat de zwangere uit angst geen punctie liet doen?

Bovenstaande vragen hebben een overwegend moreel karakter. Dat betekent niet dat zij er niet toe doen, maar het betekent dat zij uiteindelijk leiden tot de vraag of inachtneming van de wet- en regelgeving verenigbaar is met de persoonlijke morele overtuiging. Dat is niet een vraag om in het voorbijgaan te beantwoorden. In uitzonderlijke situaties is burgerlijke ongehoorzaamheid een verdedigbare positiekeuze. Daarmee is dan wel verbonden dat de overtreding in alle openheid geschiedt en dat de overtreder zich niet probeert te onttrekken aan de gevolgen van zijn keuze. Of de feitelijke onbereikbaarheid van NIPT als screeningsinstrument voor de hulpverlener een situatie oplevert die burgerlijke ongehoorzaamheid rechtvaardigt, is een conclusie die niet te snel moet worden getrokken. Er is verschil van opvatting, er is onvrede, er is ergernis over gekunstelde redeneringen, er is frustratie in de spreekkamer en er is boosheid over de onmacht en de onwil om tot een vrouwvriendelijk aanbod van prenatale screening te

komen. Dat is allemaal waar, maar rechtvaardiging van burgerlijke ongehoorzaamheid is het nog niet.

Concluderend kan beantwoording van een verzoek om informatie over de mogelijkheden en beperkingen van prenatale diagnostiek, van patiënten met een verhoogd risico op een foetale trisomie, als onvolledig worden beschouwd als de hulpverlener de mogelijke opties en beperkingen van prenatale diagnostiek in Nederland niet noemt. Echter, de gynaecoloog dient zich, al dan niet van harte, aan de wet te houden. Om deze reden alleen al, kan men niet worden verweten de optie van NIPT in het buitenland niet aan te bieden. Dat vele hulpverleners hiermee ongelukkig zijn, betekent op zichzelf nog niet dat sprake is van een zodanig gewetensconflict dat burgerlijke ongehoorzaamheid is gerechtvaardigd. Als een patiënt daarentegen om informatie vraagt over NIPT, inclusief de mogelijkheden daartoe in het buitenland, is het wel de plicht van de hulpverlener de patiënt volledig en naar beste weten antwoord te geven.

De verzekeraar betaalt nog niet: ongelijke toegankelijkheid van zorg?

Het ene probleem roept het andere op. De zorgverzekeraars hebben desgevraagd aangegeven de NIPT, uitgevoerd door een buitenlands lab, nog niet te kunnen vergoeden, daar het ministerie van VWS heeft laten weten dat NIPT wettelijk nog niet mag.* Veel gynaecologen geven aan moeite te hebben met een zorgprogramma waarbij rijkere mensen zich betere zorg kunnen veroorloven dan mensen met minder geld. Echter, er bestaat op dit moment in Nederland al een door de politiek bepaalde ongelijke toegang tot prenatale screening, waarmee veel hulpverleners overigens niet gelukkig zijn. Vrouwen jonger dan 36 jaar krijgen wel een counselingsgesprek, maar als zij kiezen voor de combinatietest moeten zij deze zelf betalen (in 2013 154 euro). Voor vrouwen van 36 jaar en ouder wordt de test vergoed via de basisverzekering. Het is gebleken dat een eigen bijdrage invloed heeft op de besluitvorming en dat een deel van de vrouwen met weinig financiële mogelijkheden om deze reden niet de combinatietest laten uitvoeren.** Het Centraal Orgaan van het Centrum voor Bevolkingsonderzoek van het RIVM, waarin alle beroepsgroepen, de patiëntvertegenwoordigers en zorgverzekeraars vertegenwoordigd zijn, heeft het ministerie van VWS overigens een formeel verzoek gestuurd (d.d. 23-05-2012) dit te veranderen.

Wettelijke achtergronden

In de nabije toekomst verwachten we dat NIPT, uitgevoerd in Nederlandse laboratoria, een legale en in het basispakket verzekerde plaats zal krijgen in het Nederlandse screeningsprogramma. Totdat het zover is, zal de vraag naar deze test groeien, evenals de omvang van de groep gynaecologen die de test al wil aanbieden. Zowel voor de hulpverleners

als voor de zwangeren is het wenselijk om in dit 'interbellum' de kwaliteit van zorg te bewaken en adequaat in te spelen op de ontwikkelingen.
De praktijk zal zich voorlopig moeten behelpen. Antwoord geven op hulpvragen en informatie verstrekken mag altijd. Als patiënten op basis daarvan besluiten om in het buitenland hulp te zoeken, dan staat hun dat vrij. Of en in hoeverre een Nederlandse hulpverlener dat zal faciliteren, laat zich in zijn algemeenheid niet vaststellen. Er zal een weinig overzichtelijke praktijk ontstaan waaraan ooit ook weer een einde zal komen. Want het onderwerp NIPT laat zich niet van de agenda afvoeren. De problemen rond de toelating van NIPT als screeningsinstrument zijn niet nieuw. Zij hangen samen met de manier waarop bevolkingsonderzoek in Nederland is georganiseerd en hoe de verschillende verschijningsvormen ervan zijn gedefinieerd. Zij hangen ook samen met de rol die de minister van VWS speelt bij vergunningplichtig bevolkingsonderzoek. Die rol kan de besluitvorming in het politieke domein trekken en er de oorzaak van zijn dat politieke overwegingen – maar ook politieke opportuniteit – de uitkomst van het besluitvormingsproces gaat bepalen.

De moeilijkheden rond prenatale screening zijn een uitloper van het abortusdebat zoals dat in de jaren zestig en zeventig van de vorige eeuw in Nederland is gevoerd. De hoog oplopende tegenstellingen zijn toen gepacificeerd in de Wet afbreking zwangerschap van 1981. Die wet verenigt twee schijnbare onverzoenlijkheden door enerzijds een strenge inhoudelijke norm te introduceren ('de noodsituatie van de vrouw maakt afbreking van de zwangerschap onontkoombaar') en anderzijds een liberale praktijk te faciliteren waarbij de inhoudelijke norm niet of nauwelijks wordt getoetst. Daarmee zijn de kwaliteit en de toegankelijkheid van de abortushulpverlening verzekerd, een hulpverlening die zich afspeelt buiten het bereik van de openbare meningsvorming. Omdat regulering van prenatale screening zich steeds weer in het publieke domein voltrekt, wordt het debat over abortus juist in verband daarmee gevoerd. Dat heeft paradoxale trekken, omdat uitgerekend in het kader van prenatale screening niet hoeft te worden gevreesd voor luchthartige beslissingen, opportunistische overwegingen of verwijtbare achteloosheid waar het betreft het ontstaan van zwangerschappen. Bovendien moet worden bedacht dat er weliswaar een verband is tussen prenatale screening en afbreking van zwangerschappen, maar dat verband is niet het enige of eerst aangewezen gezichtspunt. Prenatale screening dient er in de eerste plaats toe om keuze-opties voor zwangeren te bieden. Dat genuanceerde gezichtspunt zal de discussie naar verwachting echter niet beslissend beïnvloeden. Voor wie afbreking van een zwangerschap onder elke omstandigheid afwijst, is elke aanleiding om het thema ter sprake te brengen even goed. Hoe steiler het principe, des te eenvoudiger de positiebepaling. Voor wie abortus een pijnlijke maar helaas niet altijd te vermijden interventie is, is de prenatale screening bij uitstek niet het onderwerp waaraan de discussie zou moeten worden opgehangen. Zolang de systematiek van de WBO-regulering ongewijzigd blijft, zullen het abortusdebat en de daarmee verbonden emoties de organisatie en

vormgeving van de prenatale screening echter blijven bepalen. De Wet afbreking zwangerschap zal niet worden gewijzigd. Daarvoor is geen politieke meerderheid te organiseren en dat hoeft niemand te betreuren. De oplossing moet dus komen van wijziging van de WBO door depolitisering van de besluitvorming met betrekking tot bevolkingsonderzoek. Dat kan even duren, maar het zal er van komen. En tot die tijd zal de praktijk, al dan niet met hindernissen, wegen vinden om de hulp te bieden die zwangere vrouwen nodig hebben.

* brief en emails aan DO
** nog ongepubliceerde studie, persoonlijke communicatie EJV

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V
OPINION

E.J.T. Verweij ● M.A. de Boer ● D. Oepkes

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CHAPTER 9

Non-invasive prenatal diagnosis for Down syndrome: no paradigm shift, just better testing. . . and it is already here!

