Washington State Health Care Authority

Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies

Draft key questions: public comment and response

August 6, 2019

Updated: *August 26, 2019*

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Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies

Draft Key Questions
Public Comment and Response

Provided by:

Center for Evidence-based Policy
Oregon Health & Science University

August 6, 2019

Updated: August 26, 2019

Responses to public comment on draft key questions

The Center for Evidence-based Policy is an independent vendor contracted to produce evidence assessment reports for the Washington Health Technology Assessment (HTA) program. For transparency, all comments received during the public comment periods are included in this response document. Comments related to program decisions, process, or other matters not pertaining to the evidence report are acknowledged through inclusion only.

Draft key question document comments received:

- Marily Rhudy, Secretary and Director, Coalition for Access to Prenatal Screening (CAPS)
- Jim Clark, State Government Affairs, Roche Diagnostics Corporation
- Kimberly Martin MD, Chief Clinical Advisor, Natera, Inc
- Deirdre E. Flannery, Senior Director, Traditional Medicaid, Quest Diagnostics

Specific responses pertaining to submitted comments are shown in Table 1.

Table 1. Responses to Comments on Draft Key Questions for Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies

Comments		Response
Commenter: Marily Rhudy, Secretary and Director, Coalition for Access to Prenatal Screening (CAPS)		
General Comments:		
The Coalition for Access to Prenatal Screening (CAPS) is pleased to submit this comment on draft key questions for the Health Technology Assessment (HTA) of Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies. According to the HTA website, HTA seeks input on whether key technology assessment questions have included the "appropriate topics to address HTA's mandate to gather evidence on safety, efficacy, and cost effectiveness relevant to coverage determinations."		Thank you for your comments. Please see detailed responses to your specific comments below.
Conclusion: In order to assess the safety and efficacy of NIPS in the general population, HTA should examine of the false negative rate, false positive rate, and positive predictive value of standard screening as it is the current standard of care for women seeking prenatal screening on the Washington fee-for-service program.		
Specific Comments:		
Comparator(s)	To guide this, the draft questions should identify "appropriate comparators" as stated on the HTA website. CAPS believes the current draft questions will not adequately compare cell-free DNA noninvasive prenatal screening (NIPS) in the general population to its comparator, standard prenatal aneuploidy screening ("standard screening") in the general population. An evaluation of the safety, efficacy and cost effectiveness of NIPS must do so in comparison to the existing standard screening.	We have clarified the comparators for trisomies and common sex chromosome aneuploidies.

Outcome(s)	An evidence review of NIPS should consider its low rate of false positives and false negatives and its high positive predictive value against the false positive rate, false negative rate, and positive predictive value of standard screening as the latter is the standard of care for women regardless of risk on the fee-for-service program in Washington.[1]
	
	To illustrate this, below is an example of the superiority of NIPS:
	
	• The positive predictive rate of NIPS in the general population for the three trisomies:[1]
	  ○ Trisomy 21: 80.9%
	  ○ Trisomy 18: 90%
	  ○ Trisomy 13: 50%
		We will be evaluating the performance of the screening tests against the relevant comparators.
		
		Measures, such as false positives and predictive values, will be reported where possible.

Comments		Response
Commenter: Marily Rhudy, Secretary and Director, Coalition for Access to Prenatal Screening (CAPS)		
	• The positive predictive rate of standard screening in the general population for the three trisomies is below:[1] ○ Trisomy 21: 3.4% ○ Trisomy 18: 14% ○ Trisomy 13: 3.4% For Trisomy 21, NIPS has a positive predictive value that is 20x more accurate than standard screening in the general population. This improvement in specificity is one of the values of NIPS, and it should be captured in the HTA review of NIPS as it is a more efficacious technology than the current standard of care available to women considered low risk of chromosomal abnormalities on the fee-for-service program.	
Supporting Evidence	Below is a summary of the findings from the 2015 study in the New England Journal of Medicine, entitled "Cell-free DNA Analysis for Noninvasive Examination of Trisomy." As the study's population was of both high risk and low risk women, we request its findings be included in the key questions document. (Figure provided, but not repeated here)	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Comparator(s)	Evaluating the potential harms of NIPS should include a comparison to the potential harms of standard screening	We will be evaluating the harms of cfDNA screening compared with other approaches, including standard screening.
Terminology	The draft questions also focus on direct harms of NIPS, described as misdiagnosis and psychosocial harms. An evidence review will identify a minor chance of misdiagnosis with NIPS as it is a screening tool and not a confirmatory diagnostic.	Thank you. We have clarified the terminology. The use of cfDNA as a screening tool, not as a diagnostic tool, is an important distinction and its performance as a screening tool will consider the rates of cases that are not confirmed with further diagnostic testing.
Outcome(s)	CAPS believes it is important to consider the direct harms (misdiagnosis, psychosocial harms) associated with the standard screening when evaluating the same consequences of NIPS. By doing so, the specificity of NIPS is given context as a vastly superior screen as NIPS would lessen the exposure to misdiagnosis and its associated harms. NIPS improves upon prenatal screening care, which is already covered for all women regardless of age by the	We will be evaluating the harms of cfDNA screening compared with other approaches, including standard screening.

Comments		Response
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	Washington Health Care Authority in the form of standard screening.	
Background	Furthermore, CAPS believes the draft key questions reference a withdrawn committee opinion from the American College of Obstetricians and Gynecologists, and if this inaccuracy is included in the final key questions, it could lead to an inaccurate evidence gathering process. Review should reference current ACOG guidance: The HCA's draft document states, "[t]he American College of Obstetricians and Gynecologists (ACOG) has stated that cfDNA screening works best for individuals who already have an increased risk of having a baby with a chromosomal disorder." The source for this information is a web page on ACOG's website featuring frequently asked questions on prenatal screening tests. CAPS believes the information on the frequently asked questions page is incorrect and inconsistent with ACOG's practice bulletins. CAPS has raised this inconsistency with ACOG as it has not updated this page after the withdrawal of an outdated committee opinion. According to ACOG Practice Bulletin No.163, "all women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age."[2] In July of 2018, ACOG Committee Opinion No. 693 formally withdraw Committee Opinion No. 640, which originally stated "conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population."[3,4] Therefore, this statement is no longer accurate and should not be used during the HTA of NIPS. CAPS requests that HTA update the draft key questions to quote the current ACOG policy statement on NIPS.	ACOG recognizes that there are limitations with cfDNA screening, including performance in lower-risk populations. ACOG Practice Bulletin 163 states that "*The sensitivity and specificity in the general obstetric population are similar to the levels previously published for the high-risk population.* *However, cell-free DNA screening cannot have the same accuracy in low-risk pregnancies (e.g., in young women) because the positive predictive value is affected by the prevalence of the disorder in the population. The positive predictive value is lower in the general obstetric population because of the lower prevalence of aneuploidy in this population.*" The recommendation on offering cfDNA screening to all women is based primarily on expert consensus and opinion. We have therefore not made the suggested changes, as we consider the text to accurately reflect ACOG's position.
Background	In addition, CAPS requests HTA include in its key questions document the American College of Medical Genetics (ACMG) 2016 Statement, which recommends "informing all pregnant women that NIPS is the most sensitive screening option for traditionally screening aneuploidies (i.e., Patau, Edwards, and Down Syndromes)."[5]	We have not added this reference to the background section. The background includes selected references and does not aim to include all relevant guidelines. We will include a review of relevant practice guideline recommendations on cfDNA screening in the full report.

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Commenter: Marily Rhudy, Secretary and Director, Coalition for Access to Prenatal Screening (CAPS)		
Background	Due to the statements from ACMG and ACOG, we request the removal of the following sentence from the key questions document: "However, clinical practice guideline authors vary in their recommendations, citing challenges with cost and the positioning of cfDNA in the screening and diagnostic pathways."	We have not removed this statement in the background section because there are different views and approaches to the use of cfDNA screening in lower-risk populations; hence, this review is being conducted.
Other	Review should include more specific information on current insurance coverage of NIPS: CAPS is pleased with the inclusion of commercial coverage of NIPS in the key questions documents. However, we recommend adding specificity with the following lines: • 60 major commercial health insurance plans cover NIPS for all women. This includes 40 Blue Cross Blue Shield plans, Cigna, Anthem, and Wellmark. • Six states, including Florida, Virginia, Minnesota, Ohio, North Dakota, and Pennsylvania now cover NIPS under state Medicaid benefits for pregnant women of all risks. 36 states, including Washington State, cover NIPS for women categorized as high-risk.	We have not added this information to the background section. We will include a review of selected coverage decisions for cfDNA screening in the full report.
Key Question(s)	We suggest the following edits to Key Question One: • What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive screening or invasive diagnostic testing) or no screening testing in pregnant individuals not known to be at high risk for chromosomal abnormalities?	We have clarified the comparators for trisomies and common sex chromosome aneuploidies, which we hope addresses your concerns.
Key Question(s)	We suggest the following addition to Key Question Two: • What direct harms are associated with standard screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using standard screening methods (instead of cfDNA screening) in pregnant individuals who are both known and not known to be at high risk for chromosomal abnormalities?	We have clarified the comparators for trisomies and common sex chromosome aneuploidies, which we hope addresses your concerns. We have not added pregnant people who are known to be at high risk because this is outside the scope of this review. However, we will report the harms of cfDNA screening in pregnant individuals known to be at high risk for chromosomal

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		abnormalities in Contextual Question 1.
Key Question(s)	We suggest the following addition to Key Question Two: • What current methods can differentiate a 'high risk' from a 'low risk' pregnancy with regard to common sex chromosome aneuploidies?	We have not added this to the questions because it is beyond the scope of the review. We will report how the included studies determine the risk for the population being studied. We will also report the definitions used to classify risk in the relevant practice guideline recommendations and selected coverage decisions included in the final report.
Key Question(s)	We suggest the following edit to Key Question Three: • Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary – as compared to other active noninvasive interventions - for the mother and fetus or infant, by: a) Maternal characteristics (e.g., age) b) Singleton or multifetal pregnancy c) Timing of screening (e.g., gestational age)	We have not added this to the question, but we have amended the comparators in Key Questions 1 and 2. Key Question 3 will then draw on the evidence addressing Key Questions 1 and 2.
Comparator(s)	We request specification of "other NIPT technologies" mentioned under inclusion criteria for Comparators as it is not obvious what other technologies this refers to.	We have clarified this to "another cfDNA screening test."
Outcome(s)	Include comparison to standard screen in evaluating safety of NIPS We recommend adding the following as a bullet: • Safety: harms directly related to screening for trisomies 21, 18, and 13 and common sex chromosomal aneuploidies using standard screening methods with a high rate of false positives and detection rates that are significantly lower than cfDNA screening, regardless of a priority risk in the tested population	We have clarified the comparators for trisomies and common sex chromosome aneuploidies in Key Question 2. We have not added this to the outcomes.
Inclusion and Exclusion Criteria	Include relevant cost-effectiveness research: We request that the review include relevant cost-effectiveness studies, by removing the exclusion of studies	We have revised the date criteria to the last 5 years, in order to balance the

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	older than two years. All U.S. based cost-effective studies on NIPS were conducted earlier than 2017 and therefore should be included in this review.	applicability of the economic evidence and its availability.
Inclusion and Exclusion Criteria	We recommend adding the following as a bullet: • Clinical experience studies that summarize real-world findings and performance characteristics of cfDNA screening tests as performed by CLIA-certified labs in the U.S.	We have not made this suggested change. We will focus on comparative studies, both randomized and nonrandomized, for effectiveness. We will also include noncomparative studies for the assessment of safety and harms. Such studies may well include real-world findings from CLIA certified labs, if they have been published in the medical literature.
Analytic Framework	Contextual Question 1: Analytic Framework We recommend adding the following to the "Intervention" section of the framework: • Standard prenatal aneuploidy screening	We have not added this statement because it reflects the use of cfDNA as an intervention, and standard screening for this framework would constitute a comparator (which is not defined in this diagram).
Supporting Evidence	Provided references	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

Notes.

1. Norton, M. et al Cell free DNA analysis for noninvasive examination of Trisomy. The New England Journal of Medicine April 2015; 372: 1589-1597
2. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. The Society for Maternal-Fetal Medicine. 2016 May. [cited 2017 Mar 23]. Available from: https://s3.amazonaws.com/cdn.smfm.org/publications/224/download-491f0e6962960848d2097447ab57a024.pdf.
3. Women's Health Care Physicians. (n.d.). Retrieved July 17, 2019, from https://www.acog.org/Clinical-Guidance-and-Publications/Withdrawn-Documents
4. Committee Opinion No. 640: Cell-Free Dna Screening For Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. 2015 September. Available from:

https://journals.lww.com/greenjournal/FullText/2015/09000/Committee_Opinion_No__640__Cell_Free_Dna_Screening.51.aspx

5. Gregg, A.R., et al. "Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics" American College of Medical Genetics. 2016 Jul. [cited 2017 Mar 23] Available from: http://www.acmg.net/docs/NIPS_AOP.pdf

Comments		Response
Commenter: Jim Clark, State Government Affairs, Roche Diagnostics Corporation		
General Comments:		
None provided		
Specific Comments:		
Key Question(s)	1. What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive or invasive) or no screening in pregnant individuals not known to be at high risk for chromosomal abnormalities? Currently, there is a vast amount of data supporting the use of cfDNA screening for trisomies 21, 18, 13, and the common sex chromosomes.	Thank you for your comment.
Supporting Evidence	There has been one head-to-head comparison study between the Harmony prenatal test and first-trimester combined screening (Norton - Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015 Dec 24;373(26):2582. (https://www.ncbi.nlm.nih.gov/pubmed/266991709) . Studies have demonstrated extremely high sensitivities and specificities for the performance of screening the common chromosome aneuploidies.	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Comparator(s)	Other than monosomy X, which may show signs of anomalies on ultrasound, there are currently no specific screening modalities for sex chromosomes aneuploidies. Invasive procedures such as chorionic villus sampling (CVS) or amniocentesis are the only methods to definitively diagnose a chromosome abnormality in pregnancy. However, these methods carry a small risk of miscarriage.	We have clarified the comparators for trisomies 21, 18, and 13 and the common sex aneuploidies to make this difference in current screening approaches more explicit.
Key Question(s)	2. What direct harms are associated with screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?	Thank you for your comment.

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	Every patient has a risk for a chromosome abnormality to occur in their pregnancy. While that risk does increase as maternal age increases, current recommendations state every patient should be offered screening from chromosome abnormalities. Every screening test has a risk for discordant results; either a false positive or false negative result. Screening with cfDNA decreases the frequency of time these discordant results may occur as compared to conventional screening methods. As with all screening tests, direct harm from screening with cfDNA other than discordant results may also include anxiety and concern with high probability results (either concordant or discordant to the pregnancy).	
Key Question(s)	3. Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by: a. Maternal characteristics (e.g., age) The performance of cfDNA screening does not change based on maternal age or characteristics. However, the incidence of disease in a population affects the positive predictive value of a particular population. For example, as maternal age increases, the risk for a chromosome abnormality also increases and the incidence of disease is more common. Therefore, the PPV of a population will be higher when the incidence of disease increases but the performance (sensitivity and specificity) of the test remains constant and this is the same for all screening tests.	Thank you for your comment.
Supporting Evidence	3. Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by: b. Singleton or multifetal pregnancy There is substantial evidence on the performance of cfDNA in singleton pregnancies. While the evidence for multifetal pregnancies is limited, studies have concluded the performance of cfDNA in twin pregnancies is similar to that of singleton pregnancies and superior to that of first-trimester screening (Gil et al. Ultrasound Obstet Gynecol 2019; 53: 734–742).	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

Comments		Response
Commenter: Jim Clark, State Government Affairs, Roche Diagnostics Corporation		
Key Question(s)	3. Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by: c. Timing of screening (e.g., gestational age) Certain factors can affect the performance of cfDNA screening. The main factor is the amount of fetal fraction (the proportion of DNA from the pregnancy in the sample) present for analysis. The lower the fetal fraction percentage, the more difficult it is to determine an accurate result. As pregnancy progresses, the amount of fetal fraction increases. Therefore, for certain samples with not enough fetal fraction, the sensitivity may suffer (Artieri et al. Prenat Diagn. 2017 May;37(5):482-490. doi: 10.1002/pd.5036. Epub 2017 Apr 26.). There are ways to prevent a lowered sensitivity and one way is to have a minimum threshold of fetal fraction. By ensuring there is enough fetal fraction in the sample, the lab can maintain the high performance of cfDNA screening (Blais, et al. (2018). Clinical biochemistry, 59, 69-77).	Thank you for your comment.
Supporting Evidence	4. What are the cost-effectiveness and other economic outcomes of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities? In the study by Fairbrother et al., NIPT identified 15% more trisomy cases than FTS and significantly reduced the amount of invasive procedures. It also found that at a NIPT unit cost of $665, the cost per case to identify trisomy is equivalent to that of FTS. In addition, at a NIPT unit cost of $453 or less, a cost savings over FTS is realized (Fairbrother et al. J Matern Fetal Neonatal med, 2016. DOI: 10.3109/14767058.2015.1038703).	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

Comments		Response
Commenter: Kimberly Martin MD, Chief Clinical Advisor, Natera, Inc		
General Comments:		
These remarks are supported by peer-reviewed references AND personal experience as an ob/gyn and clinical geneticist in academic and private practice for over 22 years.		Thank you for your comments. Please see detailed responses to your specific comments below.
Specific Comments:		
Background	1. What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive or invasive) or no screening in pregnant individuals not known to be at high risk for chromosomal abnormalities Please refer to ACOG's most recent position, enclosed in Practice Bulletin #163 (please see attached) which clearly states "all women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age." The bulletin outlines a variety of benefits and limitations of the various screening modalities, and acknowledges that the data is sufficient to conclude that screening performance for common chromosome abnormalities including Down syndrome is superior to the other methods. The following statement "The sensitivity and specificity in the general obstetric population are similar to the levels previously published for the high risk population" is also to be noted.	Thank you for your comment.
Supporting Evidence	Several cost effective publications are referenced below.	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Outcome(s)	2. What direct harms are associated with screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities? HARMS - False positive results raise anxiety, increase likelihood family will choose invasive testing, increases cost of care due to referral to MFM, additional ultrasounds, consults, perhaps even surveillance as high risk pregnancy based upon serum analyte levels (no data to suggest serum	Thank you for your comment.

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Commenter: Kimberly Martin MD, Chief Clinical Advisor, Natera, Inc		
	analytes should be used to screen for adverse pregnancy outcome, or that ancillary testing like biophysical profiles, non-stress tests improve outcome for these pregnancies). Possible that patient may then be subjected to emergent delivery or increased risk of cesarean section due to false positive results from additional testing that was not indicated. Therefore it is critical that test with highest sensitivity and lowest false positive rate is used to minimize direct patient harm - this is clearly cell-free DNA. -False negative results - patients choose testing for these abnormalities after a discussion with their healthcare provider, who must order the test. In general, patients will want the test with the highest detection rate. BENEFITS: -Identification of a child with a serious condition allows reproductive decision making for parents. This includes interruption of pregnancy assuming results of screening are confirmed with diagnostic testing, and the family are clearly and accurately counseled by non-directive providers. Many families choose not to interrupt these pregnancies but place HIGH value on prenatal diagnosis, education and preparation which may result in various improved outcomes. 1. Serious chromosome abnormalities like Trisomy 13/18 - family may continue pregnancy, however < 10% of these infants survive to first birthday, high rate of maternal intervention (such as cesarean section for fetal distress) when NOT known before delivery. Family may meet with pediatric specialists prenatally and develop care plan directed at comfort/supportive care resulting in dramatically lower healthcare costs (NICU admission, etc) a serious chromosome abnormality like Trisomy 13/18. Even more important is that the family is prepared, including extended family and friends, and can spend the livespan of the infant with the infant NOT through an incubator. 2. Down syndrome - the detection of congenital heart disease overall is < 50% particularly when ultrasounds are performed by less-experienced providers. Highly sensitive screening like cfDNA and prenatal diagnosis should lead to fetal echocardiography, evaluation for intestinal obstruction, etc and ideally delivery of the infant in a center equipped to deal with the associated complications.	

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Supporting Evidence	3. Sex chromosome abnormalities - these were not addressed in screening until the advent of prenatal screening using cell free DNA (exception is Turner syndrome which may be identified if ultrasound features are noted). There is a paper in press, survey of parents of children with sex chromosome abnormalities, who overwhelmingly support prenatal screening for these abnormalities, they are not screened in newborn screening and many experience years trying to understand their childrens' 'differences' which can be screened for (head start, early intervention), treated (early androgen replacement for 47,XXY improves outcomes), growth hormone (turner syndrome), etc. If you have any questions suggest direct to Dr. Carole Samango-Sprouse, Executive Director and Chief at The Focus Foundation in Maryland: www.thefocusfoundation.org	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Supporting Evidence	3. What are the benefits and harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals known to be at high risk for chromosomal abnormalities? EXACTLY the same as above, the definition of a maternal age of 35 as 'high risk' is completely arbitrary. Data from attached reference Snijders: (Data provided, but not repeated here)	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Supporting Evidence	The difference between a 33, 34, 35 year old cannot be distinguished by a typical pregnant woman or her partner, therefore the direct harms and benefits are no different. Women 35 and older are more anxious because they have been 'educated' that they are high risk while women < 35 are under the impression their risk is 'zero'. This "high risk" paradigm requires a complete overhaul; it is no longer appropriate for making decision regarding what tests to offer to what patients. ACOG/SMFM has held since 2007 that ALL women are to be given ALL OPTIONS for screening AND diagnostic testing. Performance of cell free DNA is not significantly different (See Norton_NEJM_2015).	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Supporting Evidence	4. What are the cost-effectiveness and other economic outcomes of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

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	Reduction in invasive testing has been demonstrated (Warsof and Hui attached) with increased detection of aneuploid fetuses, therefore higher rate of diagnosis of affected fetuses allowing reproductive decision making AND preparation for birth of child with special needs with reduced miscarriage of unaffected fetuses due to fewer invasive procedures. With respect to modelled cost effectiveness, please see enclosed: Fairbrothers_Ariosa, Benn pone and Walker_ARUP_PLOSone.	
Supporting Evidence	Provided references	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

Comments	Response
Commenter: Deirdre E. Flannery, Senior Director, Traditional Medicaid, Quest Diagnostics	
General Comments:	
Quest Diagnostics is the world's leading provider of laboratory services, and annually serves one in three adult Americans and half of the healthcare providers and hospitals in the United States. We proudly serve Washington's Medicaid program as an enrolled independent laboratory. Quest Diagnostics appreciates the opportunity to comment on the above referenced draft key questions. We are supportive of Washington's efforts to bring improved prenatal screening to beneficiaries in Washington and would welcome the opportunity to work with the Health Care Authority as coverage policies are developed. To that end, we support cell-free DNA prenatal screening (cfDNA) for chromosomal aneuploidies for the general pregnancy population, which includes both average-risk and high-risk pregnancies.	Thank you for your comments. Please see detailed responses to your specific comments below.
According to ACOG and SMFM[i] all women should be offered the option of aneuploidy screening, regardless of maternal age. Prenatal cfDNA screening demonstrates clinical utility, allows for time to make decisions on pregnancy management and possibly decreases rates of invasive diagnostic procedures that involve fetal risk.	
Additional clinical rationale to support the position that both average-risk and high-risk pregnancies should receive cell-free DNA is detailed as an attachment. Thank you for your time and consideration of this matter. We welcome an opportunity to engage further on the clinical evidence that will be used to support the Health Technology Clinical Committee work to determine coverage of cfDNA prenatal screening for pregnant women in Washington State. Please contact our Medicaid colleague at deirdre.e.flannery@questdiagnostics.com if we can provide additional information.	

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Commenter: Kimberly Martin MD, Chief Clinical Advisor, Natera, Inc		
Fetal aneuploidy is a standard practice in clinical prenatal care with the goal of identifying individuals with increased risk of having a fetus with a chromosomal aneuploidy.[1] While various conventional screens (including first-trimester screening, sequential screening, and others) have now been available for decades, QNatal Advanced and other prenatal cfDNA screens have emerged since 2011 as highly effective tools to screen for fetal aneuploidies.[2] Prenatal cfDNA screens, such as QNatal Advanced, demonstrate clinical utility by improving screening performance beyond that of conventional screens in the general obstetric population, gaining support from professional organizations, and reducing the need for diagnostic procedures that involve fetal risk.		
In response to its clinical performance, prenatal cfDNA screens, such as QNatal Advanced, have been endorsed by professional societies as a suitable strategy for fetal aneuploidy screening in the general pregnancy population. The American College of Obstetricians and Gynecologists (ACOG), the Society for Maternal-Fetal Medicine, the American College of Medical Genetics and Genomics (ACMG), and the International Society for Prenatal Diagnosis support making prenatal cfDNA screens widely available to pregnant women as an option for prenatal screening.[1,2,10] Beyond its improved ability to screen for aneuploidy, prenatal cfDNA screening has additional advantages over conventional screening, including the option for screening to be performed from as early as 10 weeks of gestation until term.[1] The availability of prenatal cfDNA screening at an earlier time point than conventional serum screening allows for additional time to make decisions on pregnancy management. This flexibility on the timing of screening, along with effective clinical performance, have contributed to the increased incorporation of prenatal cfDNA screens, such as QNatal® Advanced, into professional society recommendations.		
In summary, prenatal cfDNA screens, such as QNatal Advanced, have superior clinical performance to that of conventional serum screening in the general pregnancy population, have been endorsed by professional organizations, and may decrease rates of invasive diagnostic procedures. References		
Specific Comments:		
Supporting Evidence	While most clinical studies have evaluated cfDNA in high risk pregnancies, more recent studies[ii] have included a general population of women. In a population that included pregnant women at average risk or high risk of fetal aneuploidy, QNatal Advanced offered by Quest Diagnostics, provided highly accurate discrimination between affected and unaffected pregnancies. A copy of the study is enclosed for your reference.	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.
Supporting Evidence	in the general pregnancy population, which includes both average-risk and high-risk pregnancies, prenatal cfDNA screens such as QNatal Advanced have overall high detection rates for the most common fetal aneuploidies while maintaining low false-positive rates (eg, for T21, detection rates range from 94.12%-100% and false-positive	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions.

Comments		Response
Commenter: Kimberly Martin MD, Chief Clinical Advisor, Natera, Inc		
	rates range from 0%-0.23%).[3-7] A 2017 meta-analysis by Gil et al analyzed prenatal cfDNA screening performance in routinely screened general populations and found that for T21 (n = 7 studies), prenatal cfDNA screens had a pooled detection rate of 99.57% and a false-positive rate of 0.05%; for T18 (n = 6 studies), prenatal cfDNA screens had a detection rate of 95.91% and false-positive rate of 0.07%.[8] Notably, these false-positive rates are substantially lower than the 5% false-positive rate that is typical of conventional serum screening (eg, the combined first-trimester screen).[2] In addition, studies of a general pregnancy population[7] and an average-risk population[9] in which each patient received both prenatal cfDNA screening and conventional aneuploidy screening found that the detection and false-positive rates and positive predictive values (PPVs) of prenatal cfDNA screening were superior to those of conventional screening. For example, in a 2017 study by Langlois et al (n = 1,165), the combined PPV for all screened aneuploidies, which indicates the probability that a positive result is a true-positive case of aneuploidy, was 75% when using prenatal cfDNA screening in a general pregnancy population.[7] In contrast, the same study found that the combined PPV was 7.4% when using conventional serum screening.[7] Therefore, prenatal cfDNA screens, such as QNatal Advanced, provide more effective assessment of risk for fetal aneuploidy than conventional screening[7] in the general pregnancy population.	
Supporting Evidence	Lastly, using prenatal cfDNA screens in the general pregnancy population may decrease rates of invasive diagnostic procedures and subsequent fetal loss.[11-13] Invasive diagnostic procedures are indicated by a positive result from aneuploidy screening in order to determine conclusively whether a fetal chromosomal aneuploidy is present, but these procedures entail risk of miscarriage.[10] Modeling analysis and preliminary clinical findings within the general population suggest that prenatal cfDNA screening can decrease the need for invasive diagnostic procedures due to its low false-positive rate.[11-13] As a result of reduced diagnostic testing, modeling analysis predicted that prenatal cfDNA screening can reduce fetal loss by 73.5%-94% as compared to conventional first-trimester screening in the general pregnancy population.[11,12] Therefore, prenatal cfDNA screens, such as QNatal Advanced, may reduce the number of invasive diagnostic procedures and thereby prevent undue fetal risk.	Articles cited in this public comment will be considered for inclusion in the evidence review using the criteria outlined in the Key Questions

Note.

i. American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. Obstet Gynecol. 2016;127:e123-e137

ii. Guy C, Haji-Sheikhi F, Rowland CM, et al. Prenatal cell-free DNA screening for fetal aneuploidy in pregnant women at average or high risk: Results from a large US clinical laboratory. Molecular Genetics & Genomic Medicine. 2019;7(3):e545.

1. American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. Obstet Gynecol. 2016;127:e123-e137
2. Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18(10):1056-1065.
3. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015;372(17):1589-1597.
4. Quezada MS, Gil MM, Francisco C, et al. Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks' gestation and the combined test at 11-13 weeks. Ultrasound Obstet Gynecol. 2015:45:36-41.
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7. Langlois S, Johnson J, Audibert F, et al. Comparison of first-tier cell-free DNA screening for common aneuploidies with conventional publically funded screening. Prenat Diagn. 2017;37:1238-1244.
8. Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017;50:302-314.
9. Song Y, Liu C, QiH, et al. Noninvasive prenatal testing of fetal aneuploidies by massively parallel sequencing in a prospective Chinese population. Prenat Diagn. 2013;33:700-706.
10. Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2015;35:725-734.
11. Benn P, Curnow KJ, Chapman §, et al. An economic analysis of cell-free DNA non-invasive prenatal testing in the US general pregnancy population. PLoS One. 2015;10:e0132313.
12. Fairbrother G, Burigo J, Sharon T, et al. Prenatal screening for fetal aneuploidies with cell-free DNA in the general pregnancy population: a cost-effectiveness analysis. J Matern Fetal Neonatal Med. 2016;29:1160- 1164.

13. Health Quality, Ontario. Noninvasive prenatal testing for trisomies 21, 18, and 13, sex chromosome aneuploidies, and microdeletions: a health technology assessment. Ont Health Technol Assess Ser. 2019;19:1-166. Received: 26 July 2018 Revised: 4 December 2018 Accepted: 5 December 2018

Public comment and response

See ***Draft key questions: public comment and response*** document published separately.

Coalition for Access to
Prenatal Screening

Tuesday, July 23, 2019

Judy Zerzan, M.D.
Chief Medical Officer
Washington State Health Care Authority
626 8th Avenue SE
Olympia, Washington 98501

Re: The Coalition for Access to Prenatal Screening comment on draft key questions for the Health Technology Assessment of cell-free DNA prenatal screening for chromosomal aneuploidies

Dear Dr. Zerzan,

The Coalition for Access to Prenatal Screening (CAPS) is pleased to submit this comment on draft key questions for the Health Technology Assessment (HTA) of Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies.

According to the HTA website, HTA seeks input on whether key technology assessment questions have included the "appropriate topics to address HTA's mandate to gather evidence on safety, efficacy, and cost effectiveness relevant to coverage determinations."

To guide this, the draft questions should identify "appropriate comparators" as stated on the HTA website. CAPS believes the current draft questions will not adequately compare cell-free DNA noninvasive prenatal screening (NIPS) in the general population to its comparator, standard prenatal aneuploidy screening ("standard screening") in the general population.

An evaluation of the safety, efficacy and cost effectiveness of NIPS must do so in comparison to the existing standard screening.

An evidence review of NIPS should consider its low rate of false positives and false negatives and its high positive predictive value against the false positive rate, false negative rate, and positive predictive value of standard screening as the latter is the standard of care for women regardless of risk on the fee-for-service program in Washington.[1]

To illustrate this, below is an example of the superiority of NIPS:

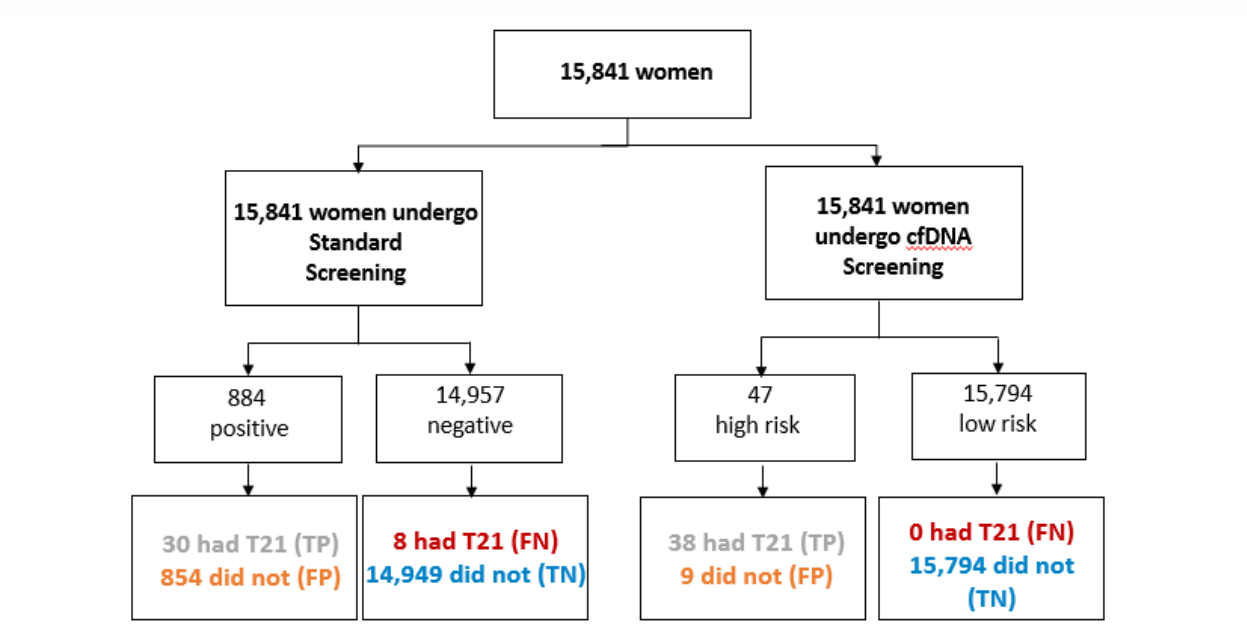
- The positive predictive rate of **NIPS** in the general population for the three trisomies:[1]
    - Trisomy 21: 80.9%
    - Trisomy 18: 90%
    - Trisomy 13: 50%

[1] Norton, M. et al Cell free DNA analysis for noninvasive examination of Trisomy. The New England Journal of Medicine April 2015; 372: 1589-1597.

- The positive predictive rate of **standard screening** in the general population for the three trisomies is below:[1]
    - Trisomy 21: 3.4%
    - Trisomy 18: 14%
    - Trisomy 13: 3.4%

For Trisomy 21, NIPS has a positive predictive value that is 20x more accurate than standard screening in the general population. This improvement in specificity is one of the values of NIPS, and it should be captured in the HTA review of NIPS as it is a more efficacious technology than the current standard of care available to women considered low risk of chromosomal abnormalities on the fee-for-service program.

Below is a summary of the findings from the 2015 study in the New England Journal of Medicine, entitled "Cell-free DNA Analysis for Noninvasive Examination of Trisomy." As the study's population was of both high risk and low risk women, we request its findings be included in the key questions document.



Evaluating the potential harms of NIPS should include a comparison to the potential harms of standard screening:

The draft questions also focus on direct harms of NIPS, described as misdiagnosis and psychosocial harms. An evidence review will identify a minor chance of misdiagnosis with NIPS as it is a screening tool and not a confirmatory diagnostic.

CAPS believes it is important to consider the direct harms (misdiagnosis, psychosocial harms) associated with the standard screening when evaluating the same consequences of NIPS. By doing so, the specificity of NIPS is given context as a vastly superior screen as NIPS

would lessen the exposure to misdiagnosis and its associated harms. NIPS improves upon prenatal screening care, which is already covered for all women regardless of age by the Washington Health Care Authority in the form of standard screening.

Furthermore, CAPS believes the draft key questions reference a withdrawn committee opinion from the American College of Obstetricians and Gynecologists, and if this inaccuracy is included in the final key questions, it could lead to an inaccurate evidence gathering process.

Below are recommendations by CAPS, organized by the respective sections in the draft key questions document:

Clinical need and target population

Review should reference current ACOG guidance:

The HCA's draft document states, "[t]he American College of Obstetricians and Gynecologists (ACOG) has stated that cfDNA screening works best for individuals who already have an increased risk of having a baby with a chromosomal disorder." The source for this information is a web page on ACOG's website featuring frequently asked questions on prenatal screening tests.

CAPS believes the information on the frequently asked questions page is incorrect and inconsistent with ACOG's practice bulletins. CAPS has raised this inconsistency with ACOG as it has not updated this page after the withdrawal of an outdated committee opinion.

According to ACOG Practice Bulletin No.163, "all women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age."[2] In July of 2018, ACOG Committee Opinion No. 693 formally withdraw Committee Opinion No. 640, which originally stated "conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population."[3,4] Therefore, this statement is no longer accurate and should not be used during the HTA of NIPS.

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[2] Practice Bulletin No. 163: Screening for Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. The Society for Maternal-Fetal Medicine. 2016 May. [cited 2017 Mar 23]. Available from: https://s3.amazonaws.com/cdn.smfm.org/publications/224/download-491f0e6962960848d2097447ab57a024.pdf.

[3] Women's Health Care Physicians. (n.d.). Retrieved July 17, 2019, from https://www.acog.org/Clinical-Guidance-and-Publications/Withdrawn-Documents

[4]Committee Opinion No. 640: Cell-Free Dna Screening For Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. 2015 September. Available from: https://journals.lww.com/greenjournal/FullText/2015/09000/Committee_Opinion_No__640__Cell_Free_Dna_Screening.51.aspx

CAPS requests that HTA update the draft key questions to quote the current ACOG policy statement on NIPS. In addition, CAPS requests HTA include in its key questions document the American College of Medical Genetics (ACMG) 2016 Statement, which recommends "informing all pregnant women that NIPS is the most sensitive screening option for traditionally screening aneuploidies (i.e., Patau, Edwards, and Down Syndromes)."[5]

Due to the statements from ACMG and ACOG, we request the removal of the following sentence from the key questions document: "However, clinical practice guideline authors vary in their recommendations, citing challenges with cost and the positioning of cfDNA in the screening and diagnostic pathways."

Review should include more specific information on current insurance coverage of NIPS:

CAPS is pleased with the inclusion of commercial coverage of NIPS in the key questions documents. However, we recommend adding specificity with the following lines:

- 60 major commercial health insurance plans cover NIPS for all women. This includes 40 Blue Cross Blue Shield plans, Cigna, Anthem, and Wellmark.
- Six states, including Florida, Virginia, Minnesota, Ohio, North Dakota, and Pennsylvania now cover NIPS under state Medicaid benefits for pregnant women of all risks. 36 states, including Washington State, cover NIPS for women categorized as high-risk.

Key Questions

We suggest the following edits to Key Question One:

- What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive screening or invasive diagnostic testing) or no screening testing in pregnant individuals not known to be at high risk for chromosomal abnormalities?

We suggest the following addition to Key Question Two:

- What direct harms are associated with standard screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using standard screening methods (instead of cfDNA screening) in pregnant individuals who are both known and not known to be at high risk for chromosomal abnormalities?
- What current methods can differentiate a 'high risk' from a 'low risk' pregnancy with regard to common sex chromosome aneuploidies?

[5]Gregg, A.R., et al. "Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics" *American College of Medical Genetics*. 2016 Jul. [cited 2017 Mar 23] Available from: http://www.acmg.net/docs/NIPS_AOP.pdf
.

We suggest the following edit to Key Question Three:

- Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary – as compared to other active noninvasive interventions - for the mother and fetus or infant, by:
    a. Maternal characteristics (e.g., age)
    b. Singleton or multifetal pregnancy
    c. Timing of screening (e.g., gestational age)

Contextual Question 1: Scope

Comparators

We request specification of "other NIPT technologies" mentioned under inclusion criteria for Comparators as it is not obvious what other technologies this refers to.

Outcomes

Include comparison to standard screen in evaluating safety of NIPS

We recommend adding the following as a bullet:

- Safety: harms directly related to screening for trisomies 21, 18, and 13 and common sex chromosomal aneuploidies using standard screening methods with a high rate of false positives and detection rates that are significantly lower than cfDNA screening, regardless of a priority risk in the tested population

Include relevant cost-effectiveness research:
We request that the review include relevant cost-effectiveness studies, by removing the exclusion of studies older than two years. All U.S. based cost-effective studies on NIPS were conducted earlier than 2017 and therefore should be included in this review.

Study Design

We recommend adding the following as a bullet:

- Clinical experience studies that summarize real-world findings and performance characteristics of cfDNA screening tests as performed by CLIA-certified labs in the U.S.

Contextual Question 1: Analytic Framework

We recommend adding the following to the "Intervention" section of the framework:

- Standard prenatal aneuploidy screening

<u>Conclusion:</u> In order to assess the safety and efficacy of NIPS in the general population, HTA should examine of the false negative rate, false positive rate, and positive predictive value of standard screening as it is the current standard of care for women seeking prenatal screening on the Washington fee-for-service program.

