Current controversies in prenatal diagnosis 1: should noninvasive DNA testing be the standard screening test for Down syndrome in all pregnant women? 

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INTRODUCTION

Over the past 2 years, noninvasive prenatal testing (NIPT) or, more precisely, noninvasive DNA testing (NIDT), using massively parallel sequencing of the circulating cell-free DNA in the plasma of pregnant women, has become rapidly incorporated into prenatal care. Scientific and technical advances, combined with intense competition between the major companies that provide the technology, have resulted in continuous additions to the testing menu. In this debate, however, we mainly considered the technology, have resulted in continuous additions to the testing menu. In this debate, however, we mainly considered NIDT only for the major autosomal aneuploidies. Given the fact that prenatal screening paradigms are currently changing in real time, this debate was quite timely.

Between the years 2008 and 2011, multiple clinical trials were performed worldwide that demonstrated consistently high sensitivities and specificities for the detection of trisomy 21,1–4 followed later by application to trisomies 18 and 13.5–8 NIDT was first offered on a clinical and commercial basis in mainland China on 1 January 2011.9 Following publication of a large scale clinical trial,4 clinical testing for trisomy 21 was first offered in the USA on 17 October 2011. Other groups later followed suit in other parts of the world. Currently, there are four companies that offer NIDT in the USA, while two offer it in China. One additional company offers the test in the German-speaking countries of Europe. NIDT is unique in the prenatal testing universe because at present, it is only available from commercial and not academic laboratories.

As clinical experience began to accumulate with these new tests, different professional societies met to consider their clinical, technological, ethical, and economic aspects. The International Society for Prenatal Diagnosis,10,11 the National Society of Genetic Counselors,12 the American College of Obstetrics and Gynecology with the Society for Maternal Fetal Medicine,13 and the Society of Obstetricians and Gynecologists of Canada14 all published recommendations that were quite similar. They stated that NIDT should be offered only in the context of adequate pre-test and post-test counseling, as an option to women carrying singleton fetuses at high risk of having autosomal aneuploidy. The pre-test counseling session should emphasize the test’s high negative predictive value, its low false positive rate, and the fact that (after 10 weeks) it does not depend on gestational age. Post-test counseling sessions need to emphasize that positive test results should be confirmed with an invasive procedure that obtains a fetal karyotype or chromosome microarray. Quality control standards need to be developed and validated. The American College of Medical Genetics and Genomics (ACMG) concurred with these statements and added a few new ones.15 They recommended that the term ‘noninvasive prenatal screening (NIPS)” be used instead of ‘noninvasive prenatal diagnosis (NIPD)” or ‘noninvasive prenatal testing (NIPT). [Note that we prefer the term ‘noninvasive DNA testing (NIDT)” because it distinguishes this specific test from other noninvasive screens or tests, such as maternal serum screening or ultrasound examination.] The ACMG also recommended giving specific educational materials on Down syndrome to women whose test results suggested that the fetus is affected. Interestingly, the ACMG did not limit their recommendations to women at high risk for fetal aneuploidy. They also recommended the establishment of a funded registry to obtain clinical outcome information and to establish both negative and positive predictive values.15 Not all areas of the world are equally enthusiastic; Japanese obstetricians, for example, have been very cautious. They are only providing NIDT in the context of a research study that involves 1000 high-risk pregnant women.16 Only centers that have obstetricians and pediatricians with expertise in prenatal diagnosis and genetics.
on site, along with genetic counselors and adequate capabilities for invasive diagnostic procedures, can participate in this study.

Recent ‘real world’ evidence from NIDT performed in obstetric practices and using certified laboratories in the USA has suggested that NIDT has a negative predictive value of 99.6%.

A primary obstetric practice compared screening for aneuploidy in ‘all risk’ women by NIDT versus serum screening, and found that the false positive rates were markedly lower with NIDT; this translated to a significant reduction in invasive procedures. Multiple groups worldwide have shown that NIDT has excellent sensitivity and specificity and extremely high negative predictive values. Therefore, we asked the question, ‘If the test is so good, then why not offer it to all pregnant women, regardless of their a priori risk for fetal aneuploidy?’

FOR

For several decades, pregnant women have been provided with the option to prenatally obtain information on their child’s health with the introduction of testing of amniotic fluid, chorionic villi, and fetal anatomy by ultrasound examination. In the vast majority of cases, such tests show reassuring results, which women greatly appreciate, although they generally understand perfectly well that no test can provide a 100% guarantee of a healthy child. In many countries, the law provides pregnant women with the right to make autonomous reproductive choices, including the choice to request termination of pregnancy, often with an upper limit of gestational age. This means that those women who receive reliable information from prenatal tests, and who carry fetuses with anomalies that they perceive to be severe, may elect to prevent the live birth of a handicapped child, in order to prevent suffering of the future child, a heavy burden for the family, or both.