We read with great interest the Opinion, 'Non-invasive prenatal diagnosis for Down syndrome: the paradigm will shift, but slowly', by Benn et al. published in the January issue of *Ultrasound in Obstetrics & Gynecology*.[1] We agree with the authors that techniques using cell-free fetal DNA in maternal plasma have great potential to improve public health and to expand reproductive choices of pregnant women. We also agree that before new tests can be introduced into clinical care, well-designed adequately powered prospective studies need to be performed. We were quite surprised, however, by a number of their negative remarks about this technology. We would like to address these, to provide an alternative view. This test is already available for pregnant women in an increasing number of countries. Thus, the test is already here!

Non-invasive prenatal testing (NIPT) for fetal trisomy: missing other diagnoses?

In many countries worldwide, pregnant women have been offered screening for fetal trisomy 21 (T21) for decades. The accepted program is first to inform women about the disease and their individual risk for T21, then to offer a sensitive screening test to select a high-risk group. This group is allowed to undergo an invasive test with karyotyping, which has a (near-) 100% accuracy to detect or exclude T21. With karyotyping, other chromosomal rearrangements, if present, are also detected. These coincidental findings are often regarded as useful, but can also be confusing, unexpected and unclear. Karyotyping is now often replaced by rapid aneuploidy detection: faster and cheaper, and logical given the fact that the woman was counseled about T21 specifically and screened using a test designed for T21. With the introduction of NIPT, we should not repeat the discussion on the estimated, really small (1:1600 to 1:4000) risk of, by abandoning full karyotyping, missing a clinically relevant anomaly that is undetectable by ultrasound. The true risk of missing such an anomaly on a population basis is actually 10–20 times lower, since it only applies to the 5% of women in whom we perform invasive testing. If we truly want to offer testing for chromosomal rearrangements and microdeletion/duplication syndromes, we should offer amniocentesis to all pregnant women, not only to the 5% who are screen-positive for T21. Even then, we would 'miss' hundreds of rare diseases, for which no genetic or ultrasound diagnosis is currently possible.

NIPT for fetal trisomy: screening or diagnosis?

Whether a test is diagnostic or for screening is not related to its accuracy. Many diagnostic tests have a far lower sensitivity and specificity than 99%. 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. The issue usually at the root of the 'diagnostic or not' debate is whether a positive NIPT result should be followed by a confirmatory invasive test.

The answer, at least for the coming years, is yes. Even with a specificity of 99.7%, in a general population with a prevalence of T21 of 1:500 (Dutch population), the positive predictive value is only around 60%.

Are there really disadvantages of NIPT?

NIPT would lead to a major reduction (around 75%) in invasive testing. NIPT now seems to work well in twins too; even if this were not the case, why would this be a reason not to offer NIPT to the 99% of women bearing singletons?[3] The equally rare confined placental mosaicism, where in 90% of cases the fetus is normal, is an interesting phenomenon to study, but would only very marginally increase the false-positive rate, and is no reason not to offer NIPT.

NIPT as first-line test or only for 'high-risk' women?

Offering NIPT as a first-line test to all pregnant women would have major advantages over current screening: not only reducing iatrogenic miscarriages of wanted, healthy children but also practically eliminating false reassurance. The current program as used in many countries (not modeling, but in the real world) results in the unexpected birth, *in women that were screened*, of a T21 child in one or two per 10 T21 cases. In addition, many women would like to know about T21 but decline what they see as the complex and uncertain serum and nuchal translucency (NT) testing, fear invasive testing, or are too late in the pregnancy for the first-trimester combined test. The only way to allow women to truly make an informed choice would be to offer a highly reliable, easy-to-explain and safe test. All that needs to be implemented, from the patient's and clinician's perspective, is adaptation of the content of counseling and arrangement of logistics to send a blood sample to a laboratory capable of performing this technology.

We do of course acknowledge the complex and highly sophisticated laboratory and biostatistical work behind NIPT. The only current restriction for offering it to all pregnant women would be cost, which will decrease with increasing numbers, advancing technology and more efficient methods, such as targeted testing.[4]

Counseling

In contrast to the current counseling on risk assessment with serum markers and NT measurement, everyone understands NIPT within minutes, including the absence of 100% certainty. This leaves time for a more in-depth discussion about T21, and whether parents are willing to accept a child with Down syndrome.

Will NIPT for trisomy replace the 11–14-week scan?

NT measurement was invented to screen for T21. Detailed first-trimester ultrasound by well-trained sonographers enables assessment of many functional and anatomical features of the fetus. Whether NT measurement will remain a cost-effective enterprise needs to be re-evaluated.

Conclusion

Finally, after decades of research, non-invasive prenatal trisomy testing is now a clinical reality. NIPT provides many advantages over current screening options. Organizational, financial and logistic aspects need to be solved. Introduction should be done after well-designed prospective clinical studies showing that expectations are truly met. NIPT is already here, and it will be increasingly hard to explain to the pregnant women under our care why we are not yet offering them this option.

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VI
SUMMARY
&
DISCUSSION

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 Bevolkings Onderzoek, 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.[2] 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).[5] 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.[2] 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.

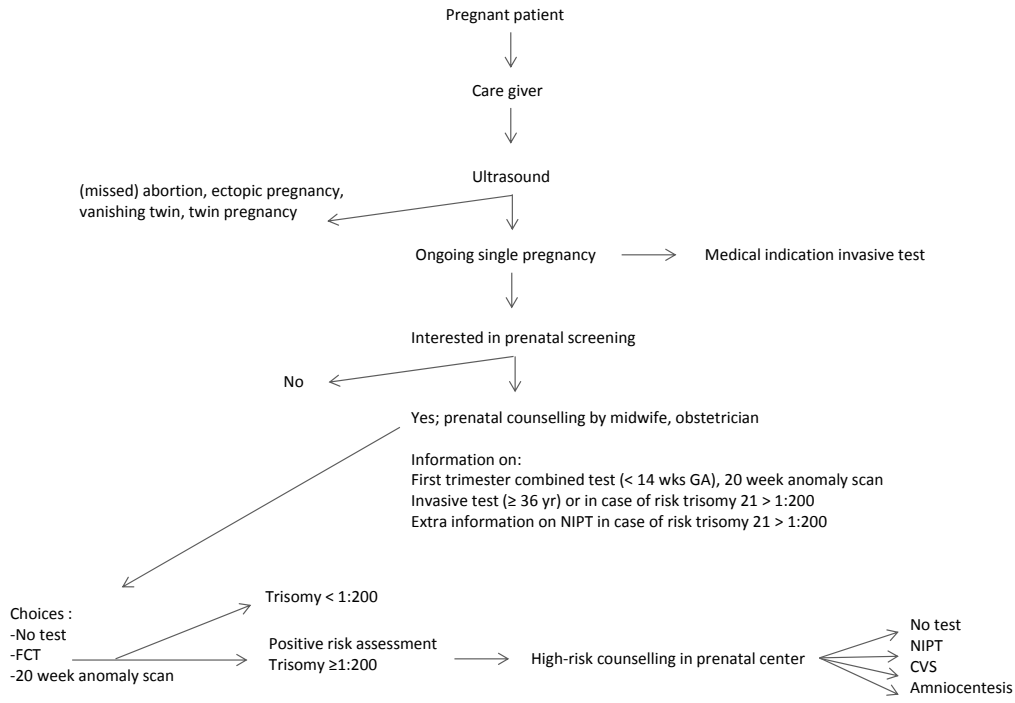

Figure 1. *The flowchart of the TRIDENT-study*

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.

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 NPT, 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.[4] 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.[6] 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.

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

	CT 1 op 200 geen NIPT	CT 1 op 200 en NIPT	CT 1 op 300 en NIPT	CT 1 op 500 en NIPT	Geen CT, direct NIPT
CT: Trisomie 21 (sensitiviteit)	170 (85%)	170 (85%)	180(90%)	194(97%)	n.v.t.
CT: foutpositief (percentage)	3.393 (3,4%)	3.393 (3,4%)	5.489 (5,5%)	9.980 (10%)	n.v.t.
NIPT (na CT): Trisomie 21	n.v.t.	169	179	193	199
NIPT: aantal foutpositief	n.v.t.	10	16	30	299
Aantal invasieve tests	3.563	179	195	223	498
PVW	5%	94%	92%	87%	40%
Detectie : miskraam[a]	10:1	189:1	184:1	173:1	80:1
Invasieve tests t.o.v. nu	0	-3214	-3198	-3170	-2895
Trisomie 21 t.o.v. nu	0	-1	+9	+23	+29

[a] Detectie-miskraamverhouding: het aantal gedetecteerde gevallen trisomie 21 op één miskraam, als de kans op iatrogenen miskraam 0,5 procent is.