Thank you for the opportunity to comment.

Sincerely,

Marily Rhudy, Secretary and Director
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Coalition for Access to Prenatal Screening

Monday, August 19, 2019

Judy Zerzan, M.D.
Chief Medical Officer
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626 8th Avenue SE
Olympia, Washington 98501

Re: Error in final key questions for the Health Technology Assessment of cell-free DNA noninvasive prenatal screening (NIPS)

Dear Dr. Zerzan,

On behalf of the Coalition for Access to Prenatal Screening (CAPS), I am writing to address an error in "Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies: Final key questions."

On Page 1, the document states, "ACOG recommends that for a woman at low risk of having a child with a chromosome disorder, conventional screening remains the most appropriate choice."

This is inaccurate. In July of 2018, ACOG Committee Opinion No. 693 formally withdrew Committee Opinion No. 640 from circulation. This opinion originally stated, "conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population."[1,2]

Because Opinion No. 640 was withdrawn, the sentence should state "all women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age." This is an accurate quote from ACOG Practice Bulletin No. 163.[3]

The Key Questions document notes the source of the inaccurate quote is "American College of Obstetricians and Gynecologists. Prenatal genetic screening tests. 2017; https://www.acog.org/Patients/FAQs/Prenatal-Genetic-Screening-Tests. Accessed June 17, 2019."

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[1] Women's Health Care Physicians. (n.d.). Retrieved July 17, 2019, from https://www.acog.org/Clinical-Guidance-and-Publications/Withdrawn-Documents

[2]Committee Opinion No. 640: Cell-Free Dna Screening For Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. 2015 September. Available from: https://journals.lww.com/greenjournal/FullText/2015/09000/Committee_Opinion_No__640__Cell_Free_Dna_Screening.51.aspx

[3] Practice Bulletin No. 163: Screening for Fetal Aneuploidy. The American College of Obstetricians and Gynecologists. The Society for Maternal-Fetal Medicine. 2016 May. [cited 2017 Mar 23]. Available from: https://s3.amazonaws.com/cdn.smfm.org/publications/224/download-491f0e6962960848d2097447ab57a024.pdf.

Coalition for Access to
Prenatal Screening

This link connects to a Frequently Asked Questions page, which has been removed by ACOG. That web page currently states, "A revised version of this FAQ is coming soon."

CAPS already requested that the HTA correct this error in our comment to the draft Key Questions, submitted on July 23, 2019. However, the final Key Questions were released without making the correction.

Please correct the reference and quote in the final Key Questions document for the review. CAPS believes it would be an arbitrary and capricious action by the state to submit the questions for analysis without correcting them for factual accuracy.

Thank you for your consideration.

Sincerely,

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Cell-free DNA Analysis for Noninvasive Examination of Trisomy

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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.)

From the University of California, San Francisco, San Francisco (M.E.N.), University of California, San Diego, San Diego (L.C.L.), Perinatal Diagnostic Center, Riverside (H.B.), and Ariosa Diagnostics, San Jose (D.H., T.J.M.) — all in California; Sahlgrenska University Hospital, Gothenburg, Sweden (B.J.); Duke University, Durham, NC (G.K.S.); Saint Peter's University Hospital, New Brunswick, NJ (A.C.R.); Northwest Perinatal Center (M.W.T.) and Oregon Health and Science University (L.P.) — both in Portland; Perinatal Quality Foundation, Oklahoma City (J.L.S.); and Columbia University, New York (H.C., R.J.W.). Address reprint requests to Dr. Norton at the Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, 550 16th St., 7th Fl., San Francisco, CA 94143, or at mary.norton@ucsf.edu.

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N Engl J Med 2015;372:1589-97.
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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 represent 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-trimester 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 aneuploidy screening at 35 centers in six countries. At enrollment, maternal blood was drawn, locally deidentified, and sent for risk assessment for trisomy 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 (Veristat). 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 collaboration with the sponsor. Representatives of the sponsor performed the analyses and interpretation 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 entered 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 conducted 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. Peripheral 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 (including 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[5] 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.[6-8] For cfDNA testing, samples were rejected if they were not collected in Cell-free DNA BCT tubes; if the tubes were broken, unfilled, 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[8] (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

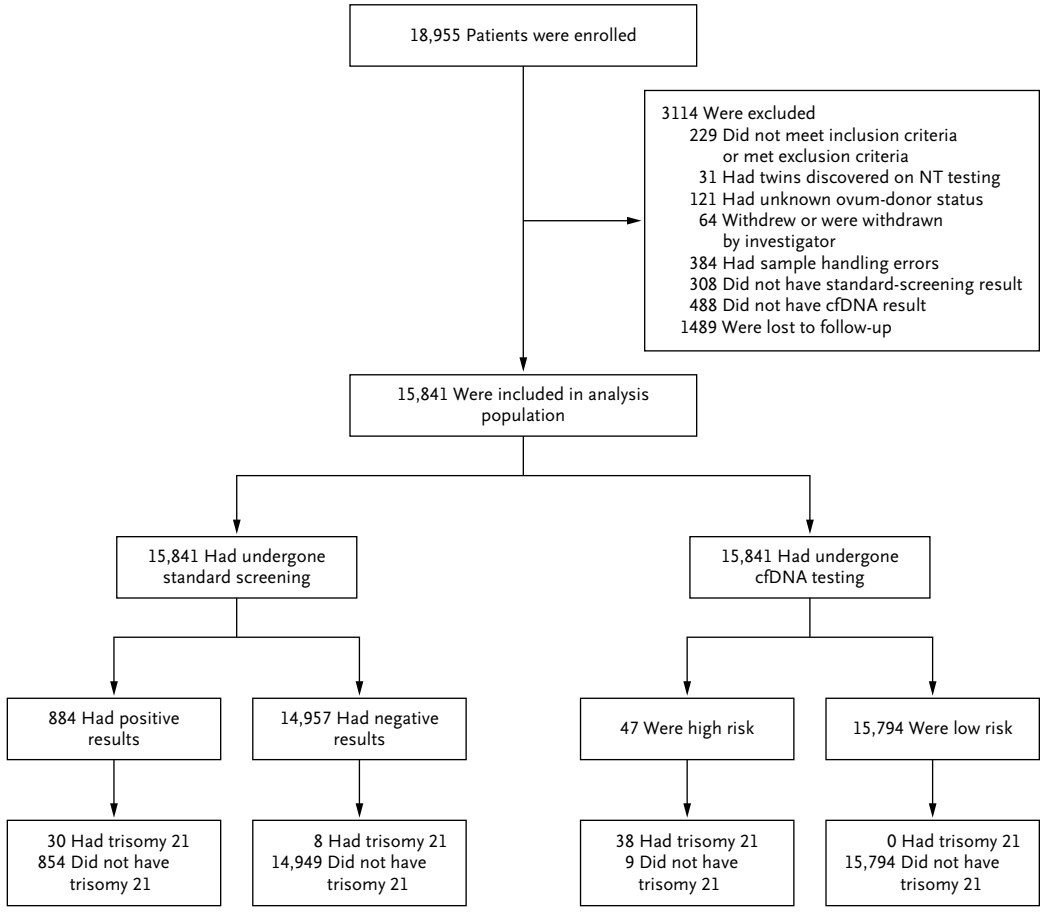

Figure 1. Enrollment and Outcomes.
The abbreviation cfDNA denotes cell-free DNA, and NT nuchal translucency.

were compared with the use of a z-test according to the method of DeLong et al.[9] 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[10] for paired comparisons in sensitivity and specificity and used the generalized score statistic[11] 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[12,13] 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.

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.

Table 1. Demographic and Clinical Characteristics of the Patients.

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

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

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

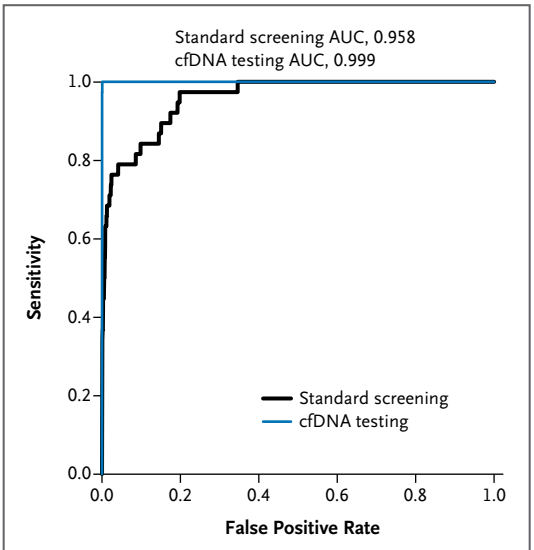

Figure 2. Primary Outcome for Trisomy 21 Screening.

The primary outcome was the area under the receiver-operating-characteristic curve (AUC) for trisomy 21 screening with cell-free DNA (cfDNA) testing versus standard screening in women with complete results for the two tests. The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (P=0.001). The use of cfDNA testing identified 38 of 38 cases of trisomy 21, for a sensitivity of 100% (95% confidence interval [CI], 90.7 to 100), as compared with 30 of 38 cases for standard screening, for a sensitivity of 78.9% (95% CI, 62.7 to 90.4; P=0.008).

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, as 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.

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

Variable	Standard Screening	Cell-free DNA Testing		
	All Patients (N=15,841)	All Patients (N=15,841)	Maternal Age <35 Yr (N=11,994)	Low Risk (N=14,957)†
True positive — no.	30	38	19	8
True negative — no.	14,949	15,794	11,969	14,941
False positive — no.	854	9	6	8
False negative — no.	8	0	0	0
Sensitivity (95% CI) — %	78.9 (62.7–90.4)	100 (90.7–100)‡	100 (82.4–100)	100 (63.1–100)
Specificity (95% CI) — %	94.6 (94.2–94.9)	99.9 (99.9–100)§	99.9 (99.9–100)	99.9 (99.9–100)
Positive predictive value (95% CI) — %	3.4 (2.3–4.8)	80.9 (66.7–90.9)§	76.0 (54.9–90.6)	50.0 (24.7–75.3)
Negative predictive value (95% CI) — %	99.9 (99.9–100)	100 (99.9–100)¶	100 (99.9–100)	100 (99.9–100)
Positive likelihood ratio	14.6	1755.9	1995.8	1868.6
Negative likelihood ratio	0.22	0	0	0

* 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<0.001
¶ P=0.005.

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

Metric	Trisomy 18		Trisomy 13	
	Standard Screening (N=15,841)	Cell-free DNA Testing (N=15,841)	Standard Screening (N=11,185)	Cell-free DNA Testing (N=11,185)
True positive — no.	8	9	1	2
True negative — no.	15,782	15,830	11,155	11,181
False positive — no.	49	1	28	2
False negative — no.	2	1	1	0
Sensitivity (95% CI) — %	80.0 (44.4–97.5)	90.0 (55.5–99.7)	50.0 (1.2–98.7)	100 (15.8–100)
Specificity (95% CI) — %	99.7 (99.6–99.8)	100 (99.9–100)†	99.7 (99.6–99.8)	100 (99.9–100)†
Positive predictive value (95% CI) — %	14.0 (6.2–25.8)	90.0 (55.5–99.7)†	3.4 (0.1–17.8)	50.0 (6.8–93.2)
Negative predictive value (95% CI) — %	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)

* Included in the trisomy 13 analysis are patients who were enrolled after September 2012.
† P<0.001 for the comparison with standard screening.

DISCUSSION

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

fraction. In previous studies, obesity was associated with a low fetal fraction,[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[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.[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.[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.[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.[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.[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 ultrasonography, 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,[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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(See also Practice Bulletin Number 162, *Prenatal Diagnostic Testing for Genetic Disorders*)

Screening for Fetal Aneuploidy

Prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. In contrast, prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. The purpose of prenatal screening for aneuploidy is to provide an assessment of the woman's risk of carrying a fetus with one of the more common fetal aneuploidies. This is in contrast to prenatal diagnostic testing for genetic disorders, in which the fetal chromosomes are evaluated for the presence or absence of abnormalities in chromosome number, deletions, and duplications, or the fetal DNA is evaluated for specific genetic disorders. The wide variety of screening test options, each offering varying levels of information and accuracy, has resulted in the need for complex counseling by the health care provider and complex decision making by the patient. No one screening test is superior to other screening tests in all test characteristics. Each test has relative advantages and disadvantages. It is important that obstetrician–gynecologists and other obstetric care providers be prepared to discuss not only the risk of aneuploidy but also the benefits, risks, and limitations of available screening tests. Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.

The purpose of this Practice Bulletin is to provide current information regarding the available screening test options for fetal aneuploidy and to review their benefits, accuracy, and limitations. For information regarding prenatal diagnostic testing for genetic disorders, refer to Practice Bulletin No. 162, Prenatal Diagnostic Testing for Genetic Disorders.

Background

Aneuploidy is defined as having one or more extra or missing chromosomes, leading to an unbalanced chromosome number in a cell. Because each chromosome consists of hundreds of genes, the loss or gain of large chromosomal segments disrupts significant amounts of genetic material and often results in a nonviable pregnancy or offspring that may not survive after birth. In the case of a surviving newborn, congenital birth defects; failure to thrive; and functional abnormalities, including mild-to-severe intellectual disability, infertility, and shortened lifespan, may occur.

Although chromosomal abnormalities occur in approximately 1 in 150 live births (1), the prevalence is greater earlier in gestation because aneuploidy accounts for a large proportion of early pregnancy loss. The incidence of fetal aneuploidy increases as a woman ages (Table 1) but can affect any woman regardless of age and is not related to race or ethnicity. Other factors that increase the risk of fetal aneuploidy include a history of a prior aneuploid fetus and the presence of fetal anomalies. Autosomal trisomies are the most common aneuploidies that are not related to sex chromosome disorders. Down syndrome (trisomy 21) is the most common of these, with a prevalence of approximately 1 in 800 live births

Committee on Practice Bulletins—Obstetrics, Committee on Genetics, and Society for Maternal–Fetal Medicine. This Practice Bulletin was developed by the American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics, Committee on Genetics, and the Society for Maternal–Fetal Medicine in collaboration with Nancy C. Rose, MD and Brian M. Mercer, MD. The information is designed to aid practitioners in making decisions about appropriate obstetric and gynecologic care. These guidelines should not be construed as dictating an exclusive course of treatment or procedure. Variations in practice may be warranted based on the needs of the individual patient, resources, and limitations unique to the institution or type of practice.

(1). The most common sex chromosome aneuploidy is Klinefelter syndrome (47,XXY) with a prevalence of 1 in 500 males. The only viable monosomy is Turner syndrome (45,X).

Down syndrome is the most common form of inherited intellectual disability, with approximately 6,000 affected infants born in the United States each year. It is estimated that 95% of cases of Down syndrome result from nondisjunction involving chromosome 21. The remaining cases result from translocations or somatic mosaicism (2). Although the clinical presentation of Down syndrome can vary, it is associated with characteristic facial features, learning disabilities, congenital heart defects (eg, atrioventricular canal defects), intestinal atresia, seizures, childhood leukemia, and early-onset Alzheimer disease. Fetuses affected with Down syndrome often do not survive pregnancy; between the first trimester and full term, an estimated 43% of pregnancies end in miscarriage or stillbirth (3). In economically developed countries, the median survival of individuals with Down syndrome is now almost 60 years (4). Factors associated with an increased risk of Down syndrome include higher maternal age, a parental translocation involving chromosome 21, a previous child with a trisomy, significant ultrasonographic findings, and a positive screening test result. After a prenatal diagnosis is made, prenatal assessment cannot predict the severity of the complications from Down syndrome.

In general, the process of aneuploidy screening identifies two groups of individuals: 1) those with a positive screening test result who have an increased risk of having a fetus with an aneuploidy and 2) those with a negative screening test result who have a lower posttest probability of the evaluated aneuploidies. Women with a positive screening test result should be counseled regarding their higher risk of aneuploidy and offered the option of diagnostic testing. Those who have a negative test result should be counseled regarding their lower adjusted risk and their lower residual risk. Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result. Even if a woman has a negative test result, she may choose diagnostic testing later in pregnancy, particularly if additional findings become evident (eg, fetal anomalies or markers of aneuploidy identified on follow-up ultrasonography).

Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit. The choice of whether to perform screening or diagnostic testing for aneuploidy depends on the woman's goals and values and her desire for informational accuracy. Although maternal age may

Table 1. Risk of Chromosomal Abnormalities Based on Maternal Age at Term

Age at Term	Risk of Trisomy 21*	Risk of Any Chromosome Abnormality†
15‡	1:1,578	1:454
16‡	1:1,572	1:475
17‡	1:1,565	1:499
18‡	1:1,556	1:525
19‡	1:1,544	1:555
20	1:1,480	1:525
21	1:1,460	1:525
22	1:1,440	1:499
23	1:1,420	1:499
24	1:1,380	1:475
25	1:1,340	1:475
26	1:1,290	1:475
27	1:1,220	1:454
28	1:1,140	1:434
29	1:1,050	1:416
30	1:940	1:384
31	1:820	1:384
32	1:700	1:322
33	1:570	1:285
34	1:456	1:243
35	1:353	1:178
36	1:267	1:148
37	1:199	1:122
38	1:148	1:104
39	1:111	1:80
40	1:85	1:62
41	1:67	1:48
42	1:54	1:38
43	1:45	1:30
44	1:39	1:23
45	1:35	1:18
46	1:31	1:14
47	1:29	1:10
48	1:27	1:8
49	1:26	1:6
50	1:25	§

*Data from Morris JK, Wald NJ, Mutton DE, Alberman E. Comparison of models of maternal age-specific risk for Down syndrome live births. Prenat Diagn 2003;23:252–8.

†Risk of any chromosomal abnormality includes the risk of trisomy 21 and trisomy 18 in addition to trisomy 13, 47,XXY, 47,XYY, Turner syndrome genotype, and other clinically significant abnormalities, 47,XXX not included. Data from Hook EB. Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 1981;58:282–5.

‡Data from Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94:387–402.

§Data not available.

be helpful in adjusting an individual woman's risk of having a fetus with aneuploidy, it should not be used as the sole determinant of whether aneuploidy screening or diagnostic testing is offered. Although the risk of aneuploidy increases with advancing maternal age, most children with Down syndrome are born to younger women because a larger proportion of all children are born to young women. An observational study of more than 38,000 women demonstrated that if all women aged 35 years and older had had diagnostic testing, the detection rate for Down syndrome would have been only 21.6% (5).

Screening tests for aneuploidy are now available for pregnant women in all trimesters of pregnancy. Among these are first-trimester, triple, quad, and penta screens; cell-free DNA; and ultrasonographic screening as single screening tests. Screening tests that are performed in the first and second trimesters include integrated, sequential, and contingent screening.

The intent of counseling for aneuploidy is to inform the pregnant woman about chromosomal disorders, provide information regarding her specific risk of carrying a fetus with aneuploidy, and review the available options so that she can make an informed choice regarding screening or diagnostic testing. After review and discussion, every patient has the right to pursue or decline screening or diagnostic testing. Pretest and posttest counseling are essential and must be a part of any screening program. When a positive or negative screening test result is obtained, the patient should be counseled regarding the adjusted likelihood of carrying a fetus with the evaluated aneuploidies. The potential for the fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should be reviewed. In the event that a prenatal diagnosis of fetal aneuploidy is made, the patient must be counseled appropriately so that she can make informed decisions regarding pregnancy management. Counseling should include family education and preparedness as well as options regarding adoption, pregnancy termination, referral to a tertiary care center for delivery of the newborn if needed, and perinatal hospice care as appropriate for a child with a condition that is incompatible with life. Patients found to have a fetus with a chromosomal abnormality often benefit from referral to a genetics professional for further detailed counseling.

Single Screening Tests

First-Trimester Screening

Typically performed when the crown–rump length measures between 38–45 mm and 84 mm (generally between 10 0/7 weeks and 13 6/7 weeks of gestation), first-trimester screening includes a nuchal translucency measurement, serum free β-hCG, or total human chorionic gonadotropin (hCG) along with pregnancy-associated plasma protein A analyte levels. A specific risk estimate for aneuploidy is calculated using these results as well as maternal factors such as maternal age, prior history of aneuploidy, weight, race, and number of fetuses.

The nuchal translucency refers to the fluid-filled space measured on the dorsal aspect of the fetal neck. An enlarged nuchal translucency (often defined as 3.0 mm or more or above the 99th percentile for the crown–rump length) is independently associated with fetal aneuploidy and structural malformations. The risk of adverse pregnancy outcome is proportional to the degree of nuchal translucency enlargement. Meticulous technique in nuchal translucency imaging is essential for accurate risk assessment because undermeasurement by even 0.5 mm can reduce the test sensitivity by 18% (6). Independent credentialing of ultrasonographers in appropriate technique is important to screening performance.

Quadruple Screen

The quadruple marker screen ("quad" screen) can be performed from approximately 15 0/7 weeks to 22 6/7 weeks of gestation; the range is dependent on the laboratory that the obstetrician–gynecologist or other obstetric care provider uses. This test does not require specialized ultrasonography for nuchal translucency measurement and gives information regarding the risk of open fetal defects in addition to aneuploidy risk assessment. The best time to perform a quad screen is from approximately 16 weeks to 18 weeks of gestation because this optimizes screening for neural tube defects. The quad screen involves the measurement of four maternal serum analytes: 1) hCG, 2) alpha fetoprotein (AFP), 3) dimeric inhibin A, and 4) unconjugated estriol, in combination with maternal factors such as age, weight, race, the presence of diabetes, and plurality to calculate a risk estimate. First-trimester and quad screening have similar detection rates for Down syndrome: more than 80% at a 5% positive result rate (Table 2) (5). Accurate gestational dating at the time of blood sampling is important because inaccurate gestational dating decreases the accuracy of the result. The later timing of this test leaves fewer options available for the patient if the results are positive.

Penta Screen

The penta screen includes hyperglycosylated hCG (also known as invasive trophoblast antigen) in addition to the quad screen markers and also is available for

Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Aneuploidy

Screening Test	Gestational Age Range for Screening (Weeks)	Detection Rate for Down Syndrome (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
First trimester[†]	11–14[‖]	82–87	5	1. Early screening 2. Single test 3. Analyte assessment of other adverse outcome	Lower DR than combined tests NT required	NT+PAPP-A and hCG
Triple screen	15–22	69	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than with first-trimester or quad screening Lowest accuracy of the single lab tests	hCG, AFP, uE3
Quad screen[†]	15–22	81	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than combined tests	hCG, AFP, uE3, DIA
Integrated[†]	11–14, then 15–22	96	5	Highest DR of combined tests Also screens for open fetal defects	Two samples needed before results are known	NT+PAPP-A, then quad screen
Sequential[‡]: Stepwise	11–14, then 15–22	95	5	First-trimester results provided; Comparable performance to integrated, but FTS results provided; also screens for open fetal defects; analyte assessment for other adverse outcomes.	Two samples needed	NT+hCG+ PAPP-A then quad screen
Contingent screening[‡]		88–94	5	First-trimester test result: Positive: diagnostic test offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed	NT+hCG+ PAPP-A, then quad screen
Serum Integrated[†]	11–14; then 15–22	88	5	1. DR compares favorably with other tests. 2. No need for NT	Two samples needed; no first-trimester results	PAPP-A+quad
Cell-free DNA[§]	10 - term	99 (in patients who receive a result)	0.5	1. Highest DR for Down syndrome 2. Can be performed at any gestational age after 10 weeks 3. Low false-positive rate in high-risk women (or women at high risk of Down syndrome)	1. NPV and PPV not clearly reported 2. Higher false-positive rate in women at low risk of Down syndrome 3. Limited information about three trisomies and fetal sex 4. Results do not always represent a fetal DNA result	Three roughly equivalent molecular methods
Nuchal Translucency[†]	11–14[‖]	64–70	5	Allows individual fetus assessment in multifetal gestations Provides additional screening for fetal anomalies and possibly for twin–twin transfusion syndrome	1. Poor screen in isolation 2. Ultrasound certification necessary	US only

Abbreviations: AFP, alpha fetoprotein; DIA, dimeric inhibin-A; DR, detection rate; DS, Down syndrome; FTS, first-trimester screening; hCG, human chorionic gonadotropin; NPV, negative predictive value; NT, nuchal translucency; NTD, neural tube defect; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; uE3, unconjugated estriol; US, ultrasonography.

*A screen positive test result includes all positive test results: the true positives and false positives.

[†]First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11 weeks, 12 weeks, and 13 weeks, respectively. Malone FASTER 2005.

[‡]Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

[§]Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group [published erratum appears in Obstet Gynecol 2012;120:957]. Obstet Gynecol 2012;119:890–901 *and* Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.

[‖]Because of variations in growth and conception timing, some fetuses at the lower and upper gestational age limits may fall outside the required crown–rump length range.

Data from Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

second-trimester screening (7) by at least one national laboratory. Although there is some evidence from one limited retrospective trial that this test may improve second-trimester screening performance, its performance has not been evaluated rigorously in prospective studies and it is not widely used. Limited data are available to compare the accuracy of the penta screen with other second-trimester screening tests.

Triple Screen

The triple marker screen measures serum hCG, AFP, and unconjugated estriol to determine a risk estimate. This test provides a lower sensitivity for the detection of Down syndrome (sensitivity of 69% at a 5% positive screening test result rate) than quad screen and first-trimester screening (5).

Combined First- and Second-Trimester Screening

Combined first- and second-trimester screening with either integrated, sequential, or contingent screening protocol provides a higher detection rate than one-step screening. Depending on the test selected, results are not available until the second trimester or possibly in the first trimester under certain circumstances.

Integrated Screening and Serum Integrated Screening

With integrated screening, the patient undergoes a first-trimester nuchal translucency measurement and analyte screening followed by a second-trimester quad screen and receives a single test result in the second trimester. In locations where a nuchal translucency measurement by a certified ultrasonographer is unavailable, or if fetal position, maternal body habitus, or imaging properties preclude an accurate nuchal translucency measurement, serum integrated screening can be offered. Serum integrated screening has a similar but slightly lower detection rate than integrated screening (Table 2). Limitations of integrated screening include the withholding of first-trimester screening test results until the second trimester and nonadherence of the second blood draw; rates of nonadherence in practice have been reported to be as high as 25% without a written reminder to complete the test (8).

Sequential Screening: the Stepwise and Contingent Screening Models

Sequential screening was developed to maintain a high detection rate using the combined first- and second-trimester screening approach while also reporting the patient's first-trimester screening test risk, which allows for earlier management options. Using stepwise sequential screening, the patient is given a preliminary risk estimate after completion of the first-trimester analytes and nuchal translucency screening. If the first-trimester screening result indicates that the risk of aneuploidy is greater than the laboratory-derived positive screening cutoff, the patient is notified and offered a diagnostic test or cell-free DNA screening, and the screening protocol is discontinued. If the patient has a lower risk than the cutoff level, she is informed that she has received a negative screening test result and proceeds to quad screening in the second trimester. Sequential screening has a detection rate of 91–93% with a positive screening test result rate of 4–5% (9, 10).

The contingent model classifies aneuploidy risk as high, intermediate, or low on the basis of the first-trimester screening test results; women at high risk are offered cell-free DNA screening or diagnostic testing with chorionic villus sampling (CVS), and for those at low risk, no further screening or testing is recommended. Only those women at intermediate risk are offered second-trimester screening and, thus, fewer women go on to second-trimester screening.

In the stepwise and contingent models, the patients at highest risk identified by first-trimester screening are offered an early diagnostic procedure. First- and second-trimester results are used together to calculate a final risk of aneuploidy in patients at lower risk in the stepwise and sequential models. The sequential approach takes advantage of the higher detection rate achieved by incorporating the first- and second-trimester screening test results with only a marginal increase in the false-positive rate. Theoretically, the contingent approach should maintain high detection rates with low false-positive rates while reducing the number of second-trimester tests performed.

The use of multiple screening tests performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver confusing risk estimates to patients. In patients who undergo first-trimester screening, if later screening for risk of neural tube defects is to be done with maternal serum alpha-fetoprotein (MSAFP), the test should be performed as an isolated screening test and not as part of a quad screen.

Ultrasonographic Screening

Although fetuses with trisomy 13 (Patau syndrome, which occurs in 1 in 10,000 births) or trisomy 18 (Edwards syndrome, which occurs in 1 in 6,000 births)

usually have major structural anomalies that are evident on ultrasonography, the ultrasonographic identification of Down syndrome is more elusive. For several decades, the second-trimester "genetic ultrasonogram" has been used to screen for Down syndrome using specific ultrasonographic findings (11). This approach seeks to identify major structural abnormalities and minor ultrasonographic "soft markers" of aneuploidy. The major structural anomalies associated with fetal Down syndrome include cardiac anomalies (such as septal defects, tetralogy of Fallot, and atrioventricular canal defects) usually identified in the second trimester and duodenal atresia, which typically is identified in the third trimester. In contrast, second- and third-trimester soft ultrasonographic markers for aneuploidy are nonspecific physical characteristics that are more common among fetuses with Down syndrome and in some cases also can reflect or progress to an overt fetal abnormality (eg, thickened nuchal fold, renal pelvis dilation, or echogenic bowel). Because soft markers for aneuploidy also are common in unaffected fetuses, it is difficult to use these findings to distinguish between pregnancies affected or unaffected by aneuploidy. As an isolated finding, an increased nuchal skinfold thickness confers the highest risk of aneuploidy. In contrast, an isolated echogenic intracardiac focus carries one of the lowest risks of fetal aneuploidy (12, 13). If an isolated low-risk marker such as a choroid plexus cyst or intracardiac echogenic focus is identified on the fetal anatomic ultrasound survey, the patient's chart should be reviewed to determine if analyte screening has been performed previously; if not, it should be offered. Additional follow-up for isolated ultrasonographic markers generally is not indicated other than for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur length (14). Patients with these markers may benefit from referral for detailed ultrasonography and follow-up. Major limitations of the use of second-trimester ultrasonographic markers include the lack of standardization in measurements and characteristics that define a positive test result, and the lack of understanding of how factors such as high maternal body mass index, multiple gestation, machine quality, and experience of the ultrasonographer and ultrasonologist affect screening performance.

Cell-free DNA Screening

Cell-free DNA screening evaluates short segments of DNA in maternal blood and can be used to screen for a variety of fetal conditions. The fetal component of cell-free DNA is released into the maternal circulation primarily from placental cells undergoing apoptosis or programmed cell death and comprises approximately 3–13% of the total cell-free DNA in maternal blood (15). This amount increases throughout gestation and is cleared from the maternal circulation within hours after childbirth (16). Several molecular methods have been developed to analyze cell-free DNA for the purpose of aneuploidy screening, and all appear to have similar detection and false-positive rates, although direct comparison trials have not been performed. Cell-free DNA screening also can be used to determine fetal sex, to identify the presence of a Rh-positive fetus in a Rh-negative mother, and to detect some paternally derived autosomal dominant genetic abnormalities (17–19). Screening can be performed from as early as 10 weeks of gestation until term and offers the highest reported detection rate for Down syndrome: more than 98% detection with positive screening rates of less than 0.5% among women with a reportable result (20). The detection rate is lower for trisomy 13 and trisomy 18 (21–27). Further, published studies have excluded those who have no reportable result, and these women are at increased risk of fetal aneuploidy (22, 23, 28). Inclusion of these women in the calculations would yield lower sensitivity for fetal aneuploidy. In addition, managing women with no reportable result as screen positive will decrease the specificity and increase the positive screening rate for this testing.

Clinical Considerations and Recommendations

▶ *Who should be offered screening for aneuploidy?*

All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age. The choice of screening test is affected by many factors, including a desire for information before delivery, prior obstetric history, family history, and the number of fetuses. Other factors to be considered include gestational age at presentation, the availability of a reliable nuchal translucency measurement, screening test sensitivity and limitations, the cost of screening, the constraints of long-term care of an affected child, and options for pregnancy care or termination for an abnormal diagnostic test result. No one test is superior for all test characteristics and not every test is available at all centers. Each test has advantages and disadvantages that should be discussed with each patient, with the appropriate test offered based on her concerns, needs, and values. Obstetrician–gynecologists and other obstetric care providers should become familiar with the available screening and diagnostic testing options for their patients within their practice and adopt a standard approach for counseling. Regardless of which screening tests are

offered, information about the detection (sensitivity) and positive screening and false-positive rates, advantages, disadvantages, and limitations should be communicated to the patient. At the time of counseling regarding aneuploidy screening, the benefits and risks of diagnostic testing (amniocentesis and chorionic villus sampling) also should be discussed (29). After counseling, patients may decline screening or diagnostic testing for any reason.

▶ *What is the role of ultrasonography in screening for fetal aneuploidy?*

In women of advanced maternal age, the absence of ultrasonographic markers has been used to decrease a woman's age-related risk of aneuploidy by greater than 80% (30, 31). However, with the exception of maternal age, second-trimester ultrasonography is the least effective primary screening test for Down syndrome, detecting only 50–60% of affected fetuses. As such, ultrasonography should not be used in isolation to diagnose or exclude Down syndrome. Ultrasonographic markers can identify other disorders, and the various soft markers have different degrees of association with Down syndrome. The risk of aneuploidy associated with each marker should be considered individually within the complete clinical context. The presence of soft ultrasonographic markers for aneuploidy warrants a targeted ultrasound to exclude other evident abnormalities and a review or offering of screening tests for fetal aneuploidy. Of the soft markers, third-trimester follow-up is only indicated for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur (14). For women who have already undergone screening for aneuploidy and have received a negative screening test result, and for those who have had normal diagnostic testing, ultrasonography should not be used as an additional screening test for aneuploidy. If aneuploidy screening has been performed before ultrasonographic evaluation, no additional evaluation is indicated if an echogenic intracardiac feature or choroid plexus cysts is the sole identified marker (Table 3). However, further detailed counseling is recommended for fetuses with a hypoplastic or absent nasal bone, echogenic bowel, or nuchal skinfold thickening (14). If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.

With regard to first-trimester imaging, an increased nuchal translucency measurement increases the risk of genetic syndromes and isolated anomalies, such as congenital heart defects, abdominal wall defects, and diaphragmatic hernia, even with normal chromosomes on diagnostic testing (32). These patients should be offered a targeted ultrasound examination and fetal echocardiography in the second trimester.

The finding of an increased nuchal translucency extending along the length of the fetus in which septations are clearly visible is referred to as a cystic hygroma. This finding is associated with a 50% likelihood of fetal aneuploidy (most commonly Down syndrome, 45,X, and trisomy 18). Of the remaining euploid fetuses, one half will have a major structural malformation, such as congenital heart defects, diaphragmatic hernia, or skeletal dysplasia, or other genetic syndromes. Less than 20% of such pregnancies will result in a healthy live-born infant at term (33). A nuchal measurement for aneuploidy risk is not necessary at the time of cell-free DNA screening in the first trimester. However, ultrasound examination is useful to confirm viability, to confirm the number of fetuses and the presence of an empty gestational sac, to assign gestational age, and to identify some major fetal anomalies for patients who choose to have cell-free DNA screening (34). Patients who choose serum integrated screening may be offered first-trimester ultrasonography for gestational dating even if nuchal translucency measurement is unavailable or cannot be obtained. If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities. Given the high risk of congenital heart disease in these fetuses, referral for fetal cardiac ultrasonography should be considered. Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.

▶ *What are the characteristics and limitations of the different screening tests?*

First-Trimester Screening

The first-trimester screening, or first-trimester combined screening, comprising nuchal translucency measurement and serum analyte measurements combined into a single test, is performed before 14 0/7 weeks of gestation (with the range determined by the laboratory accepting the sample, typically between 10 0/7 weeks and 13 6/7 weeks of gestation) and requires a crown–rump length between approximately 38–45 mm and 84 mm. Advantages of first-trimester screening are a slightly higher, but not significantly different, detection rate for Down syndrome compared with second-trimester screening. This test gives the potential for earlier diagnoses as well as the ability to screen for other fetal or placental disorders. However, first-trimester screening lacks the ability to assess the risk

Table 3. Management of Ultrasonographic Markers for Aneuploidy

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
First trimester: enlarged nuchal translucency	Certified ultrasonography measurement ≥ 3.0 mm or above the 99th percentile for the CRL	Aneuploidy risk increases with size of NT Also associated with Noonan syndrome, multiple pterygium syndrome, skeletal dysplasias, congenital heart disease, and other anomalies	1. Genetic counseling 2. Offer cfDNA or CVS 3. Second-trimester detailed anatomic survey and fetal cardiac ultrasonography
First trimester: cystic hygroma	Large single or multilocular fluid-filled cavities, in the nuchal region and can extend the length of the fetus	If septate, approximately 50% are aneuploid	1. Genetic counseling 2. Offer CVS 3. Second-trimester detailed fetal anatomic survey and fetal cardiac ultrasonography
Second trimester: echogenic intracardiac foci	Echogenic tissue in one or both ventricles of the heart seen on standard four-chamber view	LR 1.4–1.8 for Down syndrome Seen in 15–30% of Down syndrome and 4–7% euploid fetuses	1. If isolated finding, aneuploidy screening should be offered if not done previously 2. If aneuploidy screen result is negative, no further evaluation is required.
Second trimester: pyelectasis	Renal pelvis measuring ≥ 4 mm in anteroposterior diameter up to 20 weeks of gestation	LR 1.5–1.6 for Down syndrome	1. If isolated finding, aneuploidy screening should be offered if not performed previously 2. Repeat ultrasonography in third trimester for potential urinary tract obstruction
Second trimester: echogenic bowel	Fetal small bowel as echogenic as bone	LR 5.5–6.7 for Down syndrome Associated with aneuploidy, intra-amniotic bleeding, cystic fibrosis, CMV	1. Further counseling 2. Offer CMV, CF, and aneuploidy screening or diagnostic testing
Second trimester: thickened nuchal fold	≥ 6 mm from outer edge of the occipital bone to outer skin in the midline	LR 11–18.6 with 40–50% sensitivity and > 99% specificity for Down syndrome Most powerful second-trimester marker	1. Detailed anatomic survey 2. Further detailed genetic counseling and aneuploidy screening or diagnostic testing
Second trimester: mild ventriculomegaly	Lateral ventricular atrial measurement between 10–15 mm	Associated with aneuploidy LR 25 for Down syndrome	1. Genetic counseling 2. Second-trimester detailed anatomic ultrasound evaluation 3. Consider diagnostic testing for aneuploidy and CMV 4. Repeat ultrasound in third trimester
Second trimester: choroid plexus cysts	Discrete cyst(s) in one or both choroid plexus(es)	In isolation, no aneuploidy association	1. Second-trimester detailed anatomic survey and fetal cardiac ultrasound 2. No further follow-up if isolated 3. Consider aneuploidy screening or diagnostic testing if other markers are present
Second trimester: short femur length	Measurement < 2.5 percentile for gestational age	LR 1.2–2.2 for Down syndrome. Can be associated with aneuploidy, IUGR, short limb dysplasia	1. Second-trimester detailed fetal anatomic evaluation for short limb dysplasia 2. Further detailed counseling 3. Consider repeat ultrasonography in third trimester for fetal growth

Abbreviations: CF, cystic fibrosis; cfDNA, cell-free DNA; CMV, cytomegalovirus; CRL, crown–rump length; CVS, chorionic villus sampling; IUGR, intrauterine growth restriction; LR, likelihood ratio; NT, nuchal translucency.

Data from Reddy UM, Abuhamad AZ, Levine D, Saade GR. Fetal imaging: executive summary of a joint Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Institute of Ultrasound in Medicine, American College of Obstetricians and Gynecologists, American College of Radiology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound Fetal Imaging workshop. Fetal Imaging Workshop Invited Participants. Obstet Gynecol 2014;123:1070–82; Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, et al. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. FASTER Trial Research Consortium. Obstet Gynecol 2005;106:288–94; Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, et al. Role of second-trimester genetic sonography after Down syndrome screening. First and Second Trimester Evaluation of Risk Research Consortium. Obstet Gynecol 2009;114:1189–96; *and* Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ 1992;304:867–9.

of open fetal defects and relies on the availability of a certified ultrasonographer. Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.

Second-Trimester Serum Screening Tests

Second-trimester serum screening, which typically is performed between approximately 15 0/7 weeks and 22 6/7 weeks of gestation, provides an adjusted risk assessment for Down syndrome, trisomy 18, and open fetal defects. The detection rate with quad screening is similar to first-trimester screening: more than 80% detection at a 5% positive screening test result rate for Down syndrome. Some laboratories offer additional screening for rare disorders such as Smith–Lemli–Opitz syndrome and placental sulfatase deficiency if indicated by an extremely low unconjugated estriol value. Also performed in the second trimester, the triple screen is less sensitive for Down syndrome (sensitivity of 69% at a 5% positive screening test result rate). The penta screen has no prospective validation trials to determine its efficacy; using modeling, it appears to perform well with the inclusion of invasive trophoblast antigen as an additional screening marker (7). None of these screening tests require specialized ultrasonographic measurements, although accurate gestational dating improves risk accuracy determinations.

Integrated Screening

Integrated screening combines first-trimester nuchal translucency and serum analyte screening with second-trimester quad screening to give one result for aneuploidy risk, with a detection rate for Down syndrome of approximately 96% at a 5% positive screening test result rate (Table 2). In addition to having a high sensitivity for Down syndrome, integrated screening provides information that is not available from nuchal translucency assessment regarding fetal abnormalities as well as a risk assessment for open fetal defects. However, integrated screening is complex, requiring first-trimester ultrasound assessment and two different blood tests, and the final result is not available until the second trimester.

Sequential Screening: Stepwise and Contingent Screening

Like integrated screening, both forms of sequential screening have the option of first- and second-trimester testing for a combined final test result. However, the first-trimester screening result is provided to the patient when it is available and, if the patient is found to be at high risk of aneuploidy after the first test, the patient can consider further evaluation with either cell-free DNA screening or with diagnostic testing. This allows the patient to receive an abnormal result in the first trimester when more diagnostic and management options are available.

▶ *What are the limitations of cell-free DNA screening?*

Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing. A large referral-based cytogenetics laboratory reported their experience with 109 consecutive fetal samples from pregnancies that had positive screening test results for cell-free DNA screening from four different laboratories that use varied cell-free DNA screening techniques. Based on cytogenetic confirmation, the positive predictive value, or chance that a positive screening test result was a true positive, using cell-free DNA screening was 93% for Down syndrome, 64% for trisomy 18, 44% for trisomy 13, and 39% for sex chromosome aneuploidy (35). Because the test usually cannot distinguish fetal DNA from maternal DNA, a positive screening test result could represent confined placental mosaicism, a resorbing twin or, in rare instances, a maternal malignancy or maternal aneuploidy (36).

The discrimination of euploid from aneuploid pregnancies with cell-free DNA screening is more effective with larger fetal fractions. At 11–13 weeks, the median fetal fraction of cell-free DNA in maternal plasma is approximately 10% (15). Factors contributing to low fetal fraction include sampling before 10 weeks of gestation, high maternal body mass index, and fetal aneuploidy. In some laboratories, cell-free DNA fractions less than 4% are considered too low to report a result, often referred to as a "no call" result. Recent studies have demonstrated that low fetal fractions indicate a high risk of aneuploidy (22, 23, 28). In one study of more than 1,000 analyzed samples, 8% failed to obtain a result, and 22% of those were aneuploid (28). Pregnancies that initially do not return a cell-free DNA test result because of low fetal fraction can be managed with repeat cell-free DNA screening or diagnostic testing. However, if repeat cell-free DNA screening is performed, this may delay diagnosis of fetal aneuploidy, which may affect reproductive options for an abnormal result.

To date, most published experience with cell-free DNA screening is based on studies conducted on high-risk populations. Data on the performance of cell-free DNA testing in the general obstetric population are now available (22, 37–40). The sensitivity and specificity in the general obstetric population are similar to the

levels previously published for the high-risk population. However, cell-free DNA screening cannot have the same accuracy in low-risk pregnancies (eg, in young women) because the positive predictive value is affected by the prevalence of the disorder in the population. The positive predictive value is lower in the general obstetric population because of the lower prevalence of aneuploidy in this population.

In low-risk populations, there is a larger proportion of false-positive test results among the patients who receive positive screening test results. This decrease in accuracy is especially concerning when pregnancy terminations have been reported in women who have positive screening test results for aneuploidy without a confirmatory cytogenetic result (38). All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken. Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy. Even if cell-free DNA test results are a true positive, cell-free DNA cannot distinguish aneuploidy derived from a translocation or nondisjunction, and this will affect counseling and understanding of the recurrence risk. Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy (28, 39).

Cell-free DNA screening currently gives information about the three most common aneuploidies and about fetal sex but does not typically provide information about other aneuploidies. Without published clinical validation trials, some laboratories have begun to offer cell-free DNA screening for additional disorders, including two forms of aneuploidy associated with nonviable pregnancies (trisomy 16 and trisomy 22) and five or more microdeletion syndromes. A microdeletion syndrome is caused by a chromosomal deletion encompassing contiguous genes that is too small to be detected by conventional cytogenetics. Given the rarity of these disorders, it is uncertain what a positive or negative screening test result means. Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time. For women who wish to know whether their fetus has a microdeletion, the best option is to undergo prenatal diagnostic testing with microarray of fetal cells from CVS or amniocentesis (34, 41).

Cell-free DNA screening tests do not provide information regarding the potential for open fetal defects. Therefore, women who undergo cell-free DNA screening should be offered assessment for open fetal defects with ultrasonography, MSAFP screening, or both.

▶ *How should aneuploidy screening test results be interpreted and communicated?*

Positive and negative screening cutoff levels usually are defined by the different laboratories that perform these analyses. Because of these differences, and because patients interpret information differently, laboratory results should be reported as either positive or negative, and the adjusted numerical risk of aneuploidy based on the test should be provided, regardless of the screening test performed. It also is useful to contrast this risk with the patient's prescreening age-related risk and the general population risk to put the test result in context. Graphical representations of results can be helpful to some patients. After all of this information is provided, the patient's understanding of the results should be confirmed and documented.

▶ *What additional screening or diagnostic tests should be offered after a positive screening test result?*

Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing. Women found to have a positive screening test result from a serum analyte or ultrasound screening test should be offered further detailed counseling and cell-free DNA screening or diagnostic testing by CVS or amniocentesis. Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed. However, use of cell-free DNA screening as a follow-up test for patients with a positive traditional screening test result is reasonable for patients who want to avoid a diagnostic test. However, this approach may delay definitive diagnosis and management. Given that the residual risk of a chromosomal abnormality with a normal cell-free DNA screening test result after an abnormal traditional screening test has been reported to be 2%, patients should be informed of this potential limitation (42). Women with an increased risk of aneuploidy based on cell-free DNA screening should be offered diagnostic testing and should undergo ultrasonography to evaluate for fetal structural anomalies. If MSAFP has not been obtained as part of aneuploidy screening, further screening for open fetal defects with MSAFP or ultrasonography should be offered. In addition, evaluation for fetal anomalies in the second trimester is appropriate for all patients. In the first trimester, maternal serum levels of

pregnancy-associated plasma protein A below the fifth percentile are independently associated with obstetric complications, such as spontaneous fetal and neonatal loss, fetal growth restriction, preeclampsia, placental abruption, and preterm delivery (43). In the second trimester, elevated hCG, AFP, and dimeric inhibin A levels in pregnancies without structural anomalies are associated with an increased risk of fetal death, intrauterine growth restriction, and preeclampsia (44, 45). The likelihood of an adverse pregnancy outcome increases with increasing number of abnormal markers in the same screening test and with more extreme analyte values (46). Although potential management strategies for women with abnormal serum markers have been proposed, they are not evidence based (46).

If a patient conceives and has undergone preimplantation genetic screening, prenatal screening for aneuploidy still should be offered because false-negative test results can occur with preimplantation genetic screening (47). Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during pregnancy.

▶ *How does screening for aneuploidy differ in multifetal gestations?*

In multifetal gestations, the risk of fetal aneuploidy is affected by the number of fetuses and the zygosity of the pregnancy; however, data regarding the risk of aneuploidy are more limited in multiple gestations compared with singleton pregnancies. In dizygous twin pregnancies, each fetus carries a risk of aneuploidy generally similar to the mother's age-adjusted risk, but the mother carries an increased risk of having a fetus with aneuploidy because there is more than one fetus. Typically, monozygous twins will have the same karyotype, with neither or both fetuses being affected; the risk of carrying aneuploid fetuses is similar to the mother's age-adjusted risk.

No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Analysis of the risks and benefits of screening or diagnostic testing in women carrying multiple fetuses is much more complicated, given the diminished effectiveness of screening and how the prenatal identification of a single aneuploid fetus might affect the pregnancy management. Diagnostic testing may be less acceptable to women with multiple gestations because of the increased difficulty and higher potential loss rates.

Nuchal translucency measurement allows each fetus in a multifetal pregnancy to be screened independently and, therefore, can be used in twin or high-order multifetal gestations. The distribution of nuchal translucency measurements does not differ significantly between singletons and multiples, and standard cutoffs can be used (48). One study reviewed individual first-trimester screening in twin gestations and generated individual risks for each fetus with nuchal translucencies and first-trimester screening. At a 1:300 cutoff, the detection rate was 75% with a 9% positive screening rate for trisomy 21 (49). However, the review concluded that a greater reliance should be placed on nuchal translucency to evaluate the fetuses for aneuploidy. A single enlarged nuchal translucency in monochorionic twins of discordant size could be an early sign of twin–twin transfusion syndrome rather than aneuploidy (50). These patients should be evaluated further for this possibility.

Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies. First-trimester, quad, and combined serum analyte screening are options available to screen twin gestations, although few data are available from prospective studies with regard to screening. Analyte screening test results typically are provided for the entire gestation and not each individual fetus. Second-trimester serum screening of twin gestations can identify approximately 50% of fetuses affected with Down syndrome at a 5% positive screening rate (51). Because of limited evidence regarding its efficacy, cell-free DNA testing is not recommended for aneuploidy screening in women with multiple gestations (34).

In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances. The patient should be offered counseling and consider diagnostic testing instead of a screening test. The accuracy of aneuploidy screening in a multiple gestation with a fetus that has an empty gestational sac is not known.

Summary of Recommendations and Conclusions

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result.
- If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities.

- Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.
- Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.
- Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing.
- All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.
- Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
- Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing.

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time.
- Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during their pregnancy.
- No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies.

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.
- Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit.
- All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age.
- If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.
- Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy.
- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed.
- In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances.

For More Information

The American College of Obstetricians and Gynecologists has identified additional resources on topics related to this document that may be helpful for obgyns, other health care providers, and patients. You may view these resources at www.acog.org/more-info/AneuploidyScreening.

These resources are for information only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's web site, or the content of the resource. The resources may change without notice.

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Studies were reviewed and evaluated for quality according to the method outlined by the U.S. Preventive Services Task Force:

- I Evidence obtained from at least one properly designed randomized controlled trial.
- II-1 Evidence obtained from well-designed controlled trials without randomization.
- II-2 Evidence obtained from well-designed cohort or case–control analytic studies, preferably from more than one center or research group.
- II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments also could be regarded as this type of evidence.
- III Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Based on the highest level of evidence found in the data, recommendations are provided and graded according to the following categories:

Level A—Recommendations are based on good and consistent scientific evidence.

Level B—Recommendations are based on limited or inconsistent scientific evidence.

Level C—Recommendations are based primarily on consensus and expert opinion.

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ACMG STATEMENT | Genetics inMedicine

Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics

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Noninvasive prenatal screening using cell-free DNA (NIPS) has been rapidly integrated into prenatal care since the initial American College of Medical Genetics and Genomics (ACMG) statement in 2013. New evidence strongly suggests that NIPS can replace conventional screening for Patau, Edwards, and Down syndromes across the maternal age spectrum, for a continuum of gestational age beginning at 9–10 weeks, and for patients who are not significantly obese. This statement sets forth a new framework for NIPS that is supported by information from validation and clinical utility studies. Pretest counseling for NIPS remains crucial; however, it needs to go beyond discussions of Patau, Edwards, and Down syndromes. The use of NIPS to include sex chromosome aneuploidy screening and screening for selected copy-number variants (CNVs) is becoming commonplace because there are no other screening options to identify these conditions. Providers should have a more thorough understanding of patient preferences and be able to educate about the current drawbacks of NIPS across the prenatal screening spectrum. Laboratories are encouraged to meet the needs of providers and their patients by delivering meaningful screening reports and to engage in education. With health-care-provider guidance, the patient should be able to make an educated decision about the current use of NIPS and the ramifications of a positive, negative, or no-call result.

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American College of Medical Genetics and Genomics (ACMG) guidelines and statements have assisted patients seeking prenatal screening information and health-care providers responsible for providing accurate and up-to-date information to their patients.[1–3] Until recently, noninvasive prenatal screening for aneuploidy relied on measurements of maternal serum analytes and/or ultrasonography. These have a false-positive rate of approximately 5% and detection rates of 50–95%, depending on the specific screening strategy used. Advances in genomic technologies led to noninvasive prenatal screening that relies on the presence of cell-free DNA derived from the placenta but circulating in maternal blood, which is referred to here as noninvasive prenatal screening (NIPS). Massive parallel sequencing of maternal and placental (also called fetal when speaking of the fraction of this DNA in maternal blood) fragments of DNA occurs simultaneously. Sequencing with quantification can be random, targeted, and followed by quantification or exploitation of single-nucleotide polymorphisms.[4–8] Alternatively, sequencing can take place by measuring the release of hydrogen ions as nucleotides are added to a DNA template (i.e., semiconductor sequencing).[9] Microarray technology can also be used to quantify DNA.[10] Bioinformatics that enable these methodologies is complex and proprietary. Since the introduction of NIPS in 2011, health-care providers and patients have experienced marketing pressures, rapidly evolving professional practice guidelines, and confusion regarding the appropriate role of

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NIPS in prenatal practice.[11–15] This position statement replaces the 2013 "ACMG Statement on Noninvasive Prenatal Screening for Fetal Aneuploidy."[3]

We emphasize that all genetic screening has residual risk (i.e., risk of having a genetic condition even after receiving a negative or "normal" result). This concept is independent of the screening modality, condition screened, or number of conditions screened. The concept of residual risk supports our use of the acronym NIPS, where the "S" represents screening. It is important to emphasize what NIPS does not provide to patients. NIPS is not used clinically to screen for single-gene disorders (e.g., variation in the genome caused by relatively small changes in nucleotide sequence). NIPS is not used to predict late pregnancy complications. NIPS does not screen for open neural tube defects; therefore, maternal serum α-fetoprotein testing to screen for open neural tube defects should still be offered at 15–20 weeks of gestation. NIPS does not replace routine fetal anatomic screening using ultrasound.

Screening tests move through a predictable stepwise progression from laboratory development to clinical use. The ACMG recognizes this course as (i) analytical validity, (ii) clinical validity, and (iii) clinical utility. The last of these is the most complex.

- *Analytical validity* refers to whether the screening test detects the target of the test in those with the target (analytical sensitivity) without detecting it in those without the target (analytical specificity). Regarding NIPS, analytical validity asks whether various concentrations of maternal and placental DNA can be used to determine the presence or absence of fetal aneuploidy (or other conditions). Analytical validity has been established for the variety of screening methods discussed in this article.[10,16–19]
- *Clinical validity* refers to how well NIPS performs and focuses on detection rate (DR), the proportion of those who screen positive and will have the clinical condition (clinical sensitivity), and the proportion who will not (clinical specificity (SPEC)). These test metrics are independent of the prevalence of the condition being screened. Because NIPS addresses fairly uncommon conditions, validation studies are used to understand the DR and SPEC using banked or research samples. This allows overrepresentation of samples for the target condition of interest. Between 2011 and 2013, there were at least eight widely quoted validation studies spread across four laboratories offering NIPS clinically.[4–8,20–22] Validation studies reached similar conclusions. NIPS had very high DR and SPEC, reaching nearly 99% for Down syndrome caused by trisomy 21, translocations, and trisomy 21 mosaicism. The DR and SPEC were 80–100% for Edwards syndrome caused by trisomy 18 and trisomy 18 mosaicism, as well as for Patau syndrome caused by trisomy 13, translocations and trisomy 13 mosaicism. In this document, we refer to all three syndromes as "traditionally screened aneuploidies." Thus, in clinical validation studies, NIPS was shown to outperform conventional screening approaches.[23–25]
- *Clinical utility* refers to whether a screening test is reliable and useful to patients. Clinical utility studies inform patients, providers, and payers about decision making. These studies can provide objective test metrics such as positive predictive values (PPVs) and negative predictive values (NPVs). It is noteworthy that PPV and NPV can be determined for a population by modeling (using DR and SPEC as well as population prevalence) or by actual measure. Furthermore, one can establish PPV on a population basis (e.g., all women of a certain age) or individually (using information that is patient-specific). Cost efficacy in terms of dollars or cost utility measured by cost per case detected or quality-adjusted life-year is also used to describe clinical utility.[26] Because cost efficacy and cost utility studies use a high degree of modeling and assumptions (clinical care and monetary), these are at risk for bias (systematic and random). We chose not to include studies of this nature when making our recommendations.

IMPLEMENTATION OF NIPS INTO PRACTICE: GENETIC TESTING AS A MULTIFACETED CLINICAL PROCESS

Genetic testing and screening modalities used in pregnancy, such as NIPS, are offered with the aim of providing patients information that can help them optimize their pregnancy outcomes.[27] It is accepted practice, when implementing these modalities, to follow a multifaceted process in which genetic counseling is a common thread. Specific steps include: pretest education, counseling, and informed consent; the screening or testing procedure; a laboratory component that includes test interpretation; and, finally, the disclosure of results to the patient within a context that includes the appropriate education, counseling, and follow-up.

The core of genetic counseling is establishing patient desire and expectations. Genetic counseling is not merely educational; it is a patient-centered form of medical communication facilitating decisions on a course of action that are made solely by the patient once the patient has been given the necessary facts, alternatives, and anticipated consequences.[28,29] In this context, genetic counseling follows the Rogerian method, which is client-centered and nondirective.[30] ACMG recognizes it is beyond the scope of prenatal care providers to describe all genetic conditions amenable to diagnosis or screening in a pretest counseling session. However, an effort should be made to discuss in a general way the types of conditions that can (e.g., aneuploidy, translocations, microdeletions, and microduplications) and cannot (e.g., many single-gene disorders), be identified, including test limits in the case of the former, when a family history is unremarkable.[31]

Patient preferences for information should play a pivotal role in guiding the use of NIPS in prenatal care. This is in keeping with generally accepted genetic counseling tenets and respects that clinical utility may vary between patients.[28,29] Clinical utility includes test metrics (PPV and NPV), cost, and a patient's unique value system construct framed by (among other things) cultural traditions and religious beliefs. We recognize that this construct is not homogeneous across the United States. The desire for diagnostic testing or screening, the uptake of diagnostic testing, and decisions made when positive results are confirmed are influenced by a patient's value system. However, establishing a patient's value system construct can be complex and confusing. In the context of an evolving technology such as NIPS, the patient's ability to accept uncertainty with regard to possible screening outcomes should also be considered and explored as part of the pretest communication process. Cost plays a role in society's willingness to pay. Insurance coverage (private or public), responsibility for co-payments, and out-of-pocket expenses factor into the nature of follow-up diagnostic tests, availability of genetic counseling services, and reproductive decision making.

For the genetic testing and screening modalities used in pregnancy to provide patients with information that can help them optimize their pregnancy outcomes, patients must be allowed to make informed choices that occur across a time continuum. Prenatal screening and diagnostic testing target 20 weeks of gestation as an upper limit for implementation.[32] Decision making is circumscribed by state-specific laws (e.g., 20 weeks),[33] which highlights the importance of timely delivery and processing of accurate and complete information at each step. NIPS can be performed at an earlier gestational age than conventional screening, and there is no gestational age upper limit after 10 weeks of gestation. This means that patients can get the most accurate screening information at an earlier gestational age, thus enhancing informed decision making.

- ACMG recommends:
  - Providing up-to-date, balanced, and accurate information early in gestation to optimize patient decision making, independent of the screening approach used.
  - Laboratories work with public health officials, policymakers, and private payers to make NIPS, including the pre- and posttest education and counseling, accessible to all pregnant women.

For some patients the goal in prenatal screening may be to maximize the detection of fetal genetic diagnoses. In this scenario, fetal diagnostic testing (e.g., chorionic villous sampling or amniocentesis) followed by chromosomal microarray (CMA) using fetal DNA should be offered, and NIPS may not be the best choice. With diagnostic testing, whole-chromosome abnormalities, unbalanced chromosome rearrangements, small losses or gains of chromosomal material (CNVs), and in some cases single-gene disorders can be detected. An NIH study of prenatal CMA suggested the background rate of small clinically significant CNVs is 1–2%.[34] Fetal diagnostic testing carries a small risk. Pregnancy loss rates before 24 weeks of gestation for amniocentesis range from 0.1 to 0.9% (1/1,000–1/111) and for chorionic villous sampling range from 0.2 to 1.3% (1/500–1/77).[35,36] Results from these studies reflect diagnostic testing performed because of abnormal ultrasound findings, positive aneuploidy screening, or other at-risk conditions. Therefore, one can conclude that these procedure-related miscarriages are overestimates of risk compared to selecting a procedure solely for obtaining maximal information.

Patients may prefer a screening test, and there are many to choose from. Conventional screening approaches such as first-trimester screening, second-trimester screening, or combinations of both (e.g., stepwise sequential screening) have good detection rates (80–95%) but high false-positive rates (3–5%). Stepwise sequential screening has both (~95% and ~5%) but is not universally used due to the required logistics. When choosing a conventional screening approach, patients should be aware of the high false-positive rate, which may lead to diagnostic procedures and, consequently, diagnoses not detected by NIPS (e.g., some chromosome abnormalities and CNVs). For patients who prefer to avoid diagnostic testing but desire highly accurate screening for Patau, Edwards, and Down syndromes, NIPS may be preferred. There are pros and cons to any screening approach. After careful counseling, patients will ideally select the paradigm that is most aligned with their goals. Prenatal care providers should try to understand the clinical utility construct of individual patients during the informed consent and decision-making processes.

- ACMG recommends:
  - Allowing patients to select *diagnostic* or *screening* approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences.
  - Informing all pregnant women that *diagnostic* testing (CVS or amniocentesis) is an option for the detection of chromosome abnormalities and clinically significant CNVs.

SHOULD NIPS BE OFFERED TO ALL PATIENTS, INCLUDING THOSE AT LOW OR AVERAGE RISK?

In 2013, the ACMG was careful not to restrict NIPS to specific patient groups.[3] Recent clinical utility trials[23–25,37] compared NIPS to conventional screening methods for women at low risk or average risk compared to women at high risk. The DR, SPEC, PPV, and NPV for Patau, Edwards, and Down syndromes were reported. Clinical utility, measured as PPV and NPV in these studies, supports the earlier ACMG position, and several professional organizations have subsequently altered their positions.[38–40] Data from two large studies show that for "low risk women", the PPV for Down syndrome after NIPS was 50–81% (N=55,244)[24,25], and for "high risk women" this was 94% (N=72,382).[25] NIPS and conventional screening were compared and showed NIPS was superior with regards to PPV (80.9 vs. 3.4%, N=15,841).[24] The NPV approached 100% for Down

syndrome in these studies. Similarly, for Patau and Edwards syndromes, the PPVs after NIPS (Patau 33–90%, Edwards 50–70%)[24,25] were superior to those with conventional screening (Patau 14%, Edwards 3.4%)[24] and the NPV was 100% for both conditions.[23,24]

High PPV provides benefits to patients by enabling them to more easily weigh the advantages and disadvantages of follow-up diagnostic testing. Additional benefits of NIPS include earlier implementation with no gap across the gestational age spectrum, unlike conventional screening methods. This allows confirmatory diagnostic testing earlier in gestation and provides a screening option for patients who present for care any time after the first trimester. Earlier diagnosis facilitates providing up-to-date, balanced, and accurate information at a time that may enable patients to consider the broadest range of reproductive options. In some cases, patients will elect to alter the course of the pregnancy or pregnancy care; others will investigate adoption or choose to learn about the expected outcome, neonatal care, and long-term care for a child with disabilities.

- ACMG recommends:
  - Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes).
  - Referring patients to a trained genetics professional when an increased risk of aneuploidy is reported after NIPS.
  - Offering *diagnostic* testing when a positive screening test result is reported after NIPS.
  - Providing accurate, balanced, up-to-date information, at an appropriate literacy level when a fetus is *diagnosed* with a chromosomal or genomic variation in an effort to educate prospective parents about the condition of concern. These materials should reflect the medical and psychosocial implications of the diagnosis[41] (see Patient Resources).
  - Laboratories should provide readily visible and clearly stated DR, SPEC, PPV, and NPV for conditions being screened, in pretest marketing materials, and when reporting laboratory results to assist patients and providers in making decisions and interpreting results.
  - Laboratories should not offer screening for Patau, Edwards, and Down syndromes if they cannot report DR, SPEC, and PPV for these conditions.

SHOULD NIPS BE USED TO SCREEN FOR AUTOSOMAL ANEUPLOIDIES OTHER THAN PATAU, EDWARDS, AND DOWN SYNDROMES?

The expansion of NIPS to autosomes beyond 13, 18, and 21 is technically possible. Whole-chromosome fetal aneuploidy other than these common aneuploidies most often results in early fetal loss.[42] Counseling related to these rare autosomal aneuploidies is made difficult by limited case reports and variable expressivity. Confined placental mosaicism for chromosome 16 has been well described and results in a spectrum of fetal outcomes from no clinical phenotype to fetal growth restriction. In a large retrospective study of amniocentesis performed for maternal age, ultrasound findings, biochemical abnormalities, or familial indications, 1/14,830 patients had trisomy 2, 8, 12, or 22.[43] Detection of lethal chromosome abnormalities for which the natural course will be fetal loss has the potential to result in unnecessary diagnostic procedures and unnecessary pregnancy termination procedures. In addition to having a personal impact on patients, data collection in the public health sector could result in inflated pregnancy loss attributed to diagnostic procedures and maternal complications from pregnancy termination.

- ACMG does *not* recommend:
  - NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21.

HOW ARE NO-CALLS AVOIDED, INTERPRETED, AND MANAGED?

Fetal fraction

The placental fraction accounts for approximately 10% of all cell-free DNA in maternal circulation.[6,21,44] Data suggest that the lower limit of cell-free fetal DNA for a reliable result is approximately 4%. A no-call may be reported if there is not a sufficient amount of fetal cell-free DNA in the maternal blood sample. In two prospective studies including more than 16,000 pregnancies, a low fetal fraction in maternal circulation was associated with an increased risk of fetal aneuploidies.[24,45] The biologic mechanism of low fetal fraction and its association with aneuploidies is speculative. Interestingly, triploidy was most common (31%); however, trisomy 21 was seen in 23% of cases of low fetal fraction.[24] Others showed that a fetal fraction of DNA in Down syndrome cases is often the same or higher when compared to pregnancies with euploid fetuses.[46,47] Since the introduction of NIPS into clinical practice, fetal fraction has not been uniformly reported by laboratories. The described relationship between low fetal fraction and increased risk of aneuploidy adds to the importance of reporting the reason for a no-call and of indicating in the report whether a low fetal fraction was identified.

Factors that influence fetal fraction include maternal weight and gestational age.[47–49] There is no specific threshold to describe the relationship between fetal fraction and maternal weight. However, in cases of significant obesity, a no-call due to low fetal fraction should be anticipated. There is a gestational age threshold, below which results are not reliable (9 or 10 weeks depending on the laboratory used). Data suggest that before 20 weeks, fetal fraction increases less than 0.1% per week, which challenges the idea that repeating sample collection is a viable approach to overcoming a low fetal fraction.[47,49]

- ACMG recommends:
  - Offering *diagnostic* testing for a no-call NIPS result due to low fetal fraction if maternal blood for NIPS was drawn at an appropriate gestational age. A repeat blood draw is NOT appropriate.
  - Offering aneuploidy screening other than NIPS in cases of significant obesity.
  - All laboratories should include a clearly visible fetal fraction on NIPS reports.
  - All laboratories should establish and monitor analytical and clinical validity for fetal fraction.
  - All laboratories should specify the reason for a no-call when reporting NIPS results.

Long stretches of homozygosity

Single-nucleotide polymorphisms or array-based assays require adequate heterozygosity between the maternal and fetal genomes to provide meaningful data for the analysis of genomic balance and copy number. Therefore, stretches of homozygosity between the maternal and fetal genomes render any differences in copy number within that region undetectable, including small duplications or deletions. In addition to preventing in the interpretation of genomic balance, large regions of homozygosity for a single chromosome may be suggestive of uniparental disomy (UPD), whereas large regions of homozygosity dispersed over many chromosomes may be suggestive of parental consanguinity.[50]

- ACMG recommends:
  - Informing patients that a no-call result may be due to long stretches of homozygosity, which could be due to either UPD or parental consanguinity.
  - Referring patients to a trained genetics professional when a no-call result suspicious for UPD or parental consanguinity is received.
  - Offering *diagnostic* testing with CMA when a no-call result is obtained after NIPS due to possible UPD or parental consanguinity.

SHOULD NIPS BE OFFERED TO SCREEN FOR SEX CHROMOSOME ANEUPLOIDIES?

In one retrospective study of 88,970 amniocenteses, the diagnosis of any sex chromosome aneuploidy was made in 1/272 patients.[43] This was higher for women older than 35 years compared to younger women (1/210 and 1/459, respectively). Conventional screening for aneuploidies does not detect sex chromosome aneuploidies. The most common of these, monosomy X (Turner syndrome), has been estimated to occur in 1–1.5% of pregnancies[51] and is a common cause of first-trimester pregnancy loss (~23%).[52] The phenotype of individuals with a 47,XXX or 47,XYY karyotype is highly variable but may include social or cognitive deficits.[53] Klinefelter syndrome (47,XXY), however, does have a classic phenotype and is associated with sterility.[53]

The detection rate (clinical validity) of sex chromosome aneuploidies after NIPS is reported to be more than 90% and has a false-positive rate of approximately 1%.[54–57] The PPV (clinical utility) for the aggregate of sex chromosome aneuploidies among prospectively collected samples was 48.4% (range for specific aneuploidies, 30–67%).[57] A PPV in these ranges is considerably higher than those accepted for conventional screening of Patau, Edwards, and Down syndromes.

Etiologies of false-positive sex chromosome aneuploidy results have been considered, and an approach to distinguish true positives from false positives was described.[58] Maternal medical, endocrine, and fertility history can help to identify the cause of a false-positive result. This includes patients with an organ transplantation from either a 46,XY individual or unknown gender donor. Other causes of false-positive results are similar to those for traditional aneuploidies. These include confined placental mosaicism, "vanishing" twin or higher-order co-fetus, and, rarely, maternal neoplasm. For these reasons, patients should be counseled about the advantages and disadvantages of sex chromosome aneuploidy screening within the construct of their preferences for information.

- ACMG recommends:
  - Informing all pregnant women, as part of pretest counseling for NIPS, of the *availability* of the expanded use of screening for sex chromosome aneuploidies.
  - Providers should make efforts to deter patients from selecting sex chromosome aneuploidy screening for the sole purpose of biologic sex identification in the absence of a clinical indication for this information.
  - Informing patients about the causes and increased possibilities of false-positive results for sex chromosome aneuploidies as part of pretest counseling and screening for these conditions. Patients should also be informed of the potential for results of conditions that, once confirmed, may have a variable prognosis (e.g., Turner syndrome) before consenting to screening for sex chromosome aneuploidies.
  - Referring patients to a trained genetics professional when an increased risk of sex chromosome aneuploidy is reported after NIPS.
  - Offering *diagnostic* testing when a positive screening test result is reported after screening for sex chromosome aneuploidies.
  - Providing accurate, balanced, up-to-date information and materials at an appropriate literacy level when a fetus is *diagnosed* with a sex chromosome aneuploidy in an effort to educate prospective parents about the specific condition. These materials should reflect medical and psychosocial implications for the diagnosis[41] (see Patient Resources).
  - Laboratories include easily recognizable and highly visible DR, SPEC, PPV, and NPV for each sex chromosome aneuploidy when reporting results to assist patients and providers in making decisions and interpreting results.
  - Laboratories should not offer screening for sex chromosome aneuploidies if they cannot report DR, SPEC, PPV, and NPV for these conditions.

SHOULD NIPS BE OFFERED FOR DETECTION OF COPY NUMBER VARIATION (CNV)?

Conventional aneuploidy screening focuses on whole-chromosome aneuploidies that have an overall live birth frequency of 1/800 (Down syndrome)[59] to 1/30,000 (Patau syndrome). Expanding NIPS to include detection of specific conditions caused by a CNV (e.g., 22q11.2 deletion, 1p36 deletion, 15q11.2–13 deletion) is technically possible (analytical validity).[60–63] The phenotypes associated with these conditions can be severe; therefore, they may be appropriate conditions for prenatal screening. However, providers and patients must be aware that expanding the use of NIPS to include the detection of CNVs requires in-depth knowledge of the limitations of the technology, return of results, and follow-up.

Validation studies indicate a high detection rate (>97%) and low false-positive rate (<1%) can be achieved. However, there are few clinical utility studies. Therefore, PPV and NPV have been modeled.[63–65] One report showed that for a specific combination of CNVs studied, PPV ranged from 3.8 to 17%. In a large retrospective study of more than 21,000 samples, the aggregate PPV for several CNVs screened simultaneously was 18% (specific conditions: 11–48%). Methods to improve PPV have been reported.[65] Modeling PPV and NPV is made more complex for genome-wide analysis for which validation studies are limited in scope and number.[26,63] Determination of PPV and NPV is hampered by the inherent limitations of studying multiple rare conditions with variable expressivity. As greater portions of the genome are analyzed for CNVs, false positive and negative results are expected to increase. This may result in an increase in patient anxiety and fetal procedures and a burden on an already limited genetic counseling workforce.

Validation studies make the point that DR and SPEC depend on many variables (e.g., depth of read),[10,60–63] which can change the false-positive and false-negative rate when NIPS is used for prenatal detection of CNVs. Pretest and posttest counseling is further confounded by variable expressivity and penetrance of the conditions being screened, size of the deletion being screened, specific genes within the critical region of the locus interrogated, and the number of genes within the critical region being screened.

- ACMG recommends:
  - Informing all pregnant women of the *availability* of the expanded use of NIPS to screen for clinically relevant CNVs when the following conditions can also be met:
    - Obstetric care providers should discuss with their patients the desire for prenatal screening as opposed to diagnostic testing (i.e., CVS or amniocentesis).
    - Obstetric care providers should discuss with their patients the desire for maximum fetal genomic information through prenatal screening.
    - Obstetric care providers should inform their patients of the higher likelihood of false-positive and false-negative results for these conditions as compared to results obtained when NIPS is limited to common aneuploidy screening.
    - Obstetric care providers should inform their patients of the potential for results of conditions that, once confirmed, may have an uncertain prognosis.
  - Referring patients to a trained genetics professional when NIPS identifies a CNV.
  - Offering *diagnostic* testing (CVS or amniocentesis) with CMA when NIPS identifies a CNV.
  - Providing accurate, balanced, up-to-date information at an appropriate literacy level when a fetus is *diagnosed* with a CNV in an effort to educate prospective parents about the condition of concern. These materials should reflect the medical and psychosocial implications of the diagnosis[65] (see Patient Resources).
  - Laboratory requisitions and pretest counseling information should specify the DR, SPEC, PPV, and NPV of each CNV screened. This material should state whether PPV and NPV are modeled or derived from clinical utility studies (natural population or sample with known prevalence).
  - Laboratories include easily recognizable and highly visible DR, SPEC, PPV, and NPV for each CNV screened when reporting laboratory results to assist patients and providers in making decisions and interpreting results. Reports should state whether PPV and NPV are modeled or derived from clinical utility studies (natural population or sample with known prevalence). When laboratories cannot report specific DR, SPEC, PPV, and NPV, screening for those CNVs should not be performed by that laboratory.

- ACMG does *not* recommend:
  - NIPS to screen for *genome-wide* CNVs. If this level of information is desired, then diagnostic testing (e.g., chorionic villous sampling or amniocentesis) followed by CMA is recommended.

SPECIAL CONSIDERATIONS

Multiple gestation and/or donor oocytes:

There are unique challenges when NIPS is used in multiple gestation pregnancies conceived through donor oocytes. These are specific to the analytical method and bioinformatics employed by the laboratory.

- ACMG recommends:
  - In pregnancies with multiple gestations and/or donor oocytes, testing laboratories should be contacted regarding the validity of NIPS before it is offered to the patient as a screening option.

Unanticipated findings

Both constitutional and acquired forms of genomic imbalance in the mother (e.g., aneuploidy of chromosome X, microdeletions, neoplasia, chimerism due to allogenic organ

or tissue transplantation, or mosaicism) and imbalances within the fetoplacental genome (e.g., confined placental mosaicism) can give rise to identifiable bioinformatic patterns that may confound interpretations. Therefore, providers should be aware of the potential for false-positive results that may resolve after diagnostic testing. Although it is not the purpose of NIPS to identify clinically relevant maternal genomic information, patients and providers should be aware of the potential for inadvertent discovery of such information and the potential for additional follow-up testing unrelated to the pregnancy.

Given the differences in laboratory methodologies and bioinformatic processing that may be used, it is beyond the scope of this document to address considerations that might be unique to any specific method in use. It therefore remains the responsibility of each laboratory to make physician providers aware of clinically relevant features that are specific to the methodology used. This is best accomplished through educational materials and laboratory reports.

- ACMG recommends:
  - Informing patients of the possibility of identifying maternal genomic imbalances and that this possibility depends on the specific methodology used.
  - Referring patients to a trained genetics professional when NIPS identifies maternal genomic imbalances.
  - Offering aneuploidy screening other than NIPS for patients with a history of bone marrow or organ transplantation from a male donor or donor of uncertain biologic sex.
  - Discussing the possibility of discordant fetal biologic sex if maternal blood transfusion was performed <4 weeks prior to the blood draw for NIPS.

Positive and negative predictive values

Understanding the importance of PPV is paramount to screening. PPV is a screening test metric that is useful when patients screen positive. This metric is used by patients in deciding the next steps in decision making. Because the specificity is so high after NIPS for traditionally screened aneuploidies, NPV is less often the focus. However, it is one of the key features of this technology. A high NPV offers patients reassurance in the post-test setting. There are several mathematical approaches that can be used to model PPV and NPV from validation data. PPV for aneuploidy is very sensitive to prevalence/*a priori* risk, and to a lesser extent DR and SPEC, which do not fluctuate with maternal age. Maternal age is a highly important factor in determining the prevalence of Down syndrome and other aneuploidies, but it is not a factor when considering CNVs. One reason why PPV is much lower for detection of CNVs is that the prevalence and detection rate are low compared to traditionally screened aneuploidies. A common error is to interpret PPV across an entire population without taking into account patient-specific information (e.g., prevalence based on maternal age when necessary).

There are several online calculators for determining patient-specific PPV and NPV after NIPS (e.g., http://secure.itswebs.com/nsgc/niptcalculator/index.html). PPV seems irrelevant to anyone not facing a positive test result. If the PPV of each condition being considered were reported when results were negative, then there would be an excess of data cluttering a report.

- ACMG recommends:
  - Laboratories provide patient-specific PPV when reporting positive test results.
  - Laboratories provide population-derived PPV when reporting positive results in cases in which patient-specific PPV cannot be determined due to unavailable clinical information.
  - Laboratories provide modeled PPV when reporting positive results for which neither patient-specific nor population-derived PPV are possible.
  - Providers use validated online calculators to provide patient-specific PPV when results from NIPS are positive to facilitate clear and accurate communication with patients.
  - Incorporating laboratory-specific DR and SPEC to provide clear and patient-specific information when using validated online calculators.

PATIENT RESOURCES

In a consensus statement by the ACMG, the American College of Obstetricians and Gynecologists (ACOG), the National Society of Genetics Counselors (NSGC), and Down syndrome organizations, there was unanimous agreement that patient education materials about prenatal testing and associated conditions should result from "collaboration among healthcare and advocacy organizations."[41] According to Public Law 110–371 (https://www.govtrack.us/congress/bills/110/s1810/text), enacted in 2008, "partnerships between healthcare professional groups and disability advocacy organizations" were emphasized regarding the collection, synthesis, and dissemination of "current evidence-based information" related to prenatal conditions. With these charges in mind, the ACMG has identified available patient resources (listed alphabetically) that have resulted from collaborations between healthcare professional groups and advocacy organizations.

Down Syndrome Pregnancy (http://downsyndromepregnancy.org/books). This site, for expectant parents preparing for the birth of a baby with Down syndrome, provides a range of books in English and Spanish that are recommended in the "NSGC Guidelines for Communicating a Prenatal or Postnatal Diagnosis of Down Syndrome" and that have been reviewed by medical and patient advocacy experts.

Genetics Home Reference (https://ghr.nlm.nih.gov). This online reference provides information for patients and families about more than 1,000 genetic conditions. All content is written by a full-time staff with backgrounds in genetics, reviewed by outside experts, and contains input from support and advocacy

organizations. Genetics Home Reference is a service of the National Library of Medicine, which is part of the National Institutes of Health, an agency of the US Department of Health and Human Services.

Genetic Support Foundation (https://www.geneticsupportfoundation.org). This nonprofit organization, founded by genetics professionals, provides information about pregnancy and genetics and the different conditions that can be detected prenatally. It often includes instructional videos.

Lettercase/The National Center for Prenatal and Postnatal Resources (http://www.lettercase.org). Lettercase offers professionally reviewed materials about genetic conditions. Currently, "Understanding a Down Syndrome Diagnosis" and "Understanding a Turner Syndrome Diagnosis" are available in print and digital versions in several languages. The materials are intended for expectant couples who have received a prenatal diagnosis of Down or Turner syndrome but have not yet made a decision regarding their pregnancy options. The materials are prepared with assistance from the ACMG, ACOG, NSGC, and national patient advocacy organizations.

NSGC "Fact Sheet about Down Syndrome for New and Expectant Parents" (http://nsgc.org/p/cm/ld/fid=387) and **"A Patient's Guide to Understanding Noninvasive Prenatal Testing"** (http://nsgc.org/p/cm/ld/fid=385). These fact sheets on the NSGC website, which provide basic downloadable information, were reviewed by the National Society of Genetic Counselors Down Syndrome Information Act Working Group, with assistance from the National Center for Prenatal and Postnatal Resources.

PROVIDER RESOURCES

The following resources (listed alphabetically) were created by respected medical organizations or medical expert consensus and can serve as useful references for medical providers.

Delivering a diagnosis. Resources describing simulation training for healthcare professionals who deliver a prenatal diagnosis to expectant couples are available. These projects were funded by federal grants and efficacy was researched and published.[66,67]

Down syndrome healthcare guidelines. "Healthcare Supervision for Children with Down Syndrome" (http://pediatrics.aappublications.org/content/128/2/393). This was written by the Committee on Genetics of the American Academy of Pediatrics, provides guidance for healthcare professionals. Resources for parents are also listed.

GeneReviews (http://www.ncbi.nlm.nih.gov/books/NBK1116). This online resource for clinicians provides peer-reviewed information written by medical experts. Information is updated every 2 to 4 years through a formal review process. It is an excellent source of information, and physicians faced with a need to learn about common CNVs may find this resource useful.

"Care of Girls and Women with Turner Syndrome: A Guideline of the Turner Syndrome Study Group." This was written by the Turner Syndrome Consensus Study Group of the National Institutes of Health and was adopted by the American Academy of Pediatrics.[68]

22q11 deletion syndrome (DiGeorge syndrome) guidelines. Peer-reviewed expert consensus documents are available for the evaluation and management of patients with 22q11 deletion syndrome (DiGeorge syndrome).[69,70] This is the most common copy-number variation currently being offered through NIPS. Resources for other CNVs may be found in *GeneReviews*.

SUMMARY

New data and provider and patient demands require an updated position on the use of NIPS in prenatal care. We provide a framework for understanding how genetic technology moves from an idea into clinical practice. We hope this framework helps to explain ACMG's recommendations. Clinical validation strongly suggested that NIPS can replace conventional screening for Patau, Edwards, and Down syndromes. Objective measures of clinical utility support this. Test metrics support NIPS across the maternal age spectrum and continuum of gestational age beginning at 9–10 weeks as long as patients are not significantly obese. In the latter case, fetal fraction leading to an inability to make a call is limiting.

We have raised the bar for pretest counseling by expanding NIPS beyond that for Patau, Edwards, and Down syndromes. Providers should have a thorough understanding of patient preferences; efforts to educate about the limitations are not trivial. Although clinical utility studies are limited, they point to a role for NIPS in sex chromosome aneuploidy screening and screening for selected CNVs. We support these uses when the live birth frequency of conditions reaches or exceeds that of currently screened conditions and when test metrics meet or exceed those of well-established approaches to prenatal screening. Furthermore, we considered the potential for children to be impacted by early treatment. Our recommendations will affect communication between providers and patients and between providers and testing laboratories. Laboratories are encouraged to meet the needs of providers and patients by delivering meaningful screening reports, engaging in education, and identifying ways to address distributive justice, a medical ethical principle that challenges genomics-based innovative and clinically useful technologies.

ACKNOWLEDGMENT

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DISCLOSURE

B.G.S. serves on the Advisory Board of several nonprofit entities providing education about Down syndrome. The other authors declare no conflict of interest.

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1. What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive or invasive) or no screening in pregnant individuals not known to be at high risk for chromosomal abnormalities?

Currently, there is a vast amount of data supporting the use of cfDNA screening for trisomies 21, 18, 13, and the common sex chromosomes. There has been 1 head-to-head comparison study between the Harmony prenatal test and first-trimester combined screening (Norton - Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015 Dec 24;373(26):2582. https://www.ncbi.nlm.nih.gov/pubmed/26699179). Studies have demonstrated extremely high sensitivities and specificities for the performance of screening the common chromosome aneuploidies.

Other than monosomy X, which may show signs of anomalies on ultrasound, there are currently no specific screening modalities for sex chromosomes aneuploidies.

Invasive procedures such as chorionic villus sampling (CVS) or amniocentesis are the only methods to definitively diagnose a chromosome abnormality in pregnancy. However, these methods carry a small risk of miscarriage.

2. What direct harms are associated with screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

Every patient has a risk for a chromosome abnormality to occur in their pregnancy. While that risk does increase as maternal age increases, current recommendations state every patient should be offered screening from chromosome abnormalities. Every screening test has a risk for discordant results; either a false positive or false negative result. Screening with cfDNA decreases the frequency of time these discordant results may occur as compared to conventional screening methods. As with all screening tests, direct harm from screening with cfDNA other than discordant results may also include anxiety and concern with high probability results (either concordant or discordant to the pregnancy).

3. Do important efficacy/effectiveness outcomes or direct harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA vary for the mother and fetus or infant by:

a. Maternal characteristics (e.g., age)

The performance of cfDNA screening does not change based on maternal age or characteristics. However, the incidence of disease in a population affects the positive predictive value of a particular population. For example, as maternal age increases, the risk for a chromosome abnormality also increases and the incidence of disease is more common. Therefore, the PPV of a population will be higher when the incidence of disease increases but the performance (sensitivity and specificity) of the test remains constant and this is the same for all screening tests.

b. Singleton or multifetal pregnancy

There is substantial evidence on the performance of cfDNA in singleton pregnancies. While the evidence for multifetal pregnancies is limited, studies have concluded the performance of cfDNA in twin pregnancies is similar to that of singleton pregnancies and superior to that of first-trimester screening (Gil et al. Ultrasound Obstet Gynecol 2019; 53: 734–742).

c. Timing of screening (e.g., gestational age)

Certain factors can affect the performance of cfDNA screening. The main factor is the amount of fetal fraction (the proportion of DNA from the pregnancy in the sample) present for analysis. The lower the fetal fraction percentage, the more difficult it is to determine an accurate result. As pregnancy progresses, the amount of fetal fraction increases. Therefore, for certain samples with not enough fetal fraction, the sensitivity may suffer (Artieri et al. Prenat Diagn. 2017 May;37(5):482-490. doi: 10.1002/pd.5036. Epub 2017 Apr 26.). There are ways to prevent a lowered sensitivity and one way is to have a minimum threshold of fetal fraction. By ensuring there is enough fetal fraction in the sample, the lab can maintain the high performance of cfDNA screening (Blais, et al. (2018). Clinical biochemistry, 59, 69-77).

4. What are the cost-effectiveness and other economic outcomes of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

In the study by Fairbrother et al., NIPT identified 15% more trisomy cases than FTS and significantly reduced the amount of invasive procedures. It also found that at a NIPT unit cost of $665, the cost per case to identify trisomy is equivalent to that of FTS. In addition, at a NIPT unit cost of $453 or less, a cost savings over FTS is realized (Fairbrother et al. J Matern Fetal Neonatal med, 2016. DOI: 10.3109/14767058.2015.1038703).

[redacted]

From: Jennifer O'Neill <joneill@natera.com>
Sent: Tuesday, July 23, 2019 8:51 AM
To: HCA ST Health Tech Assessment Prog
Subject: Cell-free DNA Prenatal Screening for Chromosomal Aneuploidies - Public Comment Submission
Attachments: 1999 snijders down syndrome and maternal age.pdf; Hui 2017 Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy_GIM.pdf; Warsof Impact of NIPT on Diagnostic Testing PnD 2015.pdf; hui gim 2017 hui nips pop based impact.pptx; WA SHCA cfdna-draft-key-questions-20190708.pdf; Norton_NEJM_2015.pdf; Fairbrothers_Ariosa.pdf; Benn pone 0132313.pdf; Walker_ARUP_PLOSone.pdf; ACOG Practice Bulletin Number 163 May 2016.pdf

Good afternoon

I am submitting these public comments on behalf of Dr. Kimberly Martin, Chief Clinical Advisor for Natera.

Thanks in advance for the opportunity to contribute to the upcoming evidence review by Washington's Independent Health Technology Clinical Committee.

Dr. Martin can be reached directly (her contact info is listed below) should you have any further questions on these publications and comments, or feel free to reach out to me as well.

Warm Regards,

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1. What is the evidence of efficacy and effectiveness for screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA compared to other active screening interventions (noninvasive or invasive) or no screening in pregnant individuals not known to be at high risk for chromosomal abnormalities?

Please refer to ACOG's most recent position, enclosed in Practice Bulletin #163 (please see attached) which clearly states "all women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age." The bulletin outlines a variety of benefits and limitations of the various screening modalities, and acknowledges that the data is sufficient to conclude that screening performance for common chromosome abnormalities including Down syndrome is superior to the other methods. The following statement "The

sensitivity and specificity in the general obstetric population are similar to the levels previously published for the high risk population" is also to be noted. Several cost effective publications are referenced below.

2. What direct harms are associated with screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

HARMS

- False positive results raise anxiety, increase likelihood family will choose invasive testing, increases cost of care due to referral to MFM, additional ultrasounds, consults, perhaps even surveillance as high risk pregnancy based upon serum analyte levels (no data to suggest serum analytes should be used to screen for adverse pregnancy outcome, or that ancillary testing like biophysical profiles, non-stress tests improve outcome for these pregnancies). Possible that patient may then be subjected to emergent delivery or increased risk of cesarean section due to false positive results from additional testing that was not indicated. Therefore it is critical that test with highest sensitivity and lowest false positive rate is used to minimize direct patient harm - this is clearly cell-free DNA.

-False negative results - patients choose testing for these abnormalities after a discussion with their healthcare provider, who must order the test. In general, patients will want the test with the highest detection rate.

BENEFITS:

-Identification of a child with a serious condition allows reproductive decision making for parents. This includes interruption of pregnancy assuming results of screening are confirmed with diagnostic testing, and the family are clearly and accurately counseled by non-directive providers. Many families choose not to interrupt these pregnancies but place HIGH value on prenatal diagnosis, education and preparation which may result in various improved outcomes.

1. Serious chromosome abnormalities like Trisomy 13/18 - family may continue pregnancy, however < 10% of these infants survive to first birthday, high rate of maternal intervention (such as cesarean section for fetal distress) when NOT known before delivery. Family may meet with pediatric specialists prenatally and develop care plan directed at comfort/supportive care resulting in dramatically lower healthcare costs (NICU admission, etc) a serious chromosome abnormality like Trisomy 13/18. Even more important is that the family is prepared, including extended family and friends, and can spend the livespan of the infant with the infant NOT through an incubator.
2. Down syndrome - the detection of congenital heart disease overall is < 50% particularly when ultrasounds are performed by less-experienced providers. Highly sensitive screening like cfDNA and prenatal diagnosis should lead to fetal echocardiography, evaluation for intestinal obstruction, etc and ideally delivery of the infant in a center equipped to deal with the associated complications.
3. Sex chromosome abnormalities - these were not addressed in screening until the advent of prenatal screening using cell free DNA (exception is turner syndrome which may be identified if ultrasound features are noted). There is a paper in press, survey of parents of children with sex chromosome abnormalities, who overwhelmingly support prenatal screening for these abnormalities, they are not screened in newborn screening and many experience years trying to understand their childrens' 'differences' which can be screened for (head start, early intervention), treated (early androgen replacement for 47,XXY improves outcomes), growth hormone (turner syndrome), etc. If you have any questions suggest direct to Dr. Carole Samango-Sprouse, Executive Director and Chief at The Focus Foundation in Maryland: www.thefocusfoundation.org

3. What are the benefits and harms of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals known to be at high risk for chromosomal abnormalities?

EXACTLY the same as above, the definition of a maternal age of 35 as 'high risk' is completely arbitrary. Data from attached reference Snijders:

33	1/352	1/383	1/409	1/430
34	1/287	1/312	1/333	1/350
35	1/229	1/249	1/266	1/280

The difference between a 33, 34, 35 year old cannot be distinguished by a typical pregnant woman or her partner, therefore the direct harms and benefits are no different. Women 35 and older are more anxious because they have been 'educated' that they are high risk while women < 35 are under the impression their risk is 'zero'. This "high risk" paradigm requires a complete overhaul; it is no longer appropriate for making decision regarding what tests to offer to what patients. ACOG/SMFM has held since 2007 that ALL women are to be given ALL OPTIONS for screening AND diagnostic testing. Performance of cell free DNA is not significantly different (See Norton_NEJM_2015).

4. What are the cost-effectiveness and other economic outcomes of screening for trisomies 21, 18, and 13 and for common sex chromosome aneuploidies using cfDNA in pregnant individuals not known to be at high risk for chromosomal abnormalities?

Reduction in invasive testing has been demonstrated (Warsof and Hui attached) with increased detection of aneuploid fetuses, therefore higher rate of diagnosis of affected fetuses allowing reproductive decision making AND preparation for birth of child with special needs with reduced miscarriage of unaffected fetuses due to fewer invasive procedures. With respect to modelled cost effectiveness, please see enclosed: Fairbrothers_Ariosa, Benn pone and Walker_ARUP_PLOSone.

These remarks are supported by peer-reviewed references AND personal experience as a ob/gyn and clinical geneticist in academic and private practice for over 22 years.

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Maternal age- and gestation-specific risk for trisomy 21

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Key words: TRISOMY 21, CHROMOSOMAL DEFECTS, MATERNAL AGE, RISK COUNSELLING, NUCHAL TRANSLUCENCY

ABSTRACT

Objective *To provide estimates of maternal age- and gestational age-related risks for trisomy 21.*

Methods *The prevalence of trisomy 21 was examined in 57 614 women who had fetal karyotyping at 9–16 weeks of gestation for the sole indication of maternal age of 35 years or more. On the basis of the maternal age distribution and the reported maternal age-related risk for trisomy 21 at birth, the expected number of trisomy 21 cases was calculated for each gestational age subgroup (9–10 weeks, 11–14 weeks and 15–16 weeks). The ratio of the observed to expected number of cases of trisomy 21 was then calculated and regression analysis was applied to derive a smoothened curve. The formula for maternal age- and gestational age-related risk was then applied to a population of 96 127 pregnancies that were examined at 10–14 weeks to calculate the expected number of trisomy 21 pregnancies, and this number was compared to the observed number of 326.*

Results *In the 57 614 pregnancies there were 538 cases of trisomy 21. The relative prevalences of trisomy 21, compared to a prevalence of 1.0 at 40 weeks, was* $10\ exp(0.2718 \times \log_{10}(gestation)^2 - 1.023 \times \log_{10}(gestation) + 0.9425)$*. On the basis of the estimated maternal age- and gestational age-related risks, the expected number of trisomy 21 cases at 10–14 weeks of gestation in the 96 127 pregnancies was 329 (95% confidence interval 291–361), which was not significantly different from the observed number of 326 cases* ($\chi^2 = 0.02$)*.*

Conclusion *The risk for trisomy 21 increases with maternal age and decreases with gestation. The prevalence of trisomy 21 at 12 and 16 weeks of gestation is higher than the prevalence at 40 weeks by 30% and 21%, respectively.*

INTRODUCTION

Estimates of the maternal age-related risk for trisomy 21 at birth are based on two surveys with almost complete ascertainment; in a survey in South Belgium, every neonate was examined for features of trisomy 21, and, in a survey in Sweden, information was verified using several sources such as hospital notes, cytogenetic laboratories, genetic clinics and schools for the mentally handicapped[1–3].

During the past decade, with the introduction of maternal serum biochemistry and ultrasound screening for chromosomal defects at different stages of pregnancy, it has become necessary to establish maternal age- and gestational age-specific risks for chromosomal defects. Previous studies derived such estimates by comparing the birth prevalence of trisomy 21[1] to the prevalence reported in two multicenter studies on amniocentesis at 16–20 weeks of gestation[4,5] and the prevalence in small series on chorionic villus sampling at 9–14 weeks of gestation[6,7]. In this study, we revised our previous estimates by examining 57 614 pregnancies that were karyotyped at 9–16 weeks of gestation. Furthermore, we examined the accuracy of these estimates in a group of 96 127 singleton pregnancies with complete follow-up that were recruited at 10–14 weeks of gestation[8].

SUBJECTS AND METHODS

Estimate of risk

To calculate estimates of risk for trisomy 21 at different gestations, we used data from 57 614 women who had fetal karyotyping at 9–16 weeks of gestation for the sole indication of maternal age of 35 years or more. On the basis of the maternal age distribution and maternal age-related risk for trisomy 21 at birth[1], the expected

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number of trisomy 21 cases was calculated for each gestational age subgroup (9–10 weeks, 11–14 weeks and 15–16 weeks) and for each maternal age. In these calculations, the appropriate corrections were made for those women whose age in years would increase between the time of antenatal assessment at 9–16 weeks and the time of delivery. Gestational age was available in completed weeks. Pearson correlation analysis was applied to examine whether the ratio changed significantly with maternal age and/or gestation. Regression analysis was applied to ratios at 9–10 weeks, 11–14 weeks, 15–16 weeks and 40 weeks (where the ratio was set at one) to derive a smoothened curve for the decrease in ratio with gestational age.

Validation of the model

In a multicenter study of screening for trisomy 21 by combination of maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation, we obtained details on the outcome of 96 127 pregnancies[8]. This group included 326 pregnancies with trisomy 21. The new formula for maternal age- and gestational age-related risk was applied to this population, to calculate the expected number of trisomy 21 pregnancies, and this number was compared to the observed number of 326.

RESULTS

The maternal age and gestational age distributions of the 57 614 pregnancies are shown in Table 1. Trisomy 21 was diagnosed in 538 cases (Table 2). The relative prevalence of trisomy 21 (observed to expected ratio), compared to a prevalence of 1.0 at 40 weeks, was not significantly associated with maternal age ($r = 0.178$; $p = 0.31$) but decreased with gestational age ($r = 0.812$; $p < 0.01$). Data were grouped to derive the relative prevalence at different gestations (Table 3) and regression analysis was applied to derive a smoothened curve

Table 1 Maternal age and gestational age distribution of the 57 614 women who had fetal karyotyping

Maternal age (years)	*Gestational age* (weeks)								
	9	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*Total*
35	916	1832	823	233	192	1139	3 676	5 252	14 063
36	891	1736	805	258	185	971	3 101	4 484	12 431
37	744	1486	774	241	120	779	2 363	3 388	9 895
38	661	1139	629	170	109	567	1 789	2 614	7 678
39	413	905	479	164	104	364	1 211	1 740	5 380
40	278	597	339	115	60	274	749	1 147	3 559
41	168	408	231	85	48	172	459	689	2 260
42	97	219	128	59	29	96	283	357	1 268
43	45	108	84	32	13	46	135	184	647
44	23	75	29	18	1	30	64	86	326
45	4	17	16	2	8	9	24	27	107
Total	4240	8522	4337	1377	869	4447	13 854	19 968	57 614

Table 2 Maternal age and gestational age distribution of the 538 pregnancies with trisomy 21

Maternal age (years)	*Gestational age* (weeks)								
	9	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*Total*
35	5	9	5	1	0	4	14	18	56
36	6	9	4	2	1	5	16	31	74
37	7	10	7	2	0	5	15	18	64
38	8	11	5	2	1	5	14	25	71
39	7	16	8	1	1	5	14	23	75
40	3	14	8	2	1	4	14	10	56
41	4	11	4	2	1	3	10	16	51
42	4	8	3	2	1	2	7	12	39
43	2	5	4	1	1	2	5	7	27
44	1	5	1	0	0	1	3	5	16
45	0	2	2	0	1	1	1	2	9
Total	47	100	51	15	8	37	113	167	538

Table 3 Observed number of cases with trisomy 21 compared to the number expected in live births in relation to gestational age

Gestational age (weeks)	*n*	*Observed*	*Expected*	*Ratio*	*95% confidence interval*	*Regressed ratio*
9 + 0–10 + 6	12 762	147	93.6	1.57	1.32–1.82	1.55
11 + 0–14 + 6	11 030	111	82.4	1.35	1.10–1.60	1.38
15 + 0–16 + 6	33 822	280	217.8	1.29	1.14–1.44	1.27

[$\log_{10}$(relative prevalence) = $0.2718 \times \log_{10}(\text{gestation})^2 - 1.023 \times \log_{10}(\text{gestation}) + 0.9425$]. The estimated maternal age- and gestational age-related risks for trisomy 21 are given in Table 4. The estimated rates of spontaneous fetal death between different gestations and delivery at 40 weeks were derived on the basis of the relative prevalences between these gestations and 40 weeks (Table 5).

Validation of the model

The accuracy of the model was examined on the basis of findings in 96 127 pregnancies examined at 10–14 weeks of gestation. The expected number of cases with trisomy 21 was estimated to be 329 (95% confidence interval 291–361). This number is not significantly different from the observed number of 326 cases ($\chi^2 = 0.02$). There was no significant difference between the observed and expected numbers for different gestational age and maternal age subgroups (Table 6).

Table 4 Prevalence of trisomy 21 by maternal age and gestational age

Maternal age (years)	*Gestational age* (weeks)					
	10	*12*	*14*	*16*	*20*	*40*
20	1/983	1/1068	1/1140	1/1200	1/1295	1/1527
25	1/870	1/946	1/1009	1/1062	1/1147	1/1352
30	1/576	1/626	1/668	1/703	1/759	1/895
31	1/500	1/543	1/580	1/610	1/658	1/776
32	1/424	1/461	1/492	1/518	1/559	1/659
33	1/352	1/383	1/409	1/430	1/464	1/547
34	1/287	1/312	1/333	1/350	1/378	1/446
35	1/229	1/249	1/266	1/280	1/302	1/356
36	1/180	1/196	1/209	1/220	1/238	1/280
37	1/140	1/152	1/163	1/171	1/185	1/218
38	1/108	1/117	1/125	1/131	1/142	1/167
39	1/82	1/89	1/95	1/100	1/108	1/128
40	1/62	1/68	1/72	1/76	1/82	1/97
41	1/47	1/51	1/54	1/57	1/62	1/73
42	1/35	1/38	1/41	1/43	1/46	1/55
43	1/26	1/29	1/30	1/32	1/35	1/41
44	1/20	1/21	1/23	1/24	1/26	1/30
45	1/15	1/16	1/17	1/18	1/19	1/23

Table 5 Estimates for spontaneous loss rates for fetuses with trisomy 21 between various gestations and delivery at 40 weeks

Gestational age (weeks)	*Estimated loss rate* (%)
10	36
12	30
14	25
16	21

DISCUSSION

This study provides revised estimates of maternal age- and gestational age-related risk for trisomy 21. Compared to our previous report[7], in this study the number of cases with fetal karyotyping was much higher (57 614 compared to 15 793). Additionally, in this study the appropriate corrections were made for the increase in maternal age with advancing gestation.

The estimates for the rate of spontaneous fetal death for trisomy 21 are lower than in our previous report (30% compared to 41% for the loss rate between 12 and 40 weeks of gestation and 21% compared to 31% for the loss rate between 16 and 40 weeks)[7]. The main reason for this apparent discrepancy is that in the previous analysis there was no correction for the increase in maternal age with advancing gestation. This led to an underestimate for the expected number of trisomy 21 live births and thus to an overestimate of the loss rate. The new estimates of loss rates are similar to the 31% from 12 weeks and 18% from 16 weeks reported by Halliday and colleagues[9]; they compared the prevalence of trisomy 21 in 10 545 women having chorionic villus sampling or amniocentesis for the sole indication of maternal age of 36 years or more, to the prevalence in live births from 12 921 women of similar age who did not have fetal karyotyping[9].

Assessment of the model on the basis of findings in 96 127 pregnancies indicates that the estimated prevalences are accurate at least for the gestational range of 10–14 weeks; the estimates at 16 weeks and 20 weeks of gestation require validation with an independent data set.

The model makes it possible to counsel patients presenting at different stages of pregnancy concerning the risk that their fetus has trisomy 21 and the chances that the pregnancy will result in a live birth with this condition. Furthermore, the data can be used to calculate the expected

Table 6 Expected and observed number of pregnancies with trisomy 21 for different gestations and maternal age subgroups

	n	*Expected*	*Observed number*	*Observed 95% CI*	χ^2
Gestational age (weeks)					
10	4 889	15.8	11	5–18	0.86
11	34 046	119.1	137	114–160	1.26
12	42 884	146.2	141	118–164	0.09
13	14 308	48.0	37	25–49	1.43
Maternal age (years)					
< 30	39 834	46.2	36	24–48	1.03
30–34	32 489	73.9	67	51–83	0.25
35–39	20 263	128.2	125	103–147	0.02
≥ 40	3 541	80.8	98	79–117	1.51
Total	96 127	329.1	326	291–361	0.02

prevalence of trisomy 21 in any study group when new ultrasonographic or biochemical methods of screening are being evaluated.

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(See also Practice Bulletin Number 162, *Prenatal Diagnostic Testing for Genetic Disorders*)

Screening for Fetal Aneuploidy

Prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. In contrast, prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. The purpose of prenatal screening for aneuploidy is to provide an assessment of the woman's risk of carrying a fetus with one of the more common fetal aneuploidies. This is in contrast to prenatal diagnostic testing for genetic disorders, in which the fetal chromosomes are evaluated for the presence or absence of abnormalities in chromosome number, deletions, and duplications, or the fetal DNA is evaluated for specific genetic disorders. The wide variety of screening test options, each offering varying levels of information and accuracy, has resulted in the need for complex counseling by the health care provider and complex decision making by the patient. No one screening test is superior to other screening tests in all test characteristics. Each test has relative advantages and disadvantages. It is important that obstetrician–gynecologists and other obstetric care providers be prepared to discuss not only the risk of aneuploidy but also the benefits, risks, and limitations of available screening tests. Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.

The purpose of this Practice Bulletin is to provide current information regarding the available screening test options for fetal aneuploidy and to review their benefits, accuracy, and limitations. For information regarding prenatal diagnostic testing for genetic disorders, refer to Practice Bulletin No. 162, Prenatal Diagnostic Testing for Genetic Disorders.

Background

Aneuploidy is defined as having one or more extra or missing chromosomes, leading to an unbalanced chromosome number in a cell. Because each chromosome consists of hundreds of genes, the loss or gain of large chromosomal segments disrupts significant amounts of genetic material and often results in a nonviable pregnancy or offspring that may not survive after birth. In the case of a surviving newborn, congenital birth defects; failure to thrive; and functional abnormalities, including mild-to-severe intellectual disability, infertility, and shortened lifespan, may occur.

Although chromosomal abnormalities occur in approximately 1 in 150 live births (1), the prevalence is greater earlier in gestation because aneuploidy accounts for a large proportion of early pregnancy loss. The incidence of fetal aneuploidy increases as a woman ages (Table 1) but can affect any woman regardless of age and is not related to race or ethnicity. Other factors that increase the risk of fetal aneuploidy include a history of a prior aneuploid fetus and the presence of fetal anomalies. Autosomal trisomies are the most common aneuploidies that are not related to sex chromosome disorders. Down syndrome (trisomy 21) is the most common of these, with a prevalence of approximately 1 in 800 live births

Committee on Practice Bulletins—Obstetrics, Committee on Genetics, and Society for Maternal–Fetal Medicine. This Practice Bulletin was developed by the American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics, Committee on Genetics, and the Society for Maternal–Fetal Medicine in collaboration with Nancy C. Rose, MD and Brian M. Mercer, MD. The information is designed to aid practitioners in making decisions about appropriate obstetric and gynecologic care. These guidelines should not be construed as dictating an exclusive course of treatment or procedure. Variations in practice may be warranted based on the needs of the individual patient, resources, and limitations unique to the institution or type of practice.

(1). The most common sex chromosome aneuploidy is Klinefelter syndrome (47,XXY) with a prevalence of 1 in 500 males. The only viable monosomy is Turner syndrome (45,X).

Down syndrome is the most common form of inherited intellectual disability, with approximately 6,000 affected infants born in the United States each year. It is estimated that 95% of cases of Down syndrome result from nondisjunction involving chromosome 21. The remaining cases result from translocations or somatic mosaicism (2). Although the clinical presentation of Down syndrome can vary, it is associated with characteristic facial features, learning disabilities, congenital heart defects (eg, atrioventricular canal defects), intestinal atresia, seizures, childhood leukemia, and early-onset Alzheimer disease. Fetuses affected with Down syndrome often do not survive pregnancy; between the first trimester and full term, an estimated 43% of pregnancies end in miscarriage or stillbirth (3). In economically developed countries, the median survival of individuals with Down syndrome is now almost 60 years (4). Factors associated with an increased risk of Down syndrome include higher maternal age, a parental translocation involving chromosome 21, a previous child with a trisomy, significant ultrasonographic findings, and a positive screening test result. After a prenatal diagnosis is made, prenatal assessment cannot predict the severity of the complications from Down syndrome.

In general, the process of aneuploidy screening identifies two groups of individuals: 1) those with a positive screening test result who have an increased risk of having a fetus with an aneuploidy and 2) those with a negative screening test result who have a lower posttest probability of the evaluated aneuploidies. Women with a positive screening test result should be counseled regarding their higher risk of aneuploidy and offered the option of diagnostic testing. Those who have a negative test result should be counseled regarding their lower adjusted risk and their lower residual risk. Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result. Even if a woman has a negative test result, she may choose diagnostic testing later in pregnancy, particularly if additional findings become evident (eg, fetal anomalies or markers of aneuploidy identified on follow-up ultrasonography).

Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit. The choice of whether to perform screening or diagnostic testing for aneuploidy depends on the woman's goals and values and her desire for informational accuracy. Although maternal age may

Table 1. Risk of Chromosomal Abnormalities Based on Maternal Age at Term

Age at Term	Risk of Trisomy 21*	Risk of Any Chromosome Abnormality†
15‡	1:1,578	1:454
16‡	1:1,572	1:475
17‡	1:1,565	1:499
18‡	1:1,556	1:525
19‡	1:1,544	1:555
20	1:1,480	1:525
21	1:1,460	1:525
22	1:1,440	1:499
23	1:1,420	1:499
24	1:1,380	1:475
25	1:1,340	1:475
26	1:1,290	1:475
27	1:1,220	1:454
28	1:1,140	1:434
29	1:1,050	1:416
30	1:940	1:384
31	1:820	1:384
32	1:700	1:322
33	1:570	1:285
34	1:456	1:243
35	1:353	1:178
36	1:267	1:148
37	1:199	1:122
38	1:148	1:104
39	1:111	1:80
40	1:85	1:62
41	1:67	1:48
42	1:54	1:38
43	1:45	1:30
44	1:39	1:23
45	1:35	1:18
46	1:31	1:14
47	1:29	1:10
48	1:27	1:8
49	1:26	1:6
50	1:25	§

*Data from Morris JK, Wald NJ, Mutton DE, Alberman E. Comparison of models of maternal age-specific risk for Down syndrome live births. Prenat Diagn 2003;23:252–8.

†Risk of any chromosomal abnormality includes the risk of trisomy 21 and trisomy 18 in addition to trisomy 13, 47,XXY, 47,XYY, Turner syndrome genotype, and other clinically significant abnormalities, 47,XXX not included. Data from Hook EB. Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 1981;58:282–5.

‡Data from Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94:387–402.

§Data not available.

be helpful in adjusting an individual woman's risk of having a fetus with aneuploidy, it should not be used as the sole determinant of whether aneuploidy screening or diagnostic testing is offered. Although the risk of aneuploidy increases with advancing maternal age, most children with Down syndrome are born to younger women because a larger proportion of all children are born to young women. An observational study of more than 38,000 women demonstrated that if all women aged 35 years and older had had diagnostic testing, the detection rate for Down syndrome would have been only 21.6% (5).

Screening tests for aneuploidy are now available for pregnant women in all trimesters of pregnancy. Among these are first-trimester, triple, quad, and penta screens; cell-free DNA; and ultrasonographic screening as single screening tests. Screening tests that are performed in the first and second trimesters include integrated, sequential, and contingent screening.

The intent of counseling for aneuploidy is to inform the pregnant woman about chromosomal disorders, provide information regarding her specific risk of carrying a fetus with aneuploidy, and review the available options so that she can make an informed choice regarding screening or diagnostic testing. After review and discussion, every patient has the right to pursue or decline screening or diagnostic testing. Pretest and posttest counseling are essential and must be a part of any screening program. When a positive or negative screening test result is obtained, the patient should be counseled regarding the adjusted likelihood of carrying a fetus with the evaluated aneuploidies. The potential for the fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should be reviewed. In the event that a prenatal diagnosis of fetal aneuploidy is made, the patient must be counseled appropriately so that she can make informed decisions regarding pregnancy management. Counseling should include family education and preparedness as well as options regarding adoption, pregnancy termination, referral to a tertiary care center for delivery of the newborn if needed, and perinatal hospice care as appropriate for a child with a condition that is incompatible with life. Patients found to have a fetus with a chromosomal abnormality often benefit from referral to a genetics professional for further detailed counseling.

Single Screening Tests

First-Trimester Screening

Typically performed when the crown–rump length measures between 38–45 mm and 84 mm (generally between 10 0/7 weeks and 13 6/7 weeks of gestation), first-trimester screening includes a nuchal translucency measurement, serum free β-hCG, or total human chorionic gonadotropin (hCG) along with pregnancy-associated plasma protein A analyte levels. A specific risk estimate for aneuploidy is calculated using these results as well as maternal factors such as maternal age, prior history of aneuploidy, weight, race, and number of fetuses.

The nuchal translucency refers to the fluid-filled space measured on the dorsal aspect of the fetal neck. An enlarged nuchal translucency (often defined as 3.0 mm or more or above the 99th percentile for the crown–rump length) is independently associated with fetal aneuploidy and structural malformations. The risk of adverse pregnancy outcome is proportional to the degree of nuchal translucency enlargement. Meticulous technique in nuchal translucency imaging is essential for accurate risk assessment because undermeasurement by even 0.5 mm can reduce the test sensitivity by 18% (6). Independent credentialing of ultrasonographers in appropriate technique is important to screening performance.

Quadruple Screen

The quadruple marker screen ("quad" screen) can be performed from approximately 15 0/7 weeks to 22 6/7 weeks of gestation; the range is dependent on the laboratory that the obstetrician–gynecologist or other obstetric care provider uses. This test does not require specialized ultrasonography for nuchal translucency measurement and gives information regarding the risk of open fetal defects in addition to aneuploidy risk assessment. The best time to perform a quad screen is from approximately 16 weeks to 18 weeks of gestation because this optimizes screening for neural tube defects. The quad screen involves the measurement of four maternal serum analytes: 1) hCG, 2) alpha fetoprotein (AFP), 3) dimeric inhibin A, and 4) unconjugated estriol, in combination with maternal factors such as age, weight, race, the presence of diabetes, and plurality to calculate a risk estimate. First-trimester and quad screening have similar detection rates for Down syndrome: more than 80% at a 5% positive result rate (Table 2) (5). Accurate gestational dating at the time of blood sampling is important because inaccurate gestational dating decreases the accuracy of the result. The later timing of this test leaves fewer options available for the patient if the results are positive.

Penta Screen

The penta screen includes hyperglycosylated hCG (also known as invasive trophoblast antigen) in addition to the quad screen markers and also is available for

Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Aneuploidy

Screening Test	Gestational Age Range for Screening (Weeks)	Detection Rate for Down Syndrome (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
First trimester[†]	11–14[‖]	82–87	5	1. Early screening 2. Single test 3. Analyte assessment of other adverse outcome	Lower DR than combined tests NT required	NT+PAPP-A and hCG
Triple screen	15–22	69	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than with first-trimester or quad screening Lowest accuracy of the single lab tests	hCG, AFP, uE3
Quad screen[†]	15–22	81	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than combined tests	hCG, AFP, uE3, DIA
Integrated[†]	11–14, then 15–22	96	5	Highest DR of combined tests Also screens for open fetal defects	Two samples needed before results are known	NT+PAPP-A, then quad screen
Sequential[‡]: Stepwise	11–14, then 15–22	95	5	First-trimester results provided; Comparable performance to integrated, but FTS results provided; also screens for open fetal defects; analyte assessment for other adverse outcomes.	Two samples needed	NT+hCG+ PAPP-A then quad screen
Contingent screening[‡]		88–94	5	First-trimester test result: Positive: diagnostic test offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed	NT+hCG+ PAPP-A, then quad screen
Serum Integrated[†]	11–14; then 15–22	88	5	1. DR compares favorably with other tests. 2. No need for NT	Two samples needed; no first-trimester results	PAPP-A+quad
Cell-free DNA[§]	10 - term	99 (in patients who receive a result)	0.5	1. Highest DR for Down syndrome 2. Can be performed at any gestational age after 10 weeks 3. Low false-positive rate in high-risk women (or women at high risk of Down syndrome)	1. NPV and PPV not clearly reported 2. Higher false-positive rate in women at low risk of Down syndrome 3. Limited information about three trisomies and fetal sex 4. Results do not always represent a fetal DNA result	Three roughly equivalent molecular methods
Nuchal Translucency[†]	11–14[‖]	64–70	5	Allows individual fetus assessment in multifetal gestations Provides additional screening for fetal anomalies and possibly for twin–twin transfusion syndrome	1. Poor screen in isolation 2. Ultrasound certification necessary	US only

Abbreviations: AFP, alpha fetoprotein; DIA, dimeric inhibin-A; DR, detection rate; DS, Down syndrome; FTS, first-trimester screening; hCG, human chorionic gonadotropin; NPV, negative predictive value; NT, nuchal translucency; NTD, neural tube defect; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; uE3, unconjugated estriol; US, ultrasonography.

*A screen positive test result includes all positive test results: the true positives and false positives.

[†]First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11 weeks, 12 weeks, and 13 weeks, respectively. Malone FASTER 2005.

[‡]Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

[§]Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group [published erratum appears in Obstet Gynecol 2012;120:957]. Obstet Gynecol 2012;119:890–901 *and* Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.

[‖]Because of variations in growth and conception timing, some fetuses at the lower and upper gestational age limits may fall outside the required crown–rump length range.

Data from Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

second-trimester screening (7) by at least one national laboratory. Although there is some evidence from one limited retrospective trial that this test may improve second-trimester screening performance, its performance has not been evaluated rigorously in prospective studies and it is not widely used. Limited data are available to compare the accuracy of the penta screen with other second-trimester screening tests.

Triple Screen

The triple marker screen measures serum hCG, AFP, and unconjugated estriol to determine a risk estimate. This test provides a lower sensitivity for the detection of Down syndrome (sensitivity of 69% at a 5% positive screening test result rate) than quad screen and first-trimester screening (5).

Combined First- and Second-Trimester Screening

Combined first- and second-trimester screening with either integrated, sequential, or contingent screening protocol provides a higher detection rate than one-step screening. Depending on the test selected, results are not available until the second trimester or possibly in the first trimester under certain circumstances.

Integrated Screening and Serum Integrated Screening

With integrated screening, the patient undergoes a first-trimester nuchal translucency measurement and analyte screening followed by a second-trimester quad screen and receives a single test result in the second trimester. In locations where a nuchal translucency measurement by a certified ultrasonographer is unavailable, or if fetal position, maternal body habitus, or imaging properties preclude an accurate nuchal translucency measurement, serum integrated screening can be offered. Serum integrated screening has a similar but slightly lower detection rate than integrated screening (Table 2). Limitations of integrated screening include the withholding of first-trimester screening test results until the second trimester and nonadherence of the second blood draw; rates of nonadherence in practice have been reported to be as high as 25% without a written reminder to complete the test (8).

Sequential Screening: the Stepwise and Contingent Screening Models

Sequential screening was developed to maintain a high detection rate using the combined first- and second-trimester screening approach while also reporting the patient's first-trimester screening test risk, which allows for earlier management options. Using stepwise sequential screening, the patient is given a preliminary risk estimate after completion of the first-trimester analytes and nuchal translucency screening. If the first-trimester screening result indicates that the risk of aneuploidy is greater than the laboratory-derived positive screening cutoff, the patient is notified and offered a diagnostic test or cell-free DNA screening, and the screening protocol is discontinued. If the patient has a lower risk than the cutoff level, she is informed that she has received a negative screening test result and proceeds to quad screening in the second trimester. Sequential screening has a detection rate of 91–93% with a positive screening test result rate of 4–5% (9, 10).

The contingent model classifies aneuploidy risk as high, intermediate, or low on the basis of the first-trimester screening test results; women at high risk are offered cell-free DNA screening or diagnostic testing with chorionic villus sampling (CVS), and for those at low risk, no further screening or testing is recommended. Only those women at intermediate risk are offered second-trimester screening and, thus, fewer women go on to second-trimester screening.

In the stepwise and contingent models, the patients at highest risk identified by first-trimester screening are offered an early diagnostic procedure. First- and second-trimester results are used together to calculate a final risk of aneuploidy in patients at lower risk in the stepwise and sequential models. The sequential approach takes advantage of the higher detection rate achieved by incorporating the first- and second-trimester screening test results with only a marginal increase in the false-positive rate. Theoretically, the contingent approach should maintain high detection rates with low false-positive rates while reducing the number of second-trimester tests performed.

The use of multiple screening tests performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver confusing risk estimates to patients. In patients who undergo first-trimester screening, if later screening for risk of neural tube defects is to be done with maternal serum alpha-fetoprotein (MSAFP), the test should be performed as an isolated screening test and not as part of a quad screen.

Ultrasonographic Screening

Although fetuses with trisomy 13 (Patau syndrome, which occurs in 1 in 10,000 births) or trisomy 18 (Edwards syndrome, which occurs in 1 in 6,000 births)

usually have major structural anomalies that are evident on ultrasonography, the ultrasonographic identification of Down syndrome is more elusive. For several decades, the second-trimester "genetic ultrasonogram" has been used to screen for Down syndrome using specific ultrasonographic findings (11). This approach seeks to identify major structural abnormalities and minor ultrasonographic "soft markers" of aneuploidy. The major structural anomalies associated with fetal Down syndrome include cardiac anomalies (such as septal defects, tetralogy of Fallot, and atrioventricular canal defects) usually identified in the second trimester and duodenal atresia, which typically is identified in the third trimester. In contrast, second- and third-trimester soft ultrasonographic markers for aneuploidy are nonspecific physical characteristics that are more common among fetuses with Down syndrome and in some cases also can reflect or progress to an overt fetal abnormality (eg, thickened nuchal fold, renal pelvis dilation, or echogenic bowel). Because soft markers for aneuploidy also are common in unaffected fetuses, it is difficult to use these findings to distinguish between pregnancies affected or unaffected by aneuploidy. As an isolated finding, an increased nuchal skinfold thickness confers the highest risk of aneuploidy. In contrast, an isolated echogenic intracardiac focus carries one of the lowest risks of fetal aneuploidy (12, 13). If an isolated low-risk marker such as a choroid plexus cyst or intracardiac echogenic focus is identified on the fetal anatomic ultrasound survey, the patient's chart should be reviewed to determine if analyte screening has been performed previously; if not, it should be offered. Additional follow-up for isolated ultrasonographic markers generally is not indicated other than for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur length (14). Patients with these markers may benefit from referral for detailed ultrasonography and follow-up. Major limitations of the use of second-trimester ultrasonographic markers include the lack of standardization in measurements and characteristics that define a positive test result, and the lack of understanding of how factors such as high maternal body mass index, multiple gestation, machine quality, and experience of the ultrasonographer and ultrasonologist affect screening performance.

Cell-free DNA Screening

Cell-free DNA screening evaluates short segments of DNA in maternal blood and can be used to screen for a variety of fetal conditions. The fetal component of cell-free DNA is released into the maternal circulation primarily from placental cells undergoing apoptosis or programmed cell death and comprises approximately 3–13% of the total cell-free DNA in maternal blood (15). This amount increases throughout gestation and is cleared from the maternal circulation within hours after childbirth (16). Several molecular methods have been developed to analyze cell-free DNA for the purpose of aneuploidy screening, and all appear to have similar detection and false-positive rates, although direct comparison trials have not been performed. Cell-free DNA screening also can be used to determine fetal sex, to identify the presence of a Rh-positive fetus in a Rh-negative mother, and to detect some paternally derived autosomal dominant genetic abnormalities (17–19). Screening can be performed from as early as 10 weeks of gestation until term and offers the highest reported detection rate for Down syndrome: more than 98% detection with positive screening rates of less than 0.5% among women with a reportable result (20). The detection rate is lower for trisomy 13 and trisomy 18 (21–27). Further, published studies have excluded those who have no reportable result, and these women are at increased risk of fetal aneuploidy (22, 23, 28). Inclusion of these women in the calculations would yield lower sensitivity for fetal aneuploidy. In addition, managing women with no reportable result as screen positive will decrease the specificity and increase the positive screening rate for this testing.

Clinical Considerations and Recommendations

▶ *Who should be offered screening for aneuploidy?*

All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age. The choice of screening test is affected by many factors, including a desire for information before delivery, prior obstetric history, family history, and the number of fetuses. Other factors to be considered include gestational age at presentation, the availability of a reliable nuchal translucency measurement, screening test sensitivity and limitations, the cost of screening, the constraints of long-term care of an affected child, and options for pregnancy care or termination for an abnormal diagnostic test result. No one test is superior for all test characteristics and not every test is available at all centers. Each test has advantages and disadvantages that should be discussed with each patient, with the appropriate test offered based on her concerns, needs, and values. Obstetrician–gynecologists and other obstetric care providers should become familiar with the available screening and diagnostic testing options for their patients within their practice and adopt a standard approach for counseling. Regardless of which screening tests are

offered, information about the detection (sensitivity) and positive screening and false-positive rates, advantages, disadvantages, and limitations should be communicated to the patient. At the time of counseling regarding aneuploidy screening, the benefits and risks of diagnostic testing (amniocentesis and chorionic villus sampling) also should be discussed (29). After counseling, patients may decline screening or diagnostic testing for any reason.

▶ *What is the role of ultrasonography in screening for fetal aneuploidy?*

In women of advanced maternal age, the absence of ultrasonographic markers has been used to decrease a woman's age-related risk of aneuploidy by greater than 80% (30, 31). However, with the exception of maternal age, second-trimester ultrasonography is the least effective primary screening test for Down syndrome, detecting only 50–60% of affected fetuses. As such, ultrasonography should not be used in isolation to diagnose or exclude Down syndrome. Ultrasonographic markers can identify other disorders, and the various soft markers have different degrees of association with Down syndrome. The risk of aneuploidy associated with each marker should be considered individually within the complete clinical context. The presence of soft ultrasonographic markers for aneuploidy warrants a targeted ultrasound to exclude other evident abnormalities and a review or offering of screening tests for fetal aneuploidy. Of the soft markers, third-trimester follow-up is only indicated for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur (14). For women who have already undergone screening for aneuploidy and have received a negative screening test result, and for those who have had normal diagnostic testing, ultrasonography should not be used as an additional screening test for aneuploidy. If aneuploidy screening has been performed before ultrasonographic evaluation, no additional evaluation is indicated if an echogenic intracardiac feature or choroid plexus cysts is the sole identified marker (Table 3). However, further detailed counseling is recommended for fetuses with a hypoplastic or absent nasal bone, echogenic bowel, or nuchal skinfold thickening (14). If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.

With regard to first-trimester imaging, an increased nuchal translucency measurement increases the risk of genetic syndromes and isolated anomalies, such as congenital heart defects, abdominal wall defects, and diaphragmatic hernia, even with normal chromosomes on diagnostic testing (32). These patients should be offered a targeted ultrasound examination and fetal echocardiography in the second trimester.

The finding of an increased nuchal translucency extending along the length of the fetus in which septations are clearly visible is referred to as a cystic hygroma. This finding is associated with a 50% likelihood of fetal aneuploidy (most commonly Down syndrome, 45,X, and trisomy 18). Of the remaining euploid fetuses, one half will have a major structural malformation, such as congenital heart defects, diaphragmatic hernia, or skeletal dysplasia, or other genetic syndromes. Less than 20% of such pregnancies will result in a healthy live-born infant at term (33). A nuchal measurement for aneuploidy risk is not necessary at the time of cell-free DNA screening in the first trimester. However, ultrasound examination is useful to confirm viability, to confirm the number of fetuses and the presence of an empty gestational sac, to assign gestational age, and to identify some major fetal anomalies for patients who choose to have cell-free DNA screening (34). Patients who choose serum integrated screening may be offered first-trimester ultrasonography for gestational dating even if nuchal translucency measurement is unavailable or cannot be obtained. If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities. Given the high risk of congenital heart disease in these fetuses, referral for fetal cardiac ultrasonography should be considered. Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.

▶ *What are the characteristics and limitations of the different screening tests?*

First-Trimester Screening

The first-trimester screening, or first-trimester combined screening, comprising nuchal translucency measurement and serum analyte measurements combined into a single test, is performed before 14 0/7 weeks of gestation (with the range determined by the laboratory accepting the sample, typically between 10 0/7 weeks and 13 6/7 weeks of gestation) and requires a crown–rump length between approximately 38–45 mm and 84 mm. Advantages of first-trimester screening are a slightly higher, but not significantly different, detection rate for Down syndrome compared with second-trimester screening. This test gives the potential for earlier diagnoses as well as the ability to screen for other fetal or placental disorders. However, first-trimester screening lacks the ability to assess the risk

Table 3. Management of Ultrasonographic Markers for Aneuploidy

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
First trimester: enlarged nuchal translucency	Certified ultrasonography measurement ≥ 3.0 mm or above the 99th percentile for the CRL	Aneuploidy risk increases with size of NT Also associated with Noonan syndrome, multiple pterygium syndrome, skeletal dysplasias, congenital heart disease, and other anomalies	1. Genetic counseling 2. Offer cfDNA or CVS 3. Second-trimester detailed anatomic survey and fetal cardiac ultrasonography
First trimester: cystic hygroma	Large single or multilocular fluid-filled cavities, in the nuchal region and can extend the length of the fetus	If septate, approximately 50% are aneuploid	1. Genetic counseling 2. Offer CVS 3. Second-trimester detailed fetal anatomic survey and fetal cardiac ultrasonography
Second trimester: echogenic intracardiac foci	Echogenic tissue in one or both ventricles of the heart seen on standard four-chamber view	LR 1.4–1.8 for Down syndrome Seen in 15–30% of Down syndrome and 4–7% euploid fetuses	1. If isolated finding, aneuploidy screening should be offered if not done previously 2. If aneuploidy screen result is negative, no further evaluation is required.
Second trimester: pyelectasis	Renal pelvis measuring ≥ 4 mm in anteroposterior diameter up to 20 weeks of gestation	LR 1.5–1.6 for Down syndrome	1. If isolated finding, aneuploidy screening should be offered if not performed previously 2. Repeat ultrasonography in third trimester for potential urinary tract obstruction
Second trimester: echogenic bowel	Fetal small bowel as echogenic as bone	LR 5.5–6.7 for Down syndrome Associated with aneuploidy, intra-amniotic bleeding, cystic fibrosis, CMV	1. Further counseling 2. Offer CMV, CF, and aneuploidy screening or diagnostic testing
Second trimester: thickened nuchal fold	≥ 6 mm from outer edge of the occipital bone to outer skin in the midline	LR 11–18.6 with 40–50% sensitivity and > 99% specificity for Down syndrome Most powerful second-trimester marker	1. Detailed anatomic survey 2. Further detailed genetic counseling and aneuploidy screening or diagnostic testing
Second trimester: mild ventriculomegaly	Lateral ventricular atrial measurement between 10–15 mm	Associated with aneuploidy LR 25 for Down syndrome	1. Genetic counseling 2. Second-trimester detailed anatomic ultrasound evaluation 3. Consider diagnostic testing for aneuploidy and CMV 4. Repeat ultrasound in third trimester
Second trimester: choroid plexus cysts	Discrete cyst(s) in one or both choroid plexus(es)	In isolation, no aneuploidy association	1. Second-trimester detailed anatomic survey and fetal cardiac ultrasound 2. No further follow-up if isolated 3. Consider aneuploidy screening or diagnostic testing if other markers are present
Second trimester: short femur length	Measurement < 2.5 percentile for gestational age	LR 1.2–2.2 for Down syndrome. Can be associated with aneuploidy, IUGR, short limb dysplasia	1. Second-trimester detailed fetal anatomic evaluation for short limb dysplasia 2. Further detailed counseling 3. Consider repeat ultrasonography in third trimester for fetal growth

Abbreviations: CF, cystic fibrosis; cfDNA, cell-free DNA; CMV, cytomegalovirus; CRL, crown–rump length; CVS, chorionic villus sampling; IUGR, intrauterine growth restriction; LR, likelihood ratio; NT, nuchal translucency.

Data from Reddy UM, Abuhamad AZ, Levine D, Saade GR. Fetal imaging: executive summary of a joint Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Institute of Ultrasound in Medicine, American College of Obstetricians and Gynecologists, American College of Radiology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound Fetal Imaging workshop. Fetal Imaging Workshop Invited Participants. Obstet Gynecol 2014;123:1070–82; Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, et al. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. FASTER Trial Research Consortium. Obstet Gynecol 2005;106:288–94; Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, et al. Role of second-trimester genetic sonography after Down syndrome screening. First and Second Trimester Evaluation of Risk Research Consortium. Obstet Gynecol 2009;114:1189–96; *and* Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ 1992;304:867–9.

of open fetal defects and relies on the availability of a certified ultrasonographer. Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.

Second-Trimester Serum Screening Tests

Second-trimester serum screening, which typically is performed between approximately 15 0/7 weeks and 22 6/7 weeks of gestation, provides an adjusted risk assessment for Down syndrome, trisomy 18, and open fetal defects. The detection rate with quad screening is similar to first-trimester screening: more than 80% detection at a 5% positive screening test result rate for Down syndrome. Some laboratories offer additional screening for rare disorders such as Smith–Lemli–Opitz syndrome and placental sulfatase deficiency if indicated by an extremely low unconjugated estriol value. Also performed in the second trimester, the triple screen is less sensitive for Down syndrome (sensitivity of 69% at a 5% positive screening test result rate). The penta screen has no prospective validation trials to determine its efficacy; using modeling, it appears to perform well with the inclusion of invasive trophoblast antigen as an additional screening marker (7). None of these screening tests require specialized ultrasonographic measurements, although accurate gestational dating improves risk accuracy determinations.

Integrated Screening

Integrated screening combines first-trimester nuchal translucency and serum analyte screening with second-trimester quad screening to give one result for aneuploidy risk, with a detection rate for Down syndrome of approximately 96% at a 5% positive screening test result rate (Table 2). In addition to having a high sensitivity for Down syndrome, integrated screening provides information that is not available from nuchal translucency assessment regarding fetal abnormalities as well as a risk assessment for open fetal defects. However, integrated screening is complex, requiring first-trimester ultrasound assessment and two different blood tests, and the final result is not available until the second trimester.

Sequential Screening: Stepwise and Contingent Screening

Like integrated screening, both forms of sequential screening have the option of first- and second-trimester testing for a combined final test result. However, the first-trimester screening result is provided to the patient when it is available and, if the patient is found to be at high risk of aneuploidy after the first test, the patient can consider further evaluation with either cell-free DNA screening or with diagnostic testing. This allows the patient to receive an abnormal result in the first trimester when more diagnostic and management options are available.

▶ *What are the limitations of cell-free DNA screening?*

Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing. A large referral-based cytogenetics laboratory reported their experience with 109 consecutive fetal samples from pregnancies that had positive screening test results for cell-free DNA screening from four different laboratories that use varied cell-free DNA screening techniques. Based on cytogenetic confirmation, the positive predictive value, or chance that a positive screening test result was a true positive, using cell-free DNA screening was 93% for Down syndrome, 64% for trisomy 18, 44% for trisomy 13, and 39% for sex chromosome aneuploidy (35). Because the test usually cannot distinguish fetal DNA from maternal DNA, a positive screening test result could represent confined placental mosaicism, a resorbing twin or, in rare instances, a maternal malignancy or maternal aneuploidy (36).

The discrimination of euploid from aneuploid pregnancies with cell-free DNA screening is more effective with larger fetal fractions. At 11–13 weeks, the median fetal fraction of cell-free DNA in maternal plasma is approximately 10% (15). Factors contributing to low fetal fraction include sampling before 10 weeks of gestation, high maternal body mass index, and fetal aneuploidy. In some laboratories, cell-free DNA fractions less than 4% are considered too low to report a result, often referred to as a "no call" result. Recent studies have demonstrated that low fetal fractions indicate a high risk of aneuploidy (22, 23, 28). In one study of more than 1,000 analyzed samples, 8% failed to obtain a result, and 22% of those were aneuploid (28). Pregnancies that initially do not return a cell-free DNA test result because of low fetal fraction can be managed with repeat cell-free DNA screening or diagnostic testing. However, if repeat cell-free DNA screening is performed, this may delay diagnosis of fetal aneuploidy, which may affect reproductive options for an abnormal result.

To date, most published experience with cell-free DNA screening is based on studies conducted on high-risk populations. Data on the performance of cell-free DNA testing in the general obstetric population are now available (22, 37–40). The sensitivity and specificity in the general obstetric population are similar to the

levels previously published for the high-risk population. However, cell-free DNA screening cannot have the same accuracy in low-risk pregnancies (eg, in young women) because the positive predictive value is affected by the prevalence of the disorder in the population. The positive predictive value is lower in the general obstetric population because of the lower prevalence of aneuploidy in this population.

In low-risk populations, there is a larger proportion of false-positive test results among the patients who receive positive screening test results. This decrease in accuracy is especially concerning when pregnancy terminations have been reported in women who have positive screening test results for aneuploidy without a confirmatory cytogenetic result (38). All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken. Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy. Even if cell-free DNA test results are a true positive, cell-free DNA cannot distinguish aneuploidy derived from a translocation or nondisjunction, and this will affect counseling and understanding of the recurrence risk. Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy (28, 39).

Cell-free DNA screening currently gives information about the three most common aneuploidies and about fetal sex but does not typically provide information about other aneuploidies. Without published clinical validation trials, some laboratories have begun to offer cell-free DNA screening for additional disorders, including two forms of aneuploidy associated with nonviable pregnancies (trisomy 16 and trisomy 22) and five or more microdeletion syndromes. A microdeletion syndrome is caused by a chromosomal deletion encompassing contiguous genes that is too small to be detected by conventional cytogenetics. Given the rarity of these disorders, it is uncertain what a positive or negative screening test result means. Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time. For women who wish to know whether their fetus has a microdeletion, the best option is to undergo prenatal diagnostic testing with microarray of fetal cells from CVS or amniocentesis (34, 41).

Cell-free DNA screening tests do not provide information regarding the potential for open fetal defects. Therefore, women who undergo cell-free DNA screening should be offered assessment for open fetal defects with ultrasonography, MSAFP screening, or both.

▶ *How should aneuploidy screening test results be interpreted and communicated?*

Positive and negative screening cutoff levels usually are defined by the different laboratories that perform these analyses. Because of these differences, and because patients interpret information differently, laboratory results should be reported as either positive or negative, and the adjusted numerical risk of aneuploidy based on the test should be provided, regardless of the screening test performed. It also is useful to contrast this risk with the patient's prescreening age-related risk and the general population risk to put the test result in context. Graphical representations of results can be helpful to some patients. After all of this information is provided, the patient's understanding of the results should be confirmed and documented.

▶ *What additional screening or diagnostic tests should be offered after a positive screening test result?*

Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing. Women found to have a positive screening test result from a serum analyte or ultrasound screening test should be offered further detailed counseling and cell-free DNA screening or diagnostic testing by CVS or amniocentesis. Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed. However, use of cell-free DNA screening as a follow-up test for patients with a positive traditional screening test result is reasonable for patients who want to avoid a diagnostic test. However, this approach may delay definitive diagnosis and management. Given that the residual risk of a chromosomal abnormality with a normal cell-free DNA screening test result after an abnormal traditional screening test has been reported to be 2%, patients should be informed of this potential limitation (42). Women with an increased risk of aneuploidy based on cell-free DNA screening should be offered diagnostic testing and should undergo ultrasonography to evaluate for fetal structural anomalies. If MSAFP has not been obtained as part of aneuploidy screening, further screening for open fetal defects with MSAFP or ultrasonography should be offered. In addition, evaluation for fetal anomalies in the second trimester is appropriate for all patients. In the first trimester, maternal serum levels of

pregnancy-associated plasma protein A below the fifth percentile are independently associated with obstetric complications, such as spontaneous fetal and neonatal loss, fetal growth restriction, preeclampsia, placental abruption, and preterm delivery (43). In the second trimester, elevated hCG, AFP, and dimeric inhibin A levels in pregnancies without structural anomalies are associated with an increased risk of fetal death, intrauterine growth restriction, and preeclampsia (44, 45). The likelihood of an adverse pregnancy outcome increases with increasing number of abnormal markers in the same screening test and with more extreme analyte values (46). Although potential management strategies for women with abnormal serum markers have been proposed, they are not evidence based (46).

If a patient conceives and has undergone preimplantation genetic screening, prenatal screening for aneuploidy still should be offered because false-negative test results can occur with preimplantation genetic screening (47). Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during pregnancy.

▶ *How does screening for aneuploidy differ in multifetal gestations?*

In multifetal gestations, the risk of fetal aneuploidy is affected by the number of fetuses and the zygosity of the pregnancy; however, data regarding the risk of aneuploidy are more limited in multiple gestations compared with singleton pregnancies. In dizygous twin pregnancies, each fetus carries a risk of aneuploidy generally similar to the mother's age-adjusted risk, but the mother carries an increased risk of having a fetus with aneuploidy because there is more than one fetus. Typically, monozygous twins will have the same karyotype, with neither or both fetuses being affected; the risk of carrying aneuploid fetuses is similar to the mother's age-adjusted risk.

No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Analysis of the risks and benefits of screening or diagnostic testing in women carrying multiple fetuses is much more complicated, given the diminished effectiveness of screening and how the prenatal identification of a single aneuploid fetus might affect the pregnancy management. Diagnostic testing may be less acceptable to women with multiple gestations because of the increased difficulty and higher potential loss rates.

Nuchal translucency measurement allows each fetus in a multifetal pregnancy to be screened independently and, therefore, can be used in twin or high-order multifetal gestations. The distribution of nuchal translucency measurements does not differ significantly between singletons and multiples, and standard cutoffs can be used (48). One study reviewed individual first-trimester screening in twin gestations and generated individual risks for each fetus with nuchal translucencies and first-trimester screening. At a 1:300 cutoff, the detection rate was 75% with a 9% positive screening rate for trisomy 21 (49). However, the review concluded that a greater reliance should be placed on nuchal translucency to evaluate the fetuses for aneuploidy. A single enlarged nuchal translucency in monochorionic twins of discordant size could be an early sign of twin–twin transfusion syndrome rather than aneuploidy (50). These patients should be evaluated further for this possibility.

Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies. First-trimester, quad, and combined serum analyte screening are options available to screen twin gestations, although few data are available from prospective studies with regard to screening. Analyte screening test results typically are provided for the entire gestation and not each individual fetus. Second-trimester serum screening of twin gestations can identify approximately 50% of fetuses affected with Down syndrome at a 5% positive screening rate (51). Because of limited evidence regarding its efficacy, cell-free DNA testing is not recommended for aneuploidy screening in women with multiple gestations (34).

In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances. The patient should be offered counseling and consider diagnostic testing instead of a screening test. The accuracy of aneuploidy screening in a multiple gestation with a fetus that has an empty gestational sac is not known.

Summary of Recommendations and Conclusions

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result.
- If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities.

- Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.
- Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.
- Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing.
- All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.
- Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
- Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing.

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time.
- Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during their pregnancy.
- No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies.

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.
- Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit.
- All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age.
- If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.
- Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy.
- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed.
- In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances.

For More Information

The American College of Obstetricians and Gynecologists has identified additional resources on topics related to this document that may be helpful for obgyns, other health care providers, and patients. You may view these resources at www.acog.org/more-info/AneuploidyScreening.

These resources are for information only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's web site, or the content of the resource. The resources may change without notice.

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The MEDLINE database, the Cochrane Library, and the American College of Obstetricians and Gynecologists' own internal resources and documents were used to conduct a literature search to locate relevant articles published between January 1985–July 2014. The search was restricted to articles published in the English language. Priority was given to articles reporting results of original research, although review articles and commentaries also were consulted. Abstracts of research presented at symposia and scientific conferences were not considered adequate for inclusion in this document. Guidelines published by organizations or institutions such as the National Institutes of Health and the American College of Obstetricians and Gynecologists were reviewed, and additional studies were located by reviewing bibliographies of identified articles. When reliable research was not available, expert opinions from obstetrician–gynecologists were used.

Studies were reviewed and evaluated for quality according to the method outlined by the U.S. Preventive Services Task Force:

- I Evidence obtained from at least one properly designed randomized controlled trial.
- II-1 Evidence obtained from well-designed controlled trials without randomization.
- II-2 Evidence obtained from well-designed cohort or case–control analytic studies, preferably from more than one center or research group.
- II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments also could be regarded as this type of evidence.
- III Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Based on the highest level of evidence found in the data, recommendations are provided and graded according to the following categories:

Level A—Recommendations are based on good and consistent scientific evidence.

Level B—Recommendations are based on limited or inconsistent scientific evidence.

Level C—Recommendations are based primarily on consensus and expert opinion.

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RESEARCH ARTICLE

An Economic Analysis of Cell-Free DNA Non-Invasive Prenatal Testing in the US General Pregnancy Population

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Abstract

Objective

Analyze the economic value of replacing conventional fetal aneuploidy screening approaches with non-invasive prenatal testing (NIPT) in the general pregnancy population.

Methods

Using decision-analysis modeling, we compared conventional screening to NIPT with cell-free DNA (cfDNA) analysis in the annual US pregnancy population. Sensitivity and specificity for fetal aneuploidies, trisomy 21, trisomy 18, trisomy 13, and monosomy X, were estimated using published data and modeling of both first- and second trimester screening. Costs were assigned for each prenatal test component and for an affected birth. The overall cost to the healthcare system considered screening costs, the number of aneuploid cases detected, invasive procedures performed, procedure-related euploid losses, and affected pregnancies averted. Sensitivity analyses evaluated the effect of variation in parameters. Costs were reported in 2014 US Dollars.

Results

Replacing conventional screening with NIPT would reduce healthcare costs if it can be provided for $744 or less in the general pregnancy population. The most influential variables were timing of screening entry, screening costs, and pregnancy termination rates. Of the 13,176 affected pregnancies undergoing screening, NIPT detected 96.5% (12,717/13,176) of cases, compared with 85.9% (11,314/13,176) by conventional approaches. NIPT reduced invasive procedures by 60.0%, with NIPT and conventional methods resulting in 24,596 and 61,430 invasive procedures, respectively. The number of procedure-related euploid fetal losses was reduced by 73.5% (194/264) in the general screening population.

Competing Interests: The authors of this manuscript have the following competing interests: Kirsten Curnow, Steven Chapman, and Matthew Rabinowitz are or were employees of Natera, Inc. and hold stock or options to hold stock in the company. Steven Michalopoulos and John Hornberger were employees at Cedar Associates when they participated in this project. Peter Benn is a paid consultant to Natera and holds stock options in the company. This project was funded by Natera. Natera is a provider of non invasive prenatal testing and other genetic testing. Matthew Rabinowitz holds patents and other intellectual property related to this testing. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials related to this study.

Conclusion

Based on our analysis, universal application of NIPT would increase fetal aneuploidy detection rates and can be economically justified. Offering this testing to all pregnant women is associated with substantial prenatal healthcare benefits.

Introduction

There are over 4 million pregnancies annually in the United States, the majority of which undergo fetal aneuploidy testing [1]. There are many options, with test selection largely dependent on a woman's prior risk, pregnancy stage, access to healthcare services, cost, and/or patient preference. Although the American Congress of Obstetricians and Gynecologists recommends offering diagnostic testing to all women [2], many initially choose prenatal screening. Historically, first trimester screening has involved maternal serum marker evaluation plus ultrasound [2], while those entering prenatal care in the second trimester usually received maternal serum markers and some received sonographic marker evaluation. The newly-available non-invasive prenatal tests (NIPTs) offer higher detection rates and lower false positive rates than pre-existing screening methods [3]. Following validation studies in high-risk patients, NIPT was endorsed for use in high-risk women by a number of professional bodies [4, 5]. Recent data has shown that NIPT is also effective in women with low prior risk [6–10]. The American College of Medical Genetics and Genomics guideline does not specify prior risk as a factor for offering NIPT [11]. A recent position statement from the International Society for Prenatal Diagnosis recognizes that NIPT can be offered as a first line prenatal screening test for all women [12]. However, a major hurdle preventing test expansion into the general pregnancy population stems from economic concerns.

Previous studies have evaluated the circumstances where NIPT is economically justifiable [13–26]. However, these studies did not fully consider the diversity of test options utilized in the US and only one study has considered disorders other than trisomy 21 [26]. In this study, we determine the cost effectiveness of replacing conventional screening approaches with NIPT in a general US screening population taking into consideration these additional factors.

Methods

We constructed a decision-analysis model to evaluate the economic value of fetal aneuploidy screening in the annual US general screening population Screening and invasive test utilization, miscarriage, and pregnancy termination rates were all considered.

Development of the model is described in more detail in S1 Methods. Briefly, modeling was based on a theoretical cohort of 3,952,841 live births, which represented the number of births in the United States in 2012 [1, 27]. For this population, the number of births at each maternal age at delivery was known; 14.9% were to women aged 35 or older. The model was based on 70% of pregnant women in the US receiving some type of fetal aneuploidy prenatal screening; this figure was based on survey data collected prior to the introduction of NIPT [28]. We conservatively assumed that the screening utilization rate would remain at 70% with NIPT. Thus, for the purposes of the economic analysis, patients that do not undergo any screening are considered cost neutral and can be excluded from the model.

The screening strategies compared were conventional screening versus NIPT with cell-free DNA (cfDNA) analysis. This economic analysis was designed to: (a) assess the value of NIPT

as a replacement for conventional screening approaches; (b) determine the number of additional fetal trisomy 21, 18, and 13 and monosomy-X cases prenatally detected; (c) estimate the reduction in the number of affected births, and; (d) assess the reduction in the number of invasive procedure-related unaffected fetal losses. The economic analysis considered the cost of all components of screening, diagnosis, counseling, and pregnancy intervention or medical costs associated with an affected birth. The result was expressed as the per-case charge for NIPT that would be cost neutral to the healthcare system. Monosomy-X was included because there is data to show that NIPT can be effective in screening for this disorder (S2 Table) and because it is common practice in the US to provide the testing for sex chromosome abnormalities. However, we excluded other sex chromosome abnormalities from the analysis due to lack of data on screening performance and the extent of follow-up of cases with high-risk results. The additional value associated with some tests (e.g. maternal age alone, alpha fetoprotein [AFP], and nuchal translucency [NT]) for the detection of abnormalities other than the previously defined set of aneuploidies was not considered. Some aneuploidies associated with major malformations might be initially suspected through an early dating ultrasound and these were not considered. Similarly, the ability for NIPT to detect additional conditions such as microdeletion syndromes was not evaluated. Other costs of screening that can be encountered in clinical practice, non-medical costs that might be incurred by patients, the value of early reassurance to those women who receive low risk results, and the cost of any ancillary testing were excluded in this analysis. For example, the additional expense associated with chromosomal microarray analysis to detect small imbalances (in addition to aneuploidy) was excluded.

The first trimester "Combined test" (measurement of NT together with maternal serum markers, pregnancy-associated plasma protein A [PAPPA] and free beta human chorionic gonadotrophin [hCG], at 12 weeks gestational age), sometimes in combination with additional second trimester screening tests, is a recommended approach for providing Down syndrome screening to women of all ages [2]. We therefore based our analyses primarily on women entering screening in the first trimester. The model took into consideration the Combined test's efficacy for the detection of non-mosaic fetal Down syndrome, trisomy 18, trisomy 13, and monosomy-X. Additionally, this analysis considered the proportion of women: 1) subsequently receiving second trimester serum screening (i.e. integrated or sequential screening); 2) electing follow-up invasive testing (amniocentesis or chorionic villus sampling [CVS]); 3) electing pregnancy termination, and; 4) experiencing spontaneous pregnancy loss. Recognizing that a large number of women do still receive conventional screening based on the second trimester "quadruple test" (maternal serum AFP, hCG, unconjugated estriol, and inhibin-A) alone, we also constructed a subsidiary economic model that included varying proportions (up to 100%) of women entering screening in the second trimester.

The performance of conventional screening approaches was determined through multivariate simulations using SURUSS statistical parameters [29] (see S1 Methods and Table 1). The performance of NIPT for each aneuploidy was based on compiled data from validation studies (Table 1, S2 Table). Test adoption practices used in the model were derived from published studies or, if rates were unavailable, using consensus data obtained through survey of obstetricians currently providing prenatal screening and diagnosis. Full details of these procedure rates are presented in S1 Methods.

The cost estimates used in the baseline model analysis included those associated with office visits, genetic counseling, screening, invasive testing, termination procedures, and lifetime costs of delivering an affected child (S1 Table). Where possible cost estimates were derived from the literature in addition to the 2014 Centers for Medicare and Medicaid Services (CMS) Fee Schedules [31, 32]; all costs estimates were adjusted for inflation to 2014 US Dollars using the Medical Care Component of the Bureau of Labor Services Consumer Price Index. Centers

Table 1. Aneuploidy incidence rates and performance of conventional screening approaches in the first and second trimester for a general population.

	First Trimester	Second Trimester
Trisomy 21		
Prevalence	1/365	1/398
Conventional Screening^		
Sensitivity	85.3%	84.1%
Specificity	95.2%	92.5%
NIPT *		
Sensitivity	99.3%	99.3%
Specificity	99.9%	99.9%
Trisomy 18		
Prevalence	1/1208	1/1487
Conventional Screening^		
Sensitivity	95.0%	73.5%
Specificity	99.7%	99.8%
NIPT *		
Sensitivity	96.8%	96.8%
Specificity	99.9%	99.9%
Trisomy 13		
Prevalence	1/3745	1/4195
Conventional Screening^		
Sensitivity	94.5% †	16.4% ‡
Specificity §	-	-
NIPT *		
Sensitivity	87.2%	87.2%
Specificity	99.8%	99.8%
Monosomy X		
Prevalence	1/1291	1/2340
Conventional Screening^		
Sensitivity	75.0%	54.1%
Specificity §	-	-
NIPT *		
Sensitivity	89.5%	89.5%
Specificity	99.8%	99.8%

^ Conventional screening based on maternal age, nuchal translucency, maternal serum pregnancy-associated plasma protein A [PAPPA] and free beta human chorionic gonadotropin [hCG] at 12 weeks gestational age.

* NIPT sensitivity and specificity was based on pooled data from 19 published studies (see S2 Table); NIPT sensitivity and specificity was considered to be independent of pregnancy stage and maternal prior risk.

† First trimester sensitivity for trisomy 13 screening is based on the algorithm developed for trisomy 18 screening [30].

‡ Second trimester sensitivity for trisomy 13 screening is assumed to be equal to the proportion of trisomy 13 affected pregnancies serendipitously identified as a false positive in Down syndrome and trisomy 18 screening.

§ No specific screening protocols exist for trisomy 13 and monosomy X.

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for Medicare and Medicaid Services (CMS) rates were increased by 20 percent to estimate the expenditure from the perspective of private or commercial payers, as described previously [14]. The final cost of a particular test or procedure is the sum cost of all individual components. All costs were reported using 2014 US Dollars.

We also designed the analysis to show how costs would change when replacing conventional screening with NIPT in a study population restricted to women considered high-risk because of advanced maternal age (AMA; ≥35 years). The sensitivity analysis evaluated test costs from 40% below to 20% above the baseline and lifetime costs from 20% below to 20% above the baseline. To determine how differing practice patterns contribute to the value of NIPT, the sensitivity analysis evaluated key practice patterns for a range of clinically relevant inputs such as the timing of screening entry, invasive confirmation rates, and termination rates.

This study did not involve use of clinical records or any other patient information requiring approval by an ethics committee/institutional review board or data protection agency/commissioner.

Results

Based on our model, the adoption of NIPT in clinical practice by all pregnant women who receive aneuploidy screening in the US results in a per-case NIPT value of approximately $744. This number reflects the average per-case cost offset between the two screening approaches; a charge higher than $744 would represent a net increase in overall expenditure and a lower charge would constitute a savings to the system. This $744 figure includes costs for subsequent additional screening and follow-up diagnostic testing ($86), genetic counseling ($3), lifetime costs associated with affected births ($286), and the current cost of conventional screening approaches ($369). The cost of conventional screening ($369) is a build-up of all prenatal screening costs, and accounts for the proportion of patients billed for/receiving each individual cost component (see S1 Methods), and includes: $70 for a separate office visit for NT, $10 for genetic counseling, a $76 charge for ultrasound, $17 for genetic sonograms, $147 for NT, and $49 for serum analytes. The corresponding cost offset for NIPT offered only to women of advanced maternal age (AMA, ≥35 years) is $1474.

For women entering screening in the second trimester, the per-case value of NIPT was estimated to be $486. This lower figure reflects the fact that second trimester screening tests are less expensive than first trimester tests, and these women do not receive additional screening tests as part of sequential or integrated protocols (S1 Table). The somewhat inferior detection rate and higher false-positive rate of second trimester screening is also incorporated into this latter estimate. Table 2 shows estimates for various mixtures of first and second trimester screening. In contrast to conventional screening, the timing of entry for NIPT had little impact on NIPT value.

Most of the financial benefit of offering screening resides in the detection of fetal trisomy 21 rather than the other common aneuploidies. This is because trisomy 21 is the most common fetal aneuploidy and has a high survival rate, leading to high medical costs. The cost offset when trisomy 18, trisomy 13, and monosomy X are excluded is essentially unchanged at $744; financial benefits associated with the detection of these disorders are offset by the costs of testing.

The effect of variation in key inputs on the value of NIPT in a general screening population is shown in Table 2. As expected, decreasing termination rates was associated with a reduction in NIPT value. Screening costs, particularly first trimester screening, are also important variables; reimbursement levels do differ depending on the insurance carrier, and there are inter-regional differences. The range of +40% and -20% shown in Table 2 was chosen to cover these

Table 2. Model inputs and economic value of NIPT for fetal aneuploidy screening.

Model Input Variable	Baseline Value	Range	NIPT Range^	Reference
Practice Patterns				
Percent entering first trimester for conventional screening	100%	0%¥–100%	$486–744	[28]
Percent entering first trimester for NIPT	66%	0%¥–100%	$743–745	[6]; see text
Invasive testing uptake for conventional screen FPs	45%*	25%–100%	$738–747	[33]; see text
Termination Rates	65–90%†	0%–100%	$459–788	[34]
Key Cost Variables ‡				
Cost of first trimester screening	$369	$222–443	$597–818	[15, 16]; see text
Cost of sequential screening	$136	$82–164	$714–759	[15, 16]; see text
Cost of invasive testing (amniocentesis/CVS)	$835 / $892	$501–1,070	$740–747	[35]; see text
Lifetime costs of an affected child §				
Trisomy 21	$677,000	$541,600–812,400	$687–802˅	[36–38]; S1 Table
Trisomy 18	$29,307	$23,446–35,168	$687–802˅	[36–38]; S1 Table
Trisomy 13	$33,577	$26,862–40,292	$687–802˅	[36–38]; S1 Table
Monosomy X	$271,010	$216,808–325,212	$687–802˅	[36–38]; S1 Table

FP(s), false positives; CVS, chorionic villus sampling; NIPT, non-invasive prenatal testing

^ NIPT values that correspond to the range applied to each input variable. These values can be compared to the $744 value assigned to the set of baseline model inputs.

¥ Alternative scenario showing results when all initial screening is performed in the second trimester.

* Baseline invasive testing rates for true positives were 73% for trisomy 21 and 90% for trisomy 13, trisomy 18, and monosomy X. These rates were not changed when the invasive rate for false-positives was adjusted.

† Baseline termination rates for trisomy 21, trisomy 18, trisomy 13, and monosomy X were 87%, 81%, 90%, and 65%, respectively [34].

‡ The range evaluated for cost variables was baseline minus 40% to plus 20%. The cost of first trimester screening ($369) was a buildup of all the individual components (see Results).

§ The range of lifetime costs of an affected child was baseline ±20%; the upper and lower range values for each indication were modified together.

˅Most of the variability is attributable to lifetime costs for Down syndrome (see text).

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potential inter-district cost variations [32]. Changing the cost of the first trimester Combined test had the greatest had the value of NIPT; a 40% reduction in the Combined test was associated with a decrease in NIPT value of $147, and a 20% increase in the Combined test cost was associated with an increase in NIPT value of $74. Modification in the lifetime costs of an affected child up or down by 20% had a relatively small effect on the overall value of NIPT, increasing or decreasing the value by around $58, respectively.

Replacing conventional screening with NIPT would increase fetal aneuploidy detection rates, decrease the number of affected births, and lower the number of invasive tests and thereby reduce procedure-related losses of euploid fetuses (Table 3). We estimate that, replacing conventional screening with NIPT would increase the number of affected pregnancies prenatally detected by 5.9% (364/6149) in a high-risk population and 12.4% (1403/11,314) in the general screening population. Replacing conventional screening with NIPT would reduce the number of affected births by 29.9% (604/2017) and 33.4% (1213/3,629) in the high-risk and general screening populations, respectively. NIPT is associated with a 71.7% (19,037/26,555) and 60.0% (36,834/61,430) reduction in the number of invasive tests performed in the high-risk and general screening populations, respectively. As a result, the number of procedure-related euploid fetal losses is also reduced with NIPT, with a 90.9% (100/110) reduction in the high-risk population and a 73.5% (194/264) reduction in the general screening population.

Table 3. Comparison of clinical outcomes from baseline analysis of the two screening approaches in a general screening population.

	Conventional Screening	NIPT
Trisomy 21		
Affected pregnancies screened *	7836	7836
T21 affected with positive result	6687	7783
T21 births averted †	2901	4097
Invasive tests ‡	53,813	9010
Procedure-related euploid losses	246	13
Trisomy 18		
Affected pregnancies screened *	2364	2364
T18 affected with positive result	2246	2288
T18 births averted †	426	436
Invasive tests ‡	5604	4282
Procedure-related euploid losses	18	12
Trisomy 13		
Affected pregnancies screened *	763	763
T13 affected with positive result	721	665
T13 births averted †	293	268
Invasive tests ‡	614	4624
Procedure-related euploid losses	0	20
Monosomy-X		
Affected pregnancies screened *	2214	2214
MX affected with positive result	1660	1981
MX births averted †	9	41
Invasive tests ‡	1399	6680
Procedure-related euploid losses	0	25
All aneuploidies combined		
Affected pregnancies screened *	13,176	13,176
Affected with positive result	11,314	12,717
Affected births averted †	3629	4842
Invasive tests ‡	61,430	24,596
Procedure-related euploid losses	264	70

NIPT, non-invasive prenatal testing

* 70% of total affected pregnancies (30% receive no screening).

† Assuming termination rates for trisomy 21, trisomy 18, trisomy 13, and monosomy X of 87%, 81%, 90%, and 65%, respectively [34]; excludes spontaneous fetal losses.

‡ Invasive tests (amniocentesis or chorionic villus sampling) performed in true positives and false positives.

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Discussion

Our study shows that NIPT, as a universal prenatal screening test, can be introduced without increasing the net cost to the US healthcare system. The baseline model was considered to be the most appropriate estimate since it considered NIPT as an alternative to first trimester screening, which is generally accepted as the optimal time for testing. In the baseline analysis, NIPT led to increased fetal aneuploidy detection, and fewer invasive tests and procedure-related euploid fetal losses. This was expected, given NIPT's detection rates (87.2–99.3%) and

false positive rates (0.1–0.2%). Overall, the cost-neutral value of NIPT was $744 in the general pregnancy population.

Multiple studies have evaluated the financial implications of offering NIPT versus conventional screening [13–26]. Most of these studies were restricted to screening for fetal Down syndrome and direct comparison is confounded by differences in the testing components for conventional screening and diagnosis, the costs assigned to these components, screening policies, utilization rates, and maternal ages within the population. However, some conclusions can be drawn. All studies found that there was an economic advantage in offering NIPT to women at high risk for fetal Down syndrome and several studies recognized the economic benefit of extending NIPT to additional women with intermediate Down syndrome risks established by conventional screening (contingent NIPT) [17, 19, 20, 25]. Some studies have assessed the marginal benefit afforded by NIPT under the assumption that conventional screening infrastructure will not be rapidly changed [24, 25]. This assumption may be appropriate for publically funded healthcare systems that are heavily invested in conventional approaches and where there is tight control over ad-hoc use of additional testing for risk refinement. However, this is not directly applicable to the US where patient choice and market-driven forces generally result in diversity in screening approaches and the subsequent management of follow-up testing. In this study and others that have considered the lifetime costs associated with individuals with Down syndrome, the benefit of providing NIPT as a primary screening test to all pregnant women has been recognized [15, 19, 24].

A recent study by Fairbrother et al. [26] suggested a unit cost of $453 (or less) would establish NIPT as the dominant primary screening strategy over conventional screening. This value was based on the performance of conventional screening for fetal Down syndrome, trisomy 18 and trisomy 13 but not monosomy-X. Many of rates and costs used by Fairbrother et al. differ from ours. Notably, the pre-test first trimester prevalence for trisomy 21 used in that study, 1/530, was not reflective of the current US population which we estimated to be 1/365 for 2012. Fairbrother et al. assumed all testing would be completed in the first trimester with no additional cost assigned to sequential screening or other second trimester procedures. In our analyses, we included these downstream costs. We also recognized the practical reality that some women who receive high-risk conventional screening results do not pursue any additional prenatal testing and this will add to the costs associated with affected births.

As with any decision-analytic model, there were some limitations that must be considered in the interpretation of the results. The modelled performance of conventional screening was based on SURUSS parameters [28] that appear to project better screening performance than meta-analysis derived parameters [38]. If the sensitivity and specificity in actual clinical practice were worse than modeled, the benefit of using NIPT would be larger. Similarly, actual performance of NIPT could differ from that achieved in clinical trials (S2 Table). We did not consider differences in test performance between different NIPT methodologies. Test failure rates and some indirect expenses were excluded (see Methods). NIPT test failures could result in additional costs because some of these may be associated with a low fetal fraction and an increased risk for chromosome abnormality, notably trisomy 13, 18, and triploidy [10, 39–41]. In these cases, the provision of additional screening tests, ultrasound examinations and invasive testing would add to costs. However, it is not currently possible to fully evaluate this extra cost because it will depend on the policy of the laboratory with respect to measuring fetal fraction, types and prevalence of these chromosome abnormalities, and the recommended testing follow-up for cases with a test failure due to low fetal fraction. We excluded microdeletion syndromes, which are now available with some NIPTs. A separate study of lifetime costs for these latter disorders, including the costs of beneficial postnatal treatment interventions, is needed. However, it is anticipated that inclusion of clinically severe microdeletion syndromes in an

economic analysis would further increase the value of NIPT, and could provide significant cost savings to the healthcare system. In a clinical setting, there is considerable variation in gestational age at screening, use of sequential testing, genetic counseling, and ultrasound. Some of these variations are addressed in the sensitivity analyses (Table 2). It was assumed that the sensitivity and specificity of NIPT in lower risk women would be equivalent to that seen in high-risk women; there is increasing evidence to indicate that this is the case [5, 6]. We also did not analyze the consequences when some women, notably those at high a priori risk, by-pass specific components of conventional screening and proceed directly to NIPT or invasive testing. The analysis excluded the economic aspects of fetal abnormalities other than the defined aneuploidies that may be identified through testing. One of the principal concerns arising from a replacement of conventional screening with NIPT is that patients would no longer receive an NT assessment as part of routine prenatal care. As described in a recent study, the primary purpose of NT measurement is fetal aneuploidy risk assessment [42]. While NT may detect additional fetal abnormalities and congenital heart conditions, it should be noted that an enlarged NT is a relatively poor predictor for isolated congenital heart disease [42]. Most pregnant women will have a number of ultrasounds during which evaluations for fetal cardiac abnormalities might be made. If NIPT were to be introduced as a universally available prenatal screen, the value of NT would need to be assessed based on the detection of cardiac defects and other fetal abnormalities not associated with aneuploidy.

Importantly, the purpose of this analysis was not to place a monetary value on the life of an affected child. Rather, the objective was to make an economic assessment of NIPT, relative to conventional screening approaches, that could be used when establishing coverage and access policies. Ultimately, it is the parents' acceptance of screening, diagnosis, and pregnancy management options that will determine the allocation of financial resources.

As data accumulates demonstrating the superior efficacy of NIPT over conventional screening for all pregnant women, it becomes increasingly clear that NIPT does need to be offered to all patients. Intangible benefits such as reduced patient anxiety through earlier and stronger reassurance further reinforce the need for universal NIPT availability. The analysis presented here indicates that this can potentially be achieved without adding to the overall cost of prenatal healthcare.

Supporting Information

S1 Methods. Supporting methods.
(DOCX)

S1 Table. Baseline cost variables.
(DOCX)

S2 Table. Assay performance with cfDNA NIPT.
(DOCX)

Author Contributions

Conceived and designed the experiments: PB SC JH MR. Performed the experiments: N/A. Analyzed the data: PB KJC SC SNM JH MR. Wrote the paper: PB KJC SC SNM JH MR.

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ORIGINAL ARTICLE

Prenatal screening for fetal aneuploidies with cell-free DNA in the general pregnancy population: a cost-effectiveness analysis

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Abstract

Objective: To estimate the cost-effectiveness of fetal aneuploidy screening in the general pregnancy population using non-invasive prenatal testing (NIPT) as compared to first trimester combined screening (FTS) with serum markers and NT ultrasound.
Methods: Using a decision-analytic model, we estimated the number of fetal T21, T18, and T13 cases identified prenatally, the number of invasive procedures performed, corresponding normal fetus losses, and costs of screening using FTS or NIPT with cell-free DNA (cfDNA). Modeling was based on a 4 million pregnant women cohort, which represents annual births in the U.S.
Results: For the general pregnancy population, NIPT identified 15% more trisomy cases, reduced invasive procedures by 88%, and reduced iatrogenic fetal loss by 94% as compared to FTS. The cost per trisomy case identified with FTS was $497 909. At a NIPT unit, cost of $453 and below, there were cost savings as compared to FTS. Accounting for additional trisomy cases identified by NIPT, a NIPT unit cost of $665 provided the same per trisomy cost as that of FTS.
Conclusions: NIPT in the general pregnancy population leads to more prenatal identification of fetal trisomy cases as compared to FTS and is more economical at a NIPT unit cost of $453.

Keywords

Cell-free DNA, cost-effectiveness, Down syndrome, non-invasive prenatal testing, prenatal screening

History

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Introduction

Down syndrome, which is caused by trisomy 21 (T21), is the most common aneuploidy found at birth and is associated with developmental and neurocognitive delay and other medical issues. Prenatal screening for Down syndrome is a standard clinical offering in many countries and has been employed over many years [1,2]. Screening for less common aneuploidies such as trisomy 18 (T18) and trisomy 13 (T13) is often included as well [3].

Prenatal screening for T21 has evolved over the past several decades from initially using only maternal age as the criteria to the addition of serum protein markers as well as specialized ultrasound that allows for measurement of nuchal translucency (NT). First trimester combined screening (FTS) utilizes two serum proteins, beta unit of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A), in conjunction with NT measurement to provide women with a risk assessment for fetal T21. While FTS provides for early screening within the first trimester of pregnancy, it has two notable shortcomings. First, it requires ultrasound to be performed by specially trained ultrasonographers to accurately measure the NT [4]. Second, FTS identifies only about 85% of fetal T21 cases with a 5% false-positive rate [2].

Non-invasive prenatal testing (NIPT) with cell-free DNA (cfDNA) has been shown in numerous clinical studies to be highly accurate for screening of fetal trisomies with false-positive rates at 0.1% or less for each trisomy tested [5,6]. The accuracy of NIPT has been consistent in all pregnant women populations, regardless of age or risk status [7,8]. As NIPT only requires a standard blood draw without any special ultrasound assessments, it enables general Ob/Gyns as well as other primary care providers such as midwives to implement prenatal screening for fetal trisomy with high accuracy.

The objective of this study was to compare the cost-effectiveness of prenatal screening for common fetal trisomies with FTS or NIPT within a representative general pregnancy population in the U.S.

Methods

Using DATA Pro (TreeAge Software Inc., Williamston, MA), we modified a previously published decision-analytic model to compare different prenatal screening strategies for fetal T21, T18, and T13 in a general pregnancy screening population [9]. The screening strategies compared consisted of: (1) FTS which included measurement of serum proteins

This work was performed in part of a Contributor's duties as an employee of Ariosa Diagnostics, Inc.

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β-hCG and PAPP-A as well as ultrasound assessment for NT measurement and (2) NIPT with cfDNA. For both FTS and NIPT, we assumed both received the same standard obstetrical ultrasounds during pregnancy. However, as only FTS requires NT, which is a specialized ultrasound measurement, we assumed a proportion of patients would need to be referred from their primary care provider to complete screening with FTS.

We searched MEDLINE from 1997 to 2014 for English-language literature using the terms Down syndrome, trisomy 21, trisomy 18, trisomy 13, prenatal screening, non-invasive prenatal diagnosis, NIPT, non-invasive prenatal screening and cell-free DNA analysis. In addition, we reviewed abstracts from national meetings, data from Medicare, and relevant data from companies offering NIPT tests.

For the analysis, we used a cohort of 4 000 000 pregnant women which represents the current estimated annual number of births in the U.S. The first trimester prevalence of each trisomy, the performance of each screening modality in terms of sensitivity and specificity, and the risk of fetal loss from invasive testing are shown in Table 1. In the base case, we assumed a 70% screening uptake for both FTS and NIPT. For those that proceed with screening, tests can result in true positives, false positives, true negatives, and false negatives. Any screen positives, whether true or false positives, were assumed to have sufficient follow-up so that any fetal trisomies from a screen positive result were detected. Fetal losses from invasive testing complications were captured.

All costs are represented in 2014 USD. Cost items, which are listed in Table 1, included those associated with screening tests, invasive testing, office visits and counseling, termination procedures, and cost of each trisomy birth. A range of unit costs for NIPT were used for the analysis. When possible, the Medicare 2014 Fee Schedule was used to estimate cost inputs. Any cost inputs relying on data prior to 2014 were adjusted taking into account inflation based on the Bureau of Labor Statistics. A range of cost values based on published literature were used for sensitivity analysis. The cost for screening and invasive testing was based on the total cost which included any expected payments by insurance as well as patient co-pays. For the base case, we assumed 35% of FTS would require referral from a primary care provider to a specialist to perform the NT, which would incur the additional cost of an office visit. We did not assume any other downstream additional costs from the specialist referral. All screen positive tests were assumed to have follow-up counseling, which generated an office visit cost. For cost analysis, the cost of screening was inclusive of the screening test(s) and any associated office visits. Invasive testing costs included the cost of the invasive procedure as well as any terminations. The baseline cost for a given trisomy birth was estimated based on direct medical costs as well as indirect costs.

The primary outcomes of the analyses were separated into clinical and economic outcomes. For the clinical outcomes, the number of fetal trisomies detected based on confirmatory testing and number of normal fetus losses due to invasive procedures for each screening strategy was determined. For the economic outcomes, the NIPT unit cost at which it was cost savings and cost equivalent on a per trisomy case as compared to FTS was determined. Sensitivity analyses were performed on all cost and effectiveness variables over the ranges specified in Table 1.

Table 1. Probability and cost variables.

	Base case	Range	References
Variables			
T21 prevalence, 1st trimester	1 in 530	(1 in 450 to 1 in 600)	[18]
T18 prevalence, 1st trimester	1 in 1100	(1 in 900 to 1 in 1500)	[19]
T13 prevalence, 1st trimester	1 in 3500	(1 in 2500 to 1 in 5000)	[19]
FTS performance			
Cumulative false positive rate	5%	(3 7%)	[2]
Sensitivity, T21	85%	(75 90%)	[2]
Sensitivity, T18	84%	(80 90%)	[3]
Sensitivity, T13	84%	(80 90%)	[3]
% patients referred out for screening	35%	(25 50%)	Data on file
NIPT performance			
Cumulative false positive rate	0.3%	(0.1 0.5%)	[6]
Sensitivity, T21	99.0%	(98.0 99.9%)	[6]
Sensitivity, T18	96.8%	(90.0 99.9%)	[6]
Sensitivity, T13	92.1%	(85.0 95.0%)	[6]
Termination rate for T21	75%	(60 90%)	[20]
Termination rate for T18	90%	(80 95%)	[21]
Termination rate for T13	90%	(80 95%)	[21]
Procedure related miscarriage	0.5%	(0.2 2%)	[9,22]
Costs			
NIPT	$400 700	($400 $700)	n/a
1st trimester serum	$48.30	($30 $100)	see text
NT	$122.51	($100 $300)	see text
Invasive procedure	$1300	($500 $2500)	[9]
Office visit with counseling	$120	($80 $200)	[9]
T21 birth	$850 000	($600 000 $1 000 000)	[23]
T18 birth	$50 000	($30 000 $70 000)	[23]
T13 birth	$38 000	($25 000 $50 000)	[23]
Termination	$600	($400 $1000)	[9]

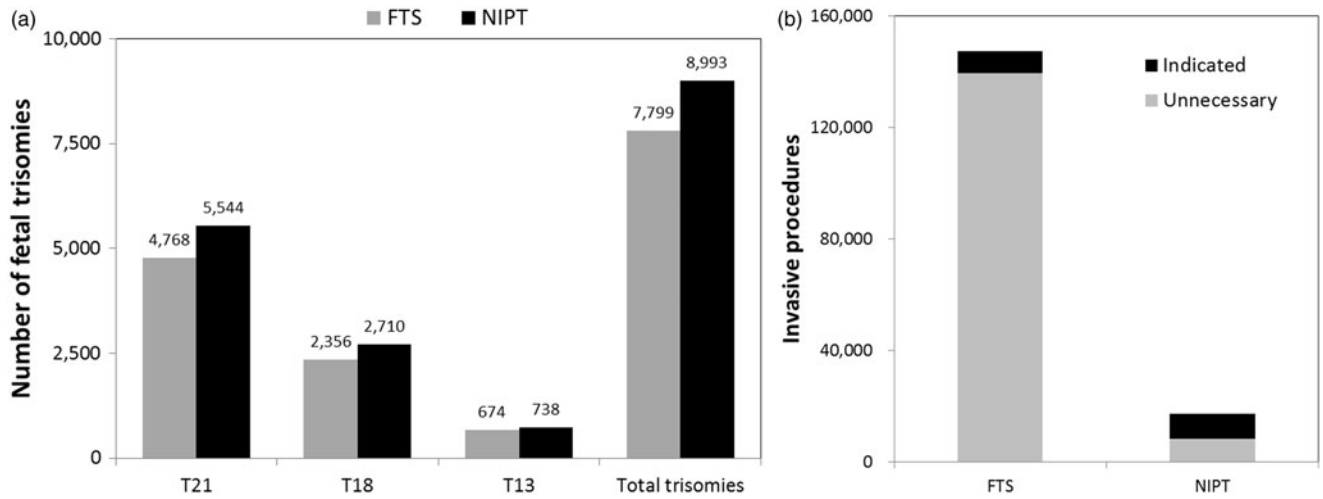
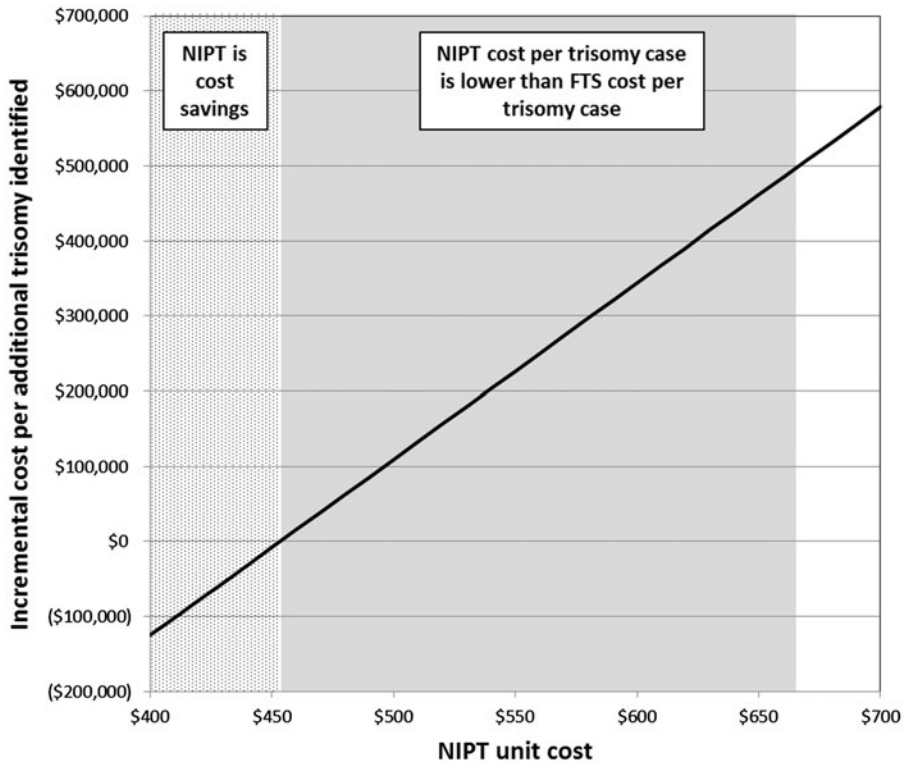


Figure 1. (a) Number of trisomies cases identified with FTS or NIPT by trisomy type and total. (b) Number of invasive procedures that were indicated or unnecessary due to false positive results with FTS or NIPT.

Figure 2. NIPT unit cost analysis. The dotted area shows NIPT unit costs at which total costs are less compared to FTS. The shaded area shows NIPT unit costs at which the cost per trisomy case identified with NIPT is equivalent or lower than that of FTS, but the total overall costs are higher.



Results

Based on a theoretical general pregnancy population of 4 million women, which represents the annual number of U.S. births, we assumed a 70% screening uptake representing 2.8 million women undergoing screening with either FTS or NIPT. NIPT led to the identification of 8993 trisomy cases, of which 5544 were T21 whereas FTS led to the identification of 7799 trisomy cases of which 4768 were T21 (Figure 1a). The total number of invasive procedures with NIPT was 17 303 of which 8342 were unnecessary due to false-positive NIPT screening results. This led to 42 normal fetal losses. With FTS, the total number of invasive procedures was 147 311 of which 139 540 were unnecessary due to false-positive FTS screening results (Figure 1b). This led to 698 normal fetal losses. As compared to FTS, NIPT identified 15% more trisomy cases, reduced invasive procedures by 88%, and reduced iatrogenic normal fetal loss by 94%.

The total costs of screening the cohort with FTS was $3.88B with each trisomy case identified costing $497 909. Taking into account only costs, at a NIPT unit cost of $453 and less, NIPT demonstrated cost savings over FTS. When accounting for the additional trisomy cases identified with NIPT, at a NIPT unit cost of $665, the cost per trisomy case identified was equivalent to that of FTS (Figure 2). No economic value was assigned in the model for any normal fetus losses averted.

Sensitivity analysis was performed on key variables using the ranges shown in Table 1. In one-way sensitivity analysis, NIPT remained the dominant strategy over FTS in all

analyses, except when the cost of NIPT exceeded $453. A two-way sensitivity analysis was performed looking at improved adherence to screening with NIPT over FTS and NIPT cost. We evaluated NIPT screening adherence at 70% (baseline, same as FTS) and at 5% increased increments at 75%, 80%, and 85% while keeping FTS screening adherence at 70%. At increased screening adherence with NIPT of 75%, 80%, and 85%, NIPT remained cost savings over FTS at a NIPT unit cost up to $490, $522, and $550, respectively.

Discussion

For the general pregnancy population, NIPT at the appropriate cost is the preferred and dominant primary screening strategy for fetal trisomies. We decided to compare NIPT to FTS as both can be performed in the first trimester of pregnancy and therefore provide earlier information to best manage the pregnancy. In our study, NIPT was able to identify 15% more trisomy cases than FTS as well as significantly reducing the amount of invasive procedures and as a consequence leading to 656 normal fetuses being saved annually, the benefits of which were not quantified economically in the model. The clinical superiority of NIPT is expected, given its higher accuracy as compared to FTS [2,6].

The initial implementation of NIPT has primarily been in pregnant women classified as ''high risk'' based on maternal age or other risk factors. ACOG issued a statement in 2012 that supported NIPT as an option only in ''high risk'' women [10]. Since then, numerous clinical studies have validated the performance of NIPT in ''average risk'' or ''low risk'' women and professional groups have supported NIPT as an option in any pregnant woman, regardless of age or risk [7,8,11,12]. The primary barrier to adoption of NIPT in the general pregnancy population appears to be one of cost. Our analysis shows that at a NIPT unit cost of $453 or less, it is cost savings over FTS. At this NIPT unit cost, NIPT is clearly the dominant screening strategy since the overall costs are lower with additional clinical benefits. The cost analysis could also be evaluated based on the cost per trisomy case identified. As NIPT identifies more fetal trisomies than FTS, a NIPT unit cost of $665 allows a cost per trisomy case identified to be equivalent to that of FTS. However, in this latter case, the total overall costs with NIPT are higher than that of FTS.

Conventional screening methods, such as FTS, not only have less accuracy than NIPT, but can be more cumbersome to implement. FTS requires the assessment of both blood serum protein markers as well as NT. As NT is a specialized ultrasound procedure, primary care providers including Ob/Gyns may need to refer their pregnant patients to a specialist to carry out screening. Specialist referral leads to additional costs to the healthcare system as well as placing an inconvenience to patients, especially those who live in less populated areas and therefore may need to travel considerable distances to obtain the NT. NIPT allows for all pregnant women to have equal access to a highly accurate screening test for fetal trisomies and also provides a means for screening to be performed by primary care providers.

Given the higher accuracy and ease of implementation, we performed a sensitivity analysis in which we assumed higher uptake of screening with NIPT as compared to FTS. Studies suggest that NIPT may lead to higher uptake of prenatal screening [13,14]. Our analysis showed that NIPT could remain cost savings up to a unit price of $550, if NIPT allowed for improved screening adherence to 85%, a 15% improvement over the base case assumption for FTS.

Several recent cost-effectiveness analyses on NIPT in the general pregnancy population have been published highlighting the broader use of NIPT in all pregnant women as a timely topic [15 17]. These studies looked at screening for Down syndrome only and compared various conventional screening methods, but all found NIPT to be clinically superior. None of these studies directly compared NIPT to FTS nor took into account the additional costs of specialist referral for FTS to perform NT. The cost of NIPT appeared to be the primary open issue in these other published studies. One study found NIPT to be more costly, but also assumed a NIPT unit cost of $1000 [15]. At a lower unit cost of $453, it is probable that NIPT would have been found to be cost savings.

As with any cost-effectiveness analysis, there are limitations. The analysis is based on a theoretical cohort of women as well as assumptions on screening performance, uptake, and cost. The analysis was also performed based on a U.S. population. Screening practices and costs can be quite different in other countries and so the findings here may not be generalizable outside the U.S. Our analysis also focused only on fetal trisomies 21, 18, and 13. We decided to focus on these conditions as they are the ones commonly being screened for today and supported by clinical standards. While both FTS and NIPT have the possibility to pick up other rare medical conditions, we are not aware of any analysis that demonstrates clinical utility or supports assessment of these other medical conditions for screening the general pregnancy population.

NIPT represents a technological advance in prenatal screening that has high accuracy for prenatal assessment of fetal trisomies. Based on our cost-effectiveness model looking at the U.S. general pregnancy population, NIPT can identify more fetal trisomy cases and at the same time reduce unnecessary invasive procedures and in turn fewer related normal fetus losses. These clinical benefits are realized in the setting of also achieving cost savings at the appropriate unit cost of NIPT.

Declaration of interest

K. S. is a paid employee of Ariosa Diagnostics, Inc.

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ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy

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Purpose: To assess the population wide impact of noninvasive prenatal screening (NIPS) on combined first trimester screening (CFTS), early ultrasound (11 13 weeks), and invasive prenatal diagnosis in a state with over 73,000 births per year.

Methods: Analysis of population based data from 2000 to 2015 including (i) invasive prenatal tests, (ii) CFTS uptake, and (iii) total births. Utilization of early ultrasound was analyzed before and after NIPS (2010 2015).

Results: Invasive testing decreased significantly by 39.6% from 2012 to 2015 despite steady births. More than half of all confirmed cases of trisomy 21 were ascertained by NIPS in 2015, despite NIPS comprising only 11.7% of total indications for invasive testing. CFTS uptake declined significantly from 77.5% in 2013 to 68.1% in 2015, but 11 to 13 week ultrasounds did not. In 2015, ultrasound abnormality replaced CFTS as the most common indication for invasive testing and chromosomal microarray was performed for 85.3% of all prenatal karyotypes.

Conclusion: Prenatal testing is now unequivocally in the genomic era. NIPS is now the screening test that precedes the majority of confirmed diagnoses of trisomy 21. The contributions of NIPS, early ultrasound, and chromosome microarray have led to unprecedented detection rates of major chromosome abnormalities, now found in 20% of all invasive tests.

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Key Words: combined first trimester screening; NIPS; NIPT; noninvasive prenatal screening; prenatal diagnosis

INTRODUCTION

Voluntary screening for trisomy 21 is a standard component of prenatal care in the United States and many other countries such as the United Kingdom, Canada, Australia, China, and within the European Union. Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities, also known as cell-free DNA based screening, has been hailed as the vanguard of genomic medicine.[1] This innovative test has spread globally to over 60 countries since its introduction into clinical practice in the United States and China in 2011.[2] Based on genomic sequencing of cell-free DNA in maternal plasma, NIPS has the highest sensitivity (>99%) and specificity (>99.9%) for trisomy 21 of any prenatal screening test.[3] The importance of NIPS to current practice has led the American College of Medical Genetics and Genomics to recently update its position statement, outlining the principles of responsible implementation and importantly, endorsing NIPS as a suitable replacement for biochemical screening for trisomy 21, 18, and 13 across the maternal age spectrum.[4]

Private health insurance for this costly screening test began on a limited basis in the United States for high-risk pregnant women in 2012, and has expanded since then. The DNA sequencing technology and bioinformatics underlying NIPS are now disseminating from the private commercial sphere into the public sector. Several countries are now implementing government-funded NIPS into their national prenatal screening programs, including the United Kingdom, the Netherlands, and Denmark.[5 8]

The potential impact of NIPS on the landscape of prenatal screening is dramatic as sequencing costs decline and the use of NIPS as a primary screening test increases. Until recently, measuring the downstream effect of NIPS has been largely confined to reporting the decline in invasive testing rates in single-center or multicenter studies.[9 11] However, detailed population-based evaluation of its impact on indications for testing, diagnostic yield, and the primary methods of prenatal ascertainment of fetal aneuploidy are lacking.

We have previously reported on data from the pre-NIPS period from 1976 to 2013.[12] In this new analysis, we focus on the period during which NIPS became widely established, the "NIPS era" (2013–2015).

Our aim was to analyze population-based state data sets for (i) changes in invasive prenatal testing, including indications for testing, procedural numbers, and results; (ii) uptake of combined first-trimester screening (CFTS) and utilization of early (11–13 weeks) ultrasound; and (iii) the contributions of different screening tests to the prenatal ascertainment of trisomy 21 in the NIPS era.

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MATERIALS AND METHODS

Study population

This study analyzed prospectively collected data on prenatal screening, diagnosis, and ultrasound from the Australian state of Victoria, with approximately 73,000 births per year. In 2015, the median maternal age was 31.5 years, average fertility rate was 1.7 births per woman, and the average weekly disposable household income was AUD998 (US$744) (http://www.abs.gov.au).

Voluntary screening for fetal chromosome and structural abnormalities is offered as a standard component of prenatal care in Australia.[13] Government rebates are provided for CFTS, second-trimester serum screening ("quadruple test") (STSS), and the midtrimester morphology scan (performed at 18–22 weeks), but most tests involve a variable out-of-pocket cost to the pregnant woman. Invasive testing (amniocentesis and chorionic villus sampling (CVS)) are fully government-funded if performed in a public hospital or partially government-funded if performed in the private sector. NIPS does not currently attract any government or private health insurance subsidy and the total cost is borne by the patient. NIPS became clinically available in Victoria via overseas laboratories in 2013 at a price exceeding AUD 1000 (US$746) and a 10-day turnaround time. By 2015, the average price had fallen to about AUD 500 (US$373) and turnaround time for the locally established laboratories was 3–5 days.

Ethics approvals for this study were provided by the Human Research Ethics Committees of the Royal Children's Hospital (ref. no. 3115A) and Monash Health (ref. no. 12063B).

Data sources

Victorian Prenatal Diagnosis Database

Prenatal diagnosis data from 2000 to 2015 were obtained from the Victorian Prenatal Diagnosis Database. This period was selected to span the period of the CFTS program, which commenced in 2000, and the first 3 years of NIPS availability (2013–2015). This database included all prenatal diagnostic testing (amniocentesis and CVS) in the state, contributed by the four Victorian cytogenetic laboratories. All amniocentesis and CVS results performed prior to 25 weeks gestation on women resident in Victoria by postcode were included in the study. This gestational age limit was chosen to capture invasive testing performed after routine screening for chromosome and fetal structural abnormalities.

The data fields collected for each woman included maternal age and gestation at the time of testing, test date, type of diagnostic test, indication for test, karyotype result, and singleton or multiple pregnancy. A single record was created for twin pregnancies or women who required repeat testing in the same pregnancy.

Clinical indications for testing was based on information provided to the laboratories by the referring clinician. The indications for testing and their definitions are listed here:

1. CFTS: maternal serum levels of pregnancy-associated plasma protein-A and total human chorionic gonadotropin (sampled at 9–13^{+6} weeks gestation) in conjunction with a measurement of the nuchal translucency (NT) (11 to 13^{+6} weeks). CFTS was considered positive if the trisomy 21 risk was ≥ 1 in 300 or the trisomy 18/trisomy 13 risk was ≥ 1 in 150.

2. Ultrasound abnormalities: fetal structural abnormality (including fetal death, intrauterine growth restriction, soft ultrasound markers of aneuploidy), placental or amniotic fluid abnormalities, NT ≥ 3.5 mm if nominated by the clinician as the sole indication and not a part of high-risk CFTS result.

3. Advanced maternal age: maternal age at estimated due date > 36 years, coded as such only in the absence of other indications.

4. STSS for trisomy 21 and trisomy 18 with the quadruple test: laboratory risk reporting thresholds were ≥ 1 in 250 for trisomy 21, and ≥ 1 in 200 for trisomy 18.

5. NIPS: including any test performed for "high risk" or "failed NIPS" result.

6. Maternal history: family or personal history of a previous pregnancy with a known chromosome or genetic condition, and/or known parental chromosome rearrangement carrier status.

7. Other: women undergoing an invasive test with no increased screening risk result, no prior history of a pregnancy with a known chromosome abnormality, and not meeting the criteria for advanced maternal age. This includes women having chromosome analysis performed following an invasive test for other miscellaneous conditions including suspected congenital infection and fetal blood group testing (in the absence of an ultrasound abnormality).

8. Single-gene testing: women who underwent invasive testing due to a risk of a specific monogenic disorder.

In women with more than one indication for invasive testing (e.g., high-risk NIPS result and fetal structural abnormality) both indications were coded. The total number of indications thus exceeds that of the number of women undergoing invasive testing.

The types of genetic testing performed included G-banded karyotype, fluorescent in situ hybridization, chromosomal microarray (CMA), and DNA testing for single-gene disorders (e.g., cystic fibrosis, fragile X, thalassemia). All CMAs were performed by a central laboratory using the Affymetrix Cytoscan 750K array (Santa Clara, CA, USA; genomic resolution of 0.2 Mb). The results of fluorescent in situ hybridization and single-gene testing are not reported in this paper. Chromosome analysis performed on fetal blood samples was rare and was also excluded. Multiple tests performed in the same pregnancy (for multiple pregnancies or repeat testing) were combined into a single report.

Chromosome test results were categorized as normal or abnormal. The abnormal results were further divided into "major" and "minor" chromosome abnormalities. Major chromosome abnormalities included all cases of autosomal and sex chromosome aneuploidy, polyploidy, unbalanced rearrangements, level III mosaics, and pathogenic copy-number variants (CNVs). Minor chromosome abnormalities

included balanced rearrangements, confined placental mosaicisms, and CNVs of uncertain or unknown significance (VUS).

We defined "diagnostic yield" as the percentage of diagnostic tests that detected a major chromosome abnormality. The total abnormality rate was the total major and minor chromosome abnormalities as a percentage of the total number of invasive tests.

The total numbers of CFTS and STSS were obtained from the state central screening laboratory. This lab performs the serum biomarker testing and the aneuploidy risk calculation for all CFTS and STSS referrals in the state, using NT measurements supplied by the referring doctor. The total number of women accessing NIPS was not obtainable due to the lack of systematic data collection for this testing.

Victorian births

Annual and quarterly statistics on Victorian live births were obtained from the website of the Australian Bureau of Statistics (http://stat.data.abs.gov.au/Index.aspx?QueryId=505) to estimate uptake rates of screening and invasive testing. Australian Bureau of Statistics data do not include stillbirths or terminations of pregnancy, and have previously been calculated to underestimate total confinements by <1%.[14]

Ultrasound scans

Ultrasound scan numbers in Victoria were estimated from billing statistics from the Medicare Australia Medical Benefits Scheme database (http://medicarestatistics.humanservices.gov.au/statistics/mbs item.jsp) using item number 55707 ("pregnancy ultrasound at 11–13 weeks gestation where NT measurement is performed to assess risk of fetal abnormality"). These billing numbers do not include services provided by hospital doctors to public patients in public hospitals, which comprise a minority of prenatal screening services. While the Medical Benefits Scheme figures consistently underestimate the annual number of 11- to 13-week scans by a median of 31% when compared to total number of CFTS tests (data not shown), they are an accepted measure of general trends in community practice in prenatal testing.[15,16]

Statistical analysis

Statistical analysis was performed with PRISM 6 Version 6.0h (San Diego, CA, USA). We performed two-tailed chi-squared tests for comparison of two proportions, or chi-squared tests for trend where appropriate, with a P value of <0.05 being considered significant.

RESULTS

Annual prenatal diagnostic procedures

The total number of diagnostic procedures performed <25 weeks gestation during the 16-year study period was 62,536. The annual number of diagnostic tests declined steadily after the introduction of CFTS in 2000 (Figure 1a). Steeper reductions in annual tests occurred following the gradual incorporation of nasal bone assessment from 2011, which had the effect of reducing the screen-positive rate of

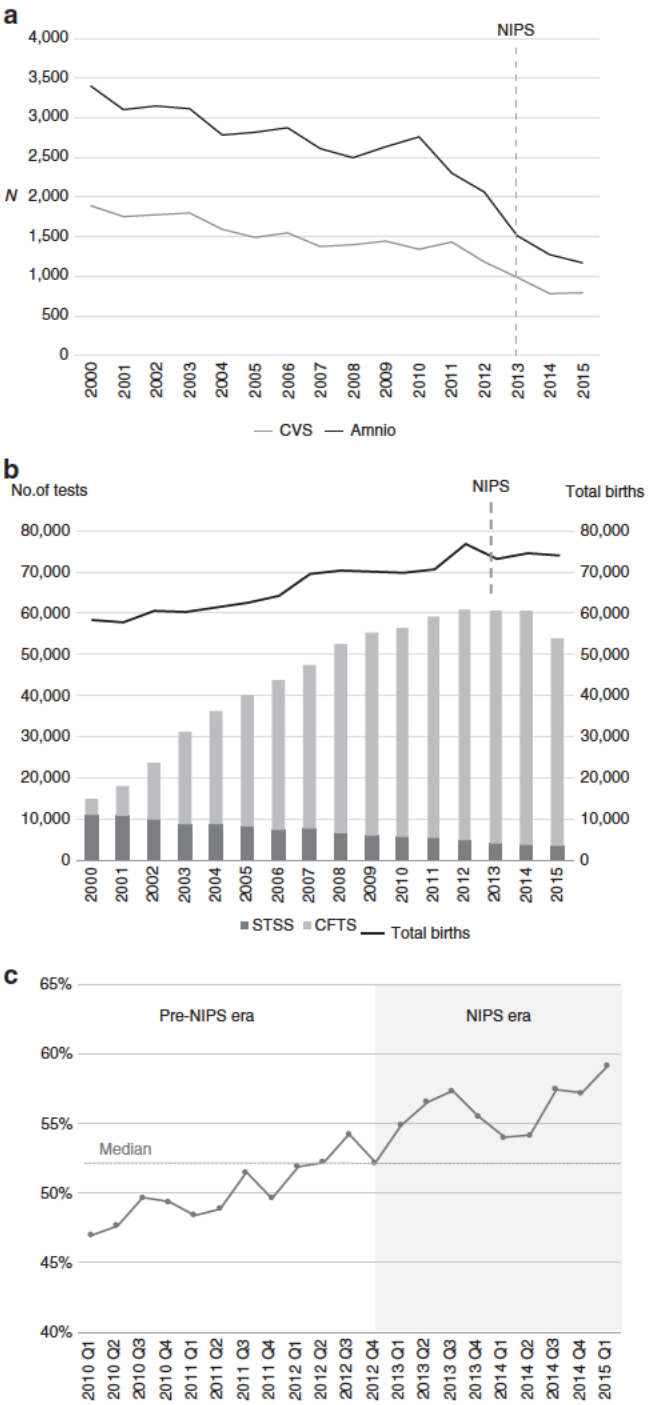

Figure 1 Trends in diagnostic procedures, serum screening, and 11 13 week ultrasounds. (**a**) Annual numbers of prenatal diagnostic tests performed <25 weeks gestation in Victoria (2000 2015). (**b**) Uptake of combined first and second trimester serum screening and annual births for 2000 2015. (**c**) Run chart of statewide trends in 11 to 13 week ultrasounds: government billings as a proportion of total births. Medicare billings for item 55707 underestimate total scans performed for combined first trimester screening by approximately 31% (data not shown). Births are taken from the period two quarters subsequent to the time of the 11 to 13 week scan to correspond to expected due date. Amnio, amniocentesis; CFTS, combined first trimester screening; CVS, chorionic villus sampling; NIPS, noninvasive prenatal screening; STSS, second trimester serum screening.

CFTS. The steepest annual decline of 22.9% was observed in 2013, the year that NIPS became available. From 2012 to 2015 there was a 39.6% reduction in invasive tests and by 2015, only 1,957 (794 CVS, 1,165 amniocenteses) were performed.

Uptake of CFTS and early ultrasound (11–13 weeks) from 2012 to 2015

The population uptake of CFTS increased annually during the first 10 years of clinical implementation and plateaued between 70.4% and 76.6% from 2009 to 2012 (Figure 1b). During the NIPS era the annual uptake rate of CFTS declined significantly for the first time, falling from 77.5% in 2013 to 68.1% in 2015 (χ^2 test for trend = 2,276, $P < 0.0001$). Meanwhile, quarterly numbers in government billing for NT ultrasounds as a percentage of births showed a steady increase in the proportion of women having an 11- to 13-week ultrasound (χ^2 for trend = 1,923.9, $P < 0.0001$) (Figure 1c).

The overall uptake of STSS continued its long-standing gradual decline and was used by <5% of women in 2015.

Indications for diagnostic testing

There were major changes in the ranking of common indications for testing in 2015 (Figure 2a). In 2015, an ultrasound abnormality was present in 35.0% (685/1,957) of all women undergoing an invasive test, replacing CFTS as the most common indication for prenatal diagnosis. An increased risk CFTS result was the indication for 31.1% (608/1,957) of invasive tests.

Of the invasive tests performed for an ultrasound abnormality in 2014–2015, 41.6% (532/1,282) were performed prior to 18 weeks gestation. NT ≥3.5 mm as a stand-alone indication (without a CFTS risk) comprised 41.0% (218/532) of indications for testing <18 weeks.

High-risk (or failed) NIPS result was the third most common indication for testing in 2015 (11.7% of all tests), showing a steep increase from 29 cases in 2013 to 229 in 2015 (Figure 2a). After many decades as the leading indication for invasive testing, advanced maternal age alone is no longer ranked among the top three indications for prenatal diagnosis, forming the sole indication in only 4.7% of tests.

Abnormal results and diagnostic yield in the NIPS era

Trisomy 21 remained the most common condition detected on prenatal diagnosis in the NIPS era (Table 1). The year 2015 was notable for the highest number of confirmed trisomy 21 cases ever recorded in Victoria ($n = 204$). Of these, cases, 105 (51.5%) had a high-risk NIPS as an indication for diagnostic testing (Figure 2b).

The diagnostic yield of testing by indication for 2013–2015 is presented in Table 2. High-risk NIPS result, parental translocation carrier, and ultrasound abnormality were the indications most likely to result in a confirmed diagnosis of a major chromosome abnormality. Of the 229 women in 2015 who had a diagnostic test following high-risk or failed NIPS result, 148 had a major abnormality confirmed, resulting in an overall positive value of 64.6%. Of these

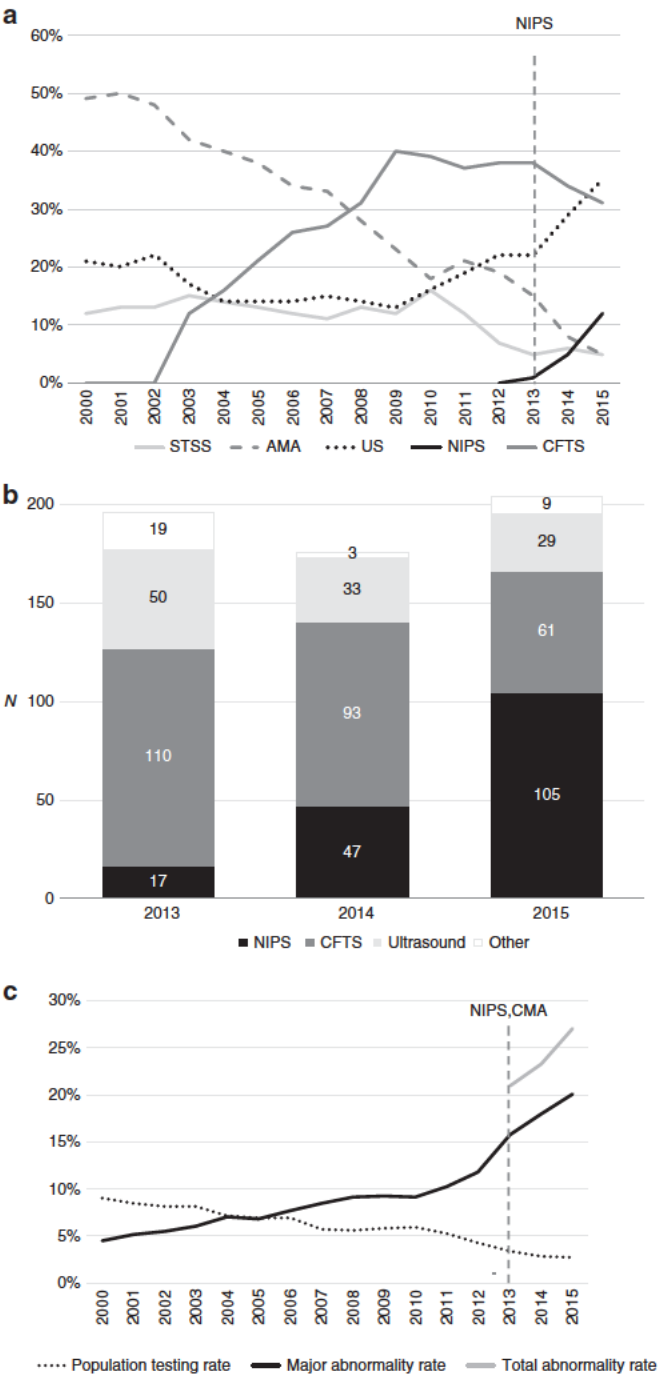

Figure 2 Trends in indications for diagnostic testing, testing rates and diagnostic yield. (**a**) Indications for invasive prenatal testing as % of all tests. (**b**) Indications for testing in confirmed cases of trisomy 21 in the noninvasive prenatal screening era (2013 2015). (**c**) Statewide trends in prenatal testing and chromosome abnormality detection rates (2000 2015). Population testing rate = total number of invasive prenatal tests <25 weeks gestation/live births. Major abnormality rate = number of major chromosome abnormalities (excluding benign variants, variants of unknown/uncertain significance)/number of diagnostic tests. Total abnormality rate = total number of chromosome abnormalities/number of diagnostic tests. AMA, advanced maternal age; CFTS, combined first trimester screening; CMA, chromosome microarray; NIPS, noninvasive prenatal screening; STSS, second trimester serum screening; US, ultrasound.

ORIGINAL RESEARCH ARTICLE

Table 1 Results of all prenatal chromosome tests by year (2013–2015)

Karyotype result	2013	2014	2015
Total tests	2,500	2,046	1,957
Normal karyotype	1,969	1,548	1,427
Major chromosome abnormalities	395 (15.8%)	369 (18.0%)	394 (20.1%)
Trisomy 21	198	176	204
Trisomy 18	61	49	42
Trisomy 13	30	21	15
Other autosomal aneuploidy, polyploidy	18	22	21
Sex chromosome aneuploidy	31	33	28
Pathogenic copy number variation	25	39	39
Other major abnormalities[a]	32	29	45
Minor chromosome abnormalities[b]	136 (5.4%)	129 (6.3%)	136 (6.9%)

[a]Includes level III mosaic, unbalanced translocation/rearrangement, and uniparental disomy. [b]Includes balanced translocations, variations of unknown/uncertain significance, and confined placental mosaicism.

Table 2 Diagnostic yield for major chromosome abnormalities by indication for testing (2013–2015)

	2013	2014	2015	Combined rate 2013 15
High risk NIPS	82.8% (24/29)	64.3% (72/112)	64.6% (148/229)	65.9% (244/370)
Known parental rearrangement	7.9% (3/38)	37.8% (14/37)	34.9% (15/43)	27.1% (32/118)
Ultrasound abnormalities	20.9% (116/554)	22.9% (137/597)	19.0% (130/685)	20.9% (383/1,836)
High risk CFTS	21.3% (203/954)	20.5% (160/781)	18.9% (115/608)	20.4% (478/2,343)
Prior pregnancy with chromosomal abnormality	2.8% (2/71)	5.6% (3/53)	8.1%% (4/49)	5.2% (9/173)
High risk second trimester screening	6.1% (7/115)	2.9% (4/139)	5.2% (5/96)	4.6% (16/350)
Advanced maternal age alone	6.8% (26/382)	1.1% (2/175)	3.3% (3/92)	4.8% (31/649)
Other	0.9% (1/112)	5.7% (5/88)	3.3% (3/91)	3.1% (9/291)

CFTS, combined first trimester screening; NIPS, noninvasive prenatal screening.
Some cases had more than one indication coded; hence, column totals may not sum to total number of tests performed by year. Testing for single gene disorders is not included in this table.

confirmed abnormalities, there were 105 cases of trisomy 21, 18 of trisomy 18 or 13, and 10 of a sex chromosome aneuploidy (**Table 3**).

Among women who had an invasive test for an ultrasound abnormality in 2014–2015, diagnostic testing prior to 18 weeks gestation was associated with a significantly higher rate of major chromosome abnormality (32.6%, 173/532), compared with testing at 18–24 weeks gestation (12.5%, 94/750) ($\chi^2 = 75.4$, $P < 0.0001$).

The NIPS era has coincided with increasing utilization of chromosome microarrays for prenatal diagnostic testing. The percentage of all tests that were submitted for CMA analysis increased from 14.2% in 2012 to 85.3% in 2015. This was accompanied by a significant increase in pathogenic CNVs as a proportion of all tests, from 1.0% in 2013 to 2.0% in 2015 ($\chi^2 = 7.6$, $P = 0.006$), but this gain was associated with an increase in the numbers of VUS from 3.9% to 6.4% over the same period.

The steady increase in the annual numbers of abnormal karyotypes identified on diagnostic testing and the decline in invasive tests have intersected to produce a historically high diagnostic yield of 20.1% in 2015. The total abnormality rate including VUS was 27.0%. Overall, the proportion of all births in Victoria undergoing prenatal diagnosis prior to 25 weeks was 2.7% (**Figure 2c**).

DISCUSSION

This study is the first to comprehensively analyze the profound impact of NIPS on prenatal screening and diagnosis on a population-wide basis. We observed a 39.6% reduction in total invasive tests in the first three years of NIPS availability, consistent with the global experience.[6 11] While CFTS was still used by the majority of women in 2015, uptake in that year significantly declined for the first time since its introduction in 2002. We attribute this decline to the increasing use of NIPS as a primary screening test, rather than an overall reduction in screening uptake. This statement is based on the observations that (i) invasive testing for NIPS increased from 29 women in 2013 to 229 in 2015, (ii) 2015 had a record number of confirmed trisomy 21 cases, and (iii) NIPS has now displaced CFTS as the most common screening test preceding a confirmed diagnosis of trisomy 21.

Importantly, there was no evidence of a trend to fewer 11- to 13-week scans in 2015. This indicates that the significant decline in CFTS uptake and the introduction of NIPS has not been accompanied by a decline in opportunities

Table 3 Results of diagnostic tests performed for noninvasive prenatal screening results

	2013	2014	2015
Total diagnostic tests for NIPS[a]	29	112	229
Karyotype results			
Normal	4 (13.8%)	37 (33.0%)	75 (32.8%)
Total major abnormalities	24 (82.8%)	72 (64.3%)	148 (64.6%)
Trisomy 21	18 (62.1%)	46 (42.0%)	105 (45.9%)
Trisomy 18	3 (10.3%)	4 (3.6%)	13(5.7%)
Trisomy 13	1	3	5
Other autosomal aneuploidy	0	0	3
Sex chromosome aneuploidy	2	12	10
Level III mosaic	0	5	12
Pathogenic CNV	0	2	2
Minor abnormalities[b]	1	3	6

CNV, copy number variant; NIPS, noninvasive prenatal screening.
[a]Includes 15 tests performed after "no call" NIPS result. [b]Includes confined placental mosaicism and variations of unknown significance.

for early structural assessment of the fetus prior to the routine midtrimester morphology scan. In fact, ultrasound abnormality is now the most common indication for invasive testing, with 41% of these procedures in 2014–2015 being performed prior to 18 weeks. This suggests that practitioners who are using NIPS as a primary aneuploidy screening test still recognize the value of an early ultrasound for a fetal structural survey. Furthermore, we observed a high diagnostic yield for invasive testing for ultrasound abnormalities prior to 18 weeks (32.6%), supporting the clinical utility of this practice.

The high sensitivity and specificity of NIPS has contributed to the overall increase in the numbers of major chromosome abnormalities detected and the decline in invasive testing to the lowest level in 30 years.[12] Over the same period, we also observed the impact of the routine adoption of CMA in a significant trend to higher detection of pathogenic CNVs. Working in parallel, these two developments in prenatal screening and diagnosis have produced historic diagnostic yields for prenatal testing, with one in five invasive tests now leading to a diagnosis of a major chromosome abnormality.

Implications for practice

These results have important implications for clinical practice. The 11- to 13-week ultrasound examination became incorporated into routine prenatal care about 15 years ago for the purpose of trisomy 21 screening. The initial role of the examination was to measure the NT thickness and crown rump length and combine these measurements with maternal serum biochemical markers for individualized aneuploidy risk assessment. However, advances in the performance of the 11- to 13-week ultrasound have seen it evolve into a detailed structural morphology survey, able to detect up to 50% of all major structural abnormalities including cardiac defects.[17] The option of using NIPS as a primary screening test for aneuploidy in the first trimester has caused the profession to reexamine the value of retaining the 11- to 13-week scan. Opinion leaders have argued that first-trimester ultrasound continues to have an important role in the NIPS era for the detection of fetal structural anomalies and prediction of obstetric complications.[18,19] Several retrospective studies examining the additional information provided by the early ultrasound in the NIPS era have emphasized its important role for accurate pregnancy dating, and diagnosis of fetal demise, multiple gestation, fetal structural anomalies, and placental and maternal pathology.[20,21] From a public health perspective, early diagnosis of fetal abnormalities may not change final pregnancy outcomes as most would be detected at the midtrimester morphology scan. However, from a patient perspective, early diagnosis of a major fetal abnormality would have substantial medical, social, and psychological benefits.

Our study also confirms the need for confirmatory diagnostic testing after a high-risk NIPS result, and demonstrates the decline in the positive predictive value of NIPS with its expansion to the general pregnant population. In the first year of availability, when Australian practitioners were predominately using NIPS in high-risk women, the rate of confirmed aneuploidy after diagnostic testing for a high-risk NIPS result was 82.9%.[22] As the offer of self-funded NIPS expanded to general-risk women, accordingly lower rates of confirmed abnormalities were observed in 2014 and 2015 (**Table 2**).

The impact of routine utilization of CMA for prenatal diagnosis is also evident in our study. Offering genome-wide diagnostic testing is now standard practice for pregnancies with fetal structural abnormalities[23] including NT measurements ≥ 3.5 mm.[24,25] Even in the presence of normal ultrasound and karyotype, there is ~1% background rate of clinically significant CNVs.[26] Recently, the Society for Maternal Fetal Medicine and the American Congress of Obstetricians and Gynecologists have supported offering the option of CMA analysis to all women undergoing prenatal diagnostic testing.[27] Our results show that expanding the use of CMA does result in significantly more diagnoses of pathogenic CNVs, but with an accompanying increase in the detection of VUS. The relatively high rate of VUS in our study may be related to the use of a high-resolution, whole-genome single-nucleotide polymorphism array, rather than a targeted prenatal array, and highlights the growing demand for genetic counseling and database annotation of genetic variants.

Strengths of the study

This is the first study to demonstrate the significant impact of NIPS on prenatal screening and diagnosis for an entire population. We had complete prenatal ascertainment of CFTS, STSS, and prenatal diagnostic tests for Victoria because of a unique long-standing collaboration with all cytogenetic laboratories in the state.[12] Prior multicenter studies of NIPS have usually been based on maternity units[6] or laboratory

services.[11] The advantages of a population-based approach are large sample sizes, and the avoidance of potential selection biases caused by tertiary referral populations, a single NIPS provider, or individual clinical practice patterns.

Limitations

We were not able to obtain data for women who had NIPS without diagnostic testing due to the fragmented nature of NIPS provision among private and nonprofit providers. We therefore cannot ascertain the total number of women who accessed NIPS during pregnancy but had to confine our analysis to the women who underwent prenatal diagnosis as a result of high-risk NIPS result. An estimate of the total numbers of women accessing NIPS in 2015 can be calculated using figures from a recent study of over 5,000 Australian women using NIPS as a primary or secondary screening test.[28] Adopting the 2.2% screen-positive rate and 73.4% invasive testing rate from this study, we estimated that 14,181 women would have used NIPS in order to lead to 229 invasive tests in 2015. This figure represents 19% of all Victorian births, which is in keeping with our impressions of local clinical practice. We were also not able to identify which women with a high-risk NIPS result had utilized this as a primary or secondary screening test.

Other limitations of our study are that we could not perform linkage to pregnancy outcomes, and hence were unable to ascertain false-negative screening results, termination of pregnancy rates, or follow-up clinical outcomes from false-positive NIPS results. Past studies on prenatal screening suggest significant differences in access according to the geographical location and maternal demographics in our population.[29] This was beyond the scope of this study, but further analysis is planned to determine whether there is any association between geographical and social disadvantage on prenatal screening and indications for invasive testing.

Conclusion

Our population-based study demonstrates the rapidly emerging importance of NIPS and the declining influence of CFTS in the genomic era—an experience with relevance for other countries adapting to NIPS from a preexisting paradigm of CFTS. The willingness of women to self-fund NIPS has led to a dramatic reduction in invasive testing over 3 years, accompanied by the highest diagnostic yield ever recorded. NIPS is now the single biggest contributor to prenatal ascertainment of T21 in our population. Ultrasound-detected abnormalities have become the most common indication for diagnostic testing, highlighting the continued importance of early ultrasound and high-resolution genomic testing in the NIPS era.

ACKNOWLEDGMENTS

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DISCLOSURE

The authors declare no conflict of interest.

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Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy

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NIPS has the highest sensitivity (>99%) and specificity (>99.9%) for trisomy 21 of any prenatal screening test.[3] The importance of NIPS to current practice has led the American College of Medical Genetics and Genomics to recently update its position statement, outlining the principles of responsible implementation and importantly, endorsing NIPS as a suitable replacement for biochemical screening for trisomy 21, 18, and 13 across the maternal age spectrum.[4]

Several countries are now implementing government-funded NIPS into their national prenatal screening programs, including the United Kingdom, the Netherlands, and Denmark.[5–8]

We have previously reported on data from the pre-NIPS period from 1976 to 2013.[12] In this new analysis, we focus on the period during which NIPS became widely established, the "NIPS era" (2013–2015).

Study population

This study analyzed prospectively collected data on prenatal screening, diagnosis, and ultrasound from the Australian state of Victoria, with approximately 73,000 births per year. In 2015, the median maternal age was 31.5 years, average fertility rate was 1.7 births per woman, and the average weekly disposable household income was AUD998 (US$744) (http://www.abs.gov.au).

Voluntary screening for fetal chromosome and structural abnormalities is offered as a standard component of prenatal care in Australia.[13] Government rebates are provided for CFTS, second-trimester serum screening ("quadruple test") (STSS), and the midtrimester morphology scan (performed at 18–22 weeks), but most tests involve a variable out-of-pocket cost to the pregnant woman. Invasive testing (amniocentesis and chorionic villus sampling (CVS)) are fully government-funded if performed in a public hospital or partially government-funded if performed in the private sector. NIPS does not currently attract any government or private health insurance subsidy and the total cost is borne by the patient. NIPS became clinically available in Victoria via overseas laboratories in 2013 at a price exceeding AUD 1000 (US$746) and a 10-day turnaround time. By 2015, the average price had fallen to about AUD 500 (US$373) and turnaround time for the locally established laboratories was 3–5 days.

Data sources

Victorian Prenatal Diagnosis Database

Prenatal diagnosis data from 2000 to 2015 were obtained from the Victorian Prenatal Diagnosis Database. This period was selected to span the period of the CFTS program, which commenced in 2000, and the first 3 years of NIPS availability (2013–2015). This database included all prenatal diagnostic testing (amniocentesis and CVS) in the state, contributed by the four Victorian cytogenetic laboratories. All amniocentesis and CVS results performed prior to 25 weeks gestation on women resident in Victoria by postcode were included in the study. This gestational age limit was chosen to capture invasive testing performed after routine screening for chromosome and fetal structural abnormalities.

The data fields collected for each woman included maternal age and gestation at the time of testing, test date, type of diagnostic test, indication for test, karyotype result, and singleton or multiple pregnancy. A single record was created for twin pregnancies or women who required repeat testing in the same pregnancy.

We defined "diagnostic yield" as the percentage of diagnostic tests that detected a major chromosome abnormality. The total abnormality rate was the total major and minor chromosome abnormalities as a percentage of the total number of invasive tests.

Annual prenatal diagnostic procedures

The total number of diagnostic procedures performed <25 weeks gestation during the 16-year study period was 62,536. The annual number of diagnostic tests declined steadily after the introduction of CFTS in 2000 (**Figure 1a**). Steeper reductions in annual tests occurred following the gradual incorporation of nasal bone assessment from 2011, which had the effect of reducing the screen-positive rate of CFTS. The steepest annual decline of 22.9% was observed in 2013, the year that NIPS became available. From 2012 to 2015 there was a 39.6% reduction in invasive tests and by 2015, only 1,957 (794 CVS, 1,165 amniocenteses) were performed.

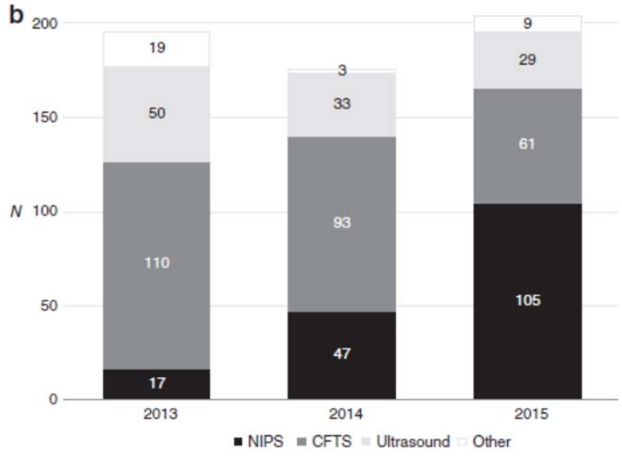
Uptake of CFTS and early ultrasound (11–13 weeks) from 2012 to 2015

The population uptake of CFTS increased annually during the first 10 years of clinical implementation and plateaued between 70.4% and 76.6% from 2009 to 2012 (**Figure 1b**). During the NIPS era the annual uptake rate of CFTS declined significantly for the first time, falling from 77.5% in 2013 to 68.1% in 2015 (χ^2 test for trend = 2,276, $P < 0.0001$). Meanwhile, quarterly numbers in government billing for NT ultrasounds as a percentage of births showed a steady increase in the proportion of women having an 11- to 13-week ultrasound (χ^2 for trend = 1,923.9, $P < 0.0001$) (**Figure 1c**).

The overall uptake of STSS continued its long-standing gradual decline and was used by <5% of women in 2015.

Abnormal results and diagnostic yield in the NIPS era

Trisomy 21 remained the most common condition detected on prenatal diagnosis in the NIPS era (**Table 1**). The year 2015 was notable for the highest number of confirmed trisomy 21 cases ever recorded in Victoria ($n = 204$). Of these, cases, 105 (51.5%) had a high-risk NIPS as an indication for diagnostic testing (**Figure 2b**).



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	2013	2014	2015	Combined rate 2013–15
High-risk NIPS	82.8% (24/29)	64.3% (72/112)	64.6% (148/229)	65.9% (244/370)
Known parental rearrangement	7.9% (3/38)	37.8% (14/37)	34.9% (15/43)	27.1% (32/118)
Ultrasound abnormalities	20.9% (116/554)	22.9% (137/597)	19.0% (130/685)	20.9% (383/1,836)
High-risk CFTS	21.3% (203/954)	20.5% (160/781)	18.9% (115/608)	20.4% (478/2,343)
Prior pregnancy with chromosomal abnormality	2.8% (2/71)	5.6% (3/53)	8.1%% (4/49)	5.2% (9/173)
High-risk second-trimester screening	6.1% (7/115)	2.9% (4/139)	5.2% (5/96)	4.6% (16/350)
Advanced maternal age alone	6.8% (26/382)	1.1% (2/175)	3.3% (3/92)	4.8% (31/649)
Other	0.9% (1/112)	5.7% (5/88)	3.3% (3/91)	3.1% (9/291)

CFTS, combined first-trimester screening; NIPS, noninvasive prenatal screening.
Some cases had more than one indication coded; hence, column totals may not sum to total number of tests performed by year. Testing for single-gene disorders is not included in this table.

Table 1 Results of all prenatal chromosome tests by year (2013–2015)

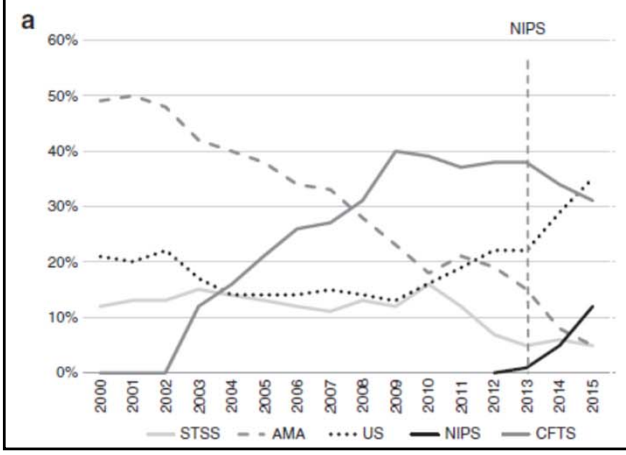
Karyotype result	2013	2014	2015
Total tests	2,500	2,046	1,957
Normal karyotype	1,969	1,548	1,427
Major chromosome abnormalities	395 (15.8%)	369 (18.0%)	394 (20.1%)
Trisomy 21	198	176	204
Trisomy 18	61	49	42
Trisomy 13	30	21	15
Other autosomal aneuploidy, polyploidy	18	22	21
Sex chromosome aneuploidy	31	33	28
Pathogenic copy-number variation	25	39	39
Other major abnormalities[a]	32	29	45
Minor chromosome abnormalities[b]	136 (5.4%)	129 (6.3%)	136 (6.9%)

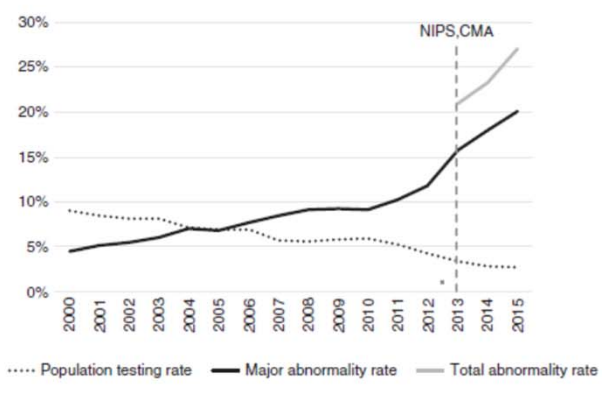
[a]Includes level III mosaic, unbalanced translocation/rearrangement, and uniparental disomy. [b]Includes balanced translocations, variations of unknown/uncertain significance, and confined placental mosaicism.

Among women who had an invasive test for an ultrasound abnormality in 2014–2015, diagnostic testing prior to 18 weeks gestation was associated with a significantly higher rate of major chromosome abnormality (32.6%, 173/532), compared with testing at 18–24 weeks gestation (12.5%, 94/750) ($\chi^2 = 75.4$, $P < 0.0001$).

The NIPS era has coincided with increasing utilization of chromosome microarrays for prenatal diagnostic testing. The percentage of all tests that were submitted for CMA analysis increased from 14.2% in 2012 to 85.3% in 2015. This was accompanied by a significant increase in pathogenic CNVs as a proportion of all tests, from 1.0% in 2013 to 2.0% in 2015 ($\chi^2 = 7.6$, $P = 0.006$), but this gain was associated with an increase in the numbers of VUS from 3.9% to 6.4% over the same period.

The steady increase in the annual numbers of abnormal karyotypes identified on diagnostic testing and the decline in invasive tests have intersected to produce a historically high diagnostic yield of 20.1% in 2015. The total abnormality rate including VUS was 27.0%. Overall, the proportion of all births in Victoria undergoing prenatal diagnosis prior to 25 weeks was 2.7% (**Figure 2c**).





DISCUSSION

This study is the first to comprehensively analyze the profound impact of NIPS on prenatal screening and diagnosis on a population-wide basis. We observed a 39.6% reduction in total invasive tests in the first three years of NIPS availability, consistent with the global experience.[6–11] While CFTS was still used by the majority of women in 2015, uptake in that year significantly declined for the first time since its introduction in 2002. We attribute this decline to the increasing use of NIPS as a primary screening test, rather than an overall reduction in screening uptake. This statement is based on the observations that (i) invasive testing for NIPS increased from 29 women in 2013 to 229 in 2015, (ii) 2015 had a record number of confirmed trisomy 21 cases, and (iii) NIPS has now displaced CFTS as the most common screening test preceding a confirmed diagnosis of trisomy 21.

for early structural assessment of the fetus prior to the routine midtrimester morphology scan. In fact, ultrasound abnormality is now the most common indication for invasive testing, with 41% of these procedures in 2014–2015 being performed prior to 18 weeks. This suggests that practitioners who are using NIPS as a primary aneuploidy screening test still recognize the value of an early ultrasound for a fetal structural survey. Furthermore, we observed a high diagnostic yield for invasive testing for ultrasound abnormalities prior to 18 weeks (32.6%), supporting the clinical utility of this practice.

Implications for practice

These results have important implications for clinical practice. The 11- to 13-week ultrasound examination became incorporated into routine prenatal care about 15 years ago for the purpose of trisomy 21 screening. The initial role of the examination was to measure the NT thickness and crown rump length and combine these measurements with maternal serum biochemical markers for individualized aneuploidy risk assessment. However, advances in the performance of the 11- to 13-week ultrasound have seen it evolve into a detailed structural morphology survey, able to detect up to 50% of all major structural abnormalities including cardiac defects.[17]

17. Rossi AC, Prefumo F. Accuracy of ultrasonography at 11–14 weeks of gestation for detection of fetal structural abnormalities. *Obstet Gynecol* 2013;122:1160–1167.

ORIGINAL ARTICLE

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

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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.)

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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 represent 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-trimester 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 aneuploidy screening at 35 centers in six countries. At enrollment, maternal blood was drawn, locally deidentified, and sent for risk assessment for trisomy 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 (Veristat). 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 collaboration with the sponsor. Representatives of the sponsor performed the analyses and interpretation 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 entered 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 conducted 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. Peripheral 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 (including 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[5] 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.[6-8] For cfDNA testing, samples were rejected if they were not collected in Cell-free DNA BCT tubes; if the tubes were broken, unfilled, 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[8] (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

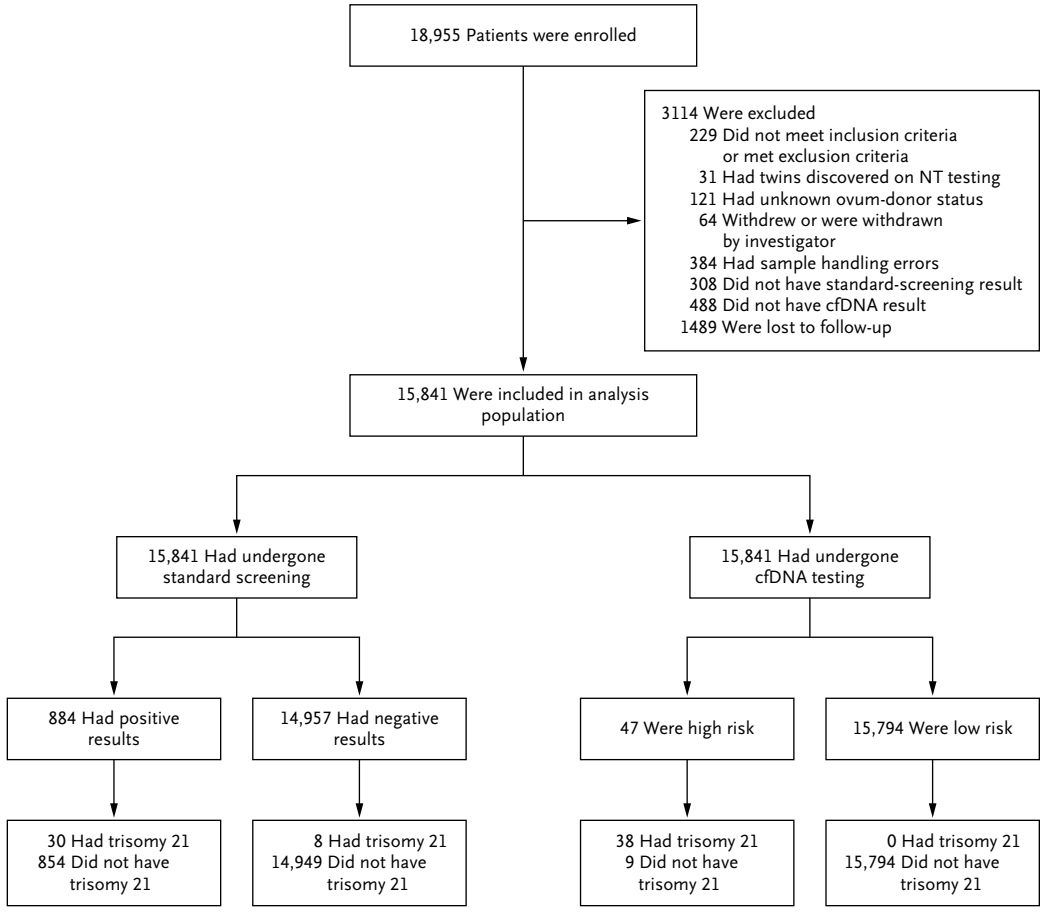

Figure 1. Enrollment and Outcomes.
The abbreviation cfDNA denotes cell-free DNA, and NT nuchal translucency.

were compared with the use of a z-test according to the method of DeLong et al.[9] 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[10] for paired comparisons in sensitivity and specificity and used the generalized score statistic[11] 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[12,13] 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.

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.

Table 1. Demographic and Clinical Characteristics of the Patients.

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

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

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

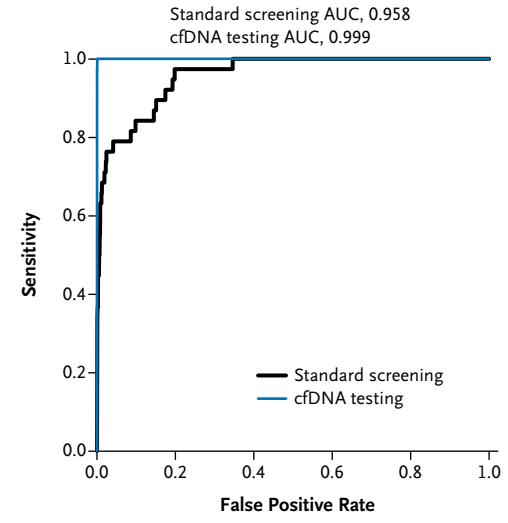

Figure 2. Primary Outcome for Trisomy 21 Screening.
The primary outcome was the area under the receiver-operating-characteristic curve (AUC) for trisomy 21 screening with cell-free DNA (cfDNA) testing versus standard screening in women with complete results for the two tests. The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (P=0.001). The use of cfDNA testing identified 38 of 38 cases of trisomy 21, for a sensitivity of 100% (95% confidence interval [CI], 90.7 to 100), as compared with 30 of 38 cases for standard screening, for a sensitivity of 78.9% (95% CI, 62.7 to 90.4; P=0.008).

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, as 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.

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

Variable	Standard Screening	Cell-free DNA Testing		
	All Patients (N=15,841)	All Patients (N=15,841)	Maternal Age <35 Yr (N=11,994)	Low Risk (N=14,957)†
True positive — no.	30	38	19	8
True negative — no.	14,949	15,794	11,969	14,941
False positive — no.	854	9	6	8
False negative — no.	8	0	0	0
Sensitivity (95% CI) — %	78.9 (62.7–90.4)	100 (90.7–100)‡	100 (82.4–100)	100 (63.1–100)
Specificity (95% CI) — %	94.6 (94.2–94.9)	99.9 (99.9–100)§	99.9 (99.9–100)	99.9 (99.9–100)
Positive predictive value (95% CI) — %	3.4 (2.3–4.8)	80.9 (66.7–90.9)§	76.0 (54.9–90.6)	50.0 (24.7–75.3)
Negative predictive value (95% CI) — %	99.9 (99.9–100)	100 (99.9–100)¶	100 (99.9–100)	100 (99.9–100)
Positive likelihood ratio	14.6	1755.9	1995.8	1868.6
Negative likelihood ratio	0.22	0	0	0

* 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<0.001
¶ P=0.005.

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

Metric	Trisomy 18		Trisomy 13	
	Standard Screening (N=15,841)	Cell-free DNA Testing (N=15,841)	Standard Screening (N=11,185)	Cell-free DNA Testing (N=11,185)
True positive — no.	8	9	1	2
True negative — no.	15,782	15,830	11,155	11,181
False positive — no.	49	1	28	2
False negative — no.	2	1	1	0
Sensitivity (95% CI) — %	80.0 (44.4–97.5)	90.0 (55.5–99.7)	50.0 (1.2–98.7)	100 (15.8–100)
Specificity (95% CI) — %	99.7 (99.6–99.8)	100 (99.9–100)†	99.7 (99.6–99.8)	100 (99.9–100)†
Positive predictive value (95% CI) — %	14.0 (6.2–25.8)	90.0 (55.5–99.7)†	3.4 (0.1–17.8)	50.0 (6.8–93.2)
Negative predictive value (95% CI) — %	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)

* Included in the trisomy 13 analysis are patients who were enrolled after September 2012.
† P<0.001 for the comparison with standard screening.

DISCUSSION

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

fraction. In previous studies, obesity was associated with a low fetal fraction,[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[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.[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.[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.[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.[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.[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 ultrasonography, 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,[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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Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy

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NIPS has the highest sensitivity (>99%) and specificity (>99.9%) for trisomy 21 of any prenatal screening test.[3] The importance of NIPS to current practice has led the American College of Medical Genetics and Genomics to recently update its position statement, outlining the principles of responsible implementation and importantly, endorsing NIPS as a suitable replacement for biochemical screening for trisomy 21, 18, and 13 across the maternal age spectrum.[4]

Several countries are now implementing government-funded NIPS into their national prenatal screening programs, including the United Kingdom, the Netherlands, and Denmark.[5–8]

We have previously reported on data from the pre-NIPS period from 1976 to 2013.[12] In this new analysis, we focus on the period during which NIPS became widely established, the "NIPS era" (2013–2015).

Study population

This study analyzed prospectively collected data on prenatal screening, diagnosis, and ultrasound from the Australian state of Victoria, with approximately 73,000 births per year. In 2015, the median maternal age was 31.5 years, average fertility rate was 1.7 births per woman, and the average weekly disposable household income was AUD998 (US$744) (http://www.abs.gov.au).

Voluntary screening for fetal chromosome and structural abnormalities is offered as a standard component of prenatal care in Australia.[13] Government rebates are provided for CFTS, second-trimester serum screening ("quadruple test") (STSS), and the midtrimester morphology scan (performed at 18–22 weeks), but most tests involve a variable out-of-pocket cost to the pregnant woman. Invasive testing (amniocentesis and chorionic villus sampling (CVS)) are fully government-funded if performed in a public hospital or partially government-funded if performed in the private sector. NIPS does not currently attract any government or private health insurance subsidy and the total cost is borne by the patient. NIPS became clinically available in Victoria via overseas laboratories in 2013 at a price exceeding AUD 1000 (US$746) and a 10-day turnaround time. By 2015, the average price had fallen to about AUD 500 (US$373) and turnaround time for the locally established laboratories was 3–5 days.

Data sources

Victorian Prenatal Diagnosis Database

Prenatal diagnosis data from 2000 to 2015 were obtained from the Victorian Prenatal Diagnosis Database. This period was selected to span the period of the CFTS program, which commenced in 2000, and the first 3 years of NIPS availability (2013–2015). This database included all prenatal diagnostic testing (amniocentesis and CVS) in the state, contributed by the four Victorian cytogenetic laboratories. All amniocentesis and CVS results performed prior to 25 weeks gestation on women resident in Victoria by postcode were included in the study. This gestational age limit was chosen to capture invasive testing performed after routine screening for chromosome and fetal structural abnormalities.

The data fields collected for each woman included maternal age and gestation at the time of testing, test date, type of diagnostic test, indication for test, karyotype result, and singleton or multiple pregnancy. A single record was created for twin pregnancies or women who required repeat testing in the same pregnancy.

We defined "diagnostic yield" as the percentage of diagnostic tests that detected a major chromosome abnormality. The total abnormality rate was the total major and minor chromosome abnormalities as a percentage of the total number of invasive tests.

Annual prenatal diagnostic procedures

The total number of diagnostic procedures performed <25 weeks gestation during the 16-year study period was 62,536. The annual number of diagnostic tests declined steadily after the introduction of CFTS in 2000 (**Figure 1a**). Steeper reductions in annual tests occurred following the gradual incorporation of nasal bone assessment from 2011, which had the effect of reducing the screen-positive rate of CFTS. The steepest annual decline of 22.9% was observed in 2013, the year that NIPS became available. From 2012 to 2015 there was a 39.6% reduction in invasive tests and by 2015, only 1,957 (794 CVS, 1,165 amniocenteses) were performed.

Uptake of CFTS and early ultrasound (11–13 weeks) from 2012 to 2015

The population uptake of CFTS increased annually during the first 10 years of clinical implementation and plateaued between 70.4% and 76.6% from 2009 to 2012 (**Figure 1b**). During the NIPS era the annual uptake rate of CFTS declined significantly for the first time, falling from 77.5% in 2013 to 68.1% in 2015 (χ^2 test for trend = 2,276, $P < 0.0001$). Meanwhile, quarterly numbers in government billing for NT ultrasounds as a percentage of births showed a steady increase in the proportion of women having an 11- to 13-week ultrasound (χ^2 for trend = 1,923.9, $P < 0.0001$) (**Figure 1c**).

The overall uptake of STSS continued its long-standing gradual decline and was used by <5% of women in 2015.

Abnormal results and diagnostic yield in the NIPS era

Trisomy 21 remained the most common condition detected on prenatal diagnosis in the NIPS era (**Table 1**). The year 2015 was notable for the highest number of confirmed trisomy 21 cases ever recorded in Victoria ($n = 204$). Of these, cases, 105 (51.5%) had a high-risk NIPS as an indication for diagnostic testing (**Figure 2b**).

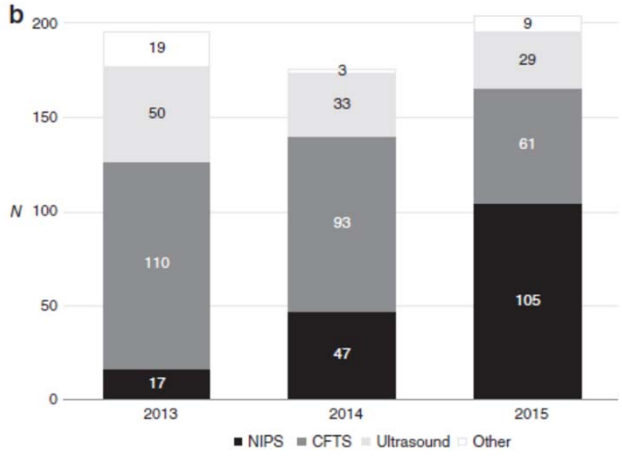

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Table 2 Diagnostic yield for major chromosome abnormalities by indication for testing (2013–2015)

	2013	2014	2015	Combined rate 2013–15
High-risk NIPS	82.8% (24/29)	64.3% (72/112)	64.6% (148/229)	65.9% (244/370)
Known parental rearrangement	7.9% (3/38)	37.8% (14/37)	34.9% (15/43)	27.1% (32/118)
Ultrasound abnormalities	20.9% (116/554)	22.9% (137/597)	19.0% (130/685)	20.9% (383/1,836)
High-risk CFTS	21.3% (203/954)	20.5% (160/781)	18.9% (115/608)	20.4% (478/2,343)
Prior pregnancy with chromosomal abnormality	2.8% (2/71)	5.6% (3/53)	8.1%% (4/49)	5.2% (9/173)
High-risk second-trimester screening	6.1% (7/115)	2.9% (4/139)	5.2% (5/96)	4.6% (16/350)
Advanced maternal age alone	6.8% (26/382)	1.1% (2/175)	3.3% (3/92)	4.8% (31/649)
Other	0.9% (1/112)	5.7% (5/88)	3.3% (3/91)	3.1% (9/291)

CFTS, combined first-trimester screening; NIPS, noninvasive prenatal screening.
Some cases had more than one indication coded; hence, column totals may not sum to total number of tests performed by year. Testing for single-gene disorders is not included in this table.

Table 1 Results of all prenatal chromosome tests by year (2013–2015)

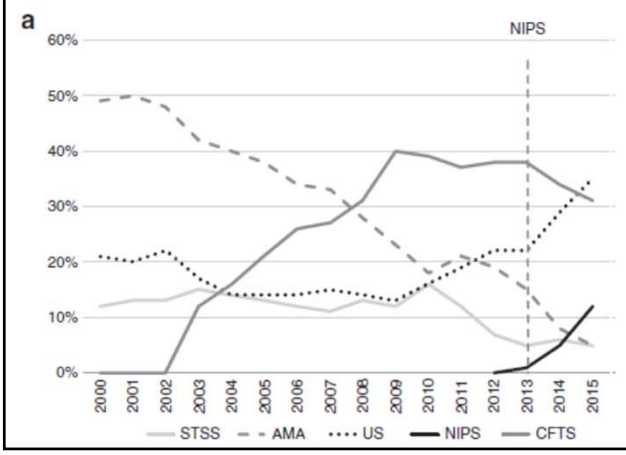
Karyotype result	2013	2014	2015
Total tests	2,500	2,046	1,957
Normal karyotype	1,969	1,548	1,427
Major chromosome abnormalities	395 (15.8%)	369 (18.0%)	394 (20.1%)
Trisomy 21	198	176	204
Trisomy 18	61	49	42
Trisomy 13	30	21	15
Other autosomal aneuploidy, polyploidy	18	22	21
Sex chromosome aneuploidy	31	33	28
Pathogenic copy-number variation	25	39	39
Other major abnormalities[a]	32	29	45
Minor chromosome abnormalities[b]	136 (5.4%)	129 (6.3%)	136 (6.9%)

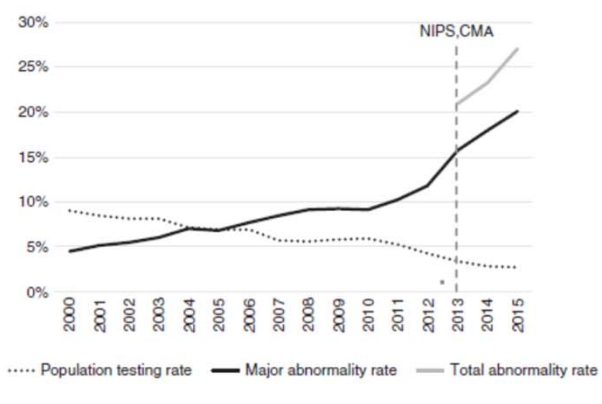
[a]Includes level III mosaic, unbalanced translocation/rearrangement, and uniparental disomy. [b]Includes balanced translocations, variations of unknown/uncertain significance, and confined placental mosaicism.

Among women who had an invasive test for an ultrasound abnormality in 2014–2015, diagnostic testing prior to 18 weeks gestation was associated with a significantly higher rate of major chromosome abnormality (32.6%, 173/532), compared with testing at 18–24 weeks gestation (12.5%, 94/750) ($\chi^2 = 75.4$, $P < 0.0001$).

The NIPS era has coincided with increasing utilization of chromosome microarrays for prenatal diagnostic testing. The percentage of all tests that were submitted for CMA analysis increased from 14.2% in 2012 to 85.3% in 2015. This was accompanied by a significant increase in pathogenic CNVs as a proportion of all tests, from 1.0% in 2013 to 2.0% in 2015 ($\chi^2 = 7.6$, $P = 0.006$), but this gain was associated with an increase in the numbers of VUS from 3.9% to 6.4% over the same period.

The steady increase in the annual numbers of abnormal karyotypes identified on diagnostic testing and the decline in invasive tests have intersected to produce a historically high diagnostic yield of 20.1% in 2015. The total abnormality rate including VUS was 27.0%. Overall, the proportion of all births in Victoria undergoing prenatal diagnosis prior to 25 weeks was 2.7% (**Figure 2c**).





DISCUSSION

This study is the first to comprehensively analyze the profound impact of NIPS on prenatal screening and diagnosis on a population-wide basis. We observed a 39.6% reduction in total invasive tests in the first three years of NIPS availability, consistent with the global experience.[6–11] While CFTS was still used by the majority of women in 2015, uptake in that year significantly declined for the first time since its introduction in 2002. We attribute this decline to the increasing use of NIPS as a primary screening test, rather than an overall reduction in screening uptake. This statement is based on the observations that (i) invasive testing for NIPS increased from 29 women in 2013 to 229 in 2015, (ii) 2015 had a record number of confirmed trisomy 21 cases, and (iii) NIPS has now displaced CFTS as the most common screening test preceding a confirmed diagnosis of trisomy 21.

for early structural assessment of the fetus prior to the routine midtrimester morphology scan. In fact, ultrasound abnormality is now the most common indication for invasive testing, with 41% of these procedures in 2014–2015 being performed prior to 18 weeks. This suggests that practitioners who are using NIPS as a primary aneuploidy screening test still recognize the value of an early ultrasound for a fetal structural survey. Furthermore, we observed a high diagnostic yield for invasive testing for ultrasound abnormalities prior to 18 weeks (32.6%), supporting the clinical utility of this practice.

Implications for practice

These results have important implications for clinical practice. The 11- to 13-week ultrasound examination became incorporated into routine prenatal care about 15 years ago for the purpose of trisomy 21 screening. The initial role of the examination was to measure the NT thickness and crown rump length and combine these measurements with maternal serum biochemical markers for individualized aneuploidy risk assessment. However, advances in the performance of the 11- to 13-week ultrasound have seen it evolve into a detailed structural morphology survey, able to detect up to 50% of all major structural abnormalities including cardiac defects.[17]

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RESEARCH ARTICLE

A Cost-Effectiveness Analysis of First Trimester Non-Invasive Prenatal Screening for Fetal Trisomies in the United States

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Abstract

Background

Non-invasive prenatal testing (NIPT) is a relatively new technology for diagnosis of fetal aneuploidies. NIPT is more accurate than conventional maternal serum screening (MSS) but is also more costly. Contingent NIPT may provide a cost-effective alternative to universal NIPT screening. Contingent screening used a two-stage process in which risk is assessed by MSS in the first stage and, based on a risk cutoff, high-risk pregnancies are referred for NIPT. The objective of this study was to (1) determine the optimum MSS risk cutoff for contingent NIPT and (2) compare the cost effectiveness of optimized contingent NIPT to universal NIPT and conventional MSS.

Study Design

Decision-analytic model using micro-simulation and probabilistic sensitivity analysis. We evaluated cost effectiveness from three perspectives: societal, governmental, and payer.

Results

From a societal perspective, universal NIPT dominated both contingent NIPT and MSS. From a government and payer perspective, contingent NIPT dominated MSS. Compared to contingent NIPT, adopting a universal NIPT would cost $203,088 for each additional case detected from a government perspective and $263,922 for each additional case detected from a payer perspective.

Conclusions

From a societal perspective, universal NIPT is a cost-effective alternative to MSS and contingent NIPT. When viewed from narrower perspectives, contingent NIPT is less costly than universal NIPT and provides a cost-effective alternative to MSS.

Introduction

Non-invasive prenatal testing (NIPT) is a relatively new screening method that provides greater prenatal detection of aneuploidies than conventional maternal serum screening (MSS). Clinical trial results show that NIPT is both sensitive and specific. For trisomy 21 (Down syndrome), NIPT has a sensitivity (detection rate) and specificity of approximately 99%.[1] Thus, NIPT provides high accuracy without the risk associated with invasive diagnostic testing (amniocentesis, chorionic villus sampling). NIPT can provide results as early as the 10th week of pregnancy.

Although NIPT is more accurate than MSS, it is also costly. Current list prices range from $500 to $2100 per test.[2,3] Despite the cost, studies have shown that universal NIPT screening (i.e., replacement of MSS by NIPT) is cost effective when viewed from a societal perspective.[4] Although the societal perspective is preferred for theoretical reasons, most decision makers actually use narrower perspectives such as governmental or payer perspective. Universal NIPT is not cost effective when viewed from these narrower perspectives.[4] NIPT-based screening policies might be acceptable if NIPT were used in a select subset of pregnancies rather than applied universally. Indeed, studies have shown that the selective use of NIPT among higher risk women (contingent NIPT) is less costly than universal NIPT.[5,6]

Contingent NIPT policies use a two-stage screening strategy. In the first stage, MSS is used to estimate the probability of an affected pregnancy. A pregnancy is categorized as "high risk" based on a risk threshold. Patients are referred for NIPT only if the probability of an affected pregnancy is greater than the risk threshold. Therefore, NIPT is *contingent* upon the results of the primary screen.

Contingent NIPT strategies are less costly than universal NIPT screening because a relatively small subset of "high-risk" patients are referred for NIPT testing.[5–7] Contingent NIPT screening policies can achieve higher detection rates than MSS by using lower risk cutoffs in the first stage. Lowering the risk cutoff in the first stage increases the number of cases classified as "high risk" so that a greater percentage of cases are referred to NIPT testing. Lowering the risk cutoff increases sensitivity but also increases false positives; however, NIPT is very specific, [1] so most false-positive results obtained in the first stage are identified in the second stage. In addition, positive results from the first stage could be followed by reflexive NIPT. When tested this way, contingent NIPT would spare women the anxiety associated with false-positive results.[7] Because it is applied to a small subset of pregnancies, contingent NIPT has the potential to reduce costs relative to universal NIPT screening with little loss of accuracy. Thus, contingent NIPT may be a cost-effective alternative to universal NIPT and MSS.

The risk cutoff of the primary screen is a key design factor for contingent NIPT policies because it affects both screening performance (sensitivity and specificity) and downstream costs. Thus, it is important to determine the best cutoff point of the primary screen to optimize the overall cost-effectiveness of a contingent screening policy. We refer to such a screening process as an *optimized contingent NIPT*. To our knowledge, the optimal cutoff has not been determined.

Several studies have examined the cost effectiveness of contingent screening policies. There have been two approaches. Some studies used risk cutoffs similar to those used in MSS [8–11] where others compared a wide range of risk cutoffs. [5–7] Neither of these approaches uses an optimized cutoff. Studies to identify the optimal risk cutoff of the primary screen are needed to compare MSS and universal NIPT to the best available contingent NIPT policy.

The optimal cutoff for contingent screening balances screening costs (cases referred to second stage NIPT) and downstream costs (medical costs, productivity, indirect costs). Some downstream costs are only relevant in particular economic perspectives (societal, government,

payer). Therefore, the optimal cutoff point of a contingent screening policy depends on the economic perspective. The evaluation of contingent NIPT relative to alternatives (MSS, universal NIPT) should be based on contingent NIPT policies that are optimized relative to a particular economic perspective.

This study compared the cost effectiveness of contingent NIPT to MSS and universal NIPT using three different economic perspectives: societal, governmental, and payer. For each perspective, we determined the optimal cutoff of the primary screen and then compared the performance of the optimized contingent NIPT policy to MSS and universal NIPT.

Methods

Design

We studied the cost effectiveness of routine use of contingent NIPT policies relative to conventional MSS and universal NIPT using a simulated population designed to represent the general population of women in the United States. We assumed that MSS and contingent NIPT used the combined serum test. We used a decision-analytic model because prenatal testing can be represented by a relatively simple sequence of decisions: Decision tree diagrams for screening protocols are provided in Figs 1–4. We based the cost effectiveness analysis upon a hypothetical cohort, and it is therefore exempt from institutional review board approval.

Perspective and time horizon

Our analysis included a societal perspective as recommended by many cost-effectiveness guidelines.[12,13] Although a societal perspective is recommended, decisions are often based on narrower perspectives and, for that reason, we also included government and payer perspectives. The societal perspective included immediate costs of screening and the direct and indirect lifetime costs. The government perspective included the immediate screening costs and direct lifetime medical and education costs. The payer perspective included only the immediate costs associated with screening.

Standard of care and comparator

We used MSS as a standard of care using the combined test (using pregnancy-associated plasma protein-A, free beta human chorionic gonadotropin and nuchal translucency ultrasound measurements). For this, we used a 2nd trimester risk cutoff of 1:270 for trisomy 21 and 1:100 for trisomy 18 and 13. We compared MSS to three alternatives: (1) universal NIPT, (2) contingent NIPT, and (3) no screening.

Optimization of risk cutoff

We determined the optimal cutoff by minimizing the expected total cost by simultaneously varying the decision thresholds for all three tests subject to the constraint that the detection rate had to be at least equivalent to conventional material screening. Because costs varied by perspective, optimal risk cutoffs were determined for each perspective. The optimization can be expressed as follows:

$$TC_i^* = TC(L_{13,i}^*\, L_{18,i}^*, L_{13,i}^*, C_i, p) = \min_{L_{13,i}, L_{18,i}, L_{21,i}} TC(L_{13,i}, L_{18,i}, L_{21,i}, C_i, p) \quad \text{1A}$$

$$s.t. \quad DR_{cont}(L_{13,i}, L_{18,i}, L_{21,i}) > DR_{MSS} \quad \text{1B}$$

Where TC_i = expected total cost under economic perspective i, $L_{j,i}$ is the decision threshold for

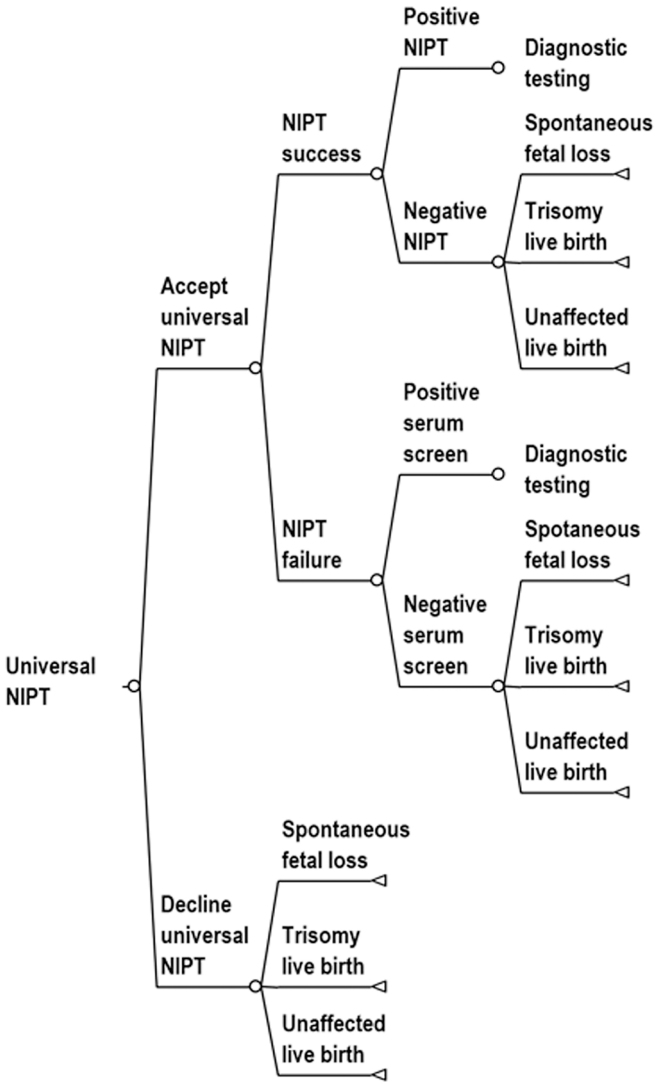

Fig 1. Decision tree diagram for universal NIPT. We assumed that women with failed NIPT would be tested with serum screening and that women with a serum screen risk greater or equal to 1:270 would be offered diagnostic testing. The decision tree is continued in the diagnostic testing tree (Fig 4).

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marker j under perspective i, C_i is the cost of an affected pregnancy under perspective i, DR_{cont} is the first stage detection rate for contingent screening, DR_{MSS} is the detection rate of conventional MSS, and p is the vector of additional parameters that are invariant to the economic perspective (e.g., uptake rates, test accuracy, etc). The asterisks designate optimal values. Because conventional MSS is the current standard of care, we reasoned that a screening policy with lower accuracy would not be acceptable. We therefore constrained the optimization of contingent NIPT to meet or exceed the detection rate of current practice. We performed the optimization using a grid search. We generated a large grid of cost and threshold combinations. The optimization was performed in stages. Once we identified the neighborhood of the optimum, we created successively finer grids to identify the optimum risk thresholds. Grids were generating using @Risk (Palisade Corp, Ithaca, NY).

All results for contingent screens were based on optimized cutoffs. For simplicity, we will refer to an optimized contingent policy as a contingent NIPT policy.

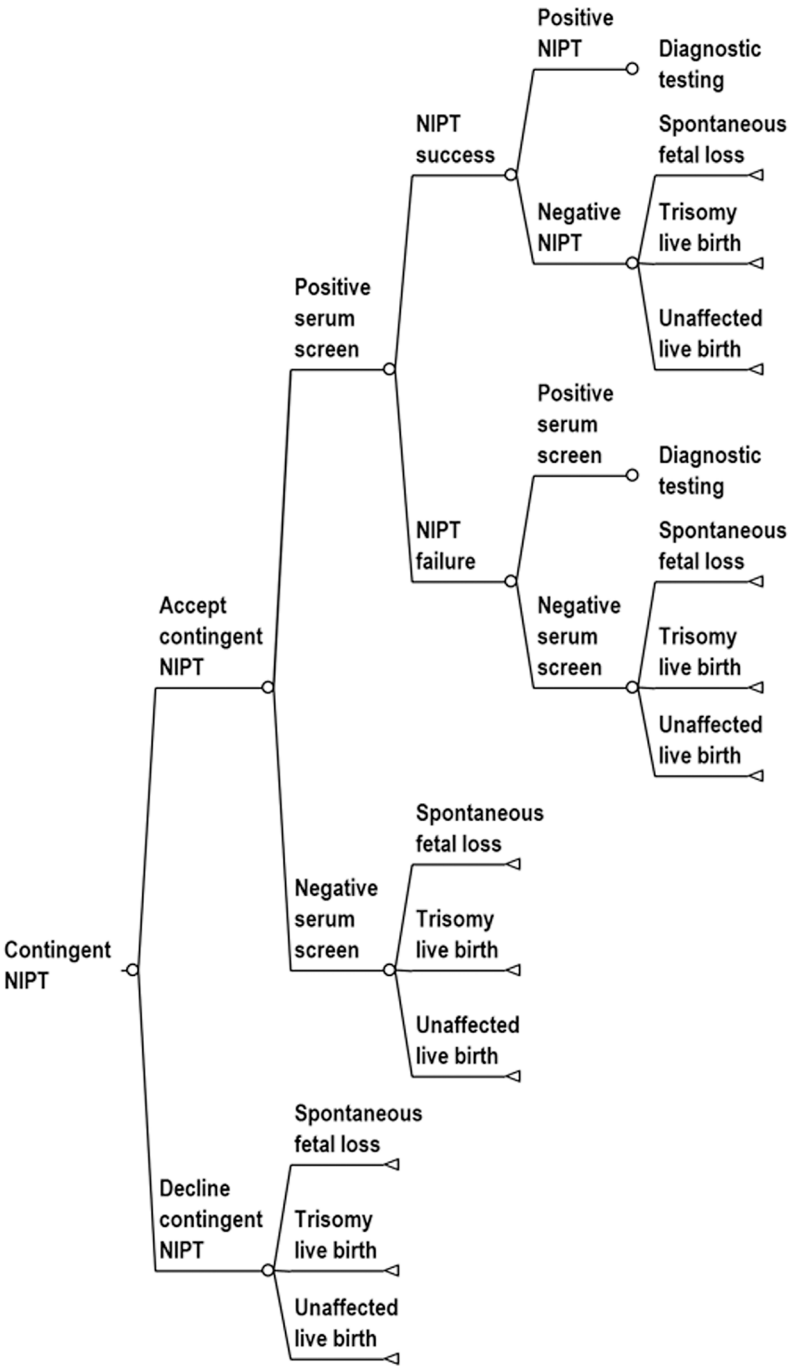

Fig 2. Decision tree diagram for contingent NIPT. We assumed that women with failed NIPT whose risk was higher than or equal to 1:270 on the initial serum screen would be offered diagnostic testing. The decision tree is continued in the diagnostic testing tree (Fig 4).

doi:10.1371/journal.pone.0131402.g002

Screening performance

We used population parameters from the Serum Urine and Ultrasound Screening Study (SURUSS) to estimate screening performance of maternal serum screening for trisomy 21[14,15] and used populations parameters from another study to estimate serum screening performance

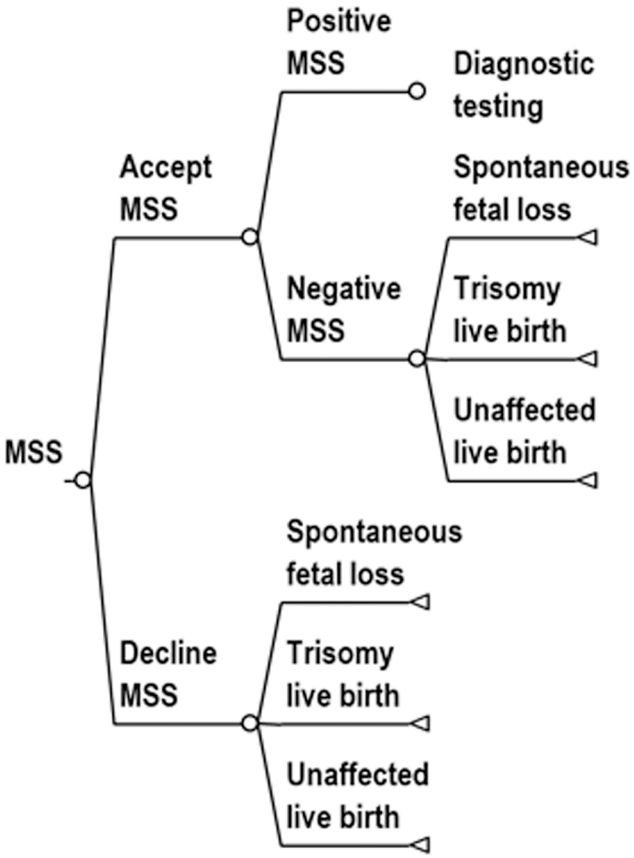

Fig 3. Decision tree diagram for MSS. The decision tree is continued in the diagnostic testing tree (Fig 4).

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for trisomy 18 and 13.[16] Age-specific detection and false positive rates of serum screening was estimated by a Monte Carlo simulation performed using Stata 12.1.[17] Multiples of median, standard deviations, correlation coefficients, and truncation limits provided by SURUSS were used to create multivariate Gaussian distribution to simulate 1,000,000 marker sets of affected and unaffected pregnancies. Post-test risks were derived from the likelihood ratios and prior risk.[18–20] For conventional maternal serum screening, a second trimester risk cutoff of 1:270 was used for trisomy 21 and a 1:100 risk cutoff was used for trisomy 18 and 13. Screen results were classified as "positive" if the post-test risk was equal to or greater than the specified risk cutoff and negative otherwise. Based on those results, age-specific detection and false-positive rates for the risk cutoffs were calculated. Detection rates of 99%, 96.8% and 92.1% were assumed for trisomy 21, 18, and 13 based on a meta-analysis of NIPT performance.[1] An overall false positive rate of 0.41% for unaffected pregnancies was assumed based upon the individual reported false positive rates for trisomy 21, 18, and 13 reported in the same study. We assumed a 2.8% failure rate for NIPT due to low fetal fraction or assay failure based on a weighted average of studies using maternal blood drawn between 10–13 weeks of pregnancy.[21–24]

Cost-effectiveness simulation

Our cost-effectiveness simulation was based on commonly used modeling practices, which incorporated a probabilistic sensitivity analysis and micro-simulation.[12] The probabilistic

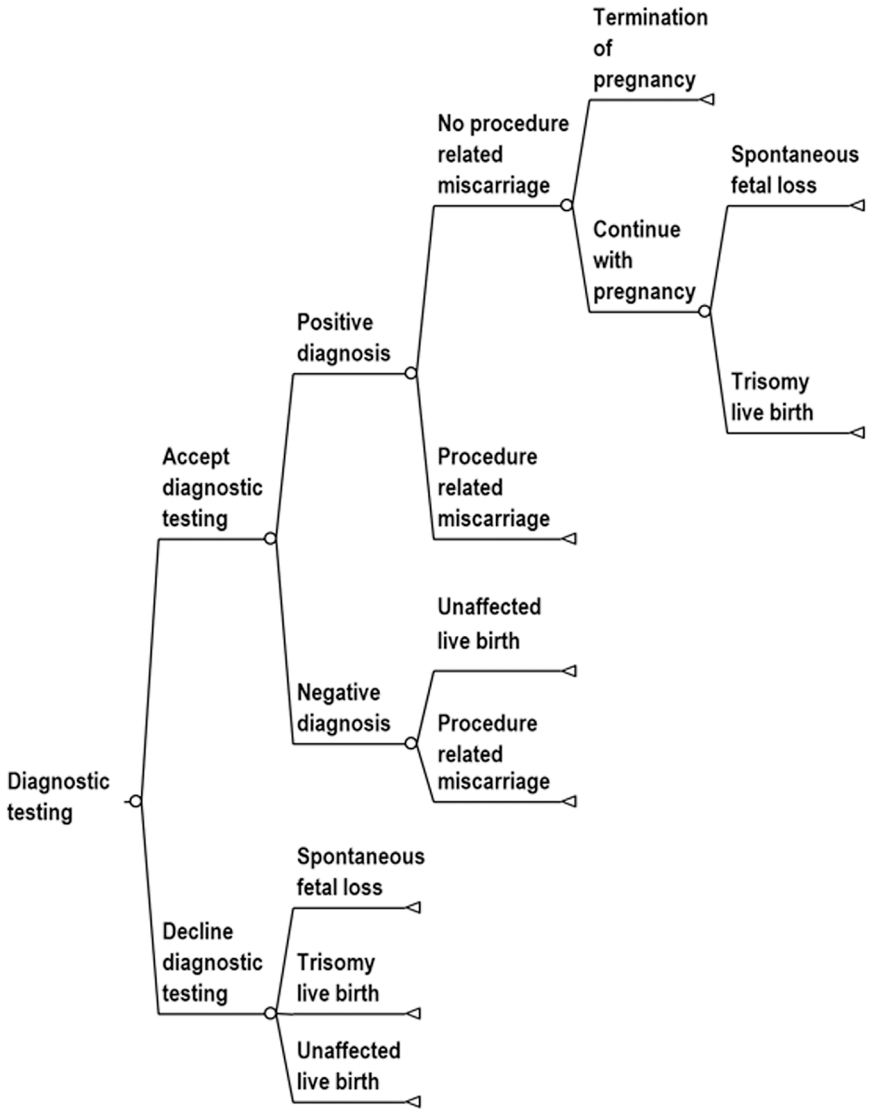

Fig 4. Decision tree diagram for diagnostic testing.

doi:10.1371/journal.pone.0131402.g004

sensitivity analysis was conducted by repeating the micro-simulation 1,000 times. During each iteration of the probabilistic sensitivity analysis, the model costs and probabilities were randomly drawn and a micro-simulation was completed using the drawn values. Following standard practice, the costs were drawn from gamma distributions while the probabilities were drawn from beta distributions.[25] The definitions of beta and gamma distributions are provided in Table 1. The micro-simulations were conducted by simulating 1,000,000 pregnant women at 12 weeks of pregnancy. For each simulated woman, a maternal age was assigned based upon the maternal age distribution reported in the 2012 National Vital Statistics birth data.[26] The individual risk of an affected pregnancy, detection rate, and false-positive rate was determined by maternal age.

The effectiveness measure was the number of affected pregnancies detected. Simulation analyses were performed with TreeAge Pro 2012 software.[27]

Table 1. Model probabilities and costs.

Probabilities	Mean	95th% CI	Parameters of beta distribution	
			alpha	beta
MSS uptake, U_{MSS}	69%	64%-74%	226.104	101.583
Increase in contingent NIPT uptake over MSS, ΔU_{cNIPT}	8.2%	4.6%-12.6%	14.606	163.516
Increase in universal NIPT uptake over MSS, ΔU_{uNIPT}	13.5%	7.6%-20.8%	13.705	87.814
Diagnostic testing uptake	66%	61%-71%	226.923	116.899
Procedure-related fetal loss	0.22%	0%-1.16%	0.447	202.595
Termination rate of trisomy 21	80%	74%-86%	135.790	33.948
Termination rate of trisomy 18	80%	73%-87%	99.552	24.888
Termination rate of trisomy 13	92%	85%-97%	71.336	6.203
NIPT detection rate of trisomy 21	99%	98.3%-99.5%	1,044.886	10.554
NIPT detection rate of trisomy 18	96.8%	95%-98.2%	448.991	14.843
NIPT detection rate of trisomy 13	92.1%	86.9%-96.1%	120.738	10.356
NIPT false positive rate	0.41%	0.29%-0.55%	36.775	8,944.280
NIPT failure rate due to low fetal fraction	2.8%	1.2%-5.1%	7.291	253.092
Costs	Mean	95th% CI	Parameters of gamma distribution	
			alpha	beta
Combined screen	$166	$95-$257	16	10.375
Cost of NIPT	$400	$229-$619	16	25
Cost of CVS	$1,010	$577-$1,562	16	63.125
Cost of genetic counseling	$160	$91-$247	16	10
Termination of pregnancy	$581	$332-$898	16	36.313
Direct lifetime costs of trisomy 21	$427,577	$244,397-$661,147	16	26,723.563
Indirect lifetime costs of trisomy 21	$1,069,195	$611,137-$1,653,257	16	66,824.688
Direct lifetime costs of trisomies 13 and 18	$37,971	$21,704-$58,713	16	2,373.188
Indirect lifetime costs of trisomies 13 and 18	$1,363,877	$779,574-$2,108,913,	16	85,242.313

A standard deviation of 25% of the mean was assumed for the percentage increases for contingent NIPT and universal NIPT uptake. As standard practice, normal distributions of probabilities were approximated with beta distributions. Normal distributions of costs were approximated with gamma distributions

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Costs

We included the costs of screening, diagnosis, and termination of pregnancy as well as the lifetime costs associated with trisomy 21, 18, and 13. Lifetime costs represent the average difference in direct medical and educational costs between trisomy and an average individual in addition to the indirect costs of lost productivity due to morbidity and mortality associated with this syndrome.

We derived lifetime and termination costs from the literature.[28,29] Lifetime costs for trisomy 21 by Waitzman et al. were updated by using more recent survival data.[30,31] We assumed that the annual costs of trisomies 13 and 18 were the same as the average annual costs of trisomy 21. We were not able to find survival data for trisomy 13 past the first year. We therefore assumed that the survival for trisomy 13 was the same as the survival for trisomy 18 past the first year.[32,33] We inflated lifetime costs and the cost of termination to reflect 2013 US dollars. We adjusted the medical portion of lifetime costs using the health care component of the personal consumption expenditure index.[34] We adjusted the non-medical direct and indirect portions of lifetime costs using the employment cost index for civilian workers.[35] As recommended, future costs incurred beyond one year were discounted to present value using

an annual rate of 3%.[12,13] Additional explanation of the lifetime cost estimates is provided in S1 Text.

The costs of serum screenings, diagnostic testing, and genetic counseling were derived from the 2013 Medicare Physician Fee Schedule (MPFS),[36] which is often used to approximate the resource value of medical care.[13] These costs included procedure costs as well as genetic counseling.

The cost of NIPT testing is uncertain. The list price can serve as an indicator of cost, but list prices show wide variation. Because NIPT providers utilize similar techniques, we would expect the underlying costs to be similar for all test providers. Therefore, the variation in price is more likely an indication of variation in profit margins rather than variation in costs. For genetic testing, these margins can be as high as 90%.[37] For that reason, we based the resource cost of NIPT on the lowest priced test (NIFTY offered by Beijing Genomics Institute in the UK for approximately $500). We assumed a conservative profit margin of 20% for a unit cost of $400.[2] This cost is consistent with cost estimates published in previous cost-effectiveness studies.[4,38]

We assumed costs had a standard deviation that was 25 percent of the mean. Gamma cost distributions were estimated using the mean and standard deviation values.[25] The costs are summarized in Table 1.

Baseline model

Baseline model inputs are shown in Table 1. The baseline uptake rate for conventional screening was assumed to be 69%.[39] For contingent and universal NIPT, we assumed screening uptake increased by 8.2% and 13.5% relative to MSS, based upon a recent study of potential uptake of NIPT.[40] Therefore, we modeled contingent and universal NIPT uptake as follows:

$$U_{cNIPT} = U_{cMSS} * (1 + \Delta U_{cNIPT}) \quad 2A$$

$$U_{uNIPT} = U_{cMSS} * (1 + \Delta U_{uNIPT}) \quad 2B$$

Where U_{cMSS} is the baseline uptake based on conventional MSS, U_{cNIPT} and U_{uNIPT} are the uptake for contingent NIPT and universal NIPT. ΔU_{cNIPT} and ΔU_{uNIPT} are the fractional change in uptake relative to U_{cMSS}. We assume that ΔU_{cNIPT} and ΔU_{uNIPT} are 0.082 and 0.135. This resulted in a baseline uptake of approximately 75% for contingent NIPT and 78% for universal NIPT.

For contingent screening, we assumed that all primary screens exceeding the risk threshold (i.e., "positive" on the primary screen) were followed by reflexive NIPT. A contingent NIPT screen was classified as positive only if the primary and secondary screens were both positive. We assumed that all positive screen results (MSS, contingent NIPT, universal NIPT) were followed by diagnostic testing at an acceptance rate of 66%.[41–46] We assumed that the termination rates for pregnancies diagnosed as positive were 80% for trisomy 21,[47–59] 80% for trisomy 18,[48,60,61] and a rate of 92% for trisomy 13.[52,60] We assumed spontaneous fetal loss rates 43%,[62] 72%,[63] and 49%[63] for trisomy 21, 18, and 13 respectively. Procedure-related fetal loss from chorionic villus sampling (CVS) was assumed to be 0.22% based on a recently published meta-analysis.[64]

We assumed that the technical failure rate of NIPT was 2.8%.[21–24] To our knowledge, there is no standard protocol for NIPT failure. For simplicity, we assumed NIPT would not be repeated in the event of an NIPT test failure. In this event, we assumed that women who elected contingent NIPT would be referred to invasive diagnostic testing based on MSS results, For those who elected universal NIPT, failures would be referred for MSS, and those who were

classified as high risk (i.e., those with a trisomy 21 risk equal to or greater than 1:270 or a trisomy 13 or 18 risk equal to or greater than 1:100) would be referred for invasive diagnostic testing.

Sensitivity analysis

Sensitivity analysis was performed using one-way and probabilistic sensitivity analysis. One-way sensitivity analyses were conducted to determine the individual impact of each input parameter value on cost-effectiveness ratios. Probabilistic sensitivity analysis was conducted to determine the overall uncertainty in the cost effectiveness due to the combined impact of uncertainty in the underlying model inputs. The parameters of the distributions are reported in Table 1. The objectives and, therefore, the parameters, of a one-way sensitivity analysis differ from the objectives of a probabilistic sensitivity analysis. For example, a one-way analysis may be designed to determine the point at which the best strategy changes (which are not necessarily plausible), whereas a probabilistic sensitivity should investigate the sensitivity over plausible ranges of the input variables.

Terminology. A screening policy is said to be *strictly dominated* by another policy if it is both more costly and more expensive.[12] A policy is *dominated by extension* if a combination of alternatives is less costly.[12] For example, policy A is dominated by extension by policies B and C if, on average, a combination of policies B and C (X% policy B, 1-X% policy C) is less expensive and more effective than policy A.

Because there is no agreed-upon willingness-to-pay threshold for trisomy screening, we deemed NIPT strategies as cost effective if they dominated MSS (the current standard of care) strictly or by extension.

Results

The prevalence at 12 weeks was approximately 1 in 301 for trisomy 21, 1 in 1,170 for trisomy 18, and 1 in 3,627 for trisomy 13. In the absence of screening, this resulted in a lower birth prevalence of 1 in 528, 1 in 4,174, and 1 in 7,084 live births for trisomy 21, 18, and 13 respectively due to spontaneous fetal loss. These rates are consistent with reported birth prevalence for these trisomies.[19] The optimal risk cutoffs, percentage of women screened receiving NIPT, detection rates, false positive rates, and number of failed NIPTs are provided in Tables 2 and 3. Total costs, cases detected, and incremental cost effectiveness ratios (ICERs) are provided in Table 4.

Societal perspective

Both direct and indirect lifetime costs were included in the analysis from a societal perspective. The optimized contingent NIPT screening policy had detection rates of 93.6%, 92.7%, and 77.7%, for trisomy 21, 18, and 13 respectively. In contrast, the detection rates were 84.4%, 75.8%, and 62.8% for conventional MSS and 98.7%, 96.4%, and 91.5% for universal NIPT. Of

Table 2. Optimal risk cutoffs and the number of women receiving NIPT for contingent NIPT policies.

	Optimal Risk Cutoff			
Perspective	**Trisomy 21**	**Trisomy 18**	**Trisomy 13**	**NIPT referral rate**
Societal	1:1515	1:1905	1:860	24.0%
Government	1:420	1:145	1:175	8.7%
Payer	1:315	1:115	1:175	7.0%

doi:10.1371/journal.pone.0131402.t002

Table 3. Detection rates, false positive rates, optimal risk cutoffs, and NIPT failure rates.

	Detection rates			False positive rates	NIPT failure rates
	Trisomy 21	Trisomy 18	Trisomy 13		
Universal NIPT	99%	96.8%	92.1%	0.4%	2.8%
MSS	84.8%	75.8%	62.8%	5.6%	0%
Contingent NIPT					
Societal perspective	93.6%	92.7%	77.7%	0.094%	0.66%
Government perspective	87%	82.1%	63.3%	0.033%	0.24%
Payer perspective	85.1%	75.6%	63.3%	0.026%	0.19%

doi:10.1371/journal.pone.0131402.t003

women screened with contingent NIPT, approximately 24% were classified as "high risk" in the primary screen and referred for NIPT. Contingent NIPT had a false positive rate of 0.09%, conventional MSS had a false positive rate of 5.6%, and universal NIPT had a false positive rate of 0.9%. Contingent NIPT also had fewer test failures than universal NIPT. Only 0.66% of those screened with contingent NIPT had a technical failure whereas 2.8% of women screened with universal NIPT failed to obtain a result.

No screening, MSS, and contingent NIPT were all dominated by universal NIPT. Out of 1,000,000 pregnancies, