

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/171862>

Please be advised that this information was generated on 2018-07-07 and may be subject to change.

ORIGINAL ARTICLE

Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I—clinical impact

Dick Oepkes¹, G. C. (Lieve) Page-Christiaens², Caroline J. Bax³, Mireille N. Bekker^{2,4}, Catia M. Bilardo⁵, Elles M. J. Boon⁶, G. Heleen Schuring-Blom⁷, Audrey B. C. Coumans⁸, Brigitte H. Faas⁹, Robert-Jan H. Galjaard¹⁰, Attie T. Go¹¹, Lidewij Henneman¹², Merryn V. E. Macville¹³, Eva Pajkrt¹⁴, Ron F. Suijkerbuijk¹⁵, Karin Huijsdens-van Amsterdam¹⁶, Diane Van Opstal¹⁰, E. J. (Joanne) Verweij¹, Marjan M. Weiss¹², Erik A. Siermans^{12*} and for the Dutch NIPT Consortium

¹Department of Obstetrics, Leiden University Medical Center, Leiden, The Netherlands

²Department of Obstetrics and Gynaecology, University Medical Center Utrecht, Utrecht, The Netherlands

³Department of Obstetrics and Gynaecology, VU University Medical Center, Amsterdam, The Netherlands

⁴Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, The Netherlands

⁵Department of Obstetrics and Gynaecology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

⁶Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

⁷Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

⁸Department of Obstetrics and Gynaecology, Maastricht UMC+, Maastricht, The Netherlands

⁹Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

¹⁰Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

¹¹Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, The Netherlands

¹²Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands

¹³Department of Clinical Genetics, Maastricht UMC+, Maastricht, The Netherlands

¹⁴Department of Obstetrics and Gynaecology, Academic Medical Centre, Amsterdam, The Netherlands

¹⁵Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

¹⁶Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands

*Correspondence to: Erik A. Siermans. E-mail: e.siermans@vumc.nl

ABSTRACT

Objective To evaluate the clinical impact of nationwide implementation of genome-wide non-invasive prenatal testing (NIPT) in pregnancies at increased risk for fetal trisomies 21, 18 and 13 (TRIDENT study).

Method Women with elevated risk based on first trimester combined testing (FCT $\geq 1:200$) or medical history, not advanced maternal age alone, were offered NIPT as contingent screening test, performed by Dutch University Medical laboratories. We analyzed uptake, test performance, redraw/failure rate, turn-around time and pregnancy outcome.

Results Between 1 April and 1 September 2014, 1413/23 232 (6%) women received a high-risk FCT result. Of these, 1211 (85.7%) chose NIPT. One hundred seventy-nine women had NIPT based on medical history. In total, 1386/1390 (99.7%) women received a result, 6 (0.4%) after redraw. Mean turn-around time was 14 days. Follow-up was available in 1376 (99.0%) pregnancies. NIPT correctly predicted 37/38 (97.4%) trisomies 21, 18 or 13 (29/30, 4/4 and 4/4 respectively); 5/1376 (0.4%) cases proved to be false positives: trisomies 21 ($n=2$), 18 ($n=1$) and 13 ($n=2$). Estimated reduction in invasive testing was 62%.

Conclusion Introduction of NIPT in the Dutch National healthcare-funded Prenatal Screening Program resulted in high uptake and a vast reduction of invasive testing. Our study supports offering NIPT to pregnant women at increased risk for fetal trisomy. © 2016 The Authors. *Prenatal Diagnosis* published by John Wiley & Sons, Ltd.

Funding sources: Supported by a grant from The Netherlands Organisation for Health Research and Development (ZonMw, No. 200340002)

Conflicts of interest: Dr. Oepkes and Verweij have previously participated in clinical research sponsored by Ariosa Diagnostics and Natara Inc. Dr. Page-Christiaens is employed as an Associated Medical Director at Illumina Inc. since January 18, 2016.

INTRODUCTION

Non-invasive prenatal testing (NIPT) for fetal trisomy using cell-free DNA analysis from maternal plasma is increasingly offered to pregnant women worldwide, reducing the number of invasive diagnostic tests.¹ Of the women eligible

for invasive testing by maternal age and first trimester combined test (FCT), more than 90% carry a healthy child. Fear for invasive testing makes many of these women refrain from further testing,^{2–4} and some even refrain from screening.^{5,6}

In March 2011, a multidisciplinary Dutch NIPT Consortium was founded as a platform for collaboration between obstetric caregivers, clinical geneticists and laboratory specialists, the national prenatal screening organization, the Dutch Genetic Alliance (VSOP), ethicists, insurance companies and policy makers. In an increasing number of countries, NIPT has been introduced commercially without governmental guidance. In the Netherlands such an introduction is subject to a governmental license under the Population Screening Act because screening is offered for untreatable disorders. The NIPT Consortium received this license as well as funding for the nationwide TRIDENT study (Trial by Dutch laboratories for Evaluation of Non-Invasive Prenatal Testing), starting 1 April 2014. The license approved NIPT as an alternative option to invasive testing for women with a $\geq 1:200$ FCT result and for women with an increased risk for trisomy 21, 18 or 13 because of medical history. Direct access to NIPT based on maternal age alone was not allowed, and the direct access to invasive testing based on maternal age alone was halted.