* Als voorbeeld worden alleen de gegevens van trisomie 21 gebruikt, omdat deze het best zijn onderbouwd.

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.[7]

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.

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CHAPTER 11

Nederlandse Samenvatting

De niet-invasieve prenatale test (NIPT) is in veel landen beschikbaar. Er zijn echter nog veel onderzoeksvragen onbeantwoord.

In dit proefschrift worden vraagstukken over de implementatie van NIPT besproken. De algemene conclusies worden eerst samengevat, gevolgd door een discussie over toekomstige mogelijkheden.

SAMENVATTING

HET HUIDIGE PRENATALE SCREENINGSPROGRAMMA

De meest voorkomende chromosoomafwijking bij levend geboren kinderen is het syndroom van Down (trisomie 21(T21)). De prevalentie van het syndroom van Down in Nederland wordt geschat op 1:500. De kans op T21 neemt toe met de leeftijd, hoe ouder een vrouw is tijdens de zwangerschap hoe hoger de kans op een aangedaan kind.
In de meeste westerse landen krijgen zwangere vrouwen prenatale screening naar T21 aangeboden. In Nederland werd tot 2007 een invasieve procedure, de vlokkentest of de vruchtwaterpunctie, aangeboden aan vrouwen van 36 jaar of ouder. Leeftijd is een relatief slechte voorspeller voor T21. Ongeveer 1% van de uitslagen van de invasieve tests was positief voor T21, terwijl door de procedure ook 0,5-1% (meestal gezonde) zwangerschappen verloren gingen. Om deze reden werd in 2007 een prenataal screenings programma gestart om de kans op T21 nauwkeuriger te bepalen. In het eerste trimester worden vrouwen op de hoogte gesteld van de mogelijkheid om de zogenaamde combinatietest (CT) te laten uitvoeren. De CT is een test waarmee de individuele kans op een foetus met T21 bepaald wordt. De kans wordt berekend met een algoritme waarin de maternale leeftijd, de uitslagen van twee maternale serummarkers en een echografisch gemeten nekplooimeting worden meegewogen. De CT kan worden uitgevoerd tussen 11 en 14 weken zwangerschap. De nekplooi is een vochtophoping achter de foetale nek. Een abnormale dikte ervan is geassocieerd met foetale trisomie en vele andere afwijkingen, zoals hartafwijkingen. De nauwkeurigheid van de meting is afhankelijk van de ervaring en de precisie van de echoscopist. Sinds 2010 wordt de CT ook aangeboden met een aangepast algoritme voor de screening op trisomie 18 (Edwards syndroom) en trisomie 13 (Patau syndroom).
De prevalentie van trisomie 18 en 13 is lager (1 op 3000 en 1 op 6000, respectievelijk). Samen met de introductie van de CT werd het Structureel Echoscopisch Onderzoek (SEO) bij 20 weken zwangerschapsduur geïntroduceerd om te screenen op neurale buisdefecten en andere structurele afwijkingen.

De invoering van de CT resulteerde in een significante vermindering van invasieve procedures en werd beschouwd als een grote stap voorwaarts. De nauwkeurigheid is mede afhankelijk van de kwaliteit van de echo. In de klinische praktijk resulteert dit in 10-25 % vals negatieve uitslagen.[1-5] Als er sprake is van een vals negatief resultaat worden vrouwen onterecht gerustgesteld maar geconfronteerd met een kind met T21 na de geboorte. De vals positieve uitslagen van de CT worden bepaald door de testkarakteristieken en de afkapwaarde, die in Nederland gesteld is op 5% (1:200). Om deze reden zal 1 op de 20 vrouwen worden doorverwezen voor een invasieve procedure , terwijl >90% van hen niet een aangedaan kind draagt. Een andere ernstige beperking van CT is het feit dat deze alleen tussen 11-14 weken zwangerschapsduur kan worden uitgevoerd. Vrouwen die te laat zijn voor hun eerste bezoek aan een arts of verloskundige, om welke reden dan ook, kunnen geen CT meer laten doen. Tot voor kort kon tot 18 weken de zogenaamde triple test worden verricht, maar de Nederlandse laboratoria bieden deze serumtest niet meer aan. Ongeveer 25% van de Nederlandse zwangere bevolking laat de CT uitvoeren. In vergelijking met andere Europese landen is dit een laag percentage.

Invasieve procedures

Als sprake is van een verhoogde kans op een foetale trisomie bij de CT wordt een invasieve test aangeboden: de vlokkentest of de vruchtwaterpunctie. Een vlokkentest is mogelijk vanaf 11 weken en kan zowel transabdominaal als transcervicaal worden uitgevoerd. De vruchtwaterpunctie is de meest gebruikte invasieve procedure en wordt uitgevoerd vanaf 15 weken. Zowel de chorion villi als de vruchtwatercellen worden onderzocht in het regionaal universitair klinisch genetisch laboratorium. Er kan een snelle uitslag volgen binnen enkele dagen (alleen voor trisomie 21, 18 en 13 en het geslacht), maar er kan ook een uitgebreid onderzoek worden gedaan waarbij uitgebreid wordt gekeken naar chromosomale afwijkingen (karyotypering of microarray). Deze invasieve testen zijn geassocieerd met een verhoogde kans op een miskraam, in studies wordt deze kans geschat op 0,5-1%.[6-7] De vlokkentest heeft, zeker in de zogenaamde lange termijn kweek, een zeer hoge nauwkeurigheid, de vruchtwaterpunctie presteert nog beter. Een negatieve of positieve uitslag betreffende trisomie 21, 18 en 13 wordt bij beide tests 100% betrouwbaar geacht. De vlokkentest mislukt soms, en geeft in 1-2% uitslagen die niet goed te interpreteren zijn, waardoor alsnog een vruchtwaterpunctie moet plaatsvinden. Volgens gepubliceerde cijfers van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) kiest slechts de helft van de vrouwen in Nederland met een verhoogde kans op trisomie na CT voor een invasieve prenatale diagnostische test. De reden voor het afzien van een invasieve procedure na een verhoogde kans uit de CT is onbekend. Het kan angst voor verlies van het kind zijn na een invasieve procedure, maar ook angst voor pijn of naalden. Of afhankelijk van de CT uitslag kan het gevoel bestaan dat de kans op een kind met T21 niet echt hoog is. Samenvattend is zowel op het gebied van de

gemiste afwijkingen, de onterecht positieve uitslagen en de veiligheid van de diagnostische tests in het huidige prenatale screeningsprogramma veel ruimte voor verbetering.

DE UITVOERING EN BETROUWBAARHEID VAN NIPT

Na tientallen jaren van onderzoek naar het bepalen van een foetale trisomie op niet-invasieve wijze is dit nu mogelijk door het sequencen van cel-vrij foetaal DNA in het plasma van de moeder. De niet-invasieve prenatale test (NIPT) maakt gebruik van kleine fragmentjes vrij foetaal DNA die, naast veel meer fragmenten maternaal DNA, vrij rondzweven in het maternaal plasma. De meest gebruikte techniek is 'massive parallel shotgun sequencing' waarbij de totale hoeveelheid cel vrij DNA-fragmenten afkomstig van de moeder en van de foetus (in feite afkomstig van de placenta, ook wel foetale fractie genoemd) wordt gesequenced. Bij T21 is er sprake van drie in plaats van twee exemplaren van chromosoom 21. Als de foetus T21 heeft is het totaal aantal sequenties afkomstig van chromosoom 21 verhoogd. Het aantal sequenties van chromosoom 21 wordt vergeleken met het aantal sequenties van een ander (referentie) chromosoom. Het maternale genoom is meestal euploïd , zodat afwijkingen in de verhoudingen afkomstig moeten zijn van het foetale genoom. NIPT kan vanaf negen à tien weken zwangerschap worden uitgevoerd, zonder bovengrens. De uitslag is na 7-21 dagen bekend.

