Health Technology Clinical Committee
DRAFT Findings and Decision

Topic: Whole exome sequencing
Meeting date: November 22, 2019
Final adoption: Pending

Meeting materials and transcript are available on the HTA website.

Number and coverage topic:

20200117A – Whole exome sequencing

HTCC coverage determination:
Whole exome sequencing is a covered benefit with conditions.

HTCC reimbursement determination:

Limitations of coverage:
Whole exome sequencing (WES) is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorders in a phenotypically affected individual when **ALL of the following** criteria are met:

1. A board-certified or board-eligible Medical Geneticist, or an Advanced Practice Nurse in Genetics (APGN) credentialed by either the Genetic Nursing Credentialing Commission (GNCC) or the American Nurses Credentialing Center (ANCC), who is not employed by a commercial genetic testing laboratory, has evaluated the patient and family history, and recommends and/or orders the test; and

2. A genetic etiology is considered the most likely explanation for the phenotype, based on **EITHER of the following**; and

   • Multiple abnormalities affecting unrelated organ systems, (e.g. multiple congenital anomalies); or

   • **TWO of the following criteria are met:**
     
     — Significant abnormality affecting at minimum, a single organ system,
     — Profound global developmental delay\(^1\) or intellectual disability\(^2\) as defined below,
     — Family history strongly suggestive of a genetic etiology, including consanguinity,
     — Period of unexplained developmental regression (unrelated to autism or epilepsy),
     — Biochemical findings suggestive of an inborn error of metabolism where targeted testing is not available;

3. Other circumstances (e.g. environmental exposures, injury, infection) do not reasonably explain the constellation of symptoms; and
4. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]) is available; and

5. The differential diagnosis list and/or phenotype warrant testing of multiple genes and **ONE of the following:**
   - WES is more efficient and economical than the separate single-gene tests or panels that would be recommended based on the differential diagnosis (e.g., genetic conditions that demonstrate a high degree of genetic heterogeneity); or
   - WES results may preclude the need for multiple invasive procedures or screening that would be recommended in the absence of testing (e.g. muscle biopsy); and

6. A standard clinical work-up has been conducted and did not lead to a diagnosis; and

7. Results will impact clinical decision-making for the individual being tested; and

8. Pre- and post-test counseling is performed by an American Board of Medical Genetics or American Board of Genetic Counseling certified genetic counselor.

**Non-covered indicators:**

WES is not covered for:

- Uncomplicated autism spectrum disorder, developmental delay, mild to moderate global developmental delay.
- Other circumstances (e.g. environmental exposures, injury, infection) that reasonably explain the constellation of symptoms.
- Carrier testing for “at risk” relatives.
- Prenatal or pre-implantation testing.

**Definitions:**

1. **Global developmental delay (GDD)** is used to categorize children who are younger than five years of age.

GDD is defined as a significant delay in two or more developmental domains, including gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living and is thought to predict a future diagnosis of ID. Such delays require accurate documentation by using norm-referenced and age appropriate standardized measures of development administered by experienced developmental specialists, or documentation of profound delays based on age appropriate developmental milestones are present.


Significant delay is typically defined as performance two standard deviations or more below the mean on age-appropriate, standardized, normal-referenced testing.

2. **Intellectual disability (ID)** is a life-long disability diagnosed at or after age five when intelligence quotient (IQ) testing is considered valid and reliable. The Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-V), defines patients with ID as having an IQ
less than 70, onset during childhood, and dysfunction or impairment in more than two areas of adaptive behavior or systems of support.

Agency contact information:

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<thead>
<tr>
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<tr>
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<tr>
<td>Washington State Medicaid</td>
<td>1-800-562-3022</td>
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</table>
HTCC coverage vote and formal action:

**Committee decision**

Based on the deliberations of key health outcomes the committee decided that it had the most complete information: a comprehensive and current evidence report, public comments, and state agency utilization information. The committee decided that the current evidence on whole exome sequencing is sufficient to make a determination on this topic. The committee discussed and voted on the evidence for the use of the test, considered the evidence and gave greatest weight to the evidence it determined, based on objective factors, to be the most valid and reliable. Based on these findings, the committee voted to cover with conditions whole exome sequencing for children and adults.

<table>
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<tr>
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<td>Whole exome sequencing 0</td>
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**Discussion**

The committee reviewed and discussed the available information and limitations of the evidence base. A majority of committee members found the evidence sufficient to determine that whole exome sequencing is more effective in some scenarios and equally safe to other similar tests. In drafting the conditions for coverage, the committee recognized a need for more information and refinement of the proposed coverage criteria. Agency staff were directed to compile the information and provide the committee a draft for consideration at the next meeting scheduled for January 17, 2020.

**Limitations**

N/A

**Action**

As noted the committee chair directed agency staff to prepare additional information for the proposed conditional criteria for whole exome sequencing to be considered by the committee at the next meeting.

At the January 17, 2020 committee meeting the committee checked for availability of a Centers for Medicare and Medicaid Services (CMS) national coverage decision (NCD). There is no Medicare NCD for WES. The committee checked for availability of clinical guidelines identified for WES. No clinical practice guidelines were identified specific to diagnostic testing with WES.

The committee chair directed HTA staff to prepare a findings and decision document on use of whole exome sequencing for public comment to be followed by consideration for final approval at the next public meeting.

**Health Technology Clinical Committee Authority:**

Washington State’s legislature believes it is important to use a science-based, clinician-centered approach for difficult and important health care benefit decisions. Pursuant to chapter 70.14 RCW, the legislature has directed the Washington State Health Care Authority (HCA), through its Health
Technology Assessment (HTA) program, to engage in an evaluation process that gathers and assesses the quality of the latest medical evidence using a scientific research company and that takes public input at all stages.

Pursuant to RCW 70.14.110 a Health Technology Clinical Committee (HTCC) composed of eleven independent health care professionals reviews all the information and renders a decision at an open public meeting. The Washington State HTCC determines how selected health technologies are covered by several state agencies (RCW 70.14.080-140). These technologies may include medical or surgical devices and procedures, medical equipment, and diagnostic tests. HTCC bases its decisions on evidence of the technology’s safety, efficacy, and cost effectiveness. Participating state agencies are required to comply with the decisions of the HTCC. HTCC decisions may be re-reviewed at the determination of the HCA Director.
Whole Exome Sequencing
Draft findings and decision
Timeline, overview and comments

The Health Technology Assessment (HTA) program received no comments in response to the posted Health Technology Clinical Committee (HTCC) draft findings and decision on whole exome sequencing.

Timeline

<table>
<thead>
<tr>
<th>Phase</th>
<th>Date</th>
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<tr>
<td>Technology recommendations published</td>
<td>March 5, 2018</td>
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<td>Public comments</td>
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<tr>
<td>Public comments</td>
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<td>Draft key questions published</td>
<td>March 19, 2019</td>
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<tr>
<td>Public comments</td>
<td>May 15 to May 28, 2019</td>
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<td>Final key questions published</td>
<td>June 17, 2019</td>
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Overview

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### Comments

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No comments received.

1.
Whole exome sequencing
HTCC final approval of coverage decision

(From page 7 of decision aid)

Next step: Proposed findings and decision and public comment

At the next public meeting the committee will review the proposed findings and decision and consider any public comments as appropriate prior to a vote for final adoption of the determination.

☐ 1) Based on public comment was evidence overlooked in the process that should be considered?

☐ 2) Does the proposed findings and decision document clearly convey the intended coverage determination based on review and consideration of the evidence?

Next step: Final determination

Following review of the proposed findings and decision document and public comments:

Final vote

☐ Does the committee approve the Findings and Decisions document with any changes noted in discussion?

   If yes, the process is concluded.

   If no, or unclear outcome (i.e., tie), chair will lead discussion to determine next steps.
Health Technology Clinical Committee
DRAFT Findings and Decision

Topic: Cell-free DNA prenatal screening for chromosomal aneuploidies
Meeting date: January 17, 2020
Final adoption: Pending

Meeting materials and transcript are available on the HTA website.

Number and coverage topic:

20200117A – Cell-free DNA prenatal screening for chromosomal aneuploidies (cfDNA)

HTCC coverage determination:
Cell-free DNA prenatal screening for chromosomal aneuploidies is a covered benefit.

HTCC reimbursement determination:

Limitations of coverage: N/A
Non-covered indicators: N/A

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HTCC coverage vote and formal action:

Committee decision

Based on the deliberations of key health outcomes the committee decided that it had the most complete information: a comprehensive and current evidence report, public comments, and state agency utilization information. The committee decided that the current evidence on cfDNA is sufficient to make a determination on this topic. The committee discussed and voted on the evidence for the use of cfDNA. The committee considered the evidence and gave greatest weight to the evidence it determined, based on objective factors, to be the most valid and reliable.

Based on these findings, the committee voted to cover cell-free DNA prenatal screening for chromosomal aneuploidies.

<table>
<thead>
<tr>
<th>Coverage Status</th>
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<td>Cell-free DNA prenatal screening for chromosomal aneuploidies</td>
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Discussion

The committee reviewed and discussed the available studies for use of cfDNA prenatal screening for chromosomal aneuploidies. Details of the screening test accuracy, outcomes and other factors including the affected volume of confirmatory testing were discussed in detail. A majority of committee members found the evidence sufficient to determine that use of cfDNA prenatal screening for chromosomal aneuploidies is safer, more effective or more cost-effective than comparators.

Limitations

N/A

Action

The committee checked for availability of a Centers for Medicare and Medicaid Services (CMS) national coverage decision (NCD). There is no Medicare NCD for cfDNA prenatal screening for chromosomal aneuploidies. The committee discussed clinical guidelines identified for cfDNA from the following organizations:

• Human Genetics Society of Australia, Royal Australian and New Zealand College of Obstetricians and Gynaecologists
• NHS Fetal Anomaly Screening Programme
• Society of Obstetricians and Gynaecologists of Canada, Canadian College of Medical Geneticists
• American College of Medical Genetics and Genomics (ACMG)
• American College of Obstetricians and Gynecologists, Society for Maternal–Fetal Medicine
• Society for Maternal–Fetal Medicine
• Austrian Society of Obstetrics and Gynecology, Austrian Society of Ultrasound in Medicine, Austrian Society of Pre- and Perinatal Medicine, Austrian Society of Human Genetics, German Society of Ultrasound in Medicine, Fetal Medicine Foundation Germany, Swiss Society of Ultrasound in Medicine
• Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis
• European Society of Human Genetics, American Society of Human Genetics
• International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)
• Israeli Society of Medical Genetics NIPT Committee
• National Society of Genetic Counselors
• Polish Gynecological Society, Polish Human Genetics Society

The committee’s determination is consistent with the identified guidelines.

The committee chair directed HTA staff to prepare a findings and decision document on use of cfDNA for public comment to be followed by consideration for final approval at the next public meeting.

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Cell-free DNA prenatal screening for chromosomal aneuploidies
Draft findings and decision
Timeline, overview and comments

The Health Technology Assessment (HTA) program received comments in response to the posted Health Technology Clinical Committee (HTCC) draft findings and decision on Cell-free DNA prenatal screening for chromosomal aneuploidies.

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## Comments

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<th>Respondents</th>
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<tr>
<td>1. Marily Rhudy, Secretary &amp; Director</td>
<td>Coalition for Access to Prenatal Screening (CAPS)</td>
<td>No</td>
</tr>
<tr>
<td>2. Kimberly Martin, Chief Clinical Advisor</td>
<td>Natera, Inc.</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Trish Brown, MS, CGC</td>
<td>Illumina, Inc.</td>
<td>Yes</td>
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</tbody>
</table>
Dear Dr. Zerzan:

On behalf of the Coalition for Access to Prenatal Screening (CAPS), I am submitting an official comment in response to Washington Agency Medical Directors’ Group (AMDG) presentation at the Health Technology Clinical Committee meeting on January 17. We were concerned by the mischaracterization of data on the efficacy and cost-effectiveness of cell-free DNA noninvasive prenatal screening (NIPS) in the AMDG’s presentation.

**Slide 12**

Evidence Summary: cfDNA v.s. Conventional Screening

- “2. CfDNA has a higher PPV (very low-quality evidence) and less unnecessary procedures (moderate quality evidence).”
  - The seven studies included on Slide 13 list the Positive Predictive Value (PPV) of NIPS.
  - As found on page 164 of the Washington Health Technology Assessment (HTA) Final Evidence Report, only one of these studies (Quezada, et al., 2015) has a high risk of bias assessment.
    - The other studies report a moderate risk of bias.
  - CAPS does not believe this justifies the label of “very low-quality evidence.”
- “3. PPV lower in low risk due to lower prevalence; PPV higher in those at high risk due to higher prevalence of the condition.”
  - CAPS believes this statement is misleading and should be removed from the AMDG materials in the record.
  - A lower PPV in a low risk cohort and a higher PPV in a high risk cohort is a phenomenon seen in both NIPS and conventional screening.
  - This is due to the natural occurrence of abnormalities and not a reflection on the effectiveness of the technology itself.
  - Multiple studies have documented PPVs for NIPS in average risk cohorts as being above 50%; whereas PPVs for standard screening in both average risk and high risk cohorts have been documented in the 5% range. That is a tenfold difference in favor of NIPS. This well-known and highly relevant difference was not highlighted in the AMDG materials.
Slide 15
Test Performance: T21, T18, T13

- “Conventional Screening: PPV 28% (95%CI, 25%-31.9%) for conventional screening (moderate quality evidence from 1 study).”
  - On Slide 14, Quezada et al. is the only study with a PPV of 28% for conventional screening.
  - The median maternal age in Quezada et al. was 36.9, putting it outside the scope of a low risk evaluation.
  - Quezada et al. is also the only study on Slide 14 with a high risk of bias, described in the Final Evidence Report as, “[s]ome significant concerns about patient representation, conflicts of interest, and overall lack of reporting.”
    - The other studies report a moderate risk of bias.
  - CAPS believes the Quezada et al. PPV should not have been included as representative of standard screening performance as it does not include the appropriate patient population, it is the only study with a high risk of bias, and it mischaracterizes the very low PPV of conventional screening.
    - The other studies on Slide 14 all report conventional screening PPVs below 14.4% for trisomies 13, 18 and 21.
    - The largest direct comparison study (>15,000 subjects, including nearly 12,000 average-risk subjects) between NIPS and standard screening (Norton et al, NEJM 2015) demonstrated PPVs for T21 of <4% for standard screening, and >75% for NIPS

Slide 16
Test Performance – Prevalence

- The low risk definition used in the table from Norton et al, 2015 does not fit within the parameters of the HTA.
  - Norton, et al: “Low risk was defined as a mid-trimester risk of trisomy 21 of less than 1 in 270 on standard screening.”
- The column entitled, “Maternal Age <35 Yr” should have been emphasized as it is the age cohort not currently covered by the Health Care Authority’s policy on NIPS.
- The comparison of the PPV of NIPS in a low risk population to the PPV of NIPS in a high risk population was not within the scope of the HTA review.
  - As described in the HTA Key Questions, the efficacy, effectiveness, and harms of NIPS were “compared to active screening approaches, including standard screening with serum biomarkers and ultrasound.”
- The following is a more appropriate comparison for Slide 16:
Slide 17
Cost-Effectiveness Studies: Varies from Less to More Costly

- Kaimal et al., 2015 used quality of life adjusted years in its assessment; therefore, CAPS does not consider it an appropriate measure of cost.
- CAPS submitted a public comment to the Draft HTA Evidence Report, recommending the exclusion of the Kaimal et al. study because it included an analysis of “copy number variants (microdeletion or duplication) or other rare chromosomal abnormalities,” and the study by Shiv et al., 2017 as the detection rate for sequential screening incorporated “all potential detectable aneuploidies.”
  - Per the HTA Key Questions, screening for other chromosomal abnormalities (outside of trisomies 21, 18, 13 or common sex chromosome aneuploidies) or genetic conditions were exclusion criteria.

While CAPS appreciates the attention of the Washington AMDG on the important topic of prenatal screening, we disagree with the suggestion that NIPS should be covered for lower risk individuals only after a positive result on standard screening. This contingency screening approach is estimated to delay diagnosis by two weeks or more and limits decision-making abilities.

We applaud the Washington Clinical Committee for voting to cover NIPS without conditions. All pregnant Washington women who choose to pursue aneuploidy screening, regardless of their risk factors, income, age or geographic location, should have access to NIPS alongside standard screening, amniocentesis, and chorionic villus sampling (CVS).

Throughout the Washington HTA process, our coalition has been grateful for the many opportunities for public comment. Thank you for your consideration of this important issue in women’s health care.
Sincerely,

Marily Rhudy, Secretary and Director
Coalition for Access to Prenatal Screening (CAPS)
info@capsprenatal.com
mrhudy@conafaygroup.com
(202) 803-4207
Invitae | Illumina | LabCorp | Myriad | Natera | Progenity | Roche
February 11, 2020

To Whom It May Concern:

I write this letter as an Ob/Gyn and Geneticist who has over 20 years of practice experience in academic/private centers. Since July 2015 I have worked with Natera, and currently am a consultant to them having retired from full-time employment. I have accepted no reimbursement for my participation in the Washington Healthcare Authority review process. I was delighted to review the DRAFT findings and decision document. Respect for autonomy in decision-making, informed consent and equal access are hallmarks of my experience in healthcare during my genetics fellowship at the University of British Columbia.

I was very grateful for the opportunity to address the committee by telephone January 17th and the interest in providing a forum for open comment. It is my professional opinion that all women who choose to have aneuploidy screening should have insurance coverage for cell-free DNA as a primary aneuploidy screening tool, regardless of insurance status, be it private or government assistance.

Having lived in Missouri since 1999, it is clear to me that women who live in rural communities are particularly disadvantaged by non-coverage for cell-free DNA. They may not have access to a certified nuchal translucency provider without travelling some distance, incurring additional cost for time off work, childcare, transportation costs, etc. I am attaching a study published in the journal Midwifery which surveyed women in the UK and estimated the “cost” with various screening approaches. Cell-free DNA was the least expensive option. One of the authors, Lynn Chitty, is an internationally recognized geneticist. In Missouri I can attest that I saw at least 2 women every week who had driven more than 1 hour in order to have access to nuchal translucency. It was distressing to me that I knew that at least 1 in 20 would need to return due to the high screen positive rate.

Dr. Cheng’s comments regarding shortfalls in education is well-taken, however this is not a new phenomenon. I have met countless numbers of women referred due to a high risk for some condition based upon all combinations of maternal serum screening and ultrasound. They presented with various levels of anxiety, but few presented with a clear understanding of the actual likelihood that they were carrying an affected infant. They frequently reported being told “my baby has Down syndrome”. While I do not know that this was the actual language used to explain high risk screening results by primary providers, it spoke to the women’s perception and response to a high risk screen.

Natera funded a study to address the practical aspects of offering cell-free DNA as a first line screen by primary obstetrical providers. It was performed independently by Glenn Palomaki’s group in Rhode Island, the publication of which is attached. The study was called DNAFirst and included an assessment of an educational program for OB providers. The conclusion was that after a 15 minute educational program for
providers the women achieved an understanding of cell-free DNA equivalent or better than previous studies evaluating their understanding of historical screening modalities. Not only has the Perinatal Quality Foundation developed an educational tool for both providers and their patients, primarily funded by grants from industry, most companies also provide genetic education at no charge. I do believe there remains a significant unmet need to provide ongoing education about genetics for obstetrical care providers, as well as the development of grade-level appropriate tools to provide basic education about heredity and reproductive risks combined with the options for screening and diagnostic tests. The inclusion of a discussion of risks, benefits and limitations is essential. I led an effort within Natera to provide branded and un-branded educational tools for both carrier and aneuploidy screening and am sure that other laboratories have similar programs. I would be proud to have the opportunity to work with collaborators to develop and implement educational tools and informed consent processes.

Regarding msAFP, this is now considered optional by ACOG, and the most recent guidelines are attached. The sensitivity of ultrasound for the detection of both anencephaly and open neural tube defects by experienced providers is higher than MSAFP. Stuart Campbell published the posterior fossa changes with open NTDs over 20 years ago and this has led to progressively fewer women having msAFP.

Finally, I would like to address what seem to be some errors or misinterpretations of data in the publicly released slide set used as a starting point for the committee.

Slide 11 – Evidence summary comparing cfDNA and conventional screening
Point 2 – cfDNA has higher PPV (very low quality evidence) and less unnecessary procedures (moderate quality evidence)
Point 3 – PPV lower in low risk…..
Slide 14 – The PPV for conventional screening of 28% based upon ‘moderate quality of evidence’ is significantly overestimated, particularly in the general obstetric population. I am attaching Norton 2015, and the FASTER trial, both NIH funded studies that clearly demonstrate general population PPVs for conventional screening under “ideal circumstances” of < 5%
Slide 15 – there are two columns circled in this slide, which are meant to illustrate that the PPVs for cfDNA are materially lower in “low risk patients”. Please note that in this study all the women were presenting for first trimester screening, therefore the predominant “pretest” risk factor was age only. Therefore to accurately compare “average risk or < 35 years old” with “high risk or ≥ 35 years old” is impossible from the data submitted because the authors chose not to present the performance in an exclusively AMA group. However, the “average risk” group is the column between the two that are circled with n=11,994. These are the women with age < 35 as a starting or pre-test risk. The group circled represent the PPV in women who had a negative first trimester screen and their PPV is still 50.0% because the sensitivity of age + first trimester screening was only 80% compared to 100% with cfDNA. So, the most accurate possible comparison is “whole population” PPV of 80.9% which includes AMA women with 76.0% for women < 35 years of age. Please note that the overall PPV for first trimester screening was only 30/884 or 3.4%.
Slides 16-19 Cost Effectiveness
With the exception the study by Kaimal, which used maternal quality of life adjusted years as the measure of cost, virtually all the cost-effectiveness publications estimate that cfDNA is cost neutral or cost savings at reimbursement rates of $400-800 compared to conventional screening. The early change in medical policy and TEC assessment by the Blues plans in 2013-2015, which I suspect involved some internal cost effectiveness analysis, suggests that the alignment of these studies may be reliable.

Conflicts of Interest
I attach two papers which the committee may find both interesting and thought-provoking. One of these papers highlights the, often hidden, conflicts of interest by service providers. The other paper by Hercher details how those of the lowest socioeconomic groups may be affected by variability in access and coverage for genetic testing in general.