Many modern societies agree that all pregnant women have the right to make such decisions and that their autonomy deserves to be respected. There is also general agreement that all women should be allowed to request prenatal testing based on adequate pre-test education regarding the test’s benefit and limitations. Our obligation as professionals is to help pregnant women to maximize their chances of a good pregnancy outcome by assisting them in the process of informed choice. The right choice for an individual pregnant woman requires the best, most accurate, and clearly explained information. Thus, prenatal testing for fetal anomalies should be as accurate as possible, and the test results should be completely clear to the pregnant woman in order for her to make her personal decisions.

Two other essential elements of prenatal testing drive the programs that are offered to pregnant women. First are the relatively small but inherent risks of fetal loss caused by invasive tests. The standard prenatal diagnostic tests used since the 1970s, chorionic villous sampling (CVS) and amniocentesis, carry a risk of 0.5% to 1% of causing fetal loss. Most often, these fetal deaths occur in completely healthy and wanted pregnancies. Almost all women who are considering prenatal testing are afraid of these complications. Even if, as some colleagues argue, the risk is actually lower than can be expected from the literature, any risk influences the uptake of testing in a major way. Declining the offer of the first screening step or deciding not to undergo follow-up testing after a positive screening test result is often based (at least in part) on the fear of losing a healthy, wanted child due to invasive testing. This means that a number of women, although they would have liked to avoid the birth of a handicapped child, actually will be confronted with such a child at birth. This is clearly an undesirable aspect of current screening programs.

The second aspect is financial. All prenatal tests cost money, and restriction of access to testing (such as only offering invasive tests to women with an increased risk for abnormal finding) was, and still is, driven by the financial consequences of large scale testing. Most societies consider their ever-expanding health care costs to be a serious problem. Cost–benefit analyses now play an important role with policy makers in deciding on whether or not to implement healthcare innovations.

To date, at least 200 000 noninvasive DNA tests have been performed in clinical practice worldwide. A rapidly expanding body of literature strongly suggests that this test, when applied for the prediction of trisomy 21, lives up to its promise and has a diagnostic accuracy above 99% in women at increased risk for trisomy 21. Based on our understanding of the nature of the test, and recent clinical studies, there seems to be little doubt that the test performance is equal in women of all ages.

There are two ways to scientifically evaluate whether NIDT deserves a place in the current screening programs offered to women in many countries. The first is to directly compare the test characteristics of NIDT with one of the tests we currently offer women and, if NIDT proves to perform better, to consider replacing the current test by NIDT. The second is to consider the overall performance of our current testing program, including evaluation of all pregnancies in which no testing is carried out, including costs, and including careful evaluation of women’s preferences, and then compare this to a newly designed program in which NIDT has a place, either as an add-on or as a replacement of one of the current steps.

The first step is quite simple. NIDT is beyond any doubt both more sensitive and more specific in the prediction of trisomy 21 than the current first trimester combined test, the most commonly used first step in screening programs. A simple graph that should convince anyone was published by Nicolaides et al., depicting in the same large cohort of patients the wide range of reported trisomy 21 risks from the first trimester combined test versus the complete separation into very high and very low risks by NIDT. Additionally, NIDT is not restricted to an upper limit of gestational age. This is an important and still under-reported advantage, especially for women who present late for their first prenatal visit. In addition, both pre-test and post-test counseling is considerably easier for NIDT as compared with the combined test. The individual components of the combined test, the meaning of a moderately increased nuchal translucency (NT) measurement, the use of likelihood ratios, and the completely random and quite illogical cut-off levels used in various countries (from 1 in 175 to 1 in 300) are rather complicated to explain to even the most intelligent patient. Then, there are the still unclear side effects, such as what to do with a low PAPP-A measurement, and the many rare diseases that can be associated with enlarged NT can make fully informed
counseling virtually impossible. The fact that the first step of selection by the combined test means that at least 1 in 10 trisomy 21 cases will be missed is often not clearly communicated. On the other hand, NIDT provides two widely separated results; it is either, and most often, highly reassuring, reducing the risk of trisomy 21 to less than 1 in 10,000, or it shows a high risk, even in low-risk settings of 50% or more, of a fetal trisomy. This takes 5 min to explain, and the remaining time can be spent on an often eliminated part of counseling, namely, discussing what the disease in question actually entails.

When compared with CVS, the risk of miscarriage associated with NIDT is very close to zero. It is not zero, because positive results will need to be confirmed by an invasive test, but it compares favorably to 0.5% to 1% for CVS, with nearly identical sensitivities and specificities. The major limitations of NIDT, including test failures, unclear results (due to mosaicism), false positive results (on the order of 0.1–0.2%), the need for repeat testing by amniocentesis, and the rare detection of confined placental mosaicism, all happen after CVS as well. Other aspects such as pain, fear, and discomfort are minimal in NIDT and common in CVS. In addition, CVS is a relatively safe technique only in the hands of well-trained, skilled professionals, a group that soon may become reduced in number. A blood sample for NIDT can be taken by any nurse or skilled health professional. When compared with amniocentesis, however, NIDT has a slightly lower accuracy, and this is likely to remain the case. A major disadvantage of amniocentesis, however, is the relatively late gestation at which results are provided. For amniocentesis, this is around 16 weeks, while an NIDT result can be available as early as 12 weeks. A very insightful study by Hill et al. suggests that many pregnant women value safety over accuracy and are willing to accept a slightly less accurate test provided it has no risk of causing a miscarriage.

In conclusion, based on the principle that it is our professional obligation to offer the best possible tests in order for pregnant women to make a truly informed choice, NIDT provides two widely separated results; it is either, and most often, highly reassuring, reducing the risk of trisomy 21 to less than 1 in 10,000, or it shows a high risk, even in low-risk settings of 50% or more, of a fetal trisomy. This takes 5 min to explain, and the remaining time can be spent on an often eliminated part of counseling, namely, discussing what the disease in question actually entails.

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In conclusion, based on the principle that it is our professional obligation to offer the best possible tests in order for pregnant women to make a truly informed choice, NIDT can replace both the first trimester combined test and CVS. For amniocentesis, the respect for autonomy would dictate that we give women a choice for either NIDT or amniocentesis. These evaluations are already widely known and understood by the general public, which explains the fast growing demand for access to NIDT by pregnant women irrespective of their age.

Policy makers, however, would like to have clear insights regarding overall economic consequences before allowing innovations to be implemented into prenatal care. From a health technology perspective, we need to perform calculations for different new scenarios, and national screening programs will need to be adapted to incorporate NIDT into prenatal care. Given the wide variety of currently available screening programs, this is not so straightforward. Different calculations will be needed for each country, depending upon whether they have nationalized or individual health insurance systems, although the general principles will be similar. One essential component of health technology assessment is a clear definition of the goals of the program. This is not as well defined as many doctors think. Reduction of disease burden is an acceptable goal; however, for trisomy 21, the burden for the family often plays a more important role in the decision making than the possible suffering of the child with the disease. As an example, in the Netherlands, the official government-approved goal of the screening program is to promote ‘informed choice’ in the reproductive health of pregnant women. If taken literally, this would mean that a new program could consist of offering all possible combination of tests, with adequate counseling on all aspects of each test, allowing the woman to decide which strategy she prefers. With the currently available knowledge, it will be difficult to make any reliable prediction on uptake, costs, or logistics required for such a program.

A more realistic scenario is that committees consisting of representatives of all stakeholders will design two or three new algorithms, integrating NIDT as an add-on or replacement of current tests, followed first by theoretical calculations of the overall effects and then testing the most acceptable scenario in a real-life prospective evaluation.

In summary, the two major reasons for serious suffering related to the inadequacies of the current screening program are the fetal losses caused by invasive tests, and the unwanted and often unanticipated births of handicapped children due to false negative test results or the decline of invasive testing due to a fear of miscarriage due to the procedure. Both can be virtually eliminated by offering NIDT to all pregnant women. Given the rapid reduction in costs of NIDT, soon expected to be in the range of 300 euros or 400 US dollars, there seems to be little impediment to starting large scale implementation trials in many countries.

AGAINST
The main goals of NIDT are to minimize anxiety surrounding multi-step screening for Down syndrome and other common trisomies, to increase their detection rate, reduce the false positive rates associated with serum screening, and to reduce exposure to invasive prenatal procedures with their associated risks. Multiple prospective studies have shown detection rates of 98% to 100% for Down syndrome, with false positive rates of 0.1% to 0.5%, but NIDT is currently considered to be an advanced screening test that should only be offered to women at high risk for fetal aneuploidies (maternal age $>$35 years, abnormal first trimester or quadruple screen, sonographic evidence of fetal anomalies, or prior aneuploid fetus); it requires invasive testing (CVS or amniocentesis) for confirmation. Moreover, because only preliminary data are available on twins, the test is currently recommended only in singletons. A baseline ultrasound examination is indicated prior to the test to confirm fetal viability and that it is a singleton pregnancy, to establish gestational dating, and to rule out obvious anomalies.

Noninvasive DNA testing is still evolving, however, and its many limitations have not been adequately highlighted and resolved. Until at least the major limitations of the test have been addressed, it should not be offered to low-risk women.

A first set of limitations is related to the technique itself. Although it is likely that NIDT will be as accurate in low-risk as it is in high-risk populations, several test performance characteristics have not been assessed in low-risk women, such as test failure rates, including unsuitable specimens,
samples with low fetal fraction, failed quality control, and need for redraws. Such characteristics have important repercussions in terms of turnaround time, costs, impact on subsequent access to first trimester invasive diagnostic tests, and methods of termination of pregnancy. Before large scale implementation of NIDT, several other methodological questions need to be answered. NIDT is performed by different commercial companies using different techniques (e.g. whole genome sequencing vs. selective amplification prior to sequencing; counting algorithms vs. genotyping algorithms). Each technique has its specific advantages (e.g., potential for detection of secondary findings with whole genome sequencing, reduced costs with selective amplification, and detection of triploidy with a genotyping algorithm), yet data are not available comparing the performance, yield, and cost of these tests directly to each other. Moreover, screening performance of NIDT is affected by fetal fraction (FF). Samples that contain less than 3% to 4% of FF are considered unsuitable, and detection rates of Down syndrome are directly correlated with FF for the counting methods used in massively parallel sequencing. Yet, FF is not reported by all of the commercially available companies. Maternal weight is the main determinant of FF; at what maternal weight cut-off would NIDT not be worth attempting? What threshold of FF is necessary to detect mosaicism for the most common aneuploidy? False positive results of NIDT due to confined placental mosaicism have been reported: what is the rate of such a finding? What are the repercussions in terms of obstetric risk? Once again, answers are needed before implementation of NIDT in the general obstetric population.

The second limitation is that comprehensive cost–benefit analyses for low-risk populations have not yet been reported. Published cost–benefit analyses have applied decision analysis type of economic modeling to determine the effects of varying degrees of relevant model inputs, but they either have focused only on high-risk populations or have not been able to include all relevant model inputs. In this part, it is due to the constantly changing landscape of NIDT. For example, a recently proposed algorithm recommends an initial ultrasound scan at about 10 weeks (when NIDT is requested) to document fetal viability and the number of fetuses, and another scan 2 weeks later (when NIDT results are discussed) to evaluate gross fetal anatomy and measure NT. This approach allows detection of a congruous proportion of chromosomal and structural fetal anomalies in addition to those detected by NIDT. Obviously, performance of an additional first trimester ultrasound scan and retention of NT as screening test for fetal anomalies would greatly affect costs. Other variables with adverse impact on costs include content and duration of pre-test and post-test counseling. Finally, cost–benefit analyses cannot take into consideration variables that are currently unknown: for example, NIDT may cause a shift in decision making, as knowledge of fetal status obtained in a noninvasive way may not necessarily lead to an intention to terminate the pregnancy, particularly in countries with supportive healthcare. Finally, outcome of ongoing patent litigation may result in fewer options of companies offering NIDT and higher costs.

Another important limitation is the current absence of guidelines for quality control and quality assurance of the laboratory testing. In the USA, existing Clinical Laboratory Improvement Amendments regulations are designed to oversee compliance at a laboratory level, not validation of a specific test. New commercial companies may start offering NIDT or existing companies may expand the indications or populations tested (e.g., fetal gender determination or twins) without adequate validation studies. In the absence of direct regulations, which organization will provide oversight? Will validation be left to the initiative of the commercial companies offering the test?

Pre-test and post-test counseling is another limitation that needs addressing. With implementation of NIDT in low-risk patients, healthcare providers will be left with the task of translating results into a language that can be understood by pregnant women. Unlike current genetic screening tests, which report results in a standardized fashion expressed as predictive values (or odds), with prior risk (e.g. by maternal age) already incorporated in the result, each company reports NIDT results in a different way. To add to the confusion, several companies provide positive NIDT results as either ‘affected’ or ‘aneuploidy detected’, leading to the potential for misunderstanding by healthcare providers and patients. Both of them, indeed, may be unfamiliar with the concept that positive predictive values are greatly affected by prevalence of the condition. For example, even assuming 99% sensitivity and 99% specificity for NIDT detection of Down syndrome, a ‘positive result’ in a woman with a prior risk of 1/800 would translate into a positive predictive value of only 57%.