De accuratesse van de test is hoog (sensitiviteit >99%). In 2011 hebben wij een systematische review uitgevoerd om de accuratesse te bepalen van NIPT voor T21 met behulp van de QUADAS richtlijnen. In deze review (**hoofdstuk 1**) concluderen we dat NIPT in de tot dan uitgevoerde retrospectieve studies een hoge diagnostische nauwkeurigheid heeft, maar dat voordat tot invoering besloten wordt, grootschalige prospectieve studies, zowel in hoog-risicogroepen als in een algemene populatie, zouden moeten worden uitgevoerd. Na onze systematische review zijn diverse grotere studies gepubliceerd over de accuratesse van NIPT, deze zijn samengevat in een review van Mersy et al.[8]

Toen NIPT klinisch beschikbaar werd, in november 2011 in de Verenigde Staten, raakten Nederlandse zwangere vrouwen hiervan snel op de hoogte. Nederlandse genetische laboratoria in verschillende universitaire medische centra waren al jaren bezig met het uitvoeren van NIPT in een onderzoekssetting. Echter, om NIPT te mogen aanbieden bleek een specifieke vergunning nodig van het ministerie van Volksgezondheid op basis van de Wet op het Bevolkingsonderzoek (WBO). Uiteindelijk heeft het nationale NIPT-consortium bijna drie jaar later de licentie verkregen (verleend per 1 april 2014).

In de tussentijd vonden duizenden Nederlandse zwangere vrouwen hun weg naar buitenlandse laboratoria om daar NIPT uit te laten voeren. In 2011, net na de lancering van NIPT, vlogen Nederlandse vrouwen naar de Verenigde Staten om daar bloed af te laten nemen. Later, in 2012 konden vrouwen in België een bloedmonster laten afnemen en werden de buizen daarvandaan naar de Amerikaanse laboratoria verstuurd. Ook opende een laboratorium in Duitsland haar deuren, waar Nederlandse vrouwen vooral in het oosten des lands via Duitse gynaecologen NIPT lieten uitvoeren.

Met de EU-NITE studie (**hoofdstuk 2**) hebben we aangetoond dat NIPT met het vervoeren van bloedmonsters over de Atlantische Oceaan naar een laboratorium in de Verenigde Staten een nauwkeurige en haalbare optie is.
Een andere belangrijke bevinding van de EU-NITE studie was een casus met een vals negatief NIPT resultaat voor T21. Dit was het eerste vals negatieve resultaat in meer dan 5000 beschreven casus met de DANSR/ FORTE methode van Ariosa Diagnostics. Het percentage foetale DNA in deze casus was laag (4%), net boven de drempel voor het laboratorium om een resultaat te geven. Hoe hoger de foetale fractie (het percentage foetale DNA in de totale hoeveelheid (maternaal en foetaal samen) circulerend cel vrij DNA), hoe nauwkeuriger het testresultaat. We begrijpen steeds beter dat de foetale fractie van groot belang is om de juistheid van NIPT te bepalen. De foetale fractie kan worden beïnvloed door bijvoorbeeld een hoge body mass index.[9] Met behulp van de huidige technieken (2013), wordt door de laboratoria die de foetale fractie bepalen een waarde <4% beschouwd als te laag om een betrouwbare uitslag te geven. In het geval dat de foetale fractie niet gemeten wordt, zal een sample waarin de foetale fractie <4% is niet worden geëxcludeerd, met een groter risico op het afgeven van een vals negatieve uitslag.

In **hoofdstuk 3** bespreken we dat NIPT voor trisomie 13 (T13) een minder goede accuratesse heeft dan voor T21, en de consequenties daarvan. Naast biologische factoren (meer Guanine en Cytosine basen in het DNA van chromosoom 13 ten opzichte van 21) speelt mogelijk ook mee dat de placenta bij doorgaande T13 zwagerschappen vaker een mozaïek is van normale en trisomie cellen. De iets lagere specificiteit voor T13 vertaald zich daardoor in een fors lagere positief voorspellende waarde van NIPT voor T13. Als direct op een positieve uitslag van NIPT voor T13 besloten zou worden tot een punctie, zullen er meer invasieve ingrepen plaatsvinden bij zwangerschappen zonder trisomie.
Bijna alle casus van T13 zijn geassocieerd met meerdere structurele afwijkingen die moeilijk te missen zijn bij een geavanceerde echo, zoals bij de routine structurele echo of vaak zelfs eerder. Met vroege NIPT voor T13 zou er eerder detectie zijn, waardoor de mogelijkheid van eerdere zwangerschapsafbreking bestaat, maar ook de kans op het verlies van een gezond kind door de invasieve diagnostische test. Gezien de ongunstige balans tussen voor- en nadelen bij

het testen op T13, raden we aan geen NIPT toe te passen voor T13 in een algemene populatie. De techniek voor het detecteren van T13 door NIPT zal waarschijnlijk worden verbeterd, waardoor er mogelijk sprake is van minder vals positieven in de toekomst. Als er sprake is van een positieve NIPT voor T13 zouden wij adviseren eerst een geavanceerde echografie uit te voeren, en alleen over te gaan tot een invasieve test wanneer afwijkingen worden gezien. Gezien de kans dat de afwijkende NIPT wordt veroorzaakt door een chromosomale afwijking die zich beperkt tot de placenta (confined placenta mosaicism) raden wij in deze situatie aan een vruchtwaterpunctie te verrichten.

BESLUITVORMING

De geïnformeerde reproductieve keuzes zijn de fundamentele basis van het prenatale screeningsprogramma. Een niet-directieve counseling door zorgverleners zal met de invoering van NIPT belangrijker zijn dan ooit.
Zorgverleners moeten zich er van bewust zijn dat het een ongewenste situatie is als prenatale testen routinematig worden uitgevoerd, ofwel dat de mogelijke gevolgen niet worden besproken met de zwangere. Met de introductie van NIPT, met eliminatie van het risico op iatrogene miskramen als gevolg van het testen, en dus afwezigheid van angst van vrouwen voor de ingreep, zal de vrouw een meer evenwichtige, autonome reproductieve keuze kunnen maken. Er rijzen nieuwe vragen: wat zal het effect zijn op het aantal zwangerschapsafbrekingen? En wat zal het effect zijn op het aantal uitgevoerde prenatale testen? Wat zullen de kosten zijn en hoe worden deze kosten vergoed?

Beëindiging van de zwangerschap

Momenteel kiest 93% van de vrouwen die een positief resultaat ontvangen na een invasieve procedure voor een zwangerschapsafbreking. Met de eliminatie van het risico op een iatrogene miskraam, zou de besluitvorming kunnen wijzigen. We onderzochten of een deel van de vrouwen die niet zou kiezen voor een zwangerschapsafbreking, toch geïnformeerd wil worden over de gezondheid van de foetus als er een veilige en betrouwbare test beschikbaar is. Deze studie staat beschreven in **hoofdstuk 4**. We vonden dat het totale percentage van de vrouwen dat kiest voor een prenatale test waarschijnlijk zal stijgen, echter de zwangerschapsafbrekingen zullen niet evenredig toenemen. We speculeren dat het belangrijkste verschil met het huidige screeningsprogramma's zal zijn dat, in tegenstelling tot nu, de meeste levend geboren kinderen met T21 geboren zullen worden in gezinnen die bewust de keuze hebben gemaakt om voor een kind met T21 te zorgen. Er zal een verschuiving ontstaan na de invoering van NIPT. De vrouwen die nu een invasieve ingreep laten verrichten zijn geselecteerd. Het zijn vrouwen die de kans op een procedure gerelateerde miskraam accepteren voor de zekerheid over de

aanwezigheid danwel afwezigheid van T21. Deze groep kiest indien er sprake is van T21 vrijwel altijd voor een zwangerschapsafbreking. Er zal na de implementatie van NIPT een meer diverse groep ontstaan waarbij meer vrouwen er voor kiezen wel de kennis te verkrijgen maar de zwangerschap te behouden.
In beide situaties, of er nu wordt gekozen de zwangerschap af te breken of te behouden, moeten de zorgverleners de vrouw goed begeleiden. Voorbereidingen treffen voor een leven met een kind met T21 vereist actuele informatie over T21, een uitleg van zeker aanwezige, maar qua ernst moeilijk voorspelbare geestelijke en lichamelijke handicaps, onzekere lange termijn prognose met bijvoorbeeld een grote kans op Alzheimer, en - indien gewenst - een verwijzing, bijvoorbeeld naar een steungroep of stichting.