I have not billed for my time in composition of this letter, or my testimony before the committee but do declare a conflict of interest. Please note, that providers who bill for the performance of invasive tests, ultrasound including nuchal translucency measurement and extended anatomic surveys do not generally declare any conflict of interest when they discuss the evidence related to coverage for various testing options. From my own clinical experience, and published data (Warsof et al, attached) invasive testing, particularly amniocentesis, began to decline rapidly in response to the availability of nuchal translucency and first trimester serum. This likely decline in revenue for maternal-fetal specialist was undoubtedly balanced by reimbursement for the nuchal translucency ultrasound. Initially virtually all nuchal translucencies were performed in specialty units. Over time, general ob providers began offering this ultrasound in their offices (reducing revenue for MFM)s however the 5% screen positive rate resulted in a steady stream of referrals for genetic counselling and ultrasound evaluation in MFM centers. The ability to screen with a sensitivity of > 98% with a single blood test in the MDs office and a < 1/200 screen positive rate undoubtedly will further reduce revenue for those depending upon historical screening methodology performance. It is essential that relevant disclosures of these potential conflicts are both acknowledged and quantified by these providers.

Thank you for taking the time to review this letter, and the attached peer-reviewed literature in support of these statements. Please do not hesitate to reach out to me personally with any questions or concerns.

Respectfully and with kind regards,

Kim

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Non-invasive prenatal testing has opened up difficult moral questions about how we treat vulnerable groups.

Few medical technologies debuted with the explosive growth of non-invasive prenatal testing (NIPT), which went from nothing to a $1.19 billion global industry in four years, according to a recent market report. With better accuracy than other prenatal screens in identifying the most common trisomies, including Down syndrome, NIPT has been embraced by women as a way to avoid both needlessly alarming false positives and significantly more invasive procedures like amniocentesis and chorionic villus sampling (CVS).

But, like all prior improvements in prenatal tests, it has also turned up the volume on objections to the implications of such tests. These are not synonymous with objections to abortion; it is perfectly possible to support an individual family’s right to make an informed decision not to have a child with a disability and, at the same time, to be concerned about the broad societal impact of many families making the same choice.

Our tests, and our angst about testing, both tend to focus on Down syndrome, not because Down syndrome is the condition that frightens us the most, but because we are well equipped to test for it — a classic case of searching for lost keys under the streetlamp. Headlines describe testing as an existential threat to people with trisomy 21, the makings of “a world without Down syndrome.” But talk about “extinction” may mask a more important point: A reduction in the absolute number of individuals with Down syndrome or any other genetic condition will not affect society or decrease our tolerance for
disability as much as a rapidly increasing division between who is and who is not at risk.

Anecdotally, genetic counselors across the country will tell you that decisions about what to do when a fetus has a chromosome abnormality vary widely — they vary by region, by ethnicity, by socioeconomic status, and by religious affiliation.

The fact is that populations vary tremendously in their access to and their use of prenatal genetic testing. The proportion of women who choose to end a pregnancy after a fetal diagnosis of Down syndrome is often quoted as 90 percent, but this is an unreliable and discredited figure that is based on a single, small, unrepresentative study done decades ago. A meta-analysis of U.S. data published in Prenatal Diagnosis in 2012 identified the mean termination rate as 67 percent, but more importantly, the authors noted that “Heterogeneity across studies suggests that a summary termination rate may not be applicable to the entire U.S. population.” In other words, it depends.

Anecdotally, genetic counselors across the country will tell you that decisions about what to do when a fetus has a chromosome abnormality vary widely — they vary by region, by ethnicity, by socioeconomic status, and by religious affiliation. These decisions reflect personal choices and local norms, but they may also reflect differences in access to prenatal care, prenatal testing, and abortion. A recent study by Caitlin Cooney, one of my graduate students, found that genetic counselors working in regions where multiple new laws
restricting abortion had come into effect were significantly more likely to report changes in practice that negatively affected patient care and that limited access to second trimester abortions from 2011 to 2013.

In January 2017, the Guttmacher Institute announced that U.S. abortion rates had reached their lowest level since the Roe v. Wade decision, a result it attributed more to increased availability of birth control than to restrictive legislation. But fundamental inequities are set to be a bigger part of the total picture, as the 2016 election has been followed by a wave of new proposed abortion restrictions, as well as by a rollback of the federal commitment to universal access to birth control. What’s more, the Guttmacher analysis does not look specifically at the availability of second-trimester abortion, which has been reduced by restrictions on specific procedures as well as by laws that limit abortion by gestational age.

Taken as a whole, these trends suggest that Down syndrome will not disappear, but may increasingly be restricted to certain communities, whether those communities are defined by socioeconomic status, ideology, culture, or region. Many people have speculated that the use of prenatal testing might bring with it a decreased tolerance for disability and difference. But there’s a threat more pressing and insidious than extinction: it is the risk that Down syndrome ceases to be something that could happen to anyone and becomes something that happens only to certain people.

**Genetic disease has always been our shared vulnerability. When one part of society can opt out of risk, will they continue to feel the same obligation to**
provide support and resources to those who remain vulnerable?

The emergence of NIPT brings home the point that Down syndrome is only one example of a genetic condition that can be identified before birth, and that many others — probably thousands — will follow. Already many NIPT companies offer tests for a range of microdeletion syndromes, which are individually more rare but collectively more common than Down syndrome. These new tests have not proved as popular as the “traditional” version of NIPT because their positive predictive value remains low: Most “positive” tests turn out to be nothing at all. Despite this, Sequenom introduced MaterniT GENOME in 2015. It’s an expanded version that examines all chromosomes for any deletion or duplication greater than seven million base pairs (a pretty big chunk of DNA that, depending on its location, is likely to harbor multiple genes). Positive predictive values cannot even be offered for this new test, because there are not enough clinical data to calculate the results; effectively, these are experiments masquerading as clinical care. They are bad tests now, but they will improve.

If current social, legal, political, and technological trends continue, the result may be the ghettoization of genetic disease: It will be confined to discrete areas delineated by geography or culture or socioeconomic status. Whatever the impact on the absolute number of cases, this represents a fundamental re-ordering of our relationship with what it means to say something is genetic. Genetic disease has always been our shared vulnerability. When one part of society can opt out of risk, will they continue to feel the same obligation to provide support and resources to those who remain vulnerable, especially if at least some of them have deliberately chosen to accept the risk?

Choice. For many of us who offer people the opportunity to reduce their risk of genetic disease, “choice” is the word on our banner. If choice means
anything, it has to include more than the right to terminate a pregnancy. We know that reproductive rights involve access to birth control, prenatal testing, and fully informed decision-making, as well as abortion. But if we are really in favor of choice, it goes well beyond supporting women to negotiate the prenatal decision tree according to their own values and best interests. It requires a commitment to individuals with genetic disease throughout their lives, and social advocacy to make sure that “rarer” does not mean “less welcome.”

As a genetics professional, the ghettoization of genetic disease frightens me because it has the potential to turn our efforts to improve the lives of individuals and families into a vehicle for social injustice. I don’t believe there is a simple answer, but I do believe that the answer begins with, first, awareness, and second, a genetics community that fights for all vulnerable individuals with as much vigor as it fights for reproductive rights.
First-Trimester or Second-Trimester Screening, or Both, for Down’s Syndrome


ABSTRACT

BACKGROUND

It is uncertain how best to screen pregnant women for the presence of fetal Down’s syndrome: to perform first-trimester screening, to perform second-trimester screening, or to use strategies incorporating measurements in both trimesters.

METHODS

Women with singleton pregnancies underwent first-trimester combined screening (measurement of nuchal translucency, pregnancy-associated plasma protein A [PAPP-A], and the free beta subunit of human chorionic gonadotropin at 10 weeks 3 days through 13 weeks 6 days of gestation) and second-trimester quadruple screening (measurement of alpha-fetoprotein, total human chorionic gonadotropin, unconjugated estriol, and inhibin A at 15 through 18 weeks of gestation). We compared the results of stepwise sequential screening (risk results provided after each test), fully integrated screening (single risk result provided), and serum integrated screening (identical to fully integrated screening, but without nuchal translucency).

RESULTS

First-trimester screening was performed in 38,167 patients; 117 had a fetus with Down’s syndrome. At a 5 percent false positive rate, the rates of detection of Down’s syndrome were as follows: with first-trimester combined screening, 87 percent, 85 percent, and 82 percent for measurements performed at 11, 12, and 13 weeks, respectively; with second-trimester quadruple screening, 81 percent; with stepwise sequential screening, 95 percent; with serum integrated screening, 88 percent; and with fully integrated screening with first-trimester measurements performed at 11 weeks, 96 percent. Paired comparisons found significant differences between the tests, except for the comparison between serum integrated screening and combined screening.

CONCLUSIONS

First-trimester combined screening at 11 weeks of gestation is better than second-trimester quadruple screening but at 13 weeks has results similar to second-trimester quadruple screening. Both stepwise sequential screening and fully integrated screening have high rates of detection of Down’s syndrome, with low false positive rates.

*The members of the FASTER Research Consortium are listed in the Appendix.
FIRST-TRIMESTER SCREENING FOR Down's syndrome that includes the use of ultrasonography to assess nuchal translucency has become widespread since its introduction by Nicolaides and colleagues in the early 1990s. The largest U.S. study of first-trimester screening to date, involving 8514 pregnancies, reported a 79 percent detection rate at a 5 percent false positive rate. Second-trimester screening remains the most common approach to assessing the risk of Down's syndrome in the United States. When inhibin A is included in second-trimester quadruple screening, the estimated detection rate for Down's syndrome is 81 percent with a 5 percent false positive rate. However, little information is available on the comparative performance of these first- and second-trimester approaches. More complex options for risk assessment have also become available, including sequential screening (performance of screening tests at different times during pregnancy, with the results provided to the patient after each test) and integrated screening (performance of screening tests at different times during pregnancy, with a single result provided to the patient only after all tests have been completed).

Accurate comparison of the performance of different screening tests conducted at different times during pregnancy remains complex because of the bias that can arise from spontaneous pregnancy losses that may occur between the first-trimester and the second-trimester screenings. We conducted the First- and Second-Trimester Evaluation of Risk (FASTER) Trial with the goal of providing direct comparative performance of these first- and second-trimester approaches. More complex options for risk assessment have also become available, including sequential screening (performance of screening tests at different times during pregnancy, with the results provided to the patient after each test) and integrated screening (performance of screening tests at different times during pregnancy, with a single result provided to the patient only after all tests have been completed).

ASSESSMENT OF RISK
Measurements of biochemical markers were converted into multiples of the median (MoM) for gestational age, adjusted for maternal weight and race or ethnicity. Nuchal-translucency MoM values were center-specific, and the mean of three measurements was used for calculation of risk. The risk of Down’s syndrome was estimated by multiplying the maternal age-specific odds of the live birth of an infant affected by Down’s syndrome by the likelihood ratio obtained from the overlapping gaussian distributions of affected and unaffected pregnancies, as previously described. These distributions were specified by using published statistical parameters. The distributions of nuchal-translucency measurements were based on all pregnancies, including those in which cystic hygromas were found. The patients were provided with two separate estimates of the risk of Down’s syndrome, with cutoff
points chosen at the start of the trial; a positive result from first-trimester screening was defined as a risk at the end of pregnancy (40 weeks) of 1 in 150, and a positive result from second-trimester screening was defined as a risk at the end of pregnancy of 1 in 300. Because second-trimester screening was considered the standard of care, the risk cutoff point was chosen so that the rate of positive screening results was similar to that of current screening practice—that is, a rate of 5 percent, given the age distribution of pregnancies in the United States. The first-trimester risk cutoff point was chosen to yield a lower rate of positive screening results (2 to 3 percent) in order to ensure that the overall rate for the study population would not be excessive. The results were provided to the patients after all screening tests were complete, and patients with positive results from either first-trimester or second-trimester screening were offered formal genetic counseling and the option of amniocentesis for genetic analysis.

SCREENING TESTS

The following screening tests for fetal Down’s syndrome were evaluated: measurement of first-trimester nuchal translucency alone; first-trimester serum screening alone (PAPP-A and ßhCG were measured); first-trimester combined screening (nuchal translucency plus PAPP-A and ßhCG); second-trimester quadruple screening (alpha-fetoprotein, total hCG, unconjugated estriol, and inhibin A); independent sequential screening (the results of combined screening were provided to the patient in the first trimester, and the results of quadruple screening in the second trimester, with both risks calculated independently); stepwise sequential screening (the results of combined screening were provided in the first trimester, and the results of quadruple screening in the second trimester; the risk in the second trimester was calculated with inclusion of the marker levels measured in the first trimester); serum integrated screening (PAPP-A was measured in the first trimester, and the results were not provided to the patient); quadruple markers were measured in the second trimester, and the risk in the second trimester was calculated with inclusion of the marker levels measured in the first trimester; and fully integrated screening (identical to serum integrated screening with the addition of first-trimester measurement of nuchal translucency). For all tests, the calculated risk took into account maternal age.

DATA COLLECTION

Research coordinators at each clinical site recorded information on patients by using a computerized tracking system to maximize the amount of data obtained. Copies of fetal and pediatric medical records were submitted for review by a single pediatric geneticist in all cases in which a possible fetal or neonatal medical problem was suspected, in all cases with a positive screening-test result but without karyotype results, and in a 10 percent random sample of all other cases in enrolled patients. Fetal chromosome status was determined by amniocentesis; by sampling neonatal cord blood in cases with a positive screening-test result in which the mother declined amniocentesis; or by tissue sampling in cases of spontaneous pregnancy loss, pregnancy termination, or stillbirth.

Completeness of ascertainment was assessed by calculating the expected number of cases of Down’s syndrome from the maternal age distribution of the enrollees and recent age-specific birth prevalence data. On the basis of these data, 112 cases of Down’s syndrome were expected in the second trimester; we identified 117 cases, suggesting that all cases were probably identified.

STATISTICAL ANALYSIS

Screening performance was based on the maternal age-specific risk of having an affected live-born child, corrected to early mid-trimester to allow for loss of fetuses with Down’s syndrome from this time until term, and applied to the U.S. standard population of births for 1999. MoM values for each pregnancy were calculated by dividing the observed marker concentration by the median value for unaffected pregnancies with the same fetal crown-rump length. The first trimester was not treated as a single time period, because MoM values of the markers in affected pregnancies change linearly with gestational age. Confidence intervals for the estimates of screening performance of the combined, quadruple, fully integrated, serum integrated, and stepwise sequential testing strategies were derived by bootstrapping with 1000 Down’s syndrome dataset replications. These confidence intervals give the range of values within which the true screening performances are likely to lie. To compare screening performances of different strategies, the difference between pairs of tests was determined for each dataset replication and the 95 percent confidence intervals of these differences were calculated.
An independent replication of the data analysis was performed by the Foundation for Blood Research, Scarborough, Maine, and the results were reported to the data-monitoring committee, which was independent of the FASTER Trial consortium. These results were consistent with those of the primary analysis.

RESULTS

A total of 42,367 patients were approached for enrollment (Fig. 1). The demographic characteristics of the 38,033 patients enrolled are summarized in Table 1. Data on pregnancy and pediatric outcomes were obtained in 36,837 cases (97 percent). One hundred two approved ultrasonographers performed all nuchal-translucency evaluations. The ultrasonographer failed to obtain an adequate nuchal-translucency image in 1727 cases (4.5 percent), and in a further 974 cases (2.6 percent) the images were rejected at central review. Adequate nuchal-translucency measurements were therefore obtained in 35,332 cases (92.9 percent). Complete first- and second-trimester screening data were available for 33,459 unaffected pregnancies and 87 pregnancies affected by Down’s syndrome. There were 117 cases of Down’s syndrome in the population of 38,167 patients (38,033 enrolled patients plus 134 patients whose fetuses had cystic hygromas). Of the 117 cases of Down’s syndrome, 25 were in the cystic-hygroma subgroup and 92 occurred among the 38,033 pregnancies described in this report.

Table 2 summarizes the performance of first- and second-trimester screening, by counting the number of detected and false positive cases above the risk cutoff levels used. Table 3 presents the median MoM values in pregnancies affected by Down’s
Screening for Down’s Syndrome

The second-trimester median MoM values for markers in affected pregnancies were 0.74 for alphafetoprotein (95 percent confidence interval, 0.67 to 0.82), 1.79 for hCG (95 percent confidence interval, 1.59 to 2.01), 0.61 for unconjugated estriol (95 percent confidence interval, 0.55 to 0.67), and 1.98 for inhibin A (95 percent confidence interval, 1.74 to 2.26). The observed median MoM value of 0.61 for unconjugated estriol was substantially lower than almost all previously published estimates.15 In a meta-analysis of 733 pregnancies affected by Down’s syndrome, the median MoM value for unconjugated estriol was 0.72 (95 percent confidence interval, 0.68 to 0.75). The effect of this unexpected finding in our study would be improved rates of detection of Down’s syndrome, at a 5 percent false positive rate, of 86 percent (instead of 81 percent) for quadruple screening and 78 percent (instead of 69 percent) for triple screening. The median MoM value of 0.61 for unconjugated estriol is likely to be an outlying low result that would tend to produce an overestimation of second-trimester screening performance, since the 95 percent confidence intervals of our observed values and the meta-analysis values do not overlap, whereas our other results are all consistent with published values. Our subsequent results are therefore based on the pooled median MoM of 0.72 for unconjugated estriol obtained from a meta-analysis.15

Table 2 shows the estimated performance of a

Table 1. Demographic Characteristics of the 38,033 Enrolled Patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>No. of Fetuses with Down’s Syndrome</th>
<th>Percent of Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at expected date of deliverya</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 Yr</td>
<td>29,834</td>
<td>28</td>
<td>78.4</td>
</tr>
<tr>
<td>≥35 Yr</td>
<td>8,199</td>
<td>64</td>
<td>21.6</td>
</tr>
<tr>
<td>Maternal race or ethnic group†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>25,459</td>
<td>65</td>
<td>66.9</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8,607</td>
<td>17</td>
<td>22.6</td>
</tr>
<tr>
<td>Black</td>
<td>2,031</td>
<td>5</td>
<td>5.3</td>
</tr>
<tr>
<td>Asian</td>
<td>1,556</td>
<td>4</td>
<td>4.1</td>
</tr>
<tr>
<td>Other</td>
<td>380</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Gestational age of fetus at first-trimester screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 wk 3 days to 10 wk 6 days</td>
<td>1,345</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>11 wk 0 days to 11 wk 6 days</td>
<td>8,583</td>
<td>19</td>
<td>22.6</td>
</tr>
<tr>
<td>12 wk 0 days to 12 wk 6 days</td>
<td>17,052</td>
<td>44</td>
<td>44.8</td>
</tr>
<tr>
<td>13 wk 0 days to 13 wk 6 days</td>
<td>11,053</td>
<td>29</td>
<td>29.1</td>
</tr>
</tbody>
</table>

a The mean (±SD) maternal age at the expected date of delivery was 30.1±5.8 years.† Race or ethnic group was self-reported.

Table 2. Directly Observed Performance Characteristics of First- and Second-Trimester Screening Tests for Down’s Syndrome.

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Risk Cutoff</th>
<th>Detection Ratea</th>
<th>False Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>percent (no. positive/total no.)</td>
<td>percent</td>
</tr>
<tr>
<td>First-trimester combined screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygroma not included</td>
<td>1:150</td>
<td>77 (71/92)</td>
<td>3.2</td>
</tr>
<tr>
<td>Hygroma included</td>
<td>1:150</td>
<td>82 (96/117)</td>
<td>3.2</td>
</tr>
<tr>
<td>First-trimester combined screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygroma not included</td>
<td>1:300</td>
<td>82 (75/92)</td>
<td>5.6</td>
</tr>
<tr>
<td>Hygroma included</td>
<td>1:300</td>
<td>86 (100/117)</td>
<td>5.6</td>
</tr>
<tr>
<td>Second-trimester quadruple screening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:300</td>
<td>85 (74/87)</td>
<td>8.5</td>
</tr>
<tr>
<td>Sequential screening in both trimesters†</td>
<td>1:150 for 1st trimester</td>
<td>94 (82/87)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1:300 for 2nd trimester</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The detection rate is subject to bias, because an unknown proportion of fetuses with hygroma might have been spontaneously aborted before the second trimester, when most cases of Down’s syndrome were ascertained.† The detection rate is based on a positive result from either the first-trimester combined screening at a risk cutoff of 1 in 150 or the second-trimester quadruple screening at a risk cutoff of 1 in 300, with both screening results being calculated independently.
variety of screening approaches, applied to the 1999 U.S. distribution of maternal ages (mean age, 27.1 years, with 13.2 percent 35 years of age or older). First-trimester serum screening and nuchal-translucency measurement perform similarly, but the combination of both is superior for detecting Down’s syndrome at 11 to 13 weeks of gestation. Serum integrated screening performs similarly to first-trimester combined screening yet does not require nuchal-translucency measurement. Fully integrated screening (including measurement of nuchal translucency) yields the highest detection rates with the lowest false positive rates as compared with other forms of screening. Quadruple screening performs better than triple screening (measurement of alpha-fetoprotein, hCG, and unconjugated estriol), with both lower false positive rates and higher detection rates. The detection rates at various false positive rates and the false positive rates at various detection rates are summarized in Table 4.

To compare the performance of different screening tests, it is not appropriate to rely on the 95 percent confidence intervals surrounding the point estimates of performance of the main screening tests, as shown in Table 4. Therefore, the performance of different screening tests was compared on the basis of many samplings from the study population. These comparisons showed that, at false positive rates of 1 percent or 5 percent, the detection rates were significantly different for the various testing strategies, except for the serum integrated and combined-screening tests, for which the detection rates were not significantly different (Table 5).

Subgroup analyses were performed of data from women 35 years of age or older and from those younger than 35 years. For women 35 or older, first-trimester combined screening had a detection rate of 95 percent at a false positive rate of 22 percent, as compared with a detection rate of 92 percent at a false positive rate of 13 percent for second-trimester quadruple screening and a detection rate of 91 percent at a false positive rate of 2.0 percent for integrated screening (with first-trimester markers measured at 11 weeks). For women under 35, first-trimester combined screening had a detection rate of 75 percent at a 5.0 percent false positive rate of 13 percent for second-trimester quadruple screening and a detection rate of 91 percent at a false positive rate of 2.0 percent for integrated screening (with first-trimester markers measured at 11 weeks). For women under 35, first-trimester combined screening had a detection rate of 75 percent at a false positive rate of 5.0 percent false positive rate of 13 percent for second-trimester quadruple screening and a detection rate of 77 percent at a 0.4 percent false positive rate for integrated screening.

Another option is stepwise sequential screen-
Table 4. Estimated Performance Characteristics of Screening Tests for Down's Syndrome with First-Trimester Markers Measured at 11, 12, and 13 Completed Weeks of Gestation and with Second-Trimester Serum Markers Measured at 15 through 17 Completed Weeks of Gestation.*

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>No. of Completed Weeks of Gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td></td>
</tr>
<tr>
<td>Nuchal translucency only</td>
<td>8.1 (3.1–11)</td>
</tr>
<tr>
<td>Serum only †</td>
<td>7.1 (3.9–10)</td>
</tr>
<tr>
<td>Combined‡</td>
<td>1.2 (0.6–2.3)</td>
</tr>
<tr>
<td>First and second trimesters</td>
<td></td>
</tr>
<tr>
<td>Serum integrated§</td>
<td>1.2 (0.6–2.7)</td>
</tr>
<tr>
<td>Fully integrated¶</td>
<td>0.2 (0.1–0.5)</td>
</tr>
<tr>
<td>Second trimester</td>
<td></td>
</tr>
<tr>
<td>Triple</td>
<td>7.0 (5.4–10)</td>
</tr>
<tr>
<td>Quadruple</td>
<td>3.1 (2.0–7.1)</td>
</tr>
</tbody>
</table>

* All calculations take into account maternal age, according to the 1999 U.S. distribution of maternal age.† Cases of septated cystic hygroma are excluded from the analysis. The figures in parentheses are 95 percent confidence intervals surrounding the point estimates of screening performance. Confidence intervals are given at 11 weeks of gestation for the major tests that measure first-trimester markers; the 95 percent confidence intervals at 12 and 13 weeks are similar to those at 11 weeks in proportion to the point estimates. Confidence intervals are given for all tests that measure only second-trimester markers.

† The serum-only test consists of measurement of pregnancy-associated plasma protein A (PAPP-A) and the free beta subunit of human chorionic gonadotropin (ßhCG).

‡ The combined test in the first trimester consists of measurement of nuchal translucency, PAPP-A, and ßhCG.

§ The serum integrated test consists of measurement of PAPP-A in the first trimester and quadruple markers (alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A) in the second trimester.

¶ The fully integrated test consists of measurement of nuchal translucency and PAPP-A in the first trimester and quadruple markers (alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A) in the second trimester. The triple test in the second trimester consists of measurement of alpha-fetoprotein, hCG, and unconjugated estriol.

¿ The quadruple test consists of measurement of alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A.
Table 5. Differences in False Positive Rates for a Given Detection Rate, and Differences in Detection Rates for a Given False Positive Rate for Specified Pairs of Screening Tests.*

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Percent Detection Rate</th>
<th>Percent False Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>percentage points of difference between false positive rates (95% CI)</td>
<td>percentage points of difference between detection rates (95% CI)</td>
</tr>
<tr>
<td>Combined — 11 vs. 12 wk†</td>
<td>-0.2 (-0.6 to 0.0)</td>
<td>-1.0 (-1.9 to -0.3)</td>
</tr>
<tr>
<td>Combined — 11 vs. 13 wk‡</td>
<td>-1.1 (-2.1 to -0.4)</td>
<td>-3.1 (-4.9 to -1.6)</td>
</tr>
<tr>
<td>Combined — 12 vs. 13 wk†</td>
<td>-0.8 (-1.5 to -0.4)</td>
<td>-2.1 (-3.1 to -1.3)</td>
</tr>
<tr>
<td>Nuchal translucency alone vs. combined‡</td>
<td>-6.9 (-10 to -2.6)</td>
<td>-16 (-23 to -9.1)</td>
</tr>
<tr>
<td>Serum only vs. combined‡‡</td>
<td>-5.9 (-8.7 to -3.2)</td>
<td>-12 (-16 to -6.9)</td>
</tr>
<tr>
<td>Combined‡ vs. quadruple‡</td>
<td>-1.9 (-6.0 to 0.6)</td>
<td>-3.5 (-12 to -0.3)</td>
</tr>
<tr>
<td>Serum integrated vs. combined‡¶</td>
<td>0.0 (-0.8 to 1.6)</td>
<td>-0.2 (-2.6 to 3.8)</td>
</tr>
<tr>
<td>Fully integrated vs. combined‡¶</td>
<td>-1.0 (-2.0 to -0.4)</td>
<td>-3.1 (-5.7 to -1.4)</td>
</tr>
<tr>
<td>Quadruple vs. triple</td>
<td>-3.9 (-7.0 to -2.3)</td>
<td>-6.3 (-12 to -3.3)</td>
</tr>
<tr>
<td>Serum integrated vs. quadruple</td>
<td>-1.9 (-4.8 to 1.3)</td>
<td>-3.7 (-8.7 to -2.3)</td>
</tr>
<tr>
<td>Fully integrated vs. quadruple</td>
<td>-3.0 (-6.8 to -1.9)</td>
<td>-6.7 (-14.2 to -4.1)</td>
</tr>
<tr>
<td>Fully integrated vs. serum integrated</td>
<td>-1.0 (-2.3 to -0.5)</td>
<td>-2.9 (-6.3 to -1.6)</td>
</tr>
</tbody>
</table>

* A 95 percent confidence interval that does not include zero suggests a significant difference between the results of the two screening tests. Significant differences were found for all pairs of tests in the table, except for the serum integrated test versus the combined test. The first-trimester markers for the combined and fully integrated tests were measured at 11 weeks of gestation, except where otherwise stated. CI denotes confidence interval.

† The combined test in the first trimester consists of measurement of nuchal translucency, pregnancy-associated plasma protein A (PAPP-A), and the free beta subunit of human chorionic gonadotropin (ßhCG).

‡ The serum-only test consists of measurement of PAPP-A and ßhCG.

§ The quadruple test consists of measurement of alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester.

¶ The serum integrated test consists of measurement of PAPP-A in the first trimester and alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester. The fully integrated test consists of measurement of nuchal translucency and PAPP-A in the first trimester and alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A in the second trimester.

**Discussion**

The FASTER Trial was designed to compare, in a single population, first-trimester screening for Down’s syndrome with second-trimester screening (the current standard of care) and with screening in both trimesters. Our results demonstrate that first-trimester screening for Down’s syndrome is highly effective, but combinations of measurements of markers from both the first and the second trimesters yield higher detection rates and lower false positive rates.

We found that using both nuchal translucency and serum markers in the first trimester is more effective in screening for Down’s syndrome than using either alone. At 11 weeks of gestation, adding PAPP-A and ßhCG determinations to measurement of nuchal translucency increases the detection rate of Down’s syndrome from 70 percent to
87 percent, at a 5 percent false positive rate (Table 4). The differences observed between combined screening and measurement of either nuchal translucency or serum markers alone are clinically significant and support the use of first-trimester combined screening for risk assessment. The only exception may be in the case of multiple gestations (which were excluded from the present study), in which serum markers are difficult to interpret and nuchal-translucency measurements may allow for fetus-specific risk calculation.

Although the effectiveness of screening by measurement of nuchal translucency or PAPP-A declined over this interval, so that screening at 11 weeks resulted in better detection rates overall. Other screening programs that use first-trimester markers, such as integrated or sequential screening, will also be subject to degradation in performance if the first-trimester component is delayed until 13 weeks. Estimates of risk based on gestational age-specific measurements will be more accurate than estimates based on measurements taken during the period from 11 through 13 weeks as a whole.

Ultrasonography for the measurement of nuchal translucency can be a difficult technique to perform consistently well, as evidenced by the 7 percent rate of failed or suboptimal imaging in our study. A recent U.S. study suggested a rate of failure to obtain an image of only 0.5 percent, but no data were provided on image quality. However, the detection rate of Down’s syndrome by measurement of nuchal translucency appeared lower than in the present study (79 percent, at a 5 percent false positive rate). This suggests that quality assurance, as performed by us, may contribute to improved screening performance.

Second-trimester quadruple screening had a higher false positive rate than first-trimester combined screening performed at 11 or 12 weeks. The estimated performance based on week-specific measurements indicated an advantage of combined screening over quadruple screening if the first-trimester measurements are obtained at 11 weeks, but not if they are obtained later.

In our study, the first-trimester results were not released until the completion of second-trimester screening so as to allow an unbiased comparison of the two approaches. Since fetuses with septated cystic hygroma are at particularly high risk for fetal aneuploidy, patients with this finding were immediately informed and offered chorionic villus sampling, and they were not included in our calculation of risks. Thus, our estimates of screening performance apply only to pregnancies without cystic hygromas.

Measurement of a combination of markers in both the first and the second trimesters provides the best screening performance. We studied the performance of two types of integrated screening (involving measurement of markers at different gestational ages, but provision of a single result after all testing is complete): the fully integrated model, which incorporates first-trimester nuchal-translucency measurements, and the serum integrated model, which does not. A single prospective nested case–control study from Europe found Down’s syndrome detection rates of 94 percent for fully integrated screening and 87 percent for serum integrated screening, at a 5 percent false positive rate. In the current study, fully integrated screening performed significantly better than either first-trimester combined screening or second-trimester quadruple screening alone. Serum integrated screening performed similarly to first-trimester combined screening and may be a useful alternative in situations in which staff appropriately trained in assessing nuchal translucency are not available. The differences between screening tests were less apparent if the false positive rate was set at 5 percent (as has been commonly adopted) rather than 1 percent, because the detection rates of all the tests are relatively high.

A major disadvantage of integrated screening is that it precludes the performance of chorionic villus sampling for early definitive diagnosis. With independent sequential screening, first-trimester combined-screening results are provided immediately, and women with positive results may choose to undergo chorionic villus sampling. Women with negative results return for quadruple screening, the results of which are interpreted without reference to the first-trimester results. Our results indicate a high false positive rate (11 percent, for a 94 percent detection rate) and reduced accuracy with such a strategy and thus suggest that it should not be used.

Stepwise sequential screening, in contrast, keeps the false positive rate low and provides early results to women with a positive test, but it combines the results of both the first-trimester and the second-trimester measurements into a final second-trimes-
ter risk assessment. With first-trimester combined screening at 11 weeks, and a false positive rate of each component set at 2.5 percent, stepwise sequential screening resulted in a high detection rate of Down’s syndrome, similar to that obtained by fully integrated screening, although with a slightly higher false positive rate. The sequential approach described here is simply one example of sequential testing. (Setting different false positive rates would result in different yields.) Further research is needed to determine the most effective method of sequential screening and to compare it with other screening programs.

In conclusion, when there is appropriate quality control for measurement of nuchal translucency, first-trimester combined screening is a powerful tool for the detection of Down’s syndrome. Stepwise sequential screening and fully integrated screening are both associated with high detection rates and acceptable false positive rates; the advantage of earlier diagnosis associated with sequential screening must be weighed against the lower false positive rate obtained with integrated screening. Consideration of the costs associated with different strategies and of patient preferences will help guide the choice between these approaches.

Supported by grants from the National Institutes of Health and the National Institute of Child Health and Human Development (RO1 HD 38652 and MO1 RR 00054).

Dr. Canick and Wald hold international and U.S. patents for unconjugated estriol as a marker in prenatal screening for Down’s syndrome for (for example, U.S. patent 5506150 and 5605843, issued on April 9, 1996, and February 25, 1997, respectively). Dr. Nyberg reports having received lecture fees from Genecare. Dr. Timor-Tritsch reports having received lecture fees from General Electric Medical Ultrasound and having received ultrasound equipment support from Philips Ultrasound. Dr. Bianchi reports holding equity ownership in, and receiving grant support from, Living Microsystems, and also reports having received lecture fees from Ross Products. Dr. Lambert-Messerlian reports having served as a consultant to Diagnostic Systems Laboratories. Dr. Wald holds patents for the integrated screening test for Down’s syndrome using first- and second-trimester markers together as a single test (integrated screening) (for example, U.S. patent 6573103, issued on June 3, 2003). Dr. Wald is also a director of Logical Medical Systems, which makes Alphapha, the interpretative software used for the Down’s syndrome risk calculation in FASTER, and he is a director of Intema, which licenses the integrated screening test. Dr. Canick reports having served as a consultant to, and having received grant support from, Diagnostic Systems Laboratories.

APPENDIX

The members of the FASTER Research Consortium were as follows: K. Welch and R. Denchy, Columbia University, New York; F. Porter, M. Belfort, B. Oshiro, L. Cannon, K. Nelson, C. Loocks, and A. Yoshimura, University of Utah, Salt Lake City, and Intermountain Health Care Perinatal Centers, Salt Lake City, Provo, and Ogden; D. Luby and S. Coe, Swedish Medical Center, Seattle; D. Schmidt and J. Esler, William Beaumont Hospital, Royal Oak, Mich.; G. Hankins, G. Saade, and J. Lee, University of Texas Medical Branch, Galveston; K. Edelmann and Y. Kharbutli, Mount Sinai Medical Center, New York; I. Merkatz and S. Carter, Montefiore Medical Center, Bronx, N.Y.; R. Biasch and L. Schultz, University of Colorado Health Science Center, Denver; M. Paidas and J. Borsuk, New York University Medical Center, New York; B. Isquith and B. Berlin, Tufts University, Boston; C. Duquette, Brown University, Providence, R.I.; R. Baughman, University of North Carolina, Chapel Hill; J. Hanson and F. de la Cruz, National Institute of Child Health and Human Development, Bethesda, Md.; T. Tripp, E. Emig, and J. Bestwick, Wolfson Institute of Preventive Medicine, London. Independent Ultrasound Quality Assurance Committee: A. Abrahams, Eastern Virginia Medical School, Norfolk; and J. Copel, Yale University School of Medicine, New Haven, Conn.

Independent Data-Monitoring Committee: J. Goldberg, California Pacific Medical Center, San Francisco; J. Haddow, Foundation for Blood Research, Scarborough, Me.; A. Hogge, Magee-Women’s Hospital, Pittsburgh; and M. Mennuti, University of Pennsylvania, Philadelphia.

REFERENCES

9. Wright D, Bradbury I, Benn P, Cuckle H, Ritchie K. Contingent screening for Down syndrome is an efficient alternative to non-
Time and travel costs incurred by women attending antenatal tests: A costing study

Talitha I. Verhoef, PhD (Research Associate)a,* Rebecca Daley, RM, BSc, MRes (Study Co-ordinator)b, Laura Vallejo-Torres, PhD (Research Associate)a, Lyn S. Chitty, PhD, MRCOG (Professor)b,c, Stephen Morris, PhD (Professor)a

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b North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
c Genetics and Genomic Medicine, Institute of Child Health, London, UK

Abstract

Objective: to estimate the costs to women, their friends and family for different antenatal tests in the Down’s syndrome (DS) screening pathway.

Design: questionnaire-based costing study.

Setting: eight maternity clinics across the UK.

Participants: pregnant women (n=574) attending an appointment for DS screening, NIPT or invasive testing between December 2013 and September 2014.

Measurements: using data collected from the questionnaires we calculated the total costs to women by multiplying the time spent at the hospital and travelling to and from it by the opportunity costs of the women and accompanying person and adding travel and childcare costs. Assumptions about the value of opportunity costs were tested in one-way sensitivity analyses. The main outcome measure was the mean cost to the women and friends/family for each test (DS screening, NIPT, and invasive testing).

Findings: mean costs to women and their family/friend were £33.96 per visit, of which £22.47 were time costs, £9.15 were travel costs and £2.34 were childcare costs. Costs were lowest for NIPT (£22), £32 for DS screening (£44 if combined with NIPT), and highest for invasive testing (£60). Sensitivity analysis revealed that variations around the value of leisure time opportunity costs had the largest influence on the results.

Key conclusions: there are considerable costs to women, their friends and family when attending different tests in the DS screening pathway.

Implications for practice: when assessing the cost-effectiveness of changes to this pathway, costs to women should be considered.

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Introduction

In the UK, all pregnant women are offered screening for Down’s syndrome (DS) and other aneuploidies. Women with a high risk of DS (≥1:150) after screening are offered diagnostic testing, which is currently invasive testing by amniocentesis or chorionic villus sampling (CVS), both of which are associated with a small risk of miscarriage. Non-invasive prenatal testing (NIPT) involves the analysis of cell free DNA in maternal plasma and allows detection of DS (and other genetic problems) in the fetus (Gil et al., 2015). NIPT is available in many parts of the world, but mostly through private sector healthcare providers (Minear et al., 2015). It is expected that if NIPT was offered to women with a high screening risk for DS, the number of invasive tests (and procedure-related miscarriages) could decrease dramatically (Warsof et al., 2015). Implementation of NIPT in the current screening programme could therefore lead to significant changes to the screening programme. Recently, the costs to the UK National Health Service (NHS) of implementing NIPT in the national screening programme were investigated (Morris et al., 2014). However, implementing NIPT may have cost implications beyond those incurred by health service providers, for example for the women taking part in the screening programme. These may include direct costs, such as travel and childcare expenses or lost pay, and indirect costs of unpaid time (Posnett and Jan, 1996). Some women might be accompanied by a friend or family member or need someone to look

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after their children. This will also have cost implications, and so an analysis fully considering costs incurred by women attending antenatal tests ought to include these costs too. Little is known about the costs to women, their friends and family for attending DS screening, NIPT or invasive testing. One study assessed women’s costs of antenatal ultrasound screening in 2002 and did not include costs to women for invasive testing or for NIPT (Henderson et al., 2002). The aim of this study was to estimate the costs to women, their friends and family of different antenatal appointments in the Down’s syndrome screening pathway.

Methods

Participants

Women attending one of eight hospitals for Down’s syndrome screening, NIPT or invasive testing during the period December 2013 to September 2014 were asked to complete a questionnaire detailing the time and money costs they incurred when attending the hospital. In these hospitals NIPT was offered as a contingent test to women with a traditional DS screening risk of $1/1000 as part of a study evaluating introduction of NIPT into the pathway (Hill et al., 2014). In two hospitals a one-stop DS screening service was in place and NIPT was usually offered on the same day as DS screening and women could therefore have a combined screening and NIPT appointment. In the other clinics, women with a screening result $1/1000 were contacted by phone and offered a further appointment for NIPT.

Questionnaire

The questionnaire (see Supplement) consisted of nine questions asking for information about the costs incurred by pregnant women, their friends and family when attending the hospital for antenatal tests. A similar questionnaire (including the same 9 questions) was used in a previous study examining the costs of antenatal ultrasound screening (Henderson et al., 2002), so a pilot was not performed for the current study. The first two questions were used to determine what the woman would have been doing if she was not attending the clinic, and, if she was working, what arrangements were made to take time off work (paid or unpaid leave, etc.). The questionnaire also asked about mode and costs of travel and the amount of time spent travelling, whether the woman was accompanied by someone during the appointment, how much time was spent at the hospital, whether it was advised to take extra time off work, and what amount of money income was lost. A question was also included about the need for childcare and associated costs.

Time costs

The opportunity costs of time lost from work (for the visit to the clinic, including travel time) was estimated using the median full-time gross weekly earnings in women in the UK (£458.80), as described in the Annual Survey of Hours and Earnings 2013 (ONS, 2013). We estimated tax, pension and national insurance contribution at 35% and assumed a 37.5-hour working week; net hourly earnings for women were therefore assumed to be £7.95. This wage rate was used for women who had unpaid absence from work or had to work additional hours in lieu of the appointment. When women attended the clinic outside work time or took annual leave, i.e., during leisure time, their time was valued at 40% of the female wage rate (£7.95). When women took paid leave from work, we assumed no opportunity costs to the woman as these costs were borne by the employer. For women not in paid employment we assumed the opportunity costs were equal to the wage rate of women in the lowest paid occupations (£4.93) (Henderson et al., 2002).

When women were accompanied during their visit, the companion could either be male or female. Therefore, we used the median adult wage rate to value their time (£8.97 (ONS, 2013)) if this person took time off work (assuming they took unpaid leave), and the median adult wage rate for the lowest paid occupations (£5.84 (ONS, 2013)) if they would not have been working otherwise.

Travel costs

When women travelled to the clinic by foot or bicycle, we assumed zero travel costs. For women who travelled to the clinic by car, we assumed a mean distance to the clinic of 16.1 km at a cost of £0.28/km (Propper et al., 2006; AA Motoring costs, 2013). Parking fees and costs of public transport/taxi were taken from the questionnaire directly.

Childcare costs

When someone was paid to look after children or other dependents, these costs were taken from the questionnaire. When someone took time off work to look after children or other dependents, we valued their opportunity costs using the median adult wage rate (£8.97).

All costs are expressed in 2013–14 UK£.

Statistical analysis

Total costs for each woman were calculated by multiplying the time spent at the hospital and travelling by the opportunity costs of the women and accompanying person and adding travel and childcare costs. The different tests in the DS screening pathway were grouped into the following categories: DS screening; NIPT; DS screening and NIPT; invasive testing; and, other. For each test we calculated the average total costs and used regression analysis to adjust for variations by centre in which the woman had her appointment.

Sensitivity analysis

We performed several sensitivity analyses. For the main analysis, we valued leisure time at 40% of the female wage rate. A value of zero to 150% of the wage rate has been used to value leisure time, based on the argument that for overtime work employers often pay a higher wage rate (Drummond et al., 2005). We therefore performed two alternative analyses; one in which the opportunity cost of leisure time was zero and another one in which these costs were 150% of the female wage rate. In another sensitivity analysis we costed the time of women not in paid employment at zero (in the main analysis we used the wage rate of women in the lowest paid occupations). Wages have a skewed distribution, the mean and median wage rates are not similar. We therefore performed an analysis based on the mean net hourly female rate (£9.26) instead of the median (£7.95).

Lastly, we calculated the costs to employers for women who took paid leave from work by valuing their time spent at the hospital and travelling using the female wage rate.

Findings

In total, 574 women completed the questionnaire, each for a single visit. The majority attended an appointment for DS
screening (364 women). Of the remaining women, 87 attended an appointment for NIPT, 46 for both DS screening and NIPT, 53 for invasive testing, and 24 for other tests (Table 1). Responses to the questionnaire are summarized in Table 2.

Time costs

If not attending the clinic, 335 women (58%) would have been in paid employment. For 164 (49%) of these women, the costs were borne by the employer, whereas 119 (36%) lost pay because they took unpaid absence or would make the time up and 52 (16%) came to the clinic outside work or took annual leave. Of the women not in paid employment, 165 (29% of all women) were looking after a child or relative, 11 (2%) were studying at school or college and 63 (11%) had leisure time. A large proportion of the women (n = 420, 73%) were accompanied by someone and almost 300 (71%) of these accompanying persons took time off work to come to the hospital with the woman. The mean time spent at the clinic was 71 minutes, but this varied by the type of test (Table 3).

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS screening</td>
<td>364</td>
<td>63%</td>
</tr>
<tr>
<td>NIPT</td>
<td>87</td>
<td>15%</td>
</tr>
<tr>
<td>DS screening &amp; NIPT</td>
<td>46</td>
<td>8%</td>
</tr>
<tr>
<td>Invasive testing</td>
<td>53</td>
<td>9%</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
<td>4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Centre</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>132</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>99</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>574</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

Responses to the questionnaire regarding employment, travel type and child-care.

<table>
<thead>
<tr>
<th>What would you have been doing today if you were not attending the clinic?</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paid Employment</td>
<td>335</td>
<td>58%</td>
<td>See below</td>
</tr>
<tr>
<td>Looking after Child/Relative</td>
<td>165</td>
<td>29%</td>
<td>Non-working opportunity costs</td>
</tr>
<tr>
<td>Studying at school/college</td>
<td>11</td>
<td>2%</td>
<td>Non-working opportunity costs</td>
</tr>
<tr>
<td>Leisure Time</td>
<td>63</td>
<td>11%</td>
<td>Lost leisure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What arrangements did you make to take time off work?</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paid Absence from work</td>
<td>164</td>
<td>29%</td>
<td>None</td>
</tr>
<tr>
<td>Unpaid absence from work</td>
<td>54</td>
<td>9%</td>
<td>Lost pay</td>
</tr>
<tr>
<td>Will make the time up</td>
<td>65</td>
<td>11%</td>
<td>Lost pay</td>
</tr>
<tr>
<td>Came to clinic outside work</td>
<td>25</td>
<td>4%</td>
<td>Lost leisure</td>
</tr>
<tr>
<td>Took Holiday</td>
<td>27</td>
<td>5%</td>
<td>Lost leisure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Did you travel here today by</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td>38</td>
<td>7%</td>
<td>None</td>
</tr>
<tr>
<td>Bicycle</td>
<td>7</td>
<td>1%</td>
<td>None</td>
</tr>
<tr>
<td>Private Car</td>
<td>333</td>
<td>58%</td>
<td>16.1 km, 28p/km</td>
</tr>
<tr>
<td>parking fees</td>
<td>236</td>
<td>41%</td>
<td>From questionnaire (mean: £3.61, range £0.00–£20.00)</td>
</tr>
<tr>
<td>Public Transport</td>
<td>187</td>
<td>33%</td>
<td>From questionnaire (mean: £3.32, range £0.00–£30.00)</td>
</tr>
<tr>
<td>Taxi</td>
<td>9</td>
<td>2%</td>
<td>From questionnaire (mean: £8.78, range £2.00–£13.0)</td>
</tr>
</tbody>
</table>

| How long did the whole journey take? | 31.48 minutes |

<table>
<thead>
<tr>
<th>Did anyone come with you to hospital, and wait for you while you received your care?</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>420</td>
<td>73%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>If yes, did they take time off work?</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>298</td>
<td>52%</td>
<td>Lost pay</td>
</tr>
<tr>
<td>No</td>
<td>117</td>
<td>20%</td>
<td>Non-working opportunity costs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>If you have children or other dependants, have you paid someone to look after them?</th>
<th>n</th>
<th>%</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>42</td>
<td>7%</td>
<td>From questionnaire (mean: £24.79, range £0.00–£75.00)</td>
</tr>
<tr>
<td>Someone has taken time off work to look after them</td>
<td>15</td>
<td>3%</td>
<td>Lost pay</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>574</td>
<td>100%</td>
</tr>
</tbody>
</table>
There are considerable costs to women and their family and friends associated with the DS screening pathway, with a mean cost of £34 per visit. Costs for NIPT were £22, for DS screening were £32 (£44 if combined with NIPT), and for invasive testing were £60. Many assumptions were made to estimate the value of the opportunity costs to women. Of these assumptions, the value of leisure time had the largest impact on the results. If leisure time was valued at 150% of the wage rate, the tests would have been more costly to women (£8–£15 extra). Other assumptions did not have such a large impact on the results.

**Discussion**

**Main findings**

The main strength of this study is that it was based on data from a large sample of women were recruited from eight different clinics across the UK. This reduces the chance of bias caused by the type of clinic or location. In view of this and the large sample size, we believe the results should be representative for most women undergoing tests in the DS screening pathway. There are several limitations. Some women may have been eligible to have their travel costs reimbursed by the Healthcare Travel Costs Scheme and women in the UK are entitled to paid leave to attend antenatal appointments (https://www.gov.uk/working-when-pregnant-your-rights). Neither of these were taken into account and thus costs may be underestimated. However, as relatively few women would be eligible for these benefits, the impact will be small. Some women or accompanying persons might have taken a day or half a day off work, instead of the duration the appointment and travel only. In this case, the costs calculated in this study would have been underestimated. This would also apply for people taking time off work to take care of the children or dependents of the women. However, the extra time taken off work could have been used in another useful way and therefore this time may not really be lost. A final limitation is that the data for this study were collected in 2013/14, and wage rates from this period were also applied; we acknowledge that the timings and wage rates may change over time.

### Table 3

**Costs to women or their family and friends of attending the clinic.**

<table>
<thead>
<tr>
<th>Test attended for</th>
<th>n (%)</th>
<th>Time at hospital (minutes)</th>
<th>Total costs ± SD (unadjusted)</th>
<th>Total costs; 95% CI (adjusted for centre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total/average</td>
<td>574</td>
<td>100%</td>
<td>70.88</td>
<td>£33.96 ± £20.52</td>
</tr>
<tr>
<td>DSS</td>
<td>364</td>
<td>63%</td>
<td>68.12</td>
<td>£3.191 ± £16.60</td>
</tr>
<tr>
<td>NIPT</td>
<td>87</td>
<td>15%</td>
<td>38.07</td>
<td>£22.77 ± £16.44</td>
</tr>
<tr>
<td>DSS&amp;NIPT</td>
<td>46</td>
<td>8%</td>
<td>115.67</td>
<td>£42.44 ± £24.12</td>
</tr>
<tr>
<td>IPD</td>
<td>53</td>
<td>9%</td>
<td>115.23</td>
<td>£58.03 ± £25.86</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
<td>4%</td>
<td>59.75</td>
<td>£36.20 ± £21.81</td>
</tr>
</tbody>
</table>

**DSS**—Down’s Syndrome Screening; **NIPT**—Non-Invasive Prenatal Testing; **IPD**—Invasive Prenatal Testing.

Mean time costs for the woman and accompanying person were £22.47 per visit. Twenty-nine women (5%) were advised to take some time off work after their visit to the clinic for mean 1.6 days. Fifty-five women (10%) said they were losing income through attending the clinic, ranging from £3 to £250.

**Travel costs**

More than half of the women (58%) came to the clinic by private car. Mean parking fees were £3.61 per attendance. Another 33% of the women came by public transport, with a mean cost of £3.32 (one way) and 2% took a taxi (mean £8.78 one way). On average, women spent 31.5 minutes travelling (each way) and mean travel costs per visit were £9.15.

**Childcare costs**

Forty-two women (7%) paid someone to look after their children or dependents, with a mean cost of £25. In 15 cases (3%) someone had taken time off work to look after them. Mean childcare costs per visit were £2.34.

**Total costs per test**

Table 3 shows the mean time spent in the hospital per test and the total costs including travel costs, time costs of the women themselves and the persons accompanying them and childcare costs. On average, women spent 71 minutes at the clinic to have their test and mean costs to the woman and her family/friends were £33.96. The shortest test was NIPT (38 minutes), DS screening took a mean time of 68 minutes, invasive testing 115 minutes and DS screening combined with NIPT 116 minutes. The costs to women and their family/friends were lowest for NIPT (£22), £32 for DS screening (£44 if combined with NIPT), and highest for invasive testing (£60). The results were adjusted by centre, though this did not affect the results appreciably.

**Sensitivity analysis**

If leisure time was valued at 0% of the female wage rate, the mean costs per visit would have been almost £5 lower than in our main analysis (£29 vs £34 (Table 4). If leisure time was valued at 150% however, mean costs for would have been around £8 higher (£42). The impact of valuing time of women not in paid employment at £0 and using the mean female wage rate was smaller. Costs to the employer were on average £5, but varied between £3 and £12 depending on the test performed (highest for DS screening combined with NIPT).

### Table 4

**Results of the sensitivity analyses.**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>DSS</th>
<th>NIPT</th>
<th>DSS&amp;NIPT</th>
<th>IPD</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main analysis</td>
<td>£33.96</td>
<td>£31.71</td>
<td>£21.75</td>
<td>£44.17</td>
<td>£59.56</td>
<td>£36.19</td>
</tr>
<tr>
<td>Leisure time valued at 0% of female wage rate</td>
<td>£29.29</td>
<td>£27.22</td>
<td>£18.03</td>
<td>£40.39</td>
<td>£51.50</td>
<td>£31.17</td>
</tr>
<tr>
<td>Leisure time valued at 150% of female wage rate</td>
<td>£42.43</td>
<td>£39.78</td>
<td>£28.47</td>
<td>£51.22</td>
<td>£74.76</td>
<td>£45.00</td>
</tr>
<tr>
<td>Time of women not in paid employment valued at £0</td>
<td>£30.67</td>
<td>£28.48</td>
<td>£19.10</td>
<td>£41.66</td>
<td>£54.31</td>
<td>£32.43</td>
</tr>
<tr>
<td>Mean female wage rate used (instead of median)</td>
<td>£34.80</td>
<td>£32.55</td>
<td>£22.46</td>
<td>£44.96</td>
<td>£60.66</td>
<td>£37.25</td>
</tr>
<tr>
<td>Costs to employers only</td>
<td>£5.31</td>
<td>£4.10</td>
<td>£4.95</td>
<td>£11.71</td>
<td>£9.76</td>
<td>£2.61</td>
</tr>
</tbody>
</table>

**DSS**—Down’s Syndrome Screening; **NIPT**—Non-Invasive Prenatal Testing; **IPD**—Invasive Prenatal Testing.
Interpretation (in light of other evidence)

To our knowledge this is the first study to calculate the costs to women of different tests in the DS screening pathway. Henderson et al. reported that the costs to women of antenatal ultrasound screening in 2002 were £12.42 (Henderson et al., 2002). When we inflate these costs to 2013/14 (approximately £20), they are similar to the costs we found for NIPT, but lower than the costs for DS screening (which may include an ultrasound scan and also phlebotomy).

A visit to the clinic for NIPT takes less time than for invasive testing and is therefore less costly to women. This means that introduction of NIPT could decrease the costs to women if fewer invasive tests are needed in the DS screening pathway. This could be relevant when assessing the cost-effectiveness of implementing NIPT in the DS screening pathway. NIPT could be a more attractive option for women compared to invasive testing, not only because the test is less invasive and lowers the risk of procedure related miscarriages, but also because the costs incurred to attend the test are lower. Further research should be undertaken to assess the cost-effectiveness of NIPT using a societal perspective.

The main implication of our study for practice is that costs to women and their families ought to be borne in mind in the DS screening pathway.

Conclusion

Our two conclusions are that, first, there are considerable costs to women, their friends and family when attending clinics for different tests in the DS screening pathway. Second, when assessing the cost and cost-effectiveness of changes to this pathway these costs should be considered.

Details of ethics approval

This was a prospective cohort study with National Research Ethics Approval (13/LO/0082) performed between 1.11.2013 and 28.2.2015.

Disclosure of interests

None of the authors have any conflicts of interest.

Acknowledgements

The participating clinics were University College London Hospitals NHS Foundation Trust (UCLH), Barking, Havering and Redbridge university hospitals NHS Trust, St George’s University Hospitals NHS Foundation Trust (SGH), Salisbury NHS Foundation Trust, University Hospital Southampton NHS Foundation Trust, NHS Tayside, Imperial College Healthcare NHS Trust, and The Whittington Hospital NHS Trust. We thank the midwives for distributing the questionnaires and the women for completing them.

This research was funded by the National Institute for Health Research (NIHR) Programme Grants for Applied Research (RP-PG-0707-10107) and the NIHR Local Comprehensive Research Network. LSC is partially funded by the Great Ormond Street Hospital Children’s Charity and the NIHR Comprehensive Biomedical Research Centre at Great Ormond Street Hospital. The research funded is independent and the views expressed in the paper are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.midw.2016.06.013.

References

ONS (Office for National Statistics), 2013. Annual survey of Hours and Earnings, Provisional Results.
ABSTRACT

BACKGROUND

Cell-free DNA (cfDNA) testing for fetal trisomy is highly effective among high-risk women. However, there have been few direct, well-powered studies comparing cfDNA testing with standard screening during the first trimester in routine prenatal populations.

METHODS

In this prospective, multicenter, blinded study conducted at 35 international centers, we assigned pregnant women presenting for aneuploidy screening at 10 to 14 weeks of gestation to undergo both standard screening (with measurement of nuchal translucency and biochemical analytes) and cfDNA testing. Participants received the results of standard screening; the results of cfDNA testing were blinded. Determination of the birth outcome was based on diagnostic genetic testing or newborn examination. The primary outcome was the area under the receiver-operating-characteristic curve (AUC) for trisomy 21 (Down’s syndrome) with cfDNA testing versus standard screening. We also evaluated cfDNA testing and standard screening to assess the risk of trisomies 18 and 13.

RESULTS

Of 18,955 women who were enrolled, results from 15,841 were available for analysis. The mean maternal age was 30.7 years, and the mean gestational age at testing was 12.5 weeks. The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (P = 0.001). Trisomy 21 was detected in 38 of 38 women (100%; 95% confidence interval [CI], 90.7 to 100) in the cfDNA-testing group, as compared with 30 of 38 women (78.9%; 95% CI, 62.7 to 90.4) in the standard-screening group (P = 0.008). False positive rates were 0.06% (95% CI, 0.03 to 0.11) in the cfDNA group and 5.4% (95% CI, 5.1 to 5.8) in the standard-screening group (P < 0.001). The positive predictive value for cfDNA testing was 80.9% (95% CI, 66.7 to 90.9), as compared with 3.4% (95% CI, 2.3 to 4.8) for standard screening (P < 0.001).

CONCLUSIONS

In this large, routine prenatal-screening population, cfDNA testing for trisomy 21 had higher sensitivity, a lower false positive rate, and higher positive predictive value than did standard screening with the measurement of nuchal translucency and biochemical analytes. (Funded by Ariosa Diagnostics and Perinatal Quality Foundation; NEXT ClinicalTrials.gov number, NCT01511458.)
SCREENING FOR FETAL ANEUPLOIDY WITH
the use of cell-free DNA (cfDNA) obtained
from maternal plasma was introduced in
2011. Such screening has been reported to have a
detection rate for trisomy 21 (Down's syndrome)
of more than 99%, with a false positive rate as low
as 0.1%.1 Thus, cfDNA testing appears to repre-
sent a substantial improvement over traditional multiple-
marker screening. In practice, the use of this test
could result in a significant reduction in diagnostic
procedures.

Although several large proof-of-principle studies
have confirmed the high sensitivity and specificity
of cfDNA testing for the detection of trisomy 21,
most of these studies have included only selected
populations of high-risk women who were sampled
before invasive testing. There are more limited
data available on the performance of cfDNA testing
in the general pregnancy population.2-4

In this blinded, prospective study, called the
Noninvasive Examination of Trisomy (NEXT)
study, we tested the hypothesis that cfDNA testing
has better performance than standard first-tri-
mester screening (with measurement of nuchal
translucency and biochemical analytes) in risk
assessment for trisomy 21 in a large, unselected
population of women presenting for aneuploidy
screening. We also evaluated the performance of
cfDNA testing and standard screening in the
assessment of risk for trisomies 18 and 13.

METHODS

STUDY CONDUCT
From March 2012 through April 2013, we enrolled
pregnant women undergoing first-trimester aneu-
ploidy screening at 35 centers in six countries. At
enrollment, maternal blood was drawn, locally
deidentified, and sent for risk assessment for tri-
somy 21 with the use of cfDNA testing (Harmony
Prenatal Test, Ariosa Diagnostics). We submitted
the results of cfDNA testing and standard screening
to an independent data-coordinating center (Veri-
stat). We then collected pregnancy outcomes for
all participants who met the eligibility criteria
and completed standard screening. The institutional
review board at each participating site approved
the study. Written informed consent was obtained
from all the participants.

STUDY OVERSIGHT
The study was a collaboration between the clinical
investigators and the sponsors (Ariosa Diagnostics
and the Perinatal Quality Foundation). The first
and last authors designed the protocol in collabora-
tion with the sponsor. Representatives of the
sponsor performed the analyses and interpreta-
tion of cfDNA data; data regarding maternal and
gestational age were required. Laboratory personnel
performed their analyses in a blinded fashion
with respect to all other clinical data, including
results of ultrasonographic and standard screening.
Research staff members at the clinical sites en-
tered clinical and laboratory data into an electronic
case-report form, which was stored in a secure
database. The data-coordinating center compiled
and analyzed the laboratory and clinical data. Ariosa
supervised data accrual, participated in the
preparation of the manuscript, and approved the
final version of the manuscript. Veristat performed
the primary analysis; secondary analyses were con-
ducted by Ariosa. The first author wrote the first
draft of the manuscript. All the authors vouch for
the accuracy of the data and fidelity of the study
to the protocol (available with the full text of this
article at NEJM.org) and approved the submission
of the manuscript for publication. There were no
confidentiality agreements among the authors,
sites, or sponsor.

STUDY POPULATION AND SAMPLE COLLECTION
Eligible patients were at least 18 years of age and
had a singleton pregnancy between 10.0 and 14.3
weeks of gestation at the time of the study blood-
sample collection. Gestational age was determined
according to the crown–rump length at the time
of the measurement of nuchal translucency.

Patients were ineligible if they were outside the
gestational-age window, had no standard screening
result, had known maternal aneuploidy or cancer,
had conceived with the use of donor oocytes, or
had a twin pregnancy or an empty gestational
sac that was identified on ultrasonography. Peri-
pheral blood was collected into two Cell-free
DNA BCT tubes (Streck) that were labeled with a
unique patient identifier. Samples were sent to
the Ariosa clinical laboratory, which is certified
according to the Clinical Laboratory Improvement
Amendments, without further processing. Results
for cfDNA testing were not available to providers
or participants.

TESTING METHODS
All patients underwent standard screening (in-
cluding the measurement of serum pregnancy-
associated plasma protein A, total or free beta
subunit (of human chorionic gonadotropin, and nuchal translucency) with the use of local laboratories. All providers of nuchal translucency were certified by the Nuchal Translucency Quality Review program, the Fetal Medicine Foundation, or other national quality-review programs. All measurements of nuchal translucency were performed and serum samples collected within the gestational age range required by the local laboratory.

For clinical risk assessment, we used local risk algorithms and cutoffs according to standard clinical practice. For study purposes, one of the authors used a standard algorithm\(^2\) to recalculate risk using serum multiples of the median (MoM) and measurements of nuchal translucency and crown–rump length. A positive result on standard screening was defined as a mid-trimester risk of at least 1 in 270 for trisomy 21 and at least 1 in 150 for trisomy 18 and trisomy 13, cutoffs that are commonly used by laboratories in the United States.

Details on Ariosa laboratory testing methods have been described previously.\(^5\) For cfDNA testing, samples were rejected if they were not collected in Cell-free DNA BCT tubes; if the tubes were broken, unfiled, or not labeled; or if the sample was grossly hemolyzed or arrived in the laboratory more than 7 days after collection. Each acceptable sample underwent plasma separation and cfDNA isolation, followed by ligation of locus-specific oligonucleotides to produce a template from selected genomic loci (Fig. S1 in the Supplementary Appendix, available at NEJM.org). We estimated the risk of aneuploidy using a previously described algorithm, including chromosome cfDNA counts, fetal fraction of cfDNA, and a priori trisomy risk based on maternal and gestational age\(^6\) (Fig. S2 in the Supplementary Appendix). A risk of 1 in 100 or higher was the laboratory-designated threshold for classifying a sample as high risk. Samples were not included in the analyses if they did not pass laboratory quality control because of a low fraction of fetal cfDNA (<4%), an inability to measure the fraction of fetal cfDNA, a high variation in cfDNA counts, or an assay failure.

**PREGNANCY AND NEWBORN OUTCOMES**

We recorded all pregnancy outcomes, including miscarriage, termination, and delivery. Results of invasive prenatal diagnostic testing and testing of products of conception (i.e., miscarriages) were collected when available. Newborn outcomes were determined by medical-record review of the physical examination at birth and any genetic testing performed. In the absence of genetic testing, a newborn with a normal physical examination was considered to be euploid. The results for women who had a miscarriage, chose to terminate the pregnancy, or had a stillbirth were included only if confirmatory genetic testing was performed; those without genetic analysis were excluded. In a blinded fashion, the first and last authors reviewed medical records of all neonates with congenital anomalies and excluded those with phenotypes suggestive of aneuploidy if no confirmatory genetic testing was performed. Results of fetal and newborn genetic testing were adjudicated by two clinical geneticists, categorized as euploid or aneuploid, and classified according to the type of abnormality.

**DATA HANDLING**

We transferred the results of cfDNA testing, standardized risk scores for standard screening, and clinical data to the independent data-coordinating center for consolidation and unblinding. The primary-analysis population included all eligible participants who had results on both cfDNA testing and standard screening and a documented normal or adjudicated newborn examination or results of prenatal or postnatal genetic testing.

**STUDY OUTCOMES**

The primary outcome was the area under the receiver-operating-characteristic (ROC) curve (AUC) for trisomy 21 screening with cfDNA testing versus standard screening in women with complete results for the two tests. Secondary outcomes included the evaluation of cfDNA testing and standard screening to assess the risk of trisomies 18 and 13. The evaluation of the performance of cfDNA testing for trisomy 13 included only patients who were enrolled after the introduction of the analysis in September 2012. We also evaluated the performance of cfDNA testing in low-risk patients, who were defined as having a maternal age of less than 35 years or a risk of trisomy 21 of less than 1 in 270 on standard screening.

**STATISTICAL ANALYSIS**

Standard screening and cfDNA testing each produces a measured value representing the risk of each aneuploidy. The ROC curve was generated by computing sensitivity and specificity at each observed cutoff for risk score. We calculated the differences between the ROC curves, taking into account the paired nature of the data. AUC values
were compared with the use of a z-test according to the method of DeLong et al. A P value of less than 0.05 was considered to indicate statistical significance. Confidence intervals were computed with the use of the Clopper–Pearson method. We used the exact binomial test for paired comparisons in sensitivity and specificity and used the generalized score statistic to analyze positive and negative predictive values. We compared the sensitivity, specificity, positive and negative predictive values, and likelihood ratios of standard screening and cfDNA testing for the detection of trisomy 21.

On the basis of results of previous studies and assumptions with respect to the performance of cfDNA testing, we determined that a sample size of 32 cases of trisomy 21 and 1500 negative controls would provide a power of 80% to determine the primary outcome at an alpha level of 0.05. To detect an increase to an AUC of 0.95 for cfDNA testing at a power of 80% and with a prevalence of 1 in 700 for trisomy 21, we estimated that 22,400 participants would be required. To account for loss to follow-up, we planned to enroll 25,000 participants. Using the maternal age of enrolled participants mid-trial, we revised the estimate of the prevalence of trisomy 21 at 1 in 500, and we reduced the required sample size to 18,700. Interim study outcomes were not unblinded or considered in the decision to stop enrollment before achieving the planned sample size.

Figure 1. Enrollment and Outcomes.
The abbreviation cfDNA denotes cell-free DNA, and NT nuchal translucency.
RESULTS

STUDY PARTICIPANTS
From March 2012 through April 2013, a total of 18,955 women at 35 centers in the United States, Canada, and Europe were enrolled. Of these women, 445 were excluded because they did not meet the eligibility criteria, were discovered to be carrying twins during ultrasonography to measure nuchal translucency, had undergone in vitro fertilization with unknown ovum-donor status, or withdrew from the study or were withdrawn by an investigator. In addition, 384 women were excluded because of a blood-collection or labeling error, 308 because of the absence of a result on standard screening, 488 because of the absence of a result on cfDNA screening, and 1489 because they were lost to follow-up. After all exclusions, the primary analysis cohort included 15,841 women (Fig. 1).

Baseline characteristics of the primary analysis cohort are outlined in Table 1. The mean maternal age was 31 years (range, 18 to 48), and the mean gestational age was 12.5 weeks (range, 10.0 to 14.3). In all, 557 women underwent invasive prenatal diagnostic testing, 52 underwent postnatal genetic testing, and 16 underwent testing on products of conception from miscarriages. For the remainder of the women, the outcome was based on examination of the newborn.

Among the 15,841 pregnancies in the primary analysis population, there were 68 chromosomal abnormalities (1 in 236 pregnancies). Of these abnormalities, 38 were trisomy 21, 10 were trisomy 18, 6 were trisomy 13, 3 were 45,X, 3 were marker chromosomes, 2 were unbalanced translocations, 2 were balanced translocations, and 1 each was deletion 7p, deletion/duplication 5p, 1q41 deletion, and isochromosome Yp. Trisomy 21 was identified in 38 of 15,841 women, for a prevalence of 1 in 417.

PRIMARY ANALYSIS
The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (P = 0.001) (Fig. 2). Of the 38 participants with trisomy 21 with a result on cfDNA testing, cfDNA identified all 38 cases, for a sensitivity of 100% (95% confidence interval [CI], 90.7 to 100). Standard screening identified 30 of 38 cases as positive, a sensitivity of 78.9% (95% CI, 62.7 to 90.4; P = 0.008). There were 9 false positives among the 15,803 women in the cfDNA-testing group without trisomy 21, for a false positive rate of 0.06% (95% CI, 0.03 to 0.11). There were 854 false positive results for trisomy 21 on standard screening, for a false positive rate of 5.4% (95% CI, 5.1 to 5.8; P < 0.001). The positive predictive value was

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>15,841</td>
</tr>
<tr>
<td>Mean maternal age (range) — yr</td>
<td>31 (18–48)</td>
</tr>
<tr>
<td>Mean gestational age at sample collection (range) — wk</td>
<td>12.5 (10.0–14.3)</td>
</tr>
<tr>
<td>Race or ethnic group — no. (%)*</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>11,235 (70.9)</td>
</tr>
<tr>
<td>Black</td>
<td>1,295 (8.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>1,659 (10.5)</td>
</tr>
<tr>
<td>Native American</td>
<td>93 (0.6)</td>
</tr>
<tr>
<td>Multiracial</td>
<td>422 (2.7)</td>
</tr>
<tr>
<td>Other</td>
<td>1,060 (6.7)</td>
</tr>
<tr>
<td>Missing data</td>
<td>77 (0.5)</td>
</tr>
<tr>
<td>Hispanic ethnic group — no. (%)*</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3,202 (20.2)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>12,639 (79.8)</td>
</tr>
<tr>
<td>Median maternal weight (range) — kg</td>
<td>65.8 (31.8–172.4)</td>
</tr>
<tr>
<td>Pregnancy through assisted reproductive technology — no. (%)</td>
<td>480 (3.0)</td>
</tr>
<tr>
<td>Current smoker — no. (%)</td>
<td>432 (2.7)</td>
</tr>
<tr>
<td>Insulin-dependent diabetes — no. (%)</td>
<td>188 (1.2)</td>
</tr>
<tr>
<td>Genetic testing — no./total no. (%)</td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>625/15,841 (3.9)</td>
</tr>
<tr>
<td>Chorionic villus sampling</td>
<td>135/625 (21.6)</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>422/625 (67.5)</td>
</tr>
<tr>
<td>Products of conception</td>
<td>16/625 (2.6)</td>
</tr>
<tr>
<td>Newborn</td>
<td>52/625 (8.3)</td>
</tr>
<tr>
<td>Pregnancy outcome — no. (%)</td>
<td></td>
</tr>
<tr>
<td>Live birth</td>
<td>15,715 (99.2)</td>
</tr>
<tr>
<td>Termination</td>
<td>62 (0.4)</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>17 (0.1)</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>24 (0.2)</td>
</tr>
<tr>
<td>Unknown†</td>
<td>23 (0.1)</td>
</tr>
</tbody>
</table>

* Race and ethnic group were self-reported.
† The birth outcome was unknown, but results of invasive prenatal testing were available.

Table 1. Demographic and Clinical Characteristics of the Patients.

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80.9% (95% CI, 66.7 to 90.9) for cfDNA testing and 3.4% (95% CI, 2.3 to 4.8) for standard screening (P<0.001) (Table 2). The median nuchal translucency for the entire cohort was 0.98 MoM, and the standard deviation of the log_{10} MoM was 0.09.

**Secondary Analyses**

**Trisomy 21**

Among the 11,994 women with low-risk pregnancies on the basis of a maternal age under 35 years, cfDNA testing identified 19 of 19 women with trisomy 21, with 6 false positive results. Among the 14,957 women for whom standard screening showed a risk of less than 1 in 270, cfDNA testing identified 8 of 8 women with trisomy 21, with 8 false positive results. The positive predictive value for cfDNA testing was 76.0% (95% CI, 54.9 to 90.6) for women under the age of 35 years and 50.0% (95% CI, 24.7 to 75.3) for those with a negative result on standard screening (Table 2).

**Trisomy 18**

There were 10 cases of trisomy 18 in the primary analysis population. Of these cases, cfDNA testing identified 9 and standard screening identified 8; cfDNA testing had 1 false positive result, for a false positive rate of 0.01% (95% CI, 0 to 0.04) and a positive predictive value of 90.0% (95% CI, 55.5 to 99.7), as compared with 49 false positive results on standard screening, for a false positive rate of 0.31% (95% CI, 0.23 to 0.41) and a positive predictive value of 14.0% (95% CI, 6.3 to 25.8) (P<0.001 for both comparisons).

**Trisomy 13**

Among the 11,185 women who underwent both cfDNA testing and standard screening for trisomy 13, there were 2 confirmed cases; of these cases, cfDNA testing identified 2 and standard screening identified 1. There was 1 false positive result on cfDNA testing and 28 false positive results on standard screening, for false positive rates of 0.02% (95% CI, 0 to 0.06) and 0.25% (95% CI, 0.17 to 0.36), respectively (P<0.001) (Table 3).

**Findings among Excluded Participants**

Of the 16,329 otherwise eligible women, 488 (3.0%) were excluded from the primary analysis because of a lack of results on cfDNA testing. In the group of 16,329 women, 192 (1.2%) had a fetal fraction of less than 4%, 83 (0.5%) had a fetal fraction that could not be measured, and 213 (1.3%) had a high assay variance or an assay failure. The median maternal weight in women with a low fetal fraction was 93.7 kg, compared with 65.8 kg in the women with a successful result on cfDNA testing (P<0.001).

In the group with no results on cfDNA testing, there were 13 aneuploidies: 3 with trisomy 21, 1 with trisomy 18, 2 with trisomy 13, 4 with triploidy, 1 with trisomy 16 mosaic, 1 with deletion 11p, and 1 with a structurally abnormal chromosome. The prevalence of aneuploidy in this group (1 in 38 [2.7%]) is higher than the prevalence of 1 in 236 (0.4%) in the overall cohort (P<0.001). Specifically, for women with a fetal fraction of less than 4%, 9 in 192 (4.7%) had aneuploidy. Among the women with the six common aneuploidies for which there was no result on cfDNA testing, each case was detected on standard screening, with risks ranging from 1 in 26 to 1 in 2.
In this large, multicenter cohort study, we found that cfDNA testing had a higher sensitivity and specificity than did standard screening for the detection of trisomy 21 in a general prenatal-screening population. The false positive rate of cfDNA testing was nearly 100 times lower than that of standard screening. Our study included pregnant women of all risk levels, and 76% were under the age of 35 years. We found that cfDNA testing was more sensitive than standard screening and yielded lower false positive rates, regardless of maternal age. Approximately 3% of cfDNA tests did not yield a result because of assay variation or a low fetal

#### Table 2. Test Performance for Trisomy 21 in the Primary Analysis Cohort, According to Maternal Age and Risk.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard Screening</th>
<th>Cell-free DNA Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Patients (N = 15,841)</td>
<td>All Patients (N = 15,841)</td>
</tr>
<tr>
<td>True positive — no.</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>True negative — no.</td>
<td>14,949</td>
<td>15,794</td>
</tr>
<tr>
<td>False positive — no.</td>
<td>854</td>
<td>9</td>
</tr>
<tr>
<td>False negative — no.</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity (95% CI) — %</td>
<td>78.9 (62.7–90.4)‡</td>
<td>100 (90.7–100)‡</td>
</tr>
<tr>
<td>Specificity (95% CI) — %</td>
<td>94.6 (94.2–94.9)‡</td>
<td>99.9 (99.9–100)‡</td>
</tr>
<tr>
<td>Positive predictive value (95% CI) — %</td>
<td>3.4 (2.3–4.8)‡</td>
<td>80.9 (66.7–90.9)‡</td>
</tr>
<tr>
<td>Negative predictive value (95% CI) — %</td>
<td>99.9 (99.9–100)¶</td>
<td>100 (99.9–100)¶</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>14.6</td>
<td>1755.9</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.22</td>
<td>0</td>
</tr>
</tbody>
</table>

*P values are for the comparison between standard screening and cell-free DNA screening in the primary analysis cohort.
† Low risk was defined as a mid-trimester risk of trisomy 21 of less than 1 in 270 on standard screening.
‡ P = 0.008
¶ P = 0.005

#### Table 3. Test Performance for Trisomy 18 and Trisomy 13.*

<table>
<thead>
<tr>
<th>Metric</th>
<th>Trisomy 18</th>
<th>Trisomy 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive — no.</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>True negative — no.</td>
<td>15,782</td>
<td>15,830</td>
</tr>
<tr>
<td>False positive — no.</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td>False negative — no.</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity (95% CI) — %</td>
<td>80.0 (44.4–97.5)</td>
<td>90.0 (55.5–99.7)</td>
</tr>
<tr>
<td>Specificity (95% CI) — %</td>
<td>99.7 (99.6–99.8)</td>
<td>100 (99.9–100)†</td>
</tr>
<tr>
<td>Positive predictive value (95% CI) — %</td>
<td>14.0 (6.2–25.8)†</td>
<td>90.0 (55.5–99.7)†</td>
</tr>
<tr>
<td>Negative predictive value (95% CI) — %</td>
<td>100 (99.9–100)</td>
<td>100 (99.9–100)</td>
</tr>
</tbody>
</table>

* Included in the trisomy 13 analysis are patients who were enrolled after September 2012.
† P<0.001 for the comparison with standard screening.
fraction. In previous studies, obesity was associated with a low fetal fraction,\textsuperscript{14,15} and we too found that such samples were obtained from participants with a higher body weight. We also observed a high frequency of aneuploidy among patients with no result on cfDNA testing. This association has been reported previously\textsuperscript{16,17} and strongly suggests that “no results” cases should be taken into account when reporting results and calculating test performance. If we had included in the “not detected” category participants with trisomy 21 who had no result on cfDNA testing, it would have lowered the detection rate of cfDNA testing. Alternatively, if we had categorized participants with no result on cfDNA testing as being high risk and requiring further investigation, it is possible that we could have determined their true status, but the percentage of women with positive results on cfDNA testing would have been higher. Further study is needed to determine the best approach in such cases, including the value of repeat testing, adjusting the initial test for maternal weight, additional screening by another approach, or the recommendation of invasive diagnostic testing to women with no results on cfDNA testing.

Although the strength of our study is the large sample size in a general prenatal screening population, a limitation is the comparison between cfDNA testing and only standard first-trimester screening, since methods such as integrated first- and second-trimester screening with nuchal translucency and biochemical analytes have higher sensitivity and specificity.\textsuperscript{13} The detection rate of standard screening for trisomy 21 was 79%, somewhat lower than the rate of 82 to 87% (at a false positive rate of 5%) that has been reported previously.\textsuperscript{13} It is possible that standard screening has lower performance in clinical practice than under the stringent experimental conditions in which previously reported data were collected. Finally, the study was powered only to compare the detection of trisomy 21 in the two study groups. Nevertheless, the lower false positive rate and higher positive predictive value support the use of cfDNA testing in risk assessment for trisomies 18 and 13.

Before cfDNA testing can be widely implemented for general prenatal aneuploidy screening, careful consideration of the screening method and costs is needed. Although the sensitivity and specificity of cfDNA testing are higher than those of standard screening, these benefits are lower when cases with no results on cfDNA testing are considered. It has been noted that the marginal cost for each additional detected case of trisomy 21 is high.\textsuperscript{18} In our study, among women with negative results on standard screening, 1868 would have needed to undergo cfDNA testing to identify one additional case of trisomy 21. However, the false positive rate of cfDNA testing is far lower than that of standard screening, which means that fewer invasive tests would have been performed to detect each case.

Clinical implementation of cfDNA testing requires consideration of expectations regarding prenatal genetic testing. For trisomy 21 and other common aneuploidies, cfDNA testing represents a highly accurate screening option, especially since it can also detect some sex chromosomal aneuploidies that are not identified on standard screening.\textsuperscript{19,20} However, maternal serum and nuchal translucency screening can identify risk for a broad array of abnormalities that are not detectable on cfDNA testing.\textsuperscript{21,22} As in other studies, cases of trisomy 21 comprised just over 50% of aneuploidies present in this population. Women who desire a comprehensive assessment may prefer diagnostic testing with karyotype or chromosomal microarray analysis. Further study is needed to address the incremental value of nuchal translucency, first-trimester ultrasound, and serum analytes for the detection of atypical aneuploidies, copy-number variants, structural anomalies, and other adverse perinatal outcomes.

In conclusion, the performance of cfDNA testing was superior to that of traditional first-trimester screening for the detection of trisomy 21 in a routine prenatal population. Although these data support the use of cfDNA testing in women regardless of age or risk status, further cost utility studies are warranted. As emphasized by professional societies,\textsuperscript{23-26} the use of cfDNA testing and other genetic tests requires an explanation of the limitations and benefits of prenatal test choices to the patient.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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REFERENCES


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The clinical utility of DNA-based screening for fetal aneuploidy by primary obstetrical care providers in the general pregnancy population

Glenn E. Palomaki, PhD1,2, Edward M. Kloza, MS, CGC1, Barbara M. O’Brien, MD3,4, Elizabeth E. Eklund, MS1 and Geralyn M. Lambert-Messerlian, PhD1,2

Objective: To assess the clinical utility of cell-free DNA (cfDNA)-based screening for aneuploidies offered through primary obstetrical care providers to a general pregnancy population.

Methods: Patient educational materials were developed and validated and providers were trained. Serum was collected for reflexive testing of cfDNA failures. Providers and patients were surveyed concerning knowledge, decision making, and satisfaction. Pregnancy outcome was determined by active or passive ascertainment.

Results: Between September 2014 and July 2015, 72 providers screened 2,691 women. The five largest participating practices increased uptake by 8 to 40%. Among 2,681 reports, 16 women (0.6%) were screen-positive for trisomy 21, 18, or 13; all saw genetic professionals. Twelve were confirmed (positive predictive value (PPV), 75%; 95% CI, 48–93%) and four were false-positives (0.15%). Of 150 failures (5.6%), 79% had a negative serum or subsequent cfDNA test; no aneuploidies were identified. Of 100 women surveyed, 99 understood that testing was optional, 96 had their questions answered, and 95 received sufficient information. Pretest information was provided by the physician/certified nurse midwife (55) or office nurse/educator (40); none was provided by genetic professionals.

Conclusion: This first clinical utility study of cfDNA screening found higher uptake rates, patient understanding of basic concepts, and easy incorporation into routine obstetrical practices. There were no reported cases of aneuploidy among cfDNA test failures.

Genet Med advance online publication 12 January 2017

Key Words: cell free DNA; clinical utility; Down syndrome; patient education; prenatal screening

INTRODUCTION

Clinical validity and clinical utility were first applied to genetic testing by the US Department of Health and Human Services’ Secretary’s Advisory Committee on Genetic Testing.¹ These concepts were further developed in projects such as the ACCE model²³ (analytic validity, clinical validity, clinical utility, and ethical, legal, and social implications; Supplementary Figure S1 online, Supplementary Table S1 online) and the Evaluation of Genomic Applications in Practice and Prevention project sponsored by the Centers for Disease Control and Prevention.⁴ Studies documenting the clinical validity of screening tests focus on quantifying the detection and false-positive rates under controlled conditions (e.g., karyotype-confirmed outcomes, case/control, or high-risk setting). Often, these studies are performed in settings that do not represent clinical testing (e.g., bulk testing of stored samples, omission of patient reporting, little or no retesting of failures). However, studies of clinical utility are designed to be performed in a clinical care setting (e.g., patients informed of testing options, clinical test results returned and used in patient decision making). In addition to verifying test performance as determined by case/control or retrospective cohort studies, clinical utility studies can also examine process-related components of implementation such as provider education and experience, patient education and knowledge, screening uptake rates, and women’s decision making. They can also explore other issues such as the economics of screening, long-term program evaluation, and availability of suitable facilities.³

Integrated screening is the most effective serum-based test for Down syndrome (90% detection rate, 3% false-positive rate), with a positive predictive value (PPV) of 6% in the general pregnancy population.⁵ In 1997, cell-free DNA (cfDNA) was found in maternal circulation,⁶ and next-generation sequencing (NGS) enabled proof-of-concept studies identifying common fetal aneuploidies in 2008.⁷⁸ In 2011, the first external clinical validation study reported 98.6% of 212 Down syndrome pregnancies were screen-positive, 0.2% of euploid pregnancies were false-positives, and 0.8% resulted in test failures (no calls) after duplicate sample testing.⁹ This test efficiency has increased uptake by 8 to 40%. Among 2,681 reports, 16 women (0.6%) were screen-positive for trisomy 21, 18, or 13; all saw genetic professionals. Twelve were confirmed (positive predictive value (PPV), 75%; 95% CI, 48–93%) and four were false-positives (0.15%). Of 150 failures (5.6%), 79% had a negative serum or subsequent cfDNA test; no aneuploidies were identified. Of 100 women surveyed, 99 understood that testing was optional, 96 had their questions answered, and 95 received sufficient information. Pretest information was provided by the physician/certified nurse midwife (55) or office nurse/educator (40); none was provided by genetic professionals.

Conclusion: This first clinical utility study of cfDNA screening found higher uptake rates, patient understanding of basic concepts, and easy incorporation into routine obstetrical practices. There were no reported cases of aneuploidy among cfDNA test failures.

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patient communications, including presentations, educational materials, individual patient reports, and surveys.

In 2012, the American College of Obstetricians and Gynecologists (ACOG) recommended offering cfDNA as secondary screening in high-risk pregnancies, with diagnostic testing offered to those with a screen-positive or failed result. ACOG and others recommended against cfDNA screening in the "lower-risk" population pending more information. At that time, the American College of Medical Genetics and Genomics did not directly address testing based on risk stratification, although their most recent recommendations suggest offering testing regardless of initial risk. To avoid the imprecision regarding testing "low-risk" or "high-risk" populations, we examined the utility of cfDNA testing with primary screening in the general pregnancy population (including the 15 to 20% of women age 35 and older). No study has yet demonstrated that a complex molecular test such as cfDNA screening can be offered successfully through primary obstetrical care offices.

Our process-oriented project aimed to document several clinical utility aspects of cfDNA screening for common aneuploidies through the implementation of a statewide program called DNAFirst. DNAFirst would be offered through primary obstetrical care providers as a routine first-line prenatal screen for the general pregnancy population. The study's funding source (Natera, San Carlos, CA) was not involved in study design, data collection or analysis, manuscript preparation, or final approval. There was no charge to patients or their insurance for the DNAFirst test (the cfDNA portion of testing was provided by Natera), ensuring that women's decisions about choice of screening test (integrated versus DNAFirst) would not be influenced by patient out-of-pocket expenses. The observed false-positive rates, PPV, and failure rates could be compared with those derived from previous clinical validity studies. Clinical utility issues addressed included comparing screening uptake rates before and after introducing DNAFirst, evaluating an innovative reflexive serum testing protocol for cfDNA failures, and exploring women's decision-making. A survey was included to document experience, knowledge, and choices made by a subset of enrolled women. Participating providers were also surveyed to assess their ability to include DNAFirst into routine practice and to identify perceived advantages and impediments.