The TRIDENT license was initially granted for two years. We expected to perform 1000–2000 NIPTs in the first year. The aim of this study was to evaluate uptake, test results, test performance and pregnancy outcomes of all pregnancies after the first 1000 participating women had delivered. Parallel to this study, we performed a questionnaire study to analyze women's preferences and decision-making. Results are reported separately (Van Schendel *et al.*⁷ Paper Part II).

METHODS

Organization of prenatal screening in the Netherlands

In the Netherlands, FCT has been available for all pregnant women since 2007, upon payment of €165. Uptake is between 25 and 30%.⁸ All follow-up testing including NIPT is currently reimbursed by the compulsory health insurance after subtraction of a deductible of 360 euros. National screening programs are coordinated and monitored by the Centre for Population Screening of the National Institute for Public Health and the Environment (RIVM/CvB), which resorts under the Ministry of Health, Welfare and Sport. This organization provides uniform patient information in different languages, guidance documents and e-learning for professionals.

Participating women and inclusion procedure

Our study group consisted of all pregnant women who chose NIPT because of an increased risk for trisomy 21, 18 or 13 based on FCT result or based on medical history (previous child with such a trisomy or a balanced translocation in one of the parents). FCT results were reported as risks for trisomy 21, 18 and 13. FCT is monitored and audited per region and reported to the RIVM/CvB. A program committee (with representatives of the participating professions and organizations) advises the CvB about the program. Eight Regional Centers (affiliated to the University Medical Centers) have been licensed for the FCT screening and are monitoring and auditing the quality of Nuchal Translucency (NT) measurements as well as the biochemical part of the testing as the final metrics of the FCT. FCT includes measurement of

PAPP-A (Pregnancy-Associated Plasma Protein A) and β -hCG (beta subunit of human Chorionic Gonadotrophin). NT measurements are performed by trained ultrasonographers according to guidelines and with requirements for a minimal number of measurements annually. Exclusion criteria for the study were: multiple pregnancies, vanishing twins, fetal nuchal translucency ≥ 3.5 mm or other structural anomalies, chromosome anomaly or history of maternal malignancy, gestational age $< 10 + 0$ weeks, women < 18 years old and inability to give informed consent.

As required in the National Prenatal Screening Protocol, all pregnant women were asked whether they wanted to be informed on options for screening for fetal anomalies at the first prenatal care visit. If so, they were counseled by a certified midwife or obstetrician on the FCT and the 20-week anomaly scan, and about NIPT as an alternative for invasive testing. As part of the TRIDENT study, counselors had received additional training on relevant aspects of NIPT. Written information and a website (www.meerovernipt.nl) were available for further reading.

Women with an increased risk based on FCT or medical history were referred for further counseling to one of the eight Centers for Prenatal Diagnosis, or one of their satellite centers. Women were given the choice between NIPT, invasive testing or no (follow-up) test. Extensive oral and written information on all tests was provided. For counseling on NIPT, performance data from a review by Mersy *et al.*⁹ were used: sensitivity and specificity around 99.0% for trisomy 21. We predicted a turn-around time (TAT) of 14–21 days. Women were informed that NIPT examines DNA fragments from the placenta cells rather than from the fetus itself, providing a biological explanation for some false positive and very rare false negative results. The study was a nationwide research study on all aspects of introducing NIPT in the prenatal screening program for trisomy 21, 13 and 18. A genome-wide approach was used allowing the detection of other chromosomal abnormalities. Women were informed that other abnormal results besides trisomy 21, 18 or 13 are occasionally found, and that any potentially clinically relevant abnormal NIPT result would be discussed with them, potentially leading to an offer of detailed ultrasound, invasive testing and/or parental testing. Women were informed that fetal sex and sex chromosomal aneuploidies would not be communicated, as the ministerial license did not allow analysis of sex chromosomes.

For counseling on invasive testing the following information was given: how the procedures are performed, risk-figures for procedure-related miscarriage (0.3–0.5%), TAT and genetic tests done by chorionic villi sampling (CVS) or amniocentesis (AS). The latter differed according to the indication and patient history and local policy and could be QF-PCR for trisomy 13, 18 and 21 only, karyotyping or array.

All women signed an informed consent form. Permission for the study was granted by the Minister of Health (350010-118701-PG). The study was also approved by local University Medical Center Ethics Committees.

Sample collection

All eight University Hospitals and 13 satellite centers participated. Women were given a unique TRIDENT study number. A custom-made TRIDENT page was integrated in the Astraia fetal medicine database (version 1.23, astraia software GmbH, Munich, Germany) and contained fields for entry of maternal characteristics, FCT results, invasive prenatal diagnosis and pregnancy outcome data.