Stijging van het aantal prenatale testen

Veel vrouwen hebben een positieve attitude ten opzichte van NIPT. Het aantal prenatale testen zal naar verwachting stijgen zoals we in **hoofdstuk 5** beschrijven. Meer dan de helft van de vrouwen die prenatale screening afwijst in het huidige programma zou NIPT kiezen indien de test beschikbaar zou zijn. De belangrijkste reden voor de stijging van het aantal vrouwen dat NIPT wenst, is de eliminatie van het iatrogene miskraamrisico.

Leeftijdsgebonden vergoeding en bereidheid te betalen

In Nederland hebben wij een volledig dekkend zorgstelsel dat gelijke zorg biedt aan elke burger. De overheid heeft echter, tot verbazing en ongenoegen van de meeste zorgprofessionals, besloten dat de CT bij vrouwen jonger dan 36 jaar niet wordt vergoed. Vrouwen onder 36 jaar moeten de persoonlijke kosten meenemen in de geïnformeerde besluitvorming. Gepubliceerde studies hebben aangetoond dat de accuratesse van NIPT niet is gerelateerd aan de leeftijd van de patient.[9] In **hoofdstuk 5** onderzochten we de waardering van vrouwen voor NIPT voor T21 door te vragen naar de bereidheid om voor deze test te betalen ('willingness-to-pay', WTP). Het gemiddelde bedrag dat vrouwen bereid waren te betalen was iets hoger dan de huidige kosten van de CT (rond de 150 euro). Sommige vrouwen waren bereid om veel meer te betalen. De WTP was gecorreleerd aan zowel leeftijd als inkomen, maar niet aan religie.

In **hoofdstuk 6** wordt de invloed van de eigen kosten op de keuze voor de CT bestudeerd. We concludeerden dat de financiële gevolgen niet onderschat moeten worden, want er was een significante vermindering in het aantal CT in een periode waarin vrouwen de test zelf moesten betalen ten op zichtte van een periode waarin de test werd vergoed.

DE WEG NAAR IMPLEMENTATIE VAN NIPT IN NEDERLAND

Nederland is een bijzonder land als het gaat om de prenatale zorg. Naast de hierboven genoemde WBO-vergunning die noodzakelijk is voor de implementatie van NIPT, hebben we ook een uniek verloskundig systeem. De meeste zwangere vrouwen krijgen zorg van een zelfstandig werkende eerstelijns verloskundige, waar ze ook de informatie krijgen over de prenatale screening. De attitude van de eerstelijns verloskundigen over de huidige prenatale screening en over NIPT was onbekend, maar is van groot belang voor de implementatie van de test.
In **hoofdstuk 7** wordt de attitude van eerstelijns verloskundigen ten aanzien van de huidige prenatale screening en NIPT beschreven. De belangrijkste conclusie is dat de meerderheid van de Nederlandse eerstelijns verloskundigen de invoering van NIPT zou verwelkomen. De eerstelijns verloskundigen zouden liever NIPT als primaire screeningstest invoeren dan als vervolg op de huidige CT. Goed geïnformeerde besluitvorming werd als belangrijkste aandachtspunt voor implementatie van NIPT genoemd.

In 2013 was het aanbieden van NIPT nog verboden in Nederland. In **hoofdstuk 8** wordt de situatie voor de verloskundige zorgverleners beschreven in afwachting van formele toestemming om de test aan te kunnen bieden in de klinische praktijk . Als vrouwen vragen om informatie over NIPT zijn de zorgverleners verplicht om patiënten te informeren over NIPT, zoals die momenteel alleen in het buitenland uitgevoerd kan worden. Echter, het ongevraagd aanbieden van NIPT is vooralsnog verboden. Deze situatie is een dilemma voor veel verloskundige zorgverleners, met name bij een gecompliceerde voorgeschiedenis op het gebied van obstetrie of fertiliteit. In dit artikel worden enkele medisch-juridische aspecten toegelicht en wordt stil gestaan bij de verloskundige praktijkvoering in afwachting tot een meer definitieve regeling.

OPINIE

In 2012 publiceerde Benn et al. een opiniestuk in Ultrasound in Obstetrics & Gynecology met de titel 'Non - invasive prenatal diagnostics for Down Syndrome; the paradigm will shift, but slowly'. In **hoofdstuk 9** debatteren we over een aantal belangrijke vraagstukken en we wijzen op de in onze ogen onterecht of overdreven negatieve opmerkingen over NIPT. We voorspelden, in tegenstelling tot Benn et al., dat het paradigma snel zou verschuiven, hetgeen ondertussen reeds gebeurd is. Een van de belangrijkste, en nog steeds herhaaldelijk terugkerende thema's is; is NIPT screening of diagnostiek? Of een test diagnostiek of screening is, is niet gerelateerd

aan de diagnostische accuratesse. Screening omvat het ongevraagd aanbieden van een test aan een bevolking(sgroep) zonder bekend verhoogd risico, terwijl een diagnostische test wordt aangeboden door een dokter in de spreekkamer, aan een patiënt met symptomen of een bekend hoog risico. Naar onze mening kan NIPT zowel een (goede) diagnostische test zijn als een (zeer goede) screeningtest, maar is dit afhankelijk van waar de test in de keten van prenatale zorg wordt geplaatst.

DE TRIDENT-STUDIE

Vanaf 1 april 2014 is er een WBO-vergunning voor de zogenaamde TRIDENT-studie. Het doel van de TRIDENT - studie is om alle aspecten van een proces van proefimplementatie van NIPT te evalueren als onderdeel van standaard verloskundige zorg. De evaluatie zal zich onder andere richten op de haalbaarheid, logistieke processen, aantallen, technische aspecten, kosten en geschiktheid van de test voor high-throughput analyse. Het aantal tests dat in dit project wordt uitgevoerd (1000-3000) is niet hoog genoeg om de testkarakteristieken nauwkeurig te bepalen, maar wel om deze getallen te vergelijken met de gepubliceerde literatuur. Veel vragen moeten tijdens de TRIDENT-studie worden beantwoord: wat is de doorlooptijd? Hoeveel testen falen, welke oorzaken worden daar voor gevonden? Wat zijn de kosten? Gedurende de parallel lopende ESPRIT-studie wordt de mening van de zwangere vrouw en partner bestudeerd.

Counseling tijdens de TRIDENT-studie

Het protocol voor de TRIDENT-studie dat werd ingediend bij de Minister was zo ontworpen dat NIPT een toevoeging zou zijn aan het huidige screeningsprogramma. Vrouwen die 36 jaar of ouder zijn, zouden direct kunnen kiezen voor NIPT. Echter, de Gezondheidsraad heeft geadviseerd om 'maternale leeftijd' te elimineren als een screeningcriterium. Er is nu toestemming voor een studie waarbij alleen vrouwen met een verhoogd risico uit de CT de NIPT aangeboden krijgen. Bij de counseling voor een CT moeten vrouwen geïnformeerd worden over de nieuwe mogelijkheid van NIPT. Patiënten dienen zich bewust te zijn van het feit dat het een studie is, en dat nog niet alle informatie over de test beschikbaar is.

Het is belangrijk om met de doelgroep alle voordelen en nadelen van NIPT, maar ook de bestaande alternatieven als optie te bespreken. Het nadeel, de aanwezige maar op zich kleine kans op een iatrogene miskraam moet worden genoemd, maar ook het voordeel van een invasieve ingreep met een zeer snel (2-3 dagen) en 100% betrouwbaar resultaat. In sommige regio's wordt naast de snelle test ook nog karyotypering of een beperkte microarray aangeboden, waarmee meer informatie over de foetale chromosomen wordt verkregen.