MATERIALS AND METHODS

Overview

The institutional review board at Women & Infants Hospital (WIH) approved the project (13-0013), which is registered with ClinicalTrials.gov (NCT01966991). The DNAFirst screen begins with cfDNA testing performed by a commercial laboratory using a SNP genotyping method (Natera) with reflexive serum/ultrasound screening in the event of cfDNA test failure. New DNAFirst patient materials specifically targeted for the general pregnancy population were developed, evaluated, and validated using an approach reported previously. Providers were offered a short in-service education program at each practice site. All pretest education was delivered to the pregnant women by primary obstetrical care providers in Rhode Island; logistics and materials resembled those of well-established serum screening protocols. Phlebotomists were trained and customized requisitions (Supplementary Figure S3 online) and reports (Supplementary Figure S4 online) were tailored for our local practices (e.g., all reports included a reminder that serum screening for open neural tube defects should be considered). The DNAFirst program focused on trisomies 21, 18, and 13, as well as monosomy X, because these are identifiable by current integrated screening. Interpreting cfDNA results for common sex trisomies (e.g., 47, XXY) is not recommended by ACOG but was included as a DNAFirst "opt-in" (including reporting the fetal sex). Women with screen-positive results were referred to the WIH Prenatal Diagnosis Center for genetic counseling and diagnostic testing. A subset of women with screen-negative or failed cfDNA tests was surveyed to learn about how DNAFirst test information was obtained, level of knowledge, satisfaction, and decision-making processes. Detailed methods are available in the supplement materials (Supplementary Methods online).

Data collection and statistical methods

Active enrollment was designed to run for at least 9 months allowing time for providers to reach a "steady state" of screening. This was also considered sufficient time to accumulate a minimum of 10 autosomal trisomies. Follow-up test results (e.g., reflexive serum testing, cfDNA testing after failure on a subsequent plasma sample, diagnostic testing results, pregnancy outcomes, newborn karyotypes) were sought for women with screen-positive results or initial cfDNA test failures. The 95% confidence intervals (CI) of proportions were based on the binomial distribution (TrueEpistat, Round Rock, TX). Significance was two-tailed at the 0.05 level.

RESULTS

Enrolling providers

Primary obstetrical care practices were approached in June 2014; five of the seven largest group practices in Rhode Island (>400 new patients per year) agreed to participate. Two declined, citing anticipated complexity and/or the 2012 ACOG recommendations against offering cfDNA screening to "low-risk" women. Subsequently, smaller practices were informed and encouraged to participate. Between September 2014 and July 2015 (11 months), 2,691 women agreed to undergo screening through 72 providers. The five large practices included 78% of all providers and accounted for 82% of the women screened. DNAFirst became their primary screen within 2 to 11 weeks after introduction (i.e., when weekly DNAFirst tests exceeded those for serum screening in the previous 6 months). All five large practices eventually exceeded historical serum screening rates by 8 to 40% (average, 18%) (Figure 1). Insufficient numbers of screened women in the smaller/solo practices precluded performing a similar analysis.
Characteristics of screened women

Figure 2 shows DNAFirst screening flow for the 2,691 women and focuses on trisomies 21, 18, and 13. Testing was not initiated for samples from 19 women, including 14 from a single lost shipment. Thirteen women submitted a second sample (68%); the remaining six did not. After cfDNA testing, four samples were ineligible due to unreported exclusion criteria (three dizygotic twins and one donated egg). Of the three twin pregnancies, one was known and submission of the sample was in error, another was unrecognized at the time, and details were unavailable for the third case. Table 1 shows characteristics of the remaining 2,681 women. Median gestational age was 12 weeks, with 1.6% collected after 20 weeks. Of the 43 initial samples collected after 20 weeks, 29 (67%) were collected by 24 weeks, which was beyond our recommended limit of 20 weeks for the study but still considered acceptable clinical practice. None of the samples collected at 25 weeks or later had an abnormal ultrasound finding as an indication. Median maternal age was 31 years, with 21% age 35 years or older—a rate similar to the 17% who underwent serum screening in the previous 6 months. Self-reported race included 85% Caucasian, 6% African American, and 4% Asian American; 15% reported being of Hispanic ethnicity. Testing indication was primary screening for 88%, advanced maternal age for 10% (these were considered part of a general pregnancy population), and history of a spontaneous loss for 1%. Requisitions for only eight women (0.3%) reported abnormal ultrasound or abnormal serum screen results, supporting our contention that this cohort represents an unscreened general pregnancy population. We honored requests outside the recommended testing protocols if reliable testing was still
The cfDNA screen-positive rate for trisomies 21, 18, and 13 was 0.6% (16/2,681; 95% CI, 0.34 to 0.97%). Of these, 11 were true positive and four were false positives; all were confirmed by invasive testing and diagnostic testing (e.g., karyotyping). The sixteenth result (screen-positive for trisomy 13) was clinically consistent with trisomy 13 (bilateral polydactyly, cystic hygroma, and spontaneous loss at 15 weeks with findings confirmed on abortus) but not karyotyped; it was also classified as a true positive. All 16 were referred to the WIH Prenatal Diagnosis Center and all were seen by genetic professionals. Nine true positives were prenatally confirmed and seven were terminated (78%). Based on maternal and gestational ages, 13.1 autosomal trisomies were expected28 (9.4, 2.8, and 0.9 for trisomies 21, 18, and 13, respectively) and 12 were identified (7, 3, and 2, respectively). The PPV was 75% (12/16; 95% CI, 0.34 to 0.97%) and the false-positive rate was 0.15% (4/2,681; 95% CI, 0.04 to 0.38%). Of the eight enrolled women with a previous abnormal ultrasound or serum screen result, one was screen-positive for monosomy X and confirmed.

Screen-negative results
The screen-negative rate was 93.8% (Figure 2; 2,515/2,681); these were subject to passive ascertainment. Review of newborn and infant karyotypes at WIH identified no additional aneuploidies and none were reported from participating providers. However, we were made aware of two monozygotic twin pregnancies with screen-negative cfDNA tests (cfDNA testing using the SNP-based methodology does not identify monozygotic twins).

Failed cfDNA testing
The initial cfDNA test failure rate was 5.6% (150/2,681; 95% CI, 4.8 to 6.5%) and all were subject to active outcome ascertainment. For the 85 plasma samples subsequently submitted for cfDNA testing, 65 (76%) results were reported; all were screen-negative (Figure 2). An additional 63 women relied on reflexive
serum/ultrasound results; 54 (86%) were screen-negative and 9 (14%) were screen-positive. Eight of these nine women delivered a normal infant (four also had a subsequent screen-negative cfDNA test). The ninth woman was diagnosed with a mosaic condition after a positive cfDNA test from another sequencing laboratory; a normal female infant was delivered. Figure 2 lists outcomes for the remaining pregnancies with test failures. Our follow-up revealed that none of these 150 women chose invasive testing. To verify provider awareness that open neural tube defect screening is indicated despite normal cfDNA test results, records from 100 women consecutively screened in May 2015 (near the end of the study) were reviewed: 72% had serum alpha-fetoprotein screening for open neural tube defects, with no screen-positive results ($\geq 2.0$ MoM). It was not possible to determine whether the 28% of women who did not undergo open neural tube defect screening were not offered serum screening, declined serum screening, or underwent alternative testing such as a level II ultrasound.

**Changes in testing over time**

The numbers of providers and women screened increased over time, most rapidly in the first 6 months (Table 2). Three-quarters of samples shipped the day of collection, with a median turnaround of 10 days (sample collection to report received by provider); 95% of results were returned within 15 days. In the first 2 months, a higher failure rate was noted in 35% of samples collected at 10 weeks. The 60 failures due to low fetal fraction occurred more frequently at 10 weeks versus 11–21 weeks (risk ratio, 2.5; 95% CI, 1.3 to 4.5; $P = 0.007$). In month 3, this prompted a recommendation that the optimal earliest time for collection would be 11 weeks although 10-week samples would be accepted. Subsequently, less than 8% of samples were collected at 10 weeks (Table 2). DNA failures were also confirmed$^{39}$ to be strongly associated with maternal weight of 80 kg or higher (risk ratio, 11.4; 95% CI, 6.3 to 21; $P < 0.001$; Supplementary Figure S5 online). In months 7 and 8, 22 additional failures at 3 weeks were attributed to a laboratory reagent problem that raised the rate to 7.1%.

**Sex chromosome screening**

All pregnancies were routinely screened for monosomy X and three (0.11%) were screen-positive (3/2,681; 95% CI, 0.03 to 0.33%). Two were true positives; both ended in spontaneous losses. The third resulted in a late-first-trimester fetal loss with no diagnostic information (Supplementary Figure S6 online). Optional sex trisomy (and fetal sex) interpretations were chosen by 91.2% of the women (2,445/2,681). Two were screen-positive for a sex trisomy; both women received posttest genetic counseling and both declined prenatal diagnostic testing. Both infants were live-born; one was confirmed by postnatal karyotype. Thirteen additional sex chromosome failures occurred (0.5%). No discrepancies regarding the predicted fetal sex were reported.

**Surveys of screened women**

The test requisitions of two-thirds of women (Table 2) included permission to be contacted (an institutional review board requirement); a pool of 140 was selected. Seven phone numbers were incorrect or out of service, and contact was unsuccessful for another 20. Of the remaining 113 women, 100 (88%) completed the 15-min survey after providing verbal consent. Interviews occurred 3 to 5 months after testing, but all women were still pregnant. This time frame was chosen to ensure that participants had completed all decision making about screening and follow-up prior to being contacted.

A complete list of responses to selected questions is shown in Table 3. Women reported receiving information from their physician or certified nurse midwife (55%) or an office nurse/educator (40%) in less than 5 min (36%) or in 5 to 9 min (39%). They reported sufficient time to talk with their provider (95%), having their questions answered (96%), and feeling that the optional nature of screening was conveyed (99%). Although 85% understood that the test identified Down syndrome, 15% thought it identified all genetic problems. Most (79%) understood that a negative result did not rule out Down syndrome but 13% thought it did. Overall, 69% knew that “the test could not tell for certain if the baby has Down syndrome”; however, 28% thought it could. Women were not nervous about testing (mean, 2.4: 1

**Table 2 Changes over time in DNAFirst test characteristics and practice patterns**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1–2</th>
<th>3–4</th>
<th>5–6</th>
<th>7–8</th>
<th>9–10</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of providers</td>
<td>27</td>
<td>44</td>
<td>57</td>
<td>61</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Number of initial screens</td>
<td>265</td>
<td>368</td>
<td>626</td>
<td>649</td>
<td>777</td>
<td>2,685</td>
</tr>
<tr>
<td>Screens per provider per month</td>
<td>4.9</td>
<td>4.2</td>
<td>5.5</td>
<td>5.3</td>
<td>5.4</td>
<td>-</td>
</tr>
<tr>
<td>Pregnancy dated by ultrasound</td>
<td>92%</td>
<td>86%</td>
<td>89%</td>
<td>91%</td>
<td>91%</td>
<td>90%</td>
</tr>
<tr>
<td>Sampled at 10 weeks</td>
<td>35%</td>
<td>7%</td>
<td>8%</td>
<td>6%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td>Shipped within 1 day</td>
<td>73%</td>
<td>72%</td>
<td>72%</td>
<td>79%</td>
<td>78%</td>
<td>76%</td>
</tr>
<tr>
<td>Turnaround time in days (median)</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Turnaround time in days (95% by)</td>
<td>20</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>cfDNA screen-positive$^a$(N, %)</td>
<td>0(0%)</td>
<td>5(1.3%)</td>
<td>3(0.5%)</td>
<td>3(0.5%)</td>
<td>5(0.6%)</td>
<td>16(0.6%)</td>
</tr>
<tr>
<td>Complete cfDNA test failures (N, %)</td>
<td>24(9.1%)</td>
<td>15(4.1%)</td>
<td>19(3.0%)</td>
<td>46(7.1%)</td>
<td>36(4.6%)</td>
<td>140(5.2%)$^b$</td>
</tr>
<tr>
<td>Provided permission to contact</td>
<td>72%</td>
<td>67%</td>
<td>74%</td>
<td>72%</td>
<td>66%</td>
<td>69%</td>
</tr>
</tbody>
</table>

$^a$Month 1 is September 2016; month 10 includes a small number of samples enrolled in July (month 11). $^b$Includes only trisomies 21, 18, and 13.
Table 3  Summary of responses to selected questions from the patient survey

<table>
<thead>
<tr>
<th>Question</th>
<th>N</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>About how you heard information concerning DNA testing</strong></td>
<td>100</td>
<td>Who explained the test to you? MD/CNM = 55; office nurse/educator = 40;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>other = 2; genetic counselor/expert = 0; can’t remember = 2; self = 1</td>
</tr>
<tr>
<td>How long did this person explain the test?</td>
<td>99</td>
<td>&lt;5 min = 36; 5–9 = 39; 10–14 = 15; ≥15 = 7; can’t remember = 2</td>
</tr>
<tr>
<td>Did you have enough time to talk with your provider?</td>
<td>100</td>
<td>Yes = 95; no = 5; can’t remember = 0</td>
</tr>
<tr>
<td><strong>About your decision to have DNA testing</strong></td>
<td>100</td>
<td>Were all of your questions answered? Yes = 96; no = 4; can’t remember = 0</td>
</tr>
<tr>
<td>Did the doctor’s office make you feel that testing was optional?</td>
<td>100</td>
<td>Yes = 99; no = 0; can’t remember = 1</td>
</tr>
<tr>
<td><strong>About your understanding of the DNA testing</strong></td>
<td>100</td>
<td>This DNA test checks for... Specific problems like Down syndrome = 85;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anything that can go wrong = 0; all genetic problems with the baby = 15</td>
</tr>
<tr>
<td>If the test is negative, then what is the risk of Down syndrome?</td>
<td>100</td>
<td>No chance = 13; small chance = 79; 50/50 chance = 3; fairly high chance = 0; don’t know = 5</td>
</tr>
<tr>
<td>This test tells for certain if the baby has Down syndrome.</td>
<td>100</td>
<td>True = 28; false = 69; don’t know = 3</td>
</tr>
<tr>
<td><strong>About your level of satisfaction with the DNA testing</strong></td>
<td>100</td>
<td>How nervous were you waiting for results? (scale 1–5) Not nervous at all (1) = 31; 2 = 23; 3 = 27; 4 = 12; very nervous (5) = 7</td>
</tr>
<tr>
<td>Did someone from the office review results with you?</td>
<td>100</td>
<td>Yes = 97; no = 2; can’t remember = 1</td>
</tr>
<tr>
<td>Would you recommend DNA testing to a friend/relative?</td>
<td>100</td>
<td>Yes = 98; no = 0; don’t know = 2</td>
</tr>
<tr>
<td>If you were pregnant again, would you have DNA testing?</td>
<td>100</td>
<td>Yes = 95; no = 1; don’t know = 4</td>
</tr>
<tr>
<td>How much would you pay out of pocket for this testing?</td>
<td>100</td>
<td>$0 = 7; $10-$100 = 38; $01-$100 = 33; $101-$200 = 10; $201-$400 = 10;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$401-$600 = 1; &gt;$600 = 1</td>
</tr>
<tr>
<td><strong>Reactions to optional sex chromosome (SC) testing</strong></td>
<td>100</td>
<td>Yes = 87; no = 13</td>
</tr>
<tr>
<td>Do you remember deciding about SC testing?</td>
<td></td>
<td>Know baby’s sex = 60; as much information as possible = 52; testing at no charge = 37; concerned about sex trisomies = 10; recommended by doctor’s office = 10; other = 2</td>
</tr>
<tr>
<td>Why did you choose/not choose to have this testing?</td>
<td>78</td>
<td>Didn’t want to know the sex of the baby = 8; not important = 1</td>
</tr>
<tr>
<td>Chose SC testing</td>
<td></td>
<td>Very important = 45; not important = 34; didn’t want to know = 19;</td>
</tr>
<tr>
<td>Chose to not have SC testing</td>
<td>9</td>
<td>didn’t know testing could reveal this = 0</td>
</tr>
<tr>
<td>How important was it to know the sex of the baby?</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

= not at all, 5 = very) and 93% rated their decision as “good” or “great” (mean, 4.2; 1 = terrible, 5 = great). Nearly all (97%) remembered reviewing DNAFirst results with office personnel, 98% would recommend testing to friends, and 95% said they would undergo the test in their next pregnancy. They reported a willingness to pay $10 to $50 (38%) or $51 to $100 (33%) out of pocket. Eighty-seven women remembered making decisions regarding sex chromosome trisomy screening/fetal sex. The 78 women who chose such screening wanted to know the baby’s sex (77%), wanted as much information as possible (67%), and liked not being required to pay (47%). Of nine women who did not choose sex chromosome trisomy testing, eight did not want to know the fetal sex. Knowing fetal sex was “very important” for 46% and “not important for 34%”; 20% “did not want to know.”

**Surveying the obstetrical care providers**

Surveys were completed by 33 of 72 providers (46%) and included 21 physicians and 8 certified nurse midwives. Among physicians, 90% reported personally discussing DNAFirst with women. An average of 6 min was spent informing and answering women’s questions (range, 2.5–15 min), which was consistent with women's estimates. Providers felt their staff was adequately prepared (83%) and that 60 to 100% of women they talked to about DNAFirst accepted screening. Respondents thought women accepted screening to reveal fetal sex (90%), receive better/more accurate results (28%), receive earlier results (14%), simplify screening (10%), and undergo testing at no charge (7%). Providers were positive about the ease of offering DNAFirst, screening program support, and test performance; however, they expressed concerns about the DNA failure rate, turnaround time, and costs of testing when the project ended.

**DISCUSSION**

This is the first report documenting multiple clinical utility aspects of a cfDNA-based prenatal screening test for common aneuploidies in a general US pregnancy population, offered through nonacademic, community-based obstetrical care practices. Patient educational materials were designed and validated specifically for use by the general population. The DNAFirst test
(cfDNA coupled with reflexive serum screening) was designed to address test failures in a population at general risk and to examine patient interest in sex chromosome screening as a test option. The associated programmatic activities were coordinated through an experienced prenatal screening program whose structure was based on ACOG recommendations promulgated in 1982 that recommended a "coordinated system of care resulting in prompt, accurate diagnoses and appropriate follow-up services."^23,30

Concerns regarding the use of cfDNA in the general pregnancy population include the reliability of PPV estimates. Among our 16 trisomy 21, 18, or 13 screen-positives, the PPV was 75% (three true positives for each false positive or 3:1). These odds are 50 times higher than the 6% (1:17) achievable by integrated screening but 33 times lower than the >99% (>98:1) reported by several commercial laboratories. Individual risks or PPV of >99% are almost certainly overestimates because they do not account for rare clinical false-positive results that may even be analytically correct (e.g., confined placental mosaicism, vanished affected twin, maternal mosaicism, maternal cancer). Such high risks also tend to undermine the "screening" nature of this testing. Our PPV is consistent with the estimates reported from controlled clinical validity studies in the general pregnancy population.31,32

When screening in the general population, DNA test failures are a major concern. In the high-risk setting, women with test failures can be offered diagnostic testing due to their existing risk. It seems inappropriate to offer diagnostic testing to all women with a test failure in the general pregnancy population. For example, the risk of aneuploidy is likely to be quite low in a 21-year-old woman weighing 250 pounds whose test result is a failure due to low fetal fraction. We report a failure rate of 5.6%, which is at the lower end of the published rates for this methodology^ but is still high. For DNAFirst, a new blood draw was required for a repeat cfDNA analysis in the event of a test failure and, although it delayed final reporting, no aneuploidies were identified. Our innovative reflexive serum testing protocol worked as intended to provide an acceptable alternative to repeat testing.

Recently, both the ACOG33 and ACMG12 recommended that genetic counseling and comprehensive ultrasound and diagnostic testing be offered after an initial cfDNA test failure for both high-risk and general pregnancy populations.33 These recommendations were based on only three published studies.21,32,34 Of these, two did not perform routine repeat testing for all failures,21,32 but the other did.34 However, this latter study did not provide pregnancy outcomes among those failures. It is critical to distinguish between cfDNA test failure rates (and associated risk of aneuploidy) when only an initial test is performed versus those same rates after a duplicate or subsequent sample has been tested. Further analyses of the usefulness of repeat testing based on all relevant published studies are warranted.

Obstetrical care providers received in-person training and had program-specific, validated, and grade-appropriate patient educational materials available. Given this, our patient survey results indicated that most women understood the basic concepts of cfDNA screening. The patient and provider survey results were unique in that they focused on pregnant women from the general population choosing cfDNA testing as a clinical test after being informed by primary obstetrical care providers during routine clinical practice. None of the women received pretest education from genetic professionals because the lack of resources made this impractical. Such a practice would also deviate from established prenatal serum screening protocols. Although not perfect, levels of knowledge were at least as good as in studies of women undergoing genetic counseling for cfDNA screening25-37 and in older studies of women’s knowledge regarding serum screening.30,39

A recent study performed in Indiana30 reported a similar patient survey. In that study, 98 women with a screen-negative cfDNA test completed a questionnaire about their understanding. Nearly half (49%) said they “agreed” or “strongly agreed” with the (false) statement that “There is no longer a chance for my baby to have Down syndrome.” This contrasts with our survey, in which a similar question resulted in only 13% incorrect responses that there is “no chance” (another 3% reported “a 50/50 chance” and 5% said “didn’t know”). Our results are even more impressive given that most women in the Indiana population had high-risk pregnancies (67% were ≥35 years old, 20% had an abnormal ultrasound result) and many had formal genetic counseling.

Our study has limitations. The size of the group tested (2,681 women) allowed a confident estimate of only the false-positive rate (upper CI, 0.38%) and combined PPV (lower CI, 48% or >1:1). Also, the fact that there was no financial cost to the patient or her insurance may have resulted in higher uptake. However, our project was designed to simulate the low financial barriers to serum screening due to broad insurance coverage in Rhode Island. Such coverage may exist for cfDNA screening in the near future. We documented an average 18% higher uptake of DNAFirst than for serum screening among five large practices; a recent survey-based study found similar results.41 Unfortunately, we could not determine the reason for this. It may be related to the higher detection and lower false-positive rates, the ability to learn the fetal sex earlier in pregnancy, the availability of testing at no charge, the simplicity of offering one test over a wide gestational age range, or a combination of these or other factors. Regardless, the findings have implications for future economic analyses. cfDNA testing may have a higher uptake than current serum screening when offered to a general pregnancy population, leading to a higher proportion of cases detected in the population. We did not have access to measures of socioeconomic status, but all the enrolled group practices accepted Medicaid recipients. In our project, 13.1 common trisomies were predicted, 12 were identified, and none were found among the 150 initial test failures. There were four spontaneous losses among these 150 women and the occurrence of an unidentified trisomic loss cannot be ruled out. Our population was 85% Caucasian; this was the most common race indicated by the 15% self-reporting Hispanic ethnicity. Thus, the
transferability to racial/ethnic groups such as blacks and Asians may be more limited.

This study contributes new information about the clinical utility of cfDNA sequencing of maternal plasma to screen for aneuploidy in the general pregnancy population (as described by the ACCE model; Supplementary Figure S1 online, Supplementary Table S1 online). We successfully implemented such screening with validated pretest educational information delivered by primary obstetrical care providers. The women were adequately informed and providers were able to integrate cfDNA screening into daily routines. The false-positive rate was confirmed to be very low and the PPV was confirmed to be much higher than that with current technologies. Test failures were adequately addressed through a combination of repeat cfDNA sampling and reflexive serum screening, and screening for neural tube defects continued successfully. We found higher failure rates at 10 weeks; this may suggest that an optimal window for general population screening is between 11 and 18 weeks of gestation, with samples at 10 or 19 weeks or later still being acceptable. Given that such a program has now been shown to be feasible, laboratories must strive to offer affordable cfDNA sequencing that third-party payers could routinely cover in order to improve access to better aneuploidy screening for the more than 2 million pregnant women in the United States currently choosing prenatal screening for Down syndrome.42

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE
The cell-free (cf) DNA tests were performed at no charge by a CLIA-approved and CAP-accredited commercial laboratory (Natera, San Carlos, CA). A sponsored research contract between Natera and Women & Infants Hospital provided partial support for study personnel and their activities. By contract, the funding source was not involved in study design, data collection or analysis, manuscript preparation, or manuscript approval. The authors and Women & Infants Hospital previously received grant funding from Sequenom (San Diego, CA) between 2008 and 2011 to perform an external validation study of their cfDNA test for common aneuploidies. G.E.P. is a statistical consultant to Beckman Coulter (Chaska, MN) and Ansh Laboratories (Webster, TX). G.E.P. and G.L.M. have performed research projects involving serum markers for PerkinElmer (Lexington, MA). All consulting and research were performed through contracts with Women & Infants Hospital. The other authors declare no conflict of interest.

REFERENCES
Utility of DNA screening in primary care | PALOMAKI et al


Current Commentary

When Guild Interests and Professional Obligations Collide

Howard Minkoff, MD, and Jeffrey Ecker, MD

Physicians who work in medical organizations are called on to fulfill two roles: guild members who work to advance physician interests (eg, lobbying for tort reform) and professional society members who work to advance patient interests (eg, developing clinical guidelines). Most often, physicians’ self-interest and their interest in patient well-being align. When they do not, members of a guild or profession may justify the prioritization of self-interest with a form of motivated reasoning (a process wherein physicians weigh data differently depending on whether it supports their a priori beliefs). This allows physicians to frame self-interest as being in the best interests of their patients (eg, tort reform makes malpractice insurance affordable and allows physicians to continue to serve their patients). When interests conflict, physicians must be cognizant of the forces at play, that is, self-interest or in-group interest on the one hand and obligations to patients on the other. This entails recognition and negation of motivated reasoning. Often the most difficult calculus is evaluating proposed actions that would disadvantage physicians but advantage patients. In such cases, the health care provider must be aware not only of the temptation to oppose the action for financial reasons, but also the equally natural temptation to frame the proposal as a threat to patient well-being. Ultimately recognizing that a central tenet of professionalism is the primacy of patient welfare should help physicians both to maintain their fidelity to patient good and to uphold their reputation for altruism.

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Physicians as citizens have civic responsibilities and rights but, in addition, as health care providers have a professional obligation to promote social justice and the best interests of their patients. In fulfilling this obligation, physician-citizens may lobby or work directly with the government to shape policies that affect both their own professional practice and the health and well-being of their patients. At times, laws may be proposed or enacted that appear potentially damaging to the practice of medicine or the patient–physician relationship by, for example, mandating scripts for physicians to use with patients, proscribing public health initiatives such as conversations about or studies of gun violence, or impeding tort reform. In such instances, physicians may feel the need to seek redress and argue for change.

At those times, physicians may enjoin the professional groups to which they belong to act. Those organizations as well as their members often fill dual roles, serving as both professional societies and as guilds, promoting patients’ interests and physicians’ self-interest, respectively. Groups such as the American College of Obstetricians and Gynecologists (the College) as well as various subspecialty societies such as the Society for Maternal-Fetal Medicine (SMFM) or the American Society for Reproductive Medicine (ASRM) are examples of groups with such dual roles. The question we address here is focused at the physician level, that is, what physicians should do when their interests as patient advocates and their interests as guild members appear to conflict. Those potential conflicts may exist not only in supporting or in opposing legislation, but more insidiously, may be evidenced in the creation and substance of professional guidelines.
Guilds arose in medieval times as groups of merchants or artisans, and controlled the activities of their members in a particular locale. These organizations persist to this day, dedicated to the mutual aid or protection of their members, typically individuals from the same profession or craft. For example, the College, SMFM, and ASRM may be thought of as guilds. The fiduciary obligations of guild members are principally to one another, although in upholding standards of workmanship, they can also advance a wider good. In contrast, professionals’ fiduciary obligations are to their clients, in the case of physicians, to their patients, and the College, SMFM, and ASRM may also be thought of as professional societies. As a result of the double nature of these organizations, physicians, who serve on panels for organizations such as the College, SMFM, and ASRM may be called on to fulfill both professional (patient-focussed) and guild (self-interest) obligations and thereby may be required to wear different hats at different times. For example, if the issue at hand is lobbying to establish standards for expert witnesses in malpractice suits, guild obligations come to the fore. Alternatively, if the issue is drafting a guideline for treatment of hypertensive crises, professionalism is invoked.

In regard to the physicians’ professional obligations, according to The Physician Charter drafted by the American Board of Internal Medicine and to which many medical organizations have subscribed, one of the three fundamental principles of professionalism, in addition to autonomy and social justice, and the one most germane to the argument here, is the primacy of patient welfare. Such primacy is what underpins patients’ belief in physicians’ altruism, a belief that nurtures the esteem in which physicians are generally held and which is the foundation of the therapeutic relationship. Although the fiduciary duties of professional societies are to the interests of patients, the actions of those societies in their capacities as guilds focus more directly on member interests. It is those instances in which professional and guild interests collide (eg, when furthering the interests of patients is detrimental to the interests of physicians) that we wish to examine here.

That guilds differ from professions is not to say that guilds are intrinsically immoral. Even in the middle ages, one of the roles of a guild, as noted previously, was to make sure that anything made by a guild member was up to standard and sold for a fair price. Furthermore, as we are reminded each time we prepare for takeoff, you have to put on your own oxygen mask before you can help others, or in the words of the hospital Chief Executive Officer’s devotional, “no margin, no mission.” For physicians to help patients, they have to have a viable practice. In addition, there are many times (maybe even most times) when the good of the guild and the good of the profession overlap. Indeed, legislation that enhances access to health services advances the goals of both patients and health care provider. However, at times the interests of guilds and professions do not align as seamlessly. Even in the example just cited (enhanced access), some guilds might not want enhanced access if it requires its members to accept what it considers to be below market, government or commercial insurance, payments for care.

When guild and professional goals differ, it can create difficulties, and not just because in cases when the guild and professional roles are folded into a single organization, members and leadership are required to consider a single issue from differing perspectives. Separate from the challenge of understanding one issue from multiple viewpoints, the guild aspects of a group’s mission often will only be advanced if it garners the good will of nonmembers, and the lay public is more likely to support the good of a profession that they believe serves them than the good of an organization that serves itself. For professionals, their sense of calling, a calling often formalized with the taking of an oath, may lead to an internalized disquiet when they feel that advancing their guild’s interests (eg, supporting legislation that limits the independence of care extenders like nurse practitioners or dental hygienists in underserved areas where physicians or dentists are in dangerously short supply) clashes with the interests of the patients they have sworn to serve. The easiest way for individuals who are members of a guild and profession to justify the prioritization of self-interested goals is with a form of motivated reasoning that allows them to frame almost all selfish goals as being in the best interests of their clients. For example, an obstetrician may argue that tort reform makes malpractice insurance affordable and thus allows him or her to continue to practice and serve his or her patients.

Motivated reasoning is a process through which an individual uses a scale to judge information that comports with already held beliefs that differs from the scale he or she uses to judge information in conflict with those beliefs. The standard for the former is “can I believe this evidence.” Using that standard, data that support a particular issue, belief, or premise are more readily accepted than data that refute what one wants to be so. For the latter, the standard is “must I believe this evidence?” Using that standard,
data often are viewed with more skepticism. The evidence for the existence of these internal biases is extensive. In one publication, for example, when participants were asked to read a fictitious study that purported to show a link between caffeine intake and breast cancer, women who were heavy coffee drinkers found more flaws than did men or women with lower levels of routine caffeine intake.4 In another study, participants licked a strip of paper to determine whether they had a serious enzyme deficiency.5 Some participants were told that a color change indicated good health, whereas others were told that color change indicated bad health. The paper in fact had no diagnostic value and once licked did not change in color. The investigators found that people in the study waited longer to see the paper change color when color change suggested good health than when they believed that color change suggested the opposite. A study that is perhaps even more to the point investigated how individuals’ weighting of policies varied depending on who they thought was promoting the policy; attitudes toward a social policy (generous or strict welfare benefits in his study) depended almost exclusively on the stated position of one’s political party.6 This effect overwhelmed the influence of both the policy’s objective content and participants’ own ideologic beliefs. For example, although liberals favored a generous policy toward welfare, they opposed it if they were told that conservatives were proposing it. Despite the apparent disconnect, participants denied having been influenced by their political group, although they believed that other individuals, especially their ideologic adversaries, would be so influenced. Thus, in the example of a guild interest cited previously (limiting care extenders’ independence), physicians may emphasize that they are protecting the public from undertrained individuals and downplay any thought that they might be influenced by concern about a competitor’s entry into the marketplace.

In light of these findings, it is worth considering the recent U.S. Preventive Services Task Force7 recommendation on the appropriateness of performing screening pelvic examinations in asymptomatic women and the fact that their recommendations differ from those of the American College of Physicians8 and the College.9 The U.S. Preventive Services Task Force found the evidence insufficient to assess the balance of benefits and harms of performing screening pelvic examinations in asymptomatic women, and the American College of Physicians found, based on a systematic review, that the evidence was sufficient to recommend that routine pelvic examinations not be performed. However, the College, while acknowledging the lack of evidence, recommended annual examinations, basing their determination on expert opinion. To the extent that annual examinations generate revenue, or otherwise comprise core elements of an obstetrician–gynecologist’s professional identity, one might appropriately wonder whether the experts, consciously or not, were protecting their guild’s interests in the face of evidence that supported limiting these visits.

How then should physicians act when their interests as guild members and their interests as professionals collide? The first step is to be cognizant of the forces at play, that is, self- or in-group interest on the one hand and obligations to patients on the other. This entails recognition and negation of motivated reasoning. Although attempting to do so is a meritorious goal, motivated reasoning is a manifestation of implicit bias, and modifying implicit bias is a difficult task.10 However, research suggests that the first step toward achieving that goal is for individuals to be aware of their biases and, second, to be concerned about the consequences of those biases.11,12 Through this process, physicians can disentangle their guild interests from their professional interests and separately interrogate them to see where they coincide and where they conflict. In the former instance, physicians can be full-throated advocates. In the latter instance, a deeper inquiry is warranted.

When considering the consequences of proposed legislation or other proposed actions for the physician and the patient, the analysis must also evaluate the weight (importance and value) of those consequences. If, as an extreme example, a new law would have minimal, albeit negative, effect on patients (eg, a 0.5% surcharge on office visits with a maximal allowable extra cost of $2) but a substantive benefit to the practice of medicine (eg, the money would be used to enable physicians to provide vaccinations in their offices), it would be reasonable to support the legislation. On the other hand, if the cost to the patient would be onerous, supporting the measure would be unreasonable even if it would provide physicians with financial gain. Often the most difficult calculus is evaluating proposed legislation or other actions and measures that would disadvantage physicians but advantage patients. In such cases, the health care provider must not mask these realities and be fully cognizant of the range of possible motivations as they consider their options. To do so, physicians must be aware not only of the temptation to oppose the law for financial reasons, but also the equally natural temptation (ie, motivated reasoning) to frame the
proposal as a threat to patient well-being. For example, if data emerged suggesting that annual health visits are not beneficial for low-risk patients, physicians should be skeptical about purely hypothetical assertions (ie, those unsupported by data) that people who skip an annual visit are prone to never return and thereby put themselves at risk for adverse health outcomes in the future.

Guilds and those examining an issue from a guild perspective can almost always raise these sorts of speculative arguments when proposed legislation would impose a financial harm to health care providers. In fact, no matter how well documented the health benefit at a population level of a given initiative, it is almost always possible to imagine how some individual patients might be harmed and to use those individual cases as an excuse to block the proposed measure, particularly if their real concern may be more mercantile than philanthropic. Thus, if data show that allowing a medication to be sold over-the-counter would prevent thousands of cases of a given problem (eg, morning after pills) but may harm a rare patient who is unaware of a contraindication, bringing that issue to light is legitimate. However, that fact should not delude physicians into mistaking their guild’s position (promoting physicians’ goals by maintaining their role as gatekeepers to medication access) for professional advocacy for patient well-being. Indeed, in reference to the example offered, many professionals and professional societies, including the College, have supported over-the-counter access to appropriate medications.

In sum, physicians and the organizations that speak for them have both guild interests and professional interests. In most circumstances, those interests align and unmitting advocacy is justified. However, physicians should always be aware of the basis for their advocacy, particularly when guild (ie, personal) and professional (ie, the interests of patients or others) interests diverge. When that happens, physicians should be guided by a purposeful consideration of patient interests, studiously unbiased reflection on the basis for their decisions, and a deep appreciation of the esteem in which they are held for their selfless acts. Ultimately, although physicians should be grateful that their guilds help them do well, they should be humbled that their professions allow them to do good.

REFERENCES


Overview of the impact of noninvasive prenatal testing on diagnostic procedures†

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†Results described in this paper have been presented at the ISPD conference in 2014.

ABSTRACT
Noninvasive prenatal testing (NIPT) has had a profound influence in the field of prenatal diagnosis since the 1997 discovery of cell-free fetal DNA in maternal blood. Research has progressed rapidly, with clinical data supporting laboratory studies showing that NIPT is highly sensitive and specific for fetal aneuploidy, resulting in marked uptake in the high-risk patient population. The superior accuracy of NIPT compared with conventional screening methods has led to significant decreases in the number of invasive diagnostic procedures, in addition to a concomitant decrease in the number of procedure-related fetal losses. Yet, NIPT has been described as a ‘disruptive innovation’ due to the considerable changes the technology has commanded on current prenatal screening and diagnostic practices. This review summarizes both institutional and global experience with NIPT uptake, its effect on reducing diagnostic invasive procedures, and the unique challenges that reduced procedural volume may have on physician and trainee proficiency, cytogenetic laboratories, and neonatal outcome. © 2015 John Wiley & Sons, Ltd.

INTRODUCTION
By the mid 1990s, the paradigm for prenatal diagnosis for aneuploidy in the United States relied on maternal age and second trimester multiple serum marker for assessment of risk. In this paradigm, the sensitivity for trisomy 21 was approximately 65% using second trimester serum markers and ultrasound estimation of the gestational age with a 5% false positive rate. However, when maternal age >35 years was also included as a screening criteria, the sensitivity increased to 80%, but the selection rate to achieve this sensitivity rose to 15–18%. The result of this was the performance of many invasive diagnostic procedures but with few positive results. After ACOG Practice Bulletin No. 77 in January 2007, the American College of Obstetricians and Gynecologists endorsed first trimester screening for aneuploidies by nuchal translucency and serum markers as an alternative approach, and combinations of the first and second trimesters screening yielded higher sensitivity approaching 90–95% with a similar 5% false positive rate. This started a dramatic trend away from diagnostic procedures and more reliance on improved screening. Following the identification of cell-free fetal DNA in maternal plasma by Lo et al. in 1997, the potential to use next generation sequencing for the identification of pregnancies at risk for aneuploidy became possible. This screening test is referred to as noninvasive prenatal testing (NIPT) and has had a significant impact on the prenatal testing paradigm leading to a marked decrease in the utilization of invasive diagnostic procedures such as amniocentesis and chorionic villus sampling (CVS).

NIPT UPTAKE
As with any new technology introduced in clinical practice, there was an initial uncertainty regarding patient and physician acceptance of NIPT. Prior to its introduction, concerns primarily centered around the relatively high cost of NIPT compared with more traditional screening techniques, lack of patient education regarding NIPT, and the increased use of health care resource needs such as genetic counseling services. A 2011 study by Sayres et al. on physician attitudes regarding NIPT reported that only 29% of respondents believed that they would offer NIPT in their clinic ‘within the next 5 years’, citing a lack of awareness and conviction as key factors affecting their decision.

Since its introduction, however, physician apprehension with NIPT has largely dissipated, which is evident by the extraordinary uptake of NIPT in the at-risk patient population. Already, more than half a million NIPTs have been performed worldwide in more than 61 countries. A study published just 2 years after the Sayres report detailed that more than 90% of maternal–fetal medicine specialists had adopted NIPT in their clinical practice, showing the remarkable interest patients and physicians alike share...
regarding this evolving noninvasive technology. Patients were equally as interested in NIPT for screening of trisomy 13 and 18 as they were for trisomy 21. As NIPT only involves a maternal blood sample, patients report that the greatest benefit of NIPT is the decreased risk of miscarriage compared with invasive procedures. Other benefits of NIPT include use in early pregnancy and the opportunity for enhanced decision-making. In contrast, physicians report test accuracy as the most beneficial feature of NIPT, emphasizing the need for effective pre-test and post-test counseling in a non-directive manner in order to allow patients to make informed decisions. The tremendous interest in NIPT prompted the International Society for Prenatal Diagnosis to release a position statement recognizing NIPT as the ‘most effective method for screening for fetal trisomy 21 and trisomy 18’ but acknowledged that the test is not a replacement for diagnostic testing using CVS or amniocentesis. In addition, the American College of Obstetricians and Gynecologists in conjunction with the Society of Maternal–Fetal Medicine published Committee Opinion No. 545 recognizing NIPT as a screening option for women with singleton pregnancies at increased risk for fetal aneuploidy. This formally recognized NIPT as a screening option in high-risk patients and provided the national guidelines that obstetricians had been requesting since the technology’s rapid introduction in clinical practice. Indeed, 70% of obstetric healthcare providers polled in an early survey of attitudes towards cell-free fetal DNA analysis reported that they would be more willing to offer NIPT if it were approved by professional societies, highlighting the critical need for further guidance from national bodies.

EFFECT OF NIPT ON ALTERNATE SCREENING PROCEDURES

The introduction of NIPT in clinical practice was unprecedented because despite functioning as a screening test, its sensitivity and specificity approached that of diagnostic testing. This resulted in a ‘paradigm shift’ in prenatal diagnosis because rather than incorporating into the traditional system where a sensitive but relatively nonspecific screen is followed by a diagnostic test, NIPT exists somewhere in the middle. Even before NIPT technology became commercially available, prenatal screening using maternal blood draws was projected to have a strong interest in high-risk patients scheduled to undergo invasive procedures. A large UK survey investigating the factors impacting prenatal screening decision-making reported that given the option, NIPT was viewed as a positive development in 88% of respondents, including high uptake in patients that would currently decline alternate screening. Following its implementation, studies in the United States have shown that given the option, NIPT is preferred (69%) over integrated screening (0.6%), direct-to-invasive testing (14.1%), or no screening (16.6%). Another study focusing on a high-risk cohort in the United States reported that in just its first year of use, NIPT decreased the number of combined first trimester screens by almost 50%. Interestingly, this same study reported that the total number of overall first trimester risk assessments, defined as NIPT plus combined first trimester screening, was not significantly different after NIPT introduction. This suggests that NIPT has not increased the total number of high-risk patients electing to undergo prenatal screening, only that given the option, high-risk patients prefer NIPT over combined first trimester screening. This observation is likely related to the higher sensitivity and lower false positive rates with NIPT as compared with combined first trimester screening and is in agreement with another study reporting major screening trends genetic counselors experienced following NIPT implementation. Figure 1 shows the yearly number of nuchal translucency measurements performed as part of the combined first trimester, integrated, or sequential screening experience at Eastern Virginia Medical School, showing a steady decline in the utilization of nuchal translucency measurements following introduction of NIPT in 2011.

EFFECT OF NIPT ON DIAGNOSTIC PROCEDURES

One of the most important effects of NIPT on clinical practice has been the profound decrease in the number of diagnostic procedures (Table 1). Even before its introduction, decision-analytic models in the United States predicted that NIPT would decrease invasive procedures in high-risk patients by more than 95% and reduce euploid fetal losses by more than 99%. Another study using patient adoption rates of various screening and diagnostic methods reported that NIPT introduction would decrease invasive testing and procedure-related losses in the United States by 72% and 66%, respectively. These early models were corroborated by clinical data. A large, retrospective review of more than 15,000 procedures performed over 9 years in one center in the United States compared annual diagnostic testing after (1) introduction of the combined first trimester screen in 2006 and (2) introduction of NIPT in 2012. Figure 2 elaborates this study’s findings and includes data on additional years since the original manuscript publication. As seen in the figure, genetic amniocentesis (Figure 2A) and CVS (Figure 2B) rates decreased by 76% and 54%, respectively, post-NIPT. Of note, genetic amniocenteses had been steadily decreasing for several years prior to NIPT introduction, continuing a trend that began with the introduction of the first trimester risk
Table 1: Review of studies investigating the effect of NIPT on diagnostic procedures

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Methodology</th>
<th>Study size</th>
<th>Principal related findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garfield et al.</td>
<td>2012</td>
<td>United States</td>
<td>Multistage transition probability model</td>
<td>Theoretical 100,000 pregnancy cohort</td>
<td>Implementing NIPT as an intermediate test following positive screen would result in a 72% decrease in invasive procedures and a 66% reduction in procedure-related miscarriages.</td>
</tr>
<tr>
<td>Chetty et al.</td>
<td>2013</td>
<td>United States</td>
<td>Retrospective cohort study</td>
<td>398 screen-positive patients over 12-month period</td>
<td>Proportion of women undergoing invasive diagnostic testing decreased from 47% to 34% after NIPT introduction.</td>
</tr>
<tr>
<td>Sang et al.</td>
<td>2013</td>
<td>United States</td>
<td>Decision-analytic model</td>
<td>Theoretical 4 million pregnancy cohort</td>
<td>Implementing NIPT in high-risk women would result in &gt;95% decrease in invasive procedures and &gt;99% decrease in euploid fetal loss.</td>
</tr>
<tr>
<td>Larion et al.</td>
<td>2014</td>
<td>United States</td>
<td>Retrospective review of prospectively collected database</td>
<td>15,418 tests over 9-year period</td>
<td>NIPT introduction resulted in a 48% decrease in FTS, 69% decrease in CVS, and 47% decrease in amniocentesis from peak years.</td>
</tr>
<tr>
<td>Wallenstein et al.</td>
<td>2014</td>
<td>United States</td>
<td>Prospective study</td>
<td>163 patients undergoing genetic counseling over 18-month period</td>
<td>Invasive testing decreased from 15% to 13% of screen-positive patients in similar period before NIPT introduction.</td>
</tr>
<tr>
<td>Petit et al.</td>
<td>2014</td>
<td>United States</td>
<td>Retrospective cohort study</td>
<td>206 patients undergoing NPT over 8-month period</td>
<td>Rate of invasive procedures per total number of patient visits decreased to 4.1% from 5.9% in similar period before NIPT introduction.</td>
</tr>
<tr>
<td>Friel et al.</td>
<td>2014</td>
<td>United States</td>
<td>Retrospective review of prospectively collected database</td>
<td>792 patients undergoing genetic counseling over 8-month period</td>
<td>NIPT introduction decreased second trimester invasive procedures from 35% to 18% and decreased first trimester FTS from 89% to 59% of all visits.</td>
</tr>
<tr>
<td>Platt et al.</td>
<td>2014</td>
<td>United States</td>
<td>Multicenter retrospective study</td>
<td>1,477 patients across 6 sites over 24 month period</td>
<td>6 of 6 centers reported a decrease in amniocentesis (from −23% to −50%) and 4 of 6 reported a decrease in CVS rates (from −14% to −66%).</td>
</tr>
<tr>
<td>Larion et al.</td>
<td>2014</td>
<td>United States</td>
<td>Retrospective review of prospectively collected database</td>
<td>9,287 tests over 51-month period</td>
<td>NIPT introduction resulted in a 49% decrease in FTS, 77% decrease in CVS, and 53% decrease in amniocentesis from pre-NIPT baseline period.</td>
</tr>
<tr>
<td>Wax et al.</td>
<td>2014</td>
<td>United States</td>
<td>Retrospective cohort study</td>
<td>2510 patients considered high risk for fetal aneuploidy</td>
<td>NIPT introduction decreased amniocentesis and CVS procedures by 49% and 17%, respectively, while increasing genetic counseling use by 23%.</td>
</tr>
<tr>
<td>Wald et al.</td>
<td>2013</td>
<td>UK</td>
<td>Hypothetical contingent screening model</td>
<td>—</td>
<td>NIPT following a positive first stage of the integrated screen would result in 3 per 1000 women undergoing amniocentesis, with 2 of 3 diagnosed with trisomy 21.</td>
</tr>
<tr>
<td>Okun et al.</td>
<td>2014</td>
<td>Canada</td>
<td>8 hypothetical screening algorithms</td>
<td>—</td>
<td>Contingent NIPT screening would decrease amniocentesis procedures by 50–91%, depending on an algorithm.</td>
</tr>
<tr>
<td>O’Leary et al.</td>
<td>2013</td>
<td>Australia</td>
<td>Decision-analytic model</td>
<td>Theoretical 32,478 pregnancy cohort based on Australia population</td>
<td>NIPT following a positive first trimester screen would result in an 88% decrease in the number of invasive diagnostic tests and procedure-related fetal losses in the high-risk patient population.</td>
</tr>
<tr>
<td>Manegold-Brauer et al.</td>
<td>2014</td>
<td>Switzerland</td>
<td>Retrospective study</td>
<td>951 patients presenting for FTS over 18-month period</td>
<td>NIPT introduction decreased invasive testing to 3.1% from 8.8% in pre-NIPT baseline period.</td>
</tr>
<tr>
<td>Neyt et al.</td>
<td>2014</td>
<td>Belgium</td>
<td>Multistage transition probability model</td>
<td>Theoretical 129,199 pregnancy cohort based on Belgium population</td>
<td>NIPT as a first or second line screen would result in a decrease in the number of procedure-related miscarriages from 76 with current screening to 26 and 34, respectively.</td>
</tr>
<tr>
<td>Morris et al.</td>
<td>2015</td>
<td>UK</td>
<td>Decision-analytic model</td>
<td>Theoretical 10,000 patients undergoing screening</td>
<td>NIPT as first-line screening would decrease invasive diagnostic testing by 86% from current screening paradigms.</td>
</tr>
<tr>
<td>Gill et al.</td>
<td>2015</td>
<td>UK</td>
<td>Prospective study</td>
<td>6651 patients who presented for FTS</td>
<td>NIPT introduction decreased invasive procedures in the high-risk (risk &gt; 1:100) patient population from 54% to 40%.</td>
</tr>
<tr>
<td>Chan et al.</td>
<td>2015</td>
<td>China</td>
<td>Retrospective study</td>
<td>1251 patients with positive screen for trisomy 21 over 28-month period</td>
<td>NIPT introduction decreased invasive testing to 67% from 92% in pre-NIPT baseline period.</td>
</tr>
</tbody>
</table>

NIPT, noninvasive prenatal testing; FTS, combined first trimester screen; CVS, chorionic villus sampling.
assessment. Other groups report similar experiences at their institutions. Chetty et al. reported a 17% reduction (47% to 39% decrease) following NIPT introduction in the proportion of women undergoing diagnostic procedures after a positive conventional screen. A retrospective study by Pettit et al. reported a 30% decrease in the rate of all invasive procedures compared with a similar period pre-NIPT. In contrast, a similar study design by Friel et al. found that only second trimester diagnostic procedures were decreased following NIPT introduction and also reported a significant decrease in combined first trimester screening in women who presented before 14 weeks of gestational age. A prospective study by Wallerstein et al. investigating NIPT uptake versus integrated screening, direct-to-invasive testing, or no first trimester screening reported a 31% decrease in amniocentesis rates after NIPT was being offered to patients. Wax et al. reported a significant decrease in women undergoing amniocentesis or CVS [adjusted odds ratio: 0.42; 95% confidence interval: 0.32–0.55; \( P < 0.0001 \)] after NIPT introduction but significantly greater reliance on genetic counseling services (adjusted odds ratio: 1.77; 95% confidence interval: 1.49–2.11; \( P < 0.0001 \)). Furthermore, a multicenter study by Platt et al. reported decreases in diagnostic procedures in several medical centers dispersed throughout the United States, suggesting that these findings are not a regional preference dictated by the local patient population but rather a trend expressed at the national level.

International studies using hypothetical models or clinical data report similar decreases in procedural volume. A Canadian population-based study investigating the hypothetical performance of an NIPT-based screening algorithm reported a 50–91% decrease in the number of amniocenteses performed if NIPT was implemented in their various screening algorithms. The group also reported that amniocentesis-related losses of non-trisomy 21 affected pregnancies would decrease by 58–100%. Another model based in Belgium reported that introducing NIPT as a first or second line screening test would result in a 55–66% decrease in the number of procedure-related miscarriages. A UK study describing a reflex DNA protocol where NIPT is performed after a positive first stage of the integrated screen found that only about three in 1000 women would need a diagnostic amniocentesis and about two in three procedures would result in a diagnosis of trisomy 21. Another UK study modeled after National Health Service data reported that invasive diagnostic testing would decrease by 86% if NIPT were offered as the first-line screening option, with increased detection of trisomy 21 but at an increased patient cost. Finally, an Australian model using a contingent NIPT protocol following a positive combined first trimester screen estimated an 88% decrease in the number of invasive diagnostic procedures in their high-risk patient population.

Clinical data supported these hypothetical models. A Swiss study reported a 67% decrease in invasive diagnostic testing from a baseline period in the first 9 months following NIPT introduction. Gil et al. reported a 27% (54% to 40%) decrease in the rate of invasive testing in UK women who were screened as high risk (risk \( > 1 : 100 \)) with the combined first trimester screen. Patients at intermediate risk (one in 101–2500 risk) also preferred NIPT (92%) more often than no further testing (8%). The most commonly reported reason for not undergoing further screening with NIPT in the intermediate group was that patients were satisfied with their maternal risk assessments from the combined screen and did not want to endure the 2-week wait for results. Finally, a Chinese study reported a 28% reduction in diagnostic testing in patients with a positive screen. Patients who screened positive with NIPT were also more likely to undergo further follow-up testing. As a major strength of NIPT is its positive predictive value in the high-risk patient population, these findings further underscore the need for effective pre-test and post-test counseling. Furthermore, as genetic counseling increases patient knowledge regarding NIPT, diagnostic testing is likely to further decrease.

It is also possible that NIPT may affect diagnostic testing due to its availability beginning in the 10th week of gestational age. Women in the first trimester who are screened positive by conventional screening methods and elect to undergo NIPT may miss the window for diagnostic testing with CVS and may ultimately undergo amniocentesis. Similarly, women in the second trimester who are screened positive by conventional screening methods may elect to bypass NIPT in favor of diagnostic testing. Therefore, the reported changes in utilization may at least in part be explained by the timing of NIPT availability.
EFFECT OF NIPT ON LIVE BORN INFANTS WITH TRISOMY 21

It is unknown whether the changes in screening and diagnostic testing as a result of NIPT introduction have affected the number of live born infants with trisomy. A recent study by Wax et al. comparing the effect of NIPT on invasive diagnostic procedures and trisomy 21 detection reported that despite the reduced number of diagnostic procedures, the rate of prenatal trisomy 21 detection was not significantly different after NIPT introduction (88% vs 100% detection, respectively; \( P = 0.86 \)). This suggests that reduced diagnostic testing post-NIPT did not affect the ability to detect fetuses with trisomy 21 in their patient population, which is similar to our own institutional experience with an NIPT-based trisomy 21 screening protocol using a prospectively maintained quality assurance database on more than 2800 NIPTs in high-risk patients (unpublished data). In the 8 years prior to NIPT introduction (2003–2011; Figure 3), our region had an average of 19.9 ± 2.4 (SD) live born infants with trisomy 21 per year, which is not significantly different from the average number of live born infants with trisomy 21 in the 3 years following NIPT implementation (19.0 ± 1.7; \( P = 0.577 \)). During this time, there were a total of 122 infants born with trisomy 21 in the Hampton Roads region of Virginia. Of these 122 infants, 15 (12%) were related to screen failures, with 12 related to second trimester screen failures and three to first trimester screen failures. There have been no screen failures in our patient population that have been attributed to false negative NIPTs for trisomy 21. The remaining 107 infants (88%) born with trisomy 21 in our region were related to the maternal choice of either forgoing aneuploidy screening or continuing the pregnancy despite having either a positive screen or diagnostic test (Table 2). Thus, our experience is similar to the Wax et al. study in that NIPT has not affected the number of live born infants born with trisomy 21 in our region. The full impact of NIPT on live born infants with trisomy 21 will not be known until NIPT becomes available to the general obstetrical population.

An interesting corollary regarding NIPT uptake and its effect on the rate of live born infants with trisomy 21 is the opportunity for mothers to use NIPT as a means of obtaining genetic information regarding the status of the fetus without undergoing the risk of invasive testing. Many mothers decline invasive testing due to the inherent risk to the fetus, and these mothers may alternatively view NIPT as a safer means for obtaining genetic information. These mothers may elect to undergo NIPT solely for information purposes only, and thus NIPT uptake among this group of patients would not affect live born rates of infants with trisomy. Electing to undergo NIPT solely for informational purposes such as postnatal management has already been described for patients with monogenic disorders such as cystic fibrosis (CF), where 95% of adult CF patients or carriers at a specialist center reported interest in NIPT for screening of CF, but only 44% would accept invasive testing. The most commonly reported reason for undergoing prenatal CF screening was ‘to prepare for the possible birth of a baby with CF’ (62%), with the most commonly reported benefit of NIPT being its decreased risk to the fetus (79%). Characteristics of patients who would undergo NIPT for information purposes only, rather than for consideration of pregnancy termination, include age less than 35 years, knowing someone with a child who has trisomy 21, and refusing to undergo invasive testing.

While larger studies are needed in the United States to determine if NIPT has affected the rate of live born infants with trisomy, epidemiological studies in Europe have shown that despite increased average maternal age between 1990 and 2009 resulting in an increase in the prevalence of pregnancies affected with trisomy 21, improved prenatal screening methods and the use of elective terminations have resulted in no significant change in the prevalence of live born infants with trisomy. These studies also report wide variation in the prevalence of infants born with trisomy 21 between individual countries, with commentary that if current trends continue, Denmark will not have a single infant born with trisomy 21 by the year 2030. Similar concerns are for the use of NIPT in fetal sex selection, as has already occurred in certain countries that place a high value on male children, including the United States. As there are significant ethical

Table 2: Review of the total number of live born infants with trisomy 21 in the Hampton Roads, Virginia region between 2003 and 2014 (\( N = 122 \))

<table>
<thead>
<tr>
<th>( N ) (%)</th>
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</thead>
<tbody>
<tr>
<td>No or late prenatal care</td>
</tr>
<tr>
<td>No trisomy 21 screening</td>
</tr>
<tr>
<td>Positive screen but no diagnostic testing</td>
</tr>
<tr>
<td>Confirmed trisomy 21 diagnosis but continued pregnancy</td>
</tr>
<tr>
<td>Screen failures a</td>
</tr>
</tbody>
</table>

aTwelve of 15 screen failures involved quadruple maternal serum screens, and three of 15 involved combined first trimester screens.

Figure 3: Yearly number of live born infants with trisomy 21 between 2003 and 2014 in the Hampton Roads, Virginia region, including after introduction of the combined first trimester screen in 2006 and noninvasive prenatal testing in 2012. FTS, combined first trimester screen; NIPT, noninvasive prenatal testing.
concerns relating to the effect of NIPT introduction on sex selection and rates of infants born with trisomy, larger population-based studies and ongoing surveillance are needed to assess the effect of NIPT on aneuploidy detection, sex selection, and pregnancy management.

UNINTENDED NEGATIVE CONSEQUENCES OF REDUCED DIAGNOSTIC TESTING

Noninvasive prenatal testing is highly specific for fetal aneuploidy, with validation studies consistently reporting true negative rates for trisomy 21 of greater than 99%.54–56 In experienced centers, the American College of Obstetricians and Gynecologists recognizes that the risk of amniocentesis or CVS-related fetal loss rate is 1 in approximately 300–500.57 Thus, one of the greatest advantages that NIPT offers is the decreased need for invasive diagnostic testing and subsequent risk to the fetus. Moreover, because of the increased specificity, diagnostic procedures performed following positive NIPT screening are more likely to be true positives, reducing the number of unnecessary diagnostic testing.58

Nevertheless, an unintended consequence of NIPT introduction is the effect that the reduced number of diagnostic procedures has on the typical clinical experience of an obstetrical or maternal–fetal medicine practice. While neither the Society of Maternal–Fetal Medicine nor the American College of Obstetricians and Gynecologists has set minimums, the Royal College of Obstetricians and Gynaecologists recommends that competency should be maintained in clinicians through the performance of at least 30 ultrasound-guided invasive procedures per year.59 The Royal College also reports that very experienced operators who perform more than 100 procedures per year have higher success rate and a lower procedure-related loss rate than less experienced operators (recommendation level: C). Similarly, the California Department of Public Health Genetic Disease Screening Program has lowered the minimum number of diagnostic procedures required to be a practitioner in its system.60 For instance, the program now mandates that practitioners must complete 25 successful amniocenteses and transabdominal or transcervical CVS procedures per year to maintain eligibility as a practitioner. Physicians who complete less than 25 of these procedures per year are placed on provisional approval status and must submit adverse neonatal outcome data on their patients. The 2014 guidelines are also lessened from 2013 minimums, in response to the significant changes in diagnostic testing trends across all its members. The dramatic reduction in diagnostic procedure rates endangers the ability of the practicing physician to maintain the operating skills necessary for the technique. In a large retrospective trial investigating miscarriage rates in Denmark, the risk of fetal loss following amniocentesis was more than twice as likely in departments that performed fewer than 500 procedures per year compared with departments that performed greater than 1500 procedures per year (odds ratio 2.2; 95% confidence interval: 1.6–3.1).61 Comparable results were noted for CVS procedures. Maternal cell contamination has also been reported to occur more frequently in operators who perform less than 50 procedures per year.62 Thus, the benefits of decreased invasive testing must unfortunately be tempered by the possible increase in the test failure and fetal loss rate attributed to the deterioration of physician experience and skill. This effect is likely to exist more commonly in smaller centers, with one possible solution being the formation of dedicated prenatal diagnosis centers clustered in high volume areas. However, this would be impractical for many patients and provide inequity of medical care in favor of more populated regions.

In addition, the Division of Maternal–Fetal Medicine of the American Board of Obstetrics and Gynecology requires fellows to have proficiency with invasive diagnostic procedures during their training. This includes sufficient experience to independently perform second and third trimester amniocenteses, as well as demonstrating an understanding of the principles of chorionic villus sampling.63 These procedures populate the operative procedures portion of the maternal–fetal medicine case list of the oral board examination, of which trainees must present 30 cases relating to genetics and fetal disorders and include eight cases involving fetal chromosomal abnormalities.64,65 Simulator or electronic guidance-based systems have been shown to improve competency among trainees and thus will likely be relied upon in the future in order to train the next generation of maternal–fetal medicine specialists in invasive diagnostic procedures.66,67 In addition, there will be a greater demand for genetic counseling services as NIPT uptake increases.27,68,69 Alternate screening procedures such as multiple serum marker and first trimester screening will likely decrease. Furthermore, the decreased need for diagnostic testing will have a significant financial impact on cytogenetic laboratories.

CONCLUSION

Noninvasive prenatal testing has been described as a ‘disruptive innovation’ due to its tremendous impact on the utilization of alternate screening and diagnostic procedures. While NIPT is currently restricted to high-risk singleton pregnancies, it is likely that NIPT will be available to the general obstetrical population in the near future, which will likely continue to decrease invasive procedural volume. As these trends will clearly impact the training and experience of obstetricians and maternal–fetal medicine specialists, a careful assessment of training guidelines is necessary to ensure a continued level of high quality patient care.
REFERENCES

February 17, 2020

Health Technology Clinical Committee
P.O. Box 42712
Olympia, WA 98504-2712

Re: HTCC coverage determination on cell-free DNA prenatal screening for chromosomal aneuploidies

Dear HTCC,

On behalf of Illumina, a leading developer and manufacturer of next generation sequencing (NGS) tools for both research and clinical use, we appreciate the opportunity to provide comments on the Proposed HTCC coverage determination on cell-free DNA prenatal screening for chromosomal aneuploidies.

We commend the HTCC on recommending that cell-free DNA prenatal screening for chromosomal aneuploidies is a covered benefit.

We would like to comment on the language in The Final Evidence report which states: “universal cfDNA testing is likely to be more expensive than conventional screening, depending on the exact costs of the cfDNA test used. Policymakers therefore need to consider the value of expanding cfDNA screening to all pregnant women and whether it is worth the additional associated costs.”

One critical value to consider is equitable access to prenatal screening. Clinical practice guidelines highlight that all women should have access to prenatal screening. As detailed below, cell-free DNA-based prenatal screening overcomes equity of access issues that are present for conventional first-trimester screening.

Cell free DNA for prenatal screening is a blood test obtained through routine venipuncture in the physician office, and does not require women to seek additional time or transportation beyond her usual routine obstetric appointment.

Conventional first trimester screening, however, requires a special ultrasound to determine the fetal nuchal translucency (NT) measurement in addition to a blood draw and associated biochemical tests. The NT must be obtained at an ultrasound center that has technicians certified by the Perinatal Quality Foundation, and is supervised by a credentialed Maternal Fetal Medicine specialist. It is not an ultrasound that can be obtained in most regular obstetric practices, so is not typically done as part of a routine obstetric appointment but is a separate appointment, often at a different location.

Unfortunately, this can limit who has access to conventional first trimester screening, as the availability of a NT measurement is dependent on the woman’s ability to access a qualified center. In the State of Washington, there are 76 NT centers in 32 zip codes per the Nuchal Translucency Quality Review site (NTQR.org). For your convenience, a map of the centers is included after the references. There are large areas of the state, representing an estimated 12% of pregnant Medicaid members (Cornell, K., 2019), where a woman would have to drive more than two hours, and as much as four hours, one way, to have an NT performed. This may limit access to conventional first trimester screening, as not all women are able to take that much time away from their regular routine, or obtain transportation to distant locations.

Additionally, in a 2015 study by Cuckle et al., when the Perinatal Quality Foundation examined 1.5 million NT scans in their monitoring program, they concluded that “even with extensive training, credentialing and monitoring, there remains considerable variability between NT providers. There was a general tendency towards under-measurement of NT.” Under-measurement could potentially increase the false negative rate.

In conclusion, not only is cell free DNA prenatal screening the most accurate test available for screening for common chromosome aneuploidies as confirmed by the Final Evidence Report, but because it only requires a routine blood draw, it ensures equitable access for all residents of the State of Washington. The value of preventing and eliminating disparities in care cannot be understated.
Thank you for the opportunity to comment on the Cell free DNA Prenatal Screening HTCC decision.

Sincerely,

Trish Brown, MS, CGC
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References:

1. Nuchal Translucency Quality Review (https://www.ntqr.org/) as accessed on February 17, 2020

Map of NT Centers in Washington State as of 2/17/2020
(From page 7 of decision aid)

**Next step: Proposed findings and decision and public comment**

At the next public meeting the committee will review the proposed findings and decision and consider any public comments as appropriate prior to a vote for final adoption of the determination.

☐ 1) Based on public comment was evidence overlooked in the process that should be considered?

☐ 2) Does the proposed findings and decision document clearly convey the intended coverage determination based on review and consideration of the evidence?

**Next step: Final determination**

Following review of the proposed findings and decision document and public comments:

**Final vote**

☐ Does the committee approve the Findings and Decisions document with any changes noted in discussion?

*If yes*, the process is concluded.

*If no*, or unclear outcome (i.e., tie), chair will lead discussion to determine next steps.