Several ethical issues have already been raised that would greatly impact expansion of NIDT to a low-risk population, including the issues of justice (the out of pocket cost of NIDT is the main reason for women not choosing the test), fetal autonomy (the potential for misuse with determination of fetal sex for social reasons), and woman’s autonomy (the potential for NIDT being performed ‘routinely’ with other prenatal tests, thus infringing on the right of women not-to-know). Direct marketing of NIDT to pregnant women, bypassing healthcare providers and counseling, raises several additional ethical issues (such as equitability and truthfulness).

Lastly, all major companies offering NIDT are currently involved in lawsuits over enforcement and infringement of patents. The results of such lawsuits have the potential to greatly affect market competition, quality assurance, and technology improvement, with implications for cost-effectiveness and quality.

In summary, although implementation of NIDT in low-risk women has obvious benefits, at the present time, they would likely be offset by the aforementioned limitations, which urgently need to be addressed.

CONCLUSIONS

As was true in the oral debate in Lisbon, both debaters are passionate about their respective perspectives, and they each make relevant points. Both agree that as a screening test, NIDT has improved sensitivity and specificity for the detection of Down syndrome in all pregnant women compared with the current standards of care. Dr. Ghidini, however, is concerned that many obstetric providers may not understand that in a low-risk pregnant woman, her reduced chance of fetal aneuploidy lowers
the positive predictive value of the test. The extremely high negative predictive value (>99%) and the low false positive rates (0.1–0.2%) mean that fewer pregnant women need to have invasive procedures. As a screening test, NIDT is safer than the current approach because it reduces the chance of an unnecessary invasive procedure with its associated small risk of miscarriage. As stated by Dr. Oeperkes, this is extremely important to most pregnant women who value safety over accuracy.

Many of the points made by Dr. Ghidini, with regard to the best technical approaches, the need for quality controls and assurance, and the effects of delayed turnaround time in limiting access to CVS, are true for both high-risk and low-risk cases. Although cost–benefit analyses are very important, they will have different meaning and implications in countries that have national versus individual health insurance systems. Lastly, while it is true that many of the performance characteristics of NIDT have not yet been assessed in low-risk pregnant women, this is an active area of research, and several relevant additional publications have already appeared in the month following the debate.\textsuperscript{8,9} Interestingly, some private obstetrical practices in the USA have already taken matters into their own hands by performing direct comparisons of NIDT versus combined screening, and they have changed their clinical practices based on the results of their own experiences.\textsuperscript{10}

What is clear is that the well-validated standard algorithms for prenatal screening of aneuploidy are being disrupted by the new technologies. The involvement of industry, along with patient education and empowerment by social media, has led to a rapid incorporation of NIDT into clinical care. While much has been said about the negative aspects of corporate involvement in this field,\textsuperscript{31} one benefit is that the companies have the resources to generate large data sets in relatively short periods of time. This undoubtedly contributes to the advancement of knowledge at a quick pace.

Noninvasive DNA testing is here to stay because it is a better screening test for whole chromosome aneuploidy. While there was no clear ‘winner’ of the debate, the ultimate winners are the pregnant women who can obtain the information they need to make their own individual reproductive choices. The benefits and limitations of NIDT for all pregnant women, whether high or low risk, will become increasingly clear over the next few years, as ongoing studies are completed and published in peer-reviewed journals. Eventually, it will become an economic issue that will be addressed differently in different countries. By that time, however, we will have likely moved on to even newer technologies, such as noninvasively sequencing the entire fetal genome.\textsuperscript{33}

\section*{WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?}
- Noninvasive DNA testing (NIDT) is recommended in many developed countries as an option for pregnant women who have already been determined to be at high risk for fetal aneuploidy.
- Over 2 years of clinical experience has been accumulated with offering NIDT as an advanced screen for fetal autosomal aneuploidy.
- At the present time, most testing is being performed by commercial organizations.

\section*{WHAT DOES THIS STUDY ADD?}
- This study provides a written transcript to accompany an oral debate that was presented at the 17\textsuperscript{th} International Conference on Prenatal Diagnosis and Therapy in Lisbon, Portugal, on 3 June 2013.
- The debaters, who are both experts in maternal–fetal medicine, consider the benefits and limitations of offering NIDT to all pregnant women regardless of their a priori risk of having a fetus with a chromosome abnormality.

\section*{REFERENCES}