Two to four EDTA anticoagulated or Cell-Free DNA BCT (Streck, Omaha NE, USA) tubes of 6 to 10-ml blood were taken and transported the same day (EDTA tubes) or within 7 days (Streck tubes) to a Clinical Genetics laboratory for analyses. The laboratories kept a dataset for cross-checking. The RIVM/CvB database Peridos, to which data on prenatal screening are uploaded from the electronic patient files of obstetric caregivers, was used to calculate uptake of NIPT in the group of women with an increased risk based on the FCT.

Massively parallel shotgun sequencing

In the years preceding the TRIDENT study, all eight University Hospital Genetic Laboratories had validated massively parallel shotgun sequencing for the prediction of fetal trisomies, and shared validation samples for quality control. During the project, six laboratories performed the testing, and all were involved in analysis, interpretation and reporting. Genome-wide sequencing was performed with the Illumina HiSeq 2500 or the Life Technologies 5500 W SOLiD sequencer. Bioinformatic analysis was performed either by *z*-score calculation, with *z*-scores above 3.0 as a cut-off value between indicative or not indicative for trisomy, or by the WISECONDOR algorithm under standard settings to call trisomies.¹⁰ As an extra check, several labs combined the two methods. WISECONDOR detects trisomies and monosomies of all autosomes, as well as deletions and duplications >20 Mb. Fetal fraction was not routinely measured. One laboratory estimated fetal fraction in male fetuses and requested a redraw when fetal fraction was lower than 4%.

Outcome variables

The primary clinical outcome data for this part of the TRIDENT study were uptake, defined as the percentage of women eligible for NIPT based on FCT result that actually chose to have NIPT, test characteristics of NIPT performed by the Dutch laboratories, failure rate and TAT (days between blood draw and reporting to the patient). As a secondary outcome, we made an inventory of abnormal findings other than full trisomy 21, 18 and 13 and their outcome. The standard follow-up procedure was a return form filled out and returned by the woman after pregnancy/birth. In case of abnormal findings, follow-up data were gathered as needed: invasive testing results, ultrasound data, genetic testing in products of conception (cord blood, placenta), birth data and data of postnatal examination.

Statistical analysis

Descriptive statistics, with mean, SD and range, were used to describe uptake, failure rate and TAT. Predictive performance was analyzed using two-by-two tables, and

calculation of sensitivity, specificity, and positive and negative predictive values.

RESULTS

Five months into the TRIDENT study, we already had included over 1000 pregnancies. We decided to report on the results between the start of the study 1 April 2014 and 1 September 2014, as soon as clinical follow-up was complete. The last woman of this group delivered in March 2015. Characteristics of the study group are given in Table 1.

During the first five months of the study, 1413/23 232 women (6.1%) who underwent FCT received a trisomy risk $\geq 1:200$. Of these, 1211 underwent NIPT; uptake in the screening group was 1211/1413 (85.7%). An additional 179 NIPT tests were done because of medical history. The uptake in this subgroup could not reliably be assessed because of an unknown denominator. In total, 1390 (1211 + 179) pregnant women had a blood sample taken.

A result was issued after a single blood draw in 1380/1390 (99.3%) cases. Of the 10 remaining cases, one woman elected to directly undergo amniocentesis, showing a normal karyotype. In the other nine cases, a redraw was done and three samples failed again (overall failure rate 0.3% (4/1390)). The failures were because of fetal fractions <4% ($n=3$), or the results of the WISECONDOR algorithm being inconclusive, most likely because of bad DNA quality ($n=1$). One woman was morbidly obese (BMI 52); the other three had a normal weight/BMI. Four children without anomalies were born.

Results were issued after a mean of 14 days (range 5–32); 95% of results were available within 21 days. TAT improved in later phases of the study because of faster equipment.

Table 1 Characteristics of 1390 pregnant women at increased risk for fetal trisomy who underwent NIPT

| Characteristics | n (%) |
|-----------------------------------|-----------|
| Maternal age (y) | |
| <36 | 603 (43) |
| ≥ 36 | 703 (51) |
| Unknown | 84 (6) |
| Parity | |
| 0 | 300 (22) |
| 1 or more | 486 (35) |
| Unknown | 604 (43) |
| Indication for NIPT | |
| FCT risk $\geq 1:200$ | 1211 (87) |
| Medical history | 179 (13) |
| FCT risk for fetal trisomy | |
| $\geq 1:10$ | 48 (3) |
| 1:11 – 1:50 | 239 (17) |
| 1:51 – 1:100 | 282 (20) |
| 1:101 – 1:200 | 482 (35) |
| Unknown | 160 (12) |

FCT, first trimester combined test.