De pre-test counseling van NIPT behoort de volgende aspecten te bevatten: (verwachte) accuratesse, positieve - en negatieve voorspellende waarde, de kans dat de test niet lukt en de doorlooptijd. Als de zwangere een hoog gewicht heeft behoort zij te weten dat de betrouwbaarheid van NIPT minder is (de Nederlandse laboratoria bepalen vooralsnog geen foetale fractie). De beperkingen van alleen het detecteren van T21, T18 en T13 moeten worden vermeld, maar ook de verminderde accuratesse van met name T13. Het geslacht of geslacht chromosomale afwijkingen worden niet vermeld. Alle vrouwen met een positief NIPT resultaat worden sterk geadviseerd een invasieve ingreep te laten verrichten, waarbij er een voorkeur is voor een vruchtwaterpunctie, die vanaf 15 weken kan worden verricht. Aangezien de NIPT pas wordt gedaan na een CT uitslag, en de test in de Nederlandse laboratoria 2-3 weken duurt, is deze termijn in de praktijk geen probleem. De post-test counseling is zeer belangrijk en moet worden geïndividualiseerd. Er dient geen zwangerschapsafbreking plaats te vinden voordat er bevestiging middels een invasieve ingreep heeft plaatsgevonden. In het geval van een echoscopische afwijkingen bij de nekplooimeting (inclusief een nekplooi van >3.5 mm) wordt aangeraden een invasieve ingreep uit te laten voeren met een microarray.
In het TRIDENT project zal maximale inspanning worden gedaan om weefsel na een miskraam of een intra-uteriene vruchtdood te testen op foetale trisomie.

Vanwege het feit dat NIPT gedurende de TRIDENT-studie pas mogelijk is na de CT, en dat zwangeren de CT indien jonger dan 36 jaar zelf moeten betalen, kan tot gevolg hebben dat veel zwangeren liever de CT overslaan. Ze geven wat meer geld uitgeven en gaan bij 10 weken naar het buitenland voor NIPT. Op dit moment (begin 2014) gaan veel vrouwen net over de grens naar België, maar wordt er ook in Nederland bloed afgenomen en naar Amerikaanse laboratoria gestuurd.

Aanbevelingen van de NVOG

Naast de voorbereiding voor de implementatie van NIPT in het kader van de TRIDENT-studie, werden andere aspecten voorbereid. Een belangrijk voorbeeld hiervan zijn de aanbevelingen van de NVOG. Zij onderstrepen het belang van NIPT.[10] Een samenvatting van de aanbevelingen:

1. Nu de Nederlandse laboratoria hebben aangegeven NIPT in de standaard prenatale zorg uit te kunnen voeren beveelt de NVOG aan om de NIPT als derde optie, naast de vlokkentest en de vruchtwaterpunctie, aan te bieden aan zwangeren met een verhoogde kans op foetale T21, onder goed omschreven voorwaarden.
Bij foetale echoscopische afwijkingen, inclusief een NT >3,5 mm, wordt uitgebreider onderzoek geadviseerd.

2. Zwangeren die op basis van de combinatietest geen verhoogde kans op T21 hebben, maar wel een verhoogde kans op T18 of T13, wordt geadviseerd om eerst in een derdelijns centrum een geavanceerd echoscopisch onderzoek te ondergaan. Hierna behoort counseling plaats te vinden over vervolgonderzoek, inclusief NIPT met aandacht voor de specifieke testeigenschappen voor deze afwijkingen, die minder goed zijn dan voor T21.

3. Tot het moment dat de Nederlandse laboratoria NIPT daadwerkelijk mogen uitvoeren, meent de NVOG dat zwangeren met een verhoogde kans op foetale trisomie recht hebben op informatie over de mogelijkheid NIPT door buitenlandse laboratoria te laten uitvoeren.

4. De NVOG acht het haar taak de patiënten informatie over NIPT door haar leden te uniformeren en te optimaliseren, ter voorkoming van ongelijkheid in toegang tot zorg van zwangeren.

5. Prenatale diagnostiek naar foetale chromosomale afwijkingen is vergunningplichtig op grond van artikel 2 van de Wet op Bijzondere Medische Verrichtingen (WBMV). Dit betekent dat de uitvoering van de test, ondanks dat deze niet invasief is, vooralsnog in een centrum voor prenatale diagnostiek moet plaatsvinden. De NVOG is van mening dat in de eerste fase van proefimplementatie, waarin wetenschappelijke en organisatorische evaluatie essentieel is, het gehele proces van counseling tot aan uitvoering ondergebracht moet worden in de erkende centra voor prenatale diagnostiek. Na een proefperiode van 2 jaar zal duidelijk zijn of het traject (of onderdelen daarvan) ook mogelijk kunnen plaatsvinden in andere centra.

6. De NVOG is van mening dat NIPT onder dezelfde voorwaarden als geldend voor de invasieve tests in het basispakket van de zorgverzekering moet worden vergoed. De NVOG is voorstander van gelijke toegang tot standaard zorg voor alle zwangeren.

DISCUSSIE OVER VERSCHILLENDE ASPECTEN BIJ DE IMPLEMENTATIE VAN NIPT IN NEDERLAND

Na onze studies, maar ook op basis van de huidige literatuur en persoonlijke mening, zal ik een aantal belangrijke onderwerpen bespreken met betrekking tot de implementatie van NIPT in Nederland. Deze onderwerpen zijn: stijgende aantallen en logistiek, kosten, counseling, samenwerking tussen eerste en tweedelijns zorg, de waarde van de eerste trimester echo en de daling van invasieve procedures.

Logistiek bij stijgende aantallen

In 2014 verwachten we de publicatie van een aantal grote studies die zijn uitgevoerd in algemene laag-risico, zwangeren populaties. We verwachten dat de resultaten vergelijkbaar zullen zijn met de gepubliceerde studies die zijn uitgevoerd in hoog-risico-populaties. Als dit het geval is, zal het eerst moeten laten uitvoeren van de CT met minder goede testkarakteristieken, namelijk 10-15% van de foetus met T21 wordt gemist, en enkele weken later pas beschikbaar zijn van de uitslag, moeilijk uit te leggen zijn. Echter de zwangere hecht mogelijk ook veel waarde aan een extra echo. In Nederland krijgen alle zwangere vrouwen een eerste trimester echo ter bepaling van de termijn, en tevens detectie van meerlingzwangerschappen en anencefalie. We verwachten dat het aantal vrouwen dat prenatale screening wenst zal stijgen met de implementatie van NIPT. Een ruwe schatting van de verwachte aantallen komt op meer dan 100.000 zwangeren per jaar in Nederland bij invoering van NIPT in een algemene populatie. De vraag is dan welke laboratoria in Nederland bereid en in staat zijn om zulke grote aantallen aan te kunnen? Wereldwijd zijn er slechts een paar NIPT - laboratoria, al neemt het aantal laboratoria snel toe. Na gesprekken met verschillende commerciële laboratoria blijkt dat niet moet worden onderschat wat de kosten zijn van een volledig geautomatiseerd high throughput topkwaliteit laboratorium, met een back-up systeem voor alle machines in geval van een error. In Nederland is het mogelijk niet nodig om in alle academische centra NIPT voor de trisomieën aan te bieden. Het is goedkoper en efficiënter om de uitvoering van NIPT te centraliseren in een of twee centra of misschien naar een extern laboratorium te sturen, zoals Sanquin, Amsterdam, zoals nu gebeurt voor de NIPT voor rhesus D. Een andere optie zou zijn om de monsters naar een van de buitenlandse laboratoria te sturen. Wellicht wordt de keuze straks bepaald door een Europese aanbesteding van de test.

Kosten

Kosten van de gezondheidszorg zijn een actueel onderwerp omdat de kosten elk jaar stijgen. Zal NIPT leiden tot hogere kosten voor de samenleving? Moeten vrouwen zelf betalen voor NIPT? Dit zijn belangrijke vragen die beantwoord moeten worden vóór de definitieve invoering van NIPT. Maar wat nemen we nog meer mee als het om kosten gaat van de implementatie van NIPT? De kosten van de test zelf zijn lager dan de kosten voor een invasieve procedure, maar hoger dan de huidige CT. De kosten van NIPT zijn erg gedaald in de afgelopen jaren (van ongeveer $2.000 tot ongeveer $400) en zouden mogelijk verder kunnen dalen. Secundaire kosten zijn moeilijk te berekenen. Een paar voorbeelden. Wat zijn de kosten van één of twee dagen niet werken, omdat een vrouw een invasieve ingreep ondergaat? Of bedrust moet houden vanwege bloeden of vruchtwaterverlies na de procedure? Wat zijn de kosten van een vrouw die haar kind verliest als gevolg van een invasieve procedure? Men zou kunnen stellen dat het verlies van een gezond kind in termen van economische waarde wordt vertaald in het verlies van 80 Quality-Adjusted Life Years. De kosten voor levenslange zorg

voor kinderen geboren met een verstandelijke handicap is een zeer gevoelige kwestie, maar we weten dat deze kosten behoren tot de hoogste in de Nederlandse gezondheidszorg. Het voelt niet ethisch correct om deze kosten te berekenen. Wat als beleidsmakers beslissen dat er geen of slechts een gedeeltelijke terugbetaling van NIPT zal zijn? Blijkbaar zijn vrouwen die nu naar het buitenland gaan voor NIPT, bereid te betalen voor de test. We kennen niet precies de karakteristieken van deze vrouwen, maar in de praktijk zien we dat dit een geselecteerde, vaak hoog opgeleide, dus financieel bevoorrechte groep vrouwen is. Op deze manier zal de toegang tot gezondheidszorg niet gelijk zijn en zal de besluitvorming beïnvloed worden. Een van de mogelijkheden om de kosten van de uitvoering van NIPT te verminderen is door bloed af te nemen voor NIPT na 12 weken zwangerschap. Zo wordt de kans verminderd dat NIPT wordt uitgevoerd voor een zwangerschap die spontaan eindigt in een miskraam. Bovendien is de kans op slagen van NIPT hoger, aangezien de foetale fractie toeneemt met de zwangerschapsduur. Uiteindelijk verwachten we dat NIPT de CT zal vervangen, maar eerst zal NIPT een aanvullende test zijn naast de invasieve ingrepen. Voor vrouwen die een positieve NIPT uitslag zullen ontvangen en die ervoor kiezen om dit te bevestigen door een invasieve test, zullen zorgen voor dubbele kosten. Deze groep is echter zeer klein, hooguit een paar honderd vrouwen per jaar in Nederland. Vrouwen die een verhoogd risico op trisomie hebben na een CT in het huidige systeem, kiezen in ongeveer 50% voor een invasieve ingreep. Als NIPT beschikbaar is, gaan we in de overgrote meerderheid er van uit dat men zal kiezen voor deze test. Hoewel de kosten van NIPT aanzienlijk lager zijn (althans de tarieven van buitenlandse laboratoria) dan de invasieve testen, zal de totale vermindering van kosten beperkt zijn. Als alle vrouwen met een verhoogd risico voor NIPT kiezen, dient NIPT minstens 50% goedkoper te zijn dan de invasieve ingrepen om financieel een gunstig alternatief te zijn. Zoals eerder beschreven zullen de aantallen stijgen. Als het aantal te testen vrouwen zal verdubbelen van 45.000 naar 90.000 vrouwen, zal dit een stijging van kosten betekenen van bijna €7.000.000.

Counseling

Hoewel NIPT een hogere accuratesse heeft dan de momenteel gebruikte screeningsmethoden, moet er, net als bij elke medische test in een screening en diagnostische setting, sprake zijn van passende pre-test en post-test counseling. De discordante uitslag in de EU-NITE studie onderstreept het belang hiervan. Vrouwen moeten de consequenties van de testresultaten, waaronder de kans op testfalen, onjuiste resultaten en bevindingen van onduidelijke betekenis vooraf kennen. Als er een discrepantie tussen de NIPT en de karyotypering wordt gevonden, is er vaak een onderliggende biologische reden, zoals mosaïcisme beperkt tot de placenta, maternaal mosaïcisme, vanishing twin of kanker.[4] NIPT geeft wel het juiste resultaat, dat wil zeggen een overmaat van DNA van een bepaald chromosoom, maar de foetus heeft niet het fenotype van de voorspelde trisomie. Om deze reden is er een voorkeur om in het kader van NIPT niet over vals negatief of vals positief te spreken maar over discordantie.

Een positief resultaat moet altijd worden bevestigd door een invasieve procedure, althans voor vrouwen die de zwangerschap willen beëindigen. Natuurlijk dient tijdens de counseling de informatie over T21, T18 en T13 compleet te zijn. Wel moeten we ons afvragen of we alle mogelijke zeldzame (0,5-1%) toevalsbevindingen moeten bespreken? Of moet het laboratorium gebruik maken van een methode waarbij toevalsbevindingen worden voorkomen? Of moet een geneticus in het laboratorium beoordelen of de afwijking klinisch relevant is of dat het een onschuldige variatie betreft? Over deze vragen wordt nog gedebatteerd, maar we hebben al tientallen jaren soortgelijke ervaring met het gebruik van het routinematig gebruik van de 20-weken echo. Als we een zwangere informeren over de 20-weken echo vertellen we dat de foetus van top tot teen wordt bekeken, en dat er in het algemeen ongeveer 5% kans is op het vinden van een foetale structurele afwijking. We geven enkele voorbeelden, zoals neurale buisdefecten, hartafwijkingen en we beantwoorden uiteraard elke specifieke vraag van de patiënt, maar we vertellen hen niet de duizenden zeldzame mogelijkheden. In het geval van een echoscopische afwijking, krijgen patiënten tijdens de post-test counseling zeer gedetailleerde informatie, zodat zij een beslissing kunnen nemen over de zwangerschap. Wij geloven dat dit niet anders is voor NIPT. De post-test begeleiding moet grondig worden uitgevoerd door een ervaren obstetricus, indien nodig ondersteund door een klinisch geneticus, net als bij elke andere abnormale prenatale uitslag.
In het huidige prenatale screening systeem is de informatie tijdens het uitleggen van de CT over het algemeen gericht op het uitleggen van de kansbepaling en niet of heel beperkt over de aandoening Down syndroom zelf. Als NIPT wordt geïntroduceerd als screeningstest, zal de counseling over de test zelf minder tijdrovend zijn waardoor de mogelijkheid bestaat de zwangere beter te informeren over Down syndroom.

Samenwerking tussen 1e en 2e lijn

Gedurende de TRIDENT-studie zal NIPT worden aangeboden aan hoog-risico patiënten. De counseling van de eerstelijns verloskundige zal niet veel veranderen, alleen zal korte, algemene informatie over NIPT worden toegevoegd.

Als NIPT geïmplementeerd wordt als een test voor de algemene zwangere populatie, zal het een mogelijkheid kunnen zijn om de samenwerking tussen de eerstelijns verloskundigen en de tweede lijn te versterken. Verschillende opties voor deze samenwerking zijn mogelijk. Een gezamenlijke project kan van start gaan om groepscounseling voor laag-risico vrouwen te organiseren. De meeste vrouwen met een medische indicatie hebben geen verhoogd risico voor trisomieën, zij zouden zich dan ook bij een counseling van een eerstelijns verloskundige kunnen aansluiten. Veel minder vrouwen zullen een hoog risico hebben na NIPT op een trisomie, waardoor verwijzingen naar de tweede lijn minder zullen voorkomen.

Vrouwen met een positieve NIPT of een andere reden voor prenatale diagnostiek, of bijvoorbeeld vrouwen met een tweelingzwangerschap, zullen counseling krijgen door een ervaren obstetricus in een van de centra voor prenatale diagnostiek. Vóór de implementatie van NIPT, moeten counseling cursussen worden georganiseerd voor zowel verloskundigen, gynaecologen (in opleiding) als prenatale artsen.

De waarde van de eerste trimester echo

De eerste trimester echo met meting van de nekplooi is oorspronkelijk ontworpen om te screenen op T21 en later op T18 en T13. Echter, het gebruik is verbreed in de afgelopen jaren. Als een 'toevalsbevinding' worden foetale structurele afwijkingen, in steeds grotere aantallen gedetecteerd. Een dikke NT is niet alleen geassocieerd met foetale trisomie maar met vele andere, vaak ernstige ziekten. De vraag is wat de waarde is van een eerste trimester echo als NIPT wordt geïmplementeerd? De waarde van de eerste trimester echoscopie bij een groep zwangerschappen waarbij foetale trisomie middels NIPT zal worden uitgesloten, moet worden bestudeerd. Wat kan worden gedetecteerd, en hoe betrouwbaar is een dergelijke diagnose? Wat is dan het management? In een aantal geselecteerde gevallen zal de diagnose voldoende duidelijk zijn om een zwangerschapsafbreking te overwegen. In andere gevallen, zal herhaling bij 16 en 20 weken nodig zijn, met weken van angst en onzekerheid voor de patiënte als gevolg. Zouden we elke zwangere waarbij een echoscopische afwijking wordt gezien een microarray moeten aanbieden? Hoeveel valse positieven heeft dit als gevolg, en hoeveel iatrogene miskramen? En zou een dergelijk programma kosteneffectief zijn? Grootschalige, prospectieve studies om deze vragen te beantwoorden dienen met enige spoed uitgevoerd te worden.