Accuracy

Of the 1386 successful NIPT samples, 1325 (95.6%) were reported as normal and 42 (3.0%) as either trisomy 21 ($n=31$), 18 ($n=5$) or 13 ($n=6$). Of these 42 cases, a full trisomy was confirmed in the fetus in 34 cases, by invasive testing ($n=33$) or karyotyping of postnatal tissues ($n=1$) (Table 2); 19/1386 cases had an abnormal result involving other trisomies or subchromosomal aberrations (see following paragraph for details). Five false positive cases were found (0.4%, $n=2$ trisomy 21, $n=1$ trisomy 18 and $n=2$ trisomy 13). In two pregnancies, NIPT showed unclear results, which were reported as very unlikely to be full T21 but mosaic could not be ruled out. In both cases, the parents opted for amniocentesis which revealed a normal karyotype; both children are alive and well. As these two events were not reported as T21, they are included in the 'normal result' group for this paper. No pregnancies with an abnormal NIPT result were terminated without confirmation by invasive testing.

We report one false negative case: trisomy 21 (46,XX,i(21)(q10)) was detected in amniotic fluid after invasive testing because of ultrasound anomalies at routine scanning. The parents chose to continue the pregnancy. Analysis of the placenta after birth showed absence of this isochromosome from the cytotrophoblast layer, whereas it was present as a mosaic (66%) in the mesenchyme layer.

Details of the discordant NIPT results are given in Table 3. Two-by-two tables and test characteristics are given in Tables 4, 5 and 6 for each of the three trisomies.

Additional findings

In 19/1386 (1.4%) cases, which is 31% of all abnormal cases, other full trisomies (1.1%, $n=15$) (T22 $n=1$, T20+T2 $n=1$, T16 $n=6$, T9 $n=1$, T8 $n=2$, T7 $n=4$) or subchromosomal aberrations (0.3%, $n=4$) (deletions $n=3$ (11p, 12q, 18p), duplications $n=1$ (7q)) were reported (Table 2). As 1076/1386 cases underwent genome-wide analysis by WISECONDOR, the frequency of additional findings is actually somewhat higher (1.7%). No sex chromosomal aneuploidies were found as the license did not allow analysis of sex chromosomes. All cases were counseled by the clinical geneticist on possible consequences and options for further testing. In two cases, the pregnancy was terminated after confirmation by invasive testing (12q and 18p deletion, both without clear ultrasound abnormalities). The 11p deletion was confirmed to originate from the healthy mother. In two of the 17 ongoing pregnancies, a child with multiple structural anomalies was born, one case of T16 NIPT result but normal genotype (array) in the child, and one case of T9 NIPT result which was mosaic in the child. In both cases, parents refrained from invasive testing. In 10

Table 2 Outcome of NIPT in pregnancies at increased risk for trisomy 21, 18 or 13

| NIPT result | All | Invasive tests (abnormal genotype) | TOP | IUFD | Live-born (abnormal genotype) |
|------------------------------|------|------------------------------------|----------------|------|-------------------------------|
| Normal | 1311 | 21 (5) | 8 ^a | 16 | 1287 (2 ^b) |
| Trisomy 21 | 31 | 29 (27) | 25 | 2 | 4 (2) |
| Trisomy 18 | 5 | 5 (4) | 3 | 1 | 1 (0) |
| Trisomy 13 | 6 | 3 (2) | 2 | 2 | 2 (0) |
| Other trisomies | 15 | 9 (1) | 0 | 0 | 15 (2) |
| Subchromosomal abnormalities | 4 | 3 (2) | 2 | 0 | 2 (0) |
| Failed | 4 | 1 (0) | 0 | 0 | 4 (0) |

Data are n . Data from 1376 pregnancies with complete outcome data.

TOP, termination of pregnancy; IUFD, intrauterine fetal demise.

^aOne for triploidy, seven for ultrasound anomalies.

^bOne trisomy 21, one 22q11.2 del.

Table 3 Outcome of pregnancies with false positive and false negative NIPT results

| NIPT | MA (y) | BMI | GA (wk) | FCT risk | z-Score | Ultrasound | Invasive test | Child phenotype | Cord blood |
|------|--------|-----|---------|-----------------------------|---------|-----------------|-----------------------------------|--------------------------------|------------------|
| T21 | 41 | 26 | 12 + 3 | 1:147 (T21) | 4.96 | NI | Amnio QF-PCR and FISH normal | NI female | |
| T21 | 29 | 22 | 13 + 5 | 1:80 (T21) | 3.29 | NI | None | NI female | |
| T18 | 39 | 29 | 15 + 0 | 1:85 (T18) | 4.40 | NI | Amnio QF-PCR and karyotype normal | NI female | |
| T13 | 39 | 22 | 15 + 4 | 1:5000 (T13) 1:35 (T21) | 5.11 | NI | None | NI male | QF-PCR normal |
| T13 | 37 | 22 | 13 + 5 | 1:6134 (T13) 1:121 (T21) | 4.4 | NI | None | NI male | Karyotype normal |
| NI | 34 | 23 | 13 + 5 | 1:140 (T21) | <3 | Markers for T21 | Amnio T21, 46,XX,i(21)(q10) | Live-born Down syndrome female | |

MA, maternal age; BMI, body mass index; GA, gestational age; FCT, first trimester combined test; NI, normal.

Table 4 Test characteristics of NIPT for fetal trisomy 21

| | Trisomy 21 | Non-trisomy 21 | Total |
|--------------------|------------|----------------|-------|
| NIPT high risk T21 | 29 | 2 | 31 |
| NIPT low risk T21 | 1 | 1354 | 1355 |
| | 30 | 1356 | 1386 |

Sensitivity 29/30 = 96.7%.

Specificity 1354/1356 = 99.9%.

Positive predictive value 29/31 = 93.5%.

Negative predictive value 1354/1355 = 99.9%.

Table 5 Test characteristics of NIPT for fetal trisomy 18

| | Trisomy 18 | Non-trisomy 18 | Total |
|--------------------|------------|----------------|-------|
| NIPT high risk T18 | 4 | 1 | 5 |
| NIPT low risk T18 | 0 | 1381 | 1381 |
| | 4 | 1382 | 1386 |

Sensitivity 4/4 = 100%.

Specificity 1381/1382 = 99.9%.

Positive predictive value 4/5 = 80.0%.

Negative predictive value 1381/1381 = 100%.

Table 6 Test characteristics of NIPT for fetal trisomy 13

| | Trisomy 13 | Non-trisomy 13 | Total |
|--------------------|------------|----------------|-------|
| NIPT high risk T13 | 4 | 2 | 6 |
| NIPT low risk T13 | 0 | 1380 | 1380 |
| | 4 | 1382 | 1386 |

Sensitivity 4/4 = 100%.

Specificity 1380/1382 = 99.9%.

Positive predictive value 4/6 = 66.7%.

Negative predictive value 1380/1380 = 100%.

additional children, a growth-restricted infant was born. A detailed report of an extended cohort with additional findings, including follow-up testing, clinical relevance and pregnancy outcomes will be published separately.

Outcome

Complete follow-up of pregnancy outcomes was obtained for 1376/1390 (99.0%) pregnancies. Three children died in the neonatal period, two related to severe prematurity without evidence of structural or chromosomal anomalies, and one because of severe cardiac malformations (all three normal arrays). One live-born child was found to have a 22q11.2 deletion, associated with diGeorge syndrome (too small to detect with the method used). Its heart defect was not detected during pregnancy. Three women with a prenatal diagnosis of trisomy 21 elected to continue the pregnancy; all three children were born alive. No other live-born child was reported to have chromosomal abnormalities.

Invasive testing

Fifty-three invasive tests were done following NIPT results (5 CVS, 46 AS, 1 CVS and AS): 37 for suspected trisomy 21, 18

or 13, two for possible mosaics, an additional 12 because of other NIPT findings and one after a failed result. In addition, invasive testing was done in 21 women with a normal NIPT result, mostly indicated by ultrasound anomalies. Of those, five showed an abnormal karyotype: the already described missed trisomy 21 isochromosome, a triploidy, a small (0.1 Mb) 9p deletion, a 1.7 Mb deletion on Xp and one mosaic trisomy 8.

A precise estimation of the reduction of the overall number of invasive procedures attributable to the availability of NIPT in the TRIDENT study is hampered by several factors. NIPT performed by foreign commercial laboratories has been available to Dutch women in neighboring countries, paid out-of-pocket, since 2012. Of the 202 women who in the study period received a high-risk result from the FCT and declined NIPT, some may have chosen to have their blood sample analyzed in a foreign laboratory. Numbers and outcome data are unavailable to us.

What we do know, however, is that in The Netherlands consistently around 50% of women with an increased FCT risk result elect to undergo invasive testing.^{8,11} In our study, 1413 women undergoing FCT received a risk $\geq 1:200$. Theoretically, had NIPT not been available, 50% or 706 of them would have opted for invasive testing. Adding the 21 who had invasive testing because of ultrasound abnormalities results in a total of 727 invasive tests. The actual number of invasive tests in our study group was 53 plus 21 is 74, plus an unknown part of the 202 women who declined our NIPT. The most conservative calculation, if all 202 women would have chosen invasive testing, results in a reduction from 727 to 276, or 62%.

DISCUSSION

Our first evaluation of the TRIDENT study confirms the expected high accuracy of NIPT in a real-world setting. The uptake of NIPT in this high-risk cohort was 86%. This led to a major reduction in the number of invasive diagnostic procedures, at the expense of one trisomy missed. No unexpected live birth of a child with a trisomy occurred in this cohort. No pregnancies were terminated after abnormal NIPT without confirmation in chorionic villi or amniotic fluid cells. Detection rates (DR) for the common trisomies (96% for trisomy 21, 100% for trisomies 13 and 18) and false positive rates (0.14% for trisomies 21 and 13, 0.07% for trisomy 18) were comparable to previous studies.¹² Our data support introduction of NIPT as a safe second-tier screening test to accurately select the small proportion of women truly at high risk for fetal trisomy, and to reliably reassure all others.

The uptake of NIPT of 86% was similar to the hypothetical interest of 82% in a previous questionnaire study,¹³ and the 88% found in a UK survey.¹⁴ Smaller prospective studies in the US have reported uptakes of NIPT by women at elevated risk ranging from 43 to 69%.^{3,15,16} However, data are difficult to interpret and compare because of ample opportunities to obtain NIPT as a first-tier screening test across the border. Also, costs and reimbursement policies, and the extent of invasive testing, are likely to have a considerable impact on choices. The latter may vary from QF-PCR testing for chromosomes 21, 18, 13, X and Y only, to traditional karyotyping or high-resolution microarray. Preferences may

change again when more extended cfDNA testing becomes available.

The true clinical value of our study is that it confirms the expected major reduction in the number of invasive procedures, which was the primary goal of NIPT development.¹⁷ It is well known that for biological reasons NIPT will never be as accurate as the analysis of fetal cells taken from amniotic fluid.¹⁸ However, according to our data, there appears to be only a small price to pay for trading some accuracy for safety. The single fetal trisomy missed by NIPT was detected at routine prenatal ultrasound. The underlying isochromosome was absent in the cytotrophoblast, which is the source of the cfDNA tested by NIPT. Although rare, false negative trisomy 21 cases have been reported.^{19,20} Adequate counseling should ensure that women understand the high but not 100% accuracy of NIPT.

Although there is still controversy on the magnitude of the fetal loss rate caused by invasive testing, the reduction of invasive test procedures is certainly perceived as better care both by health care providers and pregnant women. Moreover, costs of invasive testing, a sum of laboratory work and procedure by maternal–fetal medicine specialists, are higher than the cost of NIPT, and this gap is rapidly widening. Health economic consequences of our study will be subject of reviewing.²¹ Others found similar reductions in invasive testing after introduction of NIPT.^{1,22–24} A recent decision-analysis modeling study concluded that offering NIPT to the high-risk population would reduce the number of invasive procedures by 72%, and NIPT for the general population a reduction of 60%.²⁵ An important consequence is the rapidly declining experience of clinicians in performing invasive procedures.^{1,26} Quality assurance and training programs need to be adapted, and more centralized care seems unavoidable.

In the Netherlands, only University Genetic Laboratories are licensed to do genetic testing. Some of these laboratories already had several years of experience in fetal cfDNA analysis while others started this service especially for the TRIDENT study.^{27–29} Although the numbers are still limited, we found no evidence for an inferior performance of NIPT by our laboratories compared to other providers.⁹

A possible limitation of the method used in our study was our inability to measure fetal fraction in all cases. Only recently algorithms became available to calculate fetal fraction based on the single read next generation sequencing (NGS) data that we have used.³⁰

Our choice for whole genome sequencing entailed that other trisomies, as well as large duplications and deletions, also became detectable. In such cases, decisions on informing and advising couples were taken after multidisciplinary discussions between lab specialists, clinical geneticists and maternal–fetal medicine specialists. NIPT data on the occurrence of these anomalies in a population with an increased risk based on the FCT are slowly emerging.^{31,32} In our study, 31% of the chromosomal abnormalities found were other than trisomy 21, 13 or 18. This is higher than the number calculated by Norton *et al.*,³³ which might be because of the fact that the study populations were different because of different screening protocols. Using our protocol, many of the non-detectable

anomalies as described by Norton *et al.*³³ such as other trisomies, unbalanced rearrangements and insertions/deletions would have been detectable.

Biologically, NIPT resembles direct analysis of chorion villus cytotrophoblasts, and additional findings are likely to be comparable.^{18,27,34} As expected therefore, a number of cases of confined placental mosaicisms such as trisomy 7, 8 and 16 and one case of true fetal mosaicism leading to, respectively, false positive and a false negative result were found. Our series confirmed the previously described association between a mosaic placenta and fetal growth restriction.^{35,36} As with CVS, advance knowledge of such a risk may be beneficial to clinical management. The need to perform invasive testing in these cases, and if so either with CVS or AS, will be discussed between laboratory specialists and clinicians on a case to case base. The advice to the parents will depend on gestational age, type of chromosome anomaly, presence or absence of ultrasound findings and parental preferences.^{34,37} This is a new area of research into possible additional clinical value of NIPT beyond trisomy 21, 13 and 18. In our study, couples had no option to reject information on secondary findings, but a choice will be offered in our future studies as not all couples may want this information.

The use of NIPT as a secondary screening test does not diminish the false negative rate of the initial screening test (FCT) but mainly lowers the false positive rate. As the DR of NIPT for common aneuploidies is higher than the DR of FCT (~80–95%),^{38–40} replacement of FCT by NIPT as a first-tier test will lower the false negative rate. We expect that the indication for NIPT will broaden to a larger proportion of pregnant women in the coming years, in line with international trends. This will enable us to further invest in equipment and improve workflow, leading to higher efficiency, lower cost per test and faster TATs, an issue our pregnant women highly value. With improving technology also, the scope of testing may become wider. The overall impact of the introduction of NIPT into our national screening program will therefore need continuous monitoring with emphasis not only on technical performance but also on patient preferences and outcome data of all pregnancies.

In conclusion, the first evaluation of our TRIDENT study with full clinical follow-up confirms the clinical usefulness of NIPT as a safe and reliable alternative to invasive diagnostic testing for high-risk pregnant women. Our trial license was granted for two years and has been extended for another two years. We are continuing to follow up all cases of NIPT; we will separately publish questionnaire results on women's evaluation after receiving test-results (Van Schendel *et al.*, in preparation) and re-analyze the health economic consequences. With these combined data, we will discuss with the government and all involved stakeholders a plan of action for formal implementation of NIPT in the Dutch national prenatal screening program.

ACKNOWLEDGEMENTS

The authors thank the clinicians and staff of all participating centers who were instrumental to the data collection: Jozien Brons, Medisch Spectrum Twente, Enschede; Lia Wijnberger, Rijnstate Hospital, Arnhem; Addie Drogtop, Tweesteden

Hospital, Tilburg; Robbert Rijnders, MD, PhD, Jeroen Bosch Hospital, Den Bosch; Toon Tolenaar, Albert Schweitzer Hospital, Dordrecht; Katja de Graaf, Reinier de Graaf Gasthuis, Delft; Dimitri Papatsonis, Amphia Hospital, Breda; Martina Porath, Maxima Medical Centre, Veldhoven; Ella Wijnen-Duvekot, VieCuri Hospital, Venlo; Dick Schoot, Catherina Hospital, Eindhoven; Diederik Veersema, Diagnostiek Voor U, Eindhoven; Jos Offermans, Zuyderland Hospital, Heerlen; Hans Beekhuis, Isala Clinic, Zwolle. Birgit Sikkema-Raddatz (UMC Groningen) is acknowledged for her active role in the early stages of this project. The authors also like to thank all members of the Dutch NIPT Consortium.

REFERENCE

- Warsof SL, Larion S, Abuhamad AZ. Overview of the impact of noninvasive prenatal testing on diagnostic procedures. *Prenat Diagn* 2015;35:972–9.
- Jaques AM, Collins VR, Muggli EE, *et al.* Uptake of prenatal diagnostic testing and the effectiveness of prenatal screening for Down syndrome. *Prenat Diagn* 2010;30:522–30.
- Chetty S, Garabedian MJ, Norton ME. Uptake of noninvasive prenatal testing (NIPT) in women following positive aneuploidy screening. *Prenat Diagn* 2013;33:542–6.
- Lichtenbelt KD, Schuring-Blom GH, van der Burg N, *et al.* Factors determining uptake of invasive testing following first-trimester combined testing. *Prenat Diagn* 2013;33:328–33.
- Green JM, Hewison J, Bekker HL, *et al.* Psychosocial aspects of genetic screening of pregnant women and newborns: a systematic review. *Health Technol Assess* 2004;8iii, ix–iii, 1–109.
- Engels MA, Bhola SL, Twisk JW, *et al.* Evaluation of the introduction of the national Down syndrome screening program in the Netherlands: age-related uptake of prenatal screening and invasive diagnostic testing. *Eur J Obstet Gynecol Reprod Biol* 2014;174:59–63.
- Schendela RV, Page-Christiaens LC, Beulen L, *et al.* Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part II – women’s perspectives. *Prenat Diagn* 2016, DOI: 10.1002/pd.4941.
- Atsma F, Jansen B, Liefers J. *Monitor 2013 Screeningsprogramma Downsyndroom en Structureel Echoscopisch Onderzoek*. Radboudumc/Scientific Institute for Quality of Healthcare: Nijmegen; 2014.
- Mersy E, Smits LJM, van Winden LAAP, *et al.* Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. *Hum Reprod Update* 2013;19:318–29.
- Straver R, Sistermans EA, Holstege H, *et al.* WISECONDOR: detection of fetal aberrations from shallow sequencing maternal plasma based on a within-sample comparison scheme. *Nucleic Acids Res* 2014;42:e31.
- Gezondheidsraad (Health Council of the Netherlands). *Wet op het bevolkingsonderzoek: niet-invasieve prenatale test bij verhoogd risico op trisomie*. Den Haag; 2013. Report No.: 2013/38.
- Gil MM, Akolekar R, Quezada MS, *et al.* Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 2014;35:156–73.
- Verweij EJ, Oepkes D, de Vries M, *et al.* Non-invasive prenatal screening for trisomy 21: what women want and are willing to pay. *Patient Educ Couns* 2013;93:641–5.
- Lewis C, Hill M, Silcock C, *et al.* Non-invasive prenatal testing for trisomy 21: a cross-sectional survey of service users’ views and likely uptake. *BJOG: Int J Obstet Gyn* 2014;121:582–94.
- Wallerstein R, Jelsk A, Garabedian MJ. A new model for providing cell-free DNA and risk assessment for chromosome abnormalities in a public hospital setting. *J Pregnancy* 2014;2014:962720.
- Taylor J, Chock V, Hudgins L. NIPT in a clinical setting: an analysis of uptake in the first months of clinical availability. *J Genet Counsel* 2014;23:72–8.
- Lo YM. Noninvasive prenatal diagnosis: from dream to reality. *Clin Chem* 2015;61:32–7.
- Van Opstal D, Srebnik MI, Polak J, *et al.* False negative NIPT results: risk figures for chromosomes 13, 18 and 21 based on chorionic villi results in 5967 cases and literature review. *PLoS One* 2016;11:e0146794.
- Smith M, Lewis KM, Holmes A, Visootsak J. A case of false negative NIPT for Down syndrome—lessons learned. *Case Rep Genet* 2014;2014:823504.
- Willems PJ, Dierickx H, van den Akker E, *et al.* The first 3,000 non-invasive prenatal tests (NIPT) with the harmony test in Belgium and the Netherlands. *Facts Views Vis Obgyn* 2014;6:7–12.
- Beulen L, Grutters JP, Faas BH, *et al.* The consequences of implementing non-invasive prenatal testing in Dutch national health care: a cost-effectiveness analysis. *Eur J Obstet Gynecol Reprod Biol* 2014;182:53–61.
- Friel LA, Czerwinski JL, Singletary CN. The impact of noninvasive prenatal testing on the practice of maternal–fetal medicine. *Am J Perinatol* 2014;31:759–64.
- Larion S, Warsof SL, Romary L, *et al.* Association of combined first-trimester screen and noninvasive prenatal testing on diagnostic procedures. *Obstet Gynecol* 2014;123:1303–10.
- Manegold-Brauer G, Kang BA, Hahn S, *et al.* A new era in prenatal care: non-invasive prenatal testing in Switzerland. *Swiss Med Wkly* 2014;144:w13915.
- Benn P, Curnow KJ, Chapman S, *et al.* An economic analysis of cell-free DNA non-invasive prenatal testing in the US general pregnancy population. *PLoS One* 2015;10:e0132313.
- Hui L, The S, McCarthy EA, Walker SP. Emerging issues in invasive prenatal diagnosis: safety and competency in the post-NIPT era. *Aust N Z J Obstet Gynaecol* 2015;55:541–6.
- Faas BH, de Ligt J, Janssen I, *et al.* Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. *Expert Opin Biol Ther* 2012;12 Suppl 1: S19–26.
- van den Oever JM, Balkassmi S, Verweij EJ, *et al.* Single molecule sequencing of free DNA from maternal plasma for noninvasive trisomy 21 detection. *Clin Chem* 2012;58:699–706.
- van den Oever JM, Balkassmi S, Johansson LF, *et al.* Successful noninvasive trisomy 18 detection using single molecule sequencing. *Clin Chem* 2013;59:705–9.
- Straver R, Oudejans CB, Sistermans EA, Reinders MJ. Calculating the fetal fraction for Non Invasive Prenatal Testing based on genome-wide nucleosome profiles. *Prenat Diagn* 2016;36:614–21.
- Brady P, Brison N, Van Den Bogaert K, *et al.* Clinical implementation of NIPT—technical and biological challenges. *Clin Genet* 2016;89:523–30.
- Helgeson J, Wardrop J, Boomer T, *et al.* Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenat Diagn* 2015;35:999–1004.
- Norton ME, Jelliffe-Pawlowski LL, Currier RJ. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. *Obstet Gynecol* 2014;124:979–86.
- Grati FR, Bajaj K, Malvestiti F, *et al.* The type of feto-placental aneuploidy detected by cfDNA testing may influence the choice of confirmatory diagnostic procedure. *Prenat Diagn* 2015;35:994–8.

WHAT’S ALREADY KNOWN ABOUT THIS TOPIC?

- Non-invasive prenatal testing (NIPT) for trisomies 21, 18 and 13 is mainly offered in a commercial setting.
- Offering NIPT to pregnant women at increased risk for fetal trisomy leads to a reduction in invasive testing.

WHAT DOES THIS STUDY ADDS?

- NIPT implemented as a contingent test under the umbrella of a national health authority-supported fetal trisomy screening program facilitates optimal counseling with close to 100% follow-up, results in 86% uptake and confirms a major reduction of invasive tests.

35. Kalousek DK, Vekemans M. Confined placental mosaicism. *J Med Genet* 1996;33:529–33.
36. Wilkins-Haug L, Quade B, Morton CC. Confined placental mosaicism as a risk factor among newborns with fetal growth restriction. *Prenat Diagn* 2006;26:428–32.
37. Van Opstal D, Srebniak MI. Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration. *Expert Rev Mol Diagn* 2016;16:513–20.
38. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011;31:7–15.
39. Engels MA, Heijboer AC, Blankenstein MA, van Vugt JM. Performance of first-trimester combined test for Down syndrome in different maternal age groups: reason for adjustments in screening policy? *Prenat Diagn* 2011;31:1241–5.
40. Norton ME, Jacobsson B, Swamy GK, *et al.* Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med* 2015;372:1589–97.