De vermindering van invasieve procedures

Zoals Tabor et al. bestudeerden, hebben ervaren operateurs een hoger slagingspercentage en een lager complicatiepercentage bij het uitvoeren van invasieve procedures.[7] Als NIPT wordt geïmplementeerd, en zelfs op dit moment (omdat vrouwen naar het buitenland gaan voor NIPT) neemt het aantal invasieve procedures snel af. Net als bij vele andere procedures dient de zorgverlener voldoende aantallen per jaar uit te voeren om bekwaam te zijn en te blijven. Het lijkt vanzelfsprekend en onvermijdelijk dat (verdere) centralisatie van invasieve procedures nodig is met verplichte jaarverslagen waaronder de follow-up om de kwaliteit per centrum hoog te houden. In de 'general discussion' is een tabel opgenomen uit het Gezondheidsraad rapport over NIPT waarbij inzichtelijk wordt wat de reductie van het aantal invasieve ingrepen zal zijn.

NA DE TRIDENT-STUDIE

Met de resultaten en ervaringen van de TRIDENT - studie verwachten we dat we een volgende stap kunnen maken in de implementatie van NIPT met als doel NIPT te implementeren als screeningstest voor een (meer) algemene bevolking. Het is nog te vroeg om conclusies te trekken, maar verschillende opties voor implementatie kunnen worden overwogen:

1. *Geen CT maar NIPT als een eerstelijn screening test voor alle vrouwen*
2. *Eerst de CT en dan NIPT in het geval van een verhoogd risico ≥1:200*
3. *Eerst de CT en dan NIPT in geval van een risico van ≥1:1000 of 2500*
4. *NIPT, gevolgd door een gedetailleerde 13 weken echo, en de afname van serum screening voor de detectie van verschillende zwangerschapscomplicaties zoals preeclampsie, vroeggeboorte en foetale groeivertraging.*

Zoals we in hoofdstuk 5 en 8 laten zien hebben zowel patiënten als zorgverleners een voorkeur voor NIPT als primaire screeningstest.

Een andere vraag is, vooral voor de logistiek, maar ook voor de kosten, wat de beste zwangerschapsduur is om bloed af te nemen voor NIPT? Een groot voordeel van NIPT is dat deze kan worden uitgevoerd vanaf 10 weken zwangerschapsduur tot het einde van de zwangerschap. Met name voor vrouwen die laat in de zwangerschap voor een eerste controle komen is dit een voordeel. Verschillende implementatie-opties worden beschreven in de 'Discussion'.

Beperkingen van het proefschrift

In Nederland zijn er nog geen studies uitgevoerd waarbij het resultaat van de NIPT werd besproken met de patiënte. Er was immers nog geen WBO-vergunning. Om deze reden zijn alle studies die zijn verricht tot de implementatie hypothetisch. Een beperking van het onderzoek met vragenlijsten is de beperkte respons. Studies in een real-time omgeving na de implementatie van NIPT met een grotere steekproef of met keuze-experimenten moeten worden uitgevoerd om meer informatie over deze belangrijke aspecten te verkrijgen.

TOEKOMSTIGE ONTWIKKELINGEN?

Als mogelijkheden zich verbreden, zouden we in staat kunnen zijn om syndromen of ziekten die we kunnen behandelen te detecteren tijdens de zwangerschap. In de toekomst zullen de opties voor NIPT zich verbreden, zoals het detecteren van (micro)deleties en duplicaties en de detectie van monogenetische ziekten zoals M. Huntington.

De eerste resultaten in studieverband zijn hoopvol, maar de klinische waarde en haalbaarheid moet nog worden onderzocht. De evaluatie van testen voor zeldzame ziekten is vanzelfsprekend moeilijker.

Mogelijk zijn we in de toekomst in staat om de hielprik, nu uitgevoerd in de eerste week na geboorte, uit te voeren in de zwangerschap om zo een reeks van metabole aandoeningen te detecteren. Er kan vervolgens worden onderzocht of een dieet of bepaalde medicijnen de uitkomst van de zwangerschap zouden kunnen verbeteren.[11]

TOEKOMSTIGE ONDERZOEKSVRAGEN

Naast de vele onderzoeksvragen die we verwachten te kunnen beantwoorden tijdens de TRIDENT-studie, resteren er nog vele vragen. Wat zal het percentage zwangerschapsafbrekingen zijn na de invoering van NIPT? Wat zijn de echte veranderingen in het besluitvormingsproces? Wat is de klinische waarde en kosteneffectiviteit van de eerste trimester echo als test voor andere ziekten dan foetale trisomie? Wat zijn de werkelijke kosten van NIPT in een real-time, real-life setting? De diagnostische nauwkeurigheid voor meerlingzwangerschappen is nog niet optimaal. Is er een verschil in nauwkeurigheid tussen dichoriale en monochoriale monozygote tweelingzwangerschappen? De meest waarschijnlijke vooruitgang in de nabije toekomst, afgezien van het feit dat NIPT steeds goedkoper en sneller wordt en geslachtschromosomale afwijkingen betrouwbaar bepaald kunnen worden zijn de deleties en duplicaties. Wat er vervolgens gedetecteerd zal worden, of wat juist niet, zal aanleiding zijn voor een ethisch debat gezien de mogelijkheden die steeds groter worden. In onderzoeksverband is het gehele foetale genoom reeds gesequenced.


VII
APPENDICES

CHAPTER 12

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CHAPTER 13

Curriculum Vitae

CURRICULUM VITAE

Joanne Verweij was born on the 22nd of April 1984 nearby Thohoyandou, in a little village called Vhufuli in South Africa. The first years of her life she grew up in South Africa, Lesotho and Zimbabwe where her parents were working in several local hospitals. The family moved to the Netherlands, and she finished primary school in Maarn. In 2002 she graduated from secondary school (VWO) at the Revius Lyceum in Doorn. A year later she started medical school at the Leiden University.

During her studies she worked as an assistant (Joshua's) at the Intensive Care Unit of the Leiden University Medical Center (LUMC). She went to Derby – Western Australia for an internship at Derby Hospital and The Royal Flying Doctors Services. In Derby she saw her first labour. This was the moment the interest in Obstetrics and Gynaecology started. She became really enthusiastic about the profession during her internship in the Medical Center Haaglanden. After graduating in 2009 with honour (cum laude), she worked as a physician at the Medical Center Haaglanden and at the LUMC.

In 2011 prof. dr. D. Oepkes gave her the opportunity to start her research on the non-invasive prenatal test as a research fellow at the LUMC. Several studies were started on the implementation of NIPT in the Netherlands. In 2012 she started her residency training in Obstetrics & Gynaecology at the Reinier de Graaf Hospital (mentor: dr. H.A. Bremer). In 2013 she started at the Department of Obstetrics & Gynaecology at the LUMC, Leiden (Head of Department: prof. dr. J.M.M. van Lith).

CHAPTER 14

Dankwoord


DANK!
Crispijn
papa & mama
Dick • Marjon
Rose, Miek & Sander
collega's
opa's & oma's
leescommissie
Suus, Marie-Louise & Femke
co-auteurs
Ivanka & Jenny
(schoon)familie
vrienden & vriendinnen
Anne-Marie, Anna, Eva, Hanna,
Ike, Jessica, Juke, Leonie, Lot,
Ludmilla, Marie-Louise & Merijn

CHAPTER 15

List of Abbreviations

LIST OF ABBREVIATIONS

Cff-DNA	cell-free fetal DNA
CVS	chorion villus biopsy
DANSR	digital analysis of selected regions
FCT	first trimester combined test
FORTE	fetal-fraction optimized risk of trisomy evaluation
IGZ	Inspectie voor de Gezondheidszorg
MPS	massively parallel genomic sequencing
NIPT	non-invasive prenatal testing
NT	nuchal translucency measurement
PND	prenatal diagnosis
PNS	prenatal screening
PSA	Population Screening Act ('WBO-vergunning')
RAD	rapid aneuploidy detection
RIVM	National Institute for Public Health and the Environment
TOP	termination of pregnancy
T21	trisomy 21, Down Syndrome
T18	trisomy 18, Edwards Syndrome
T13	trisomy 13, Patau Syndrome
VAS	visual analogue scale
VWS	Ministerie van Volksgezondheid, Welzijn & Sport
WBO	Wet op Bevolkingsonderzoek
WTP	willingness-to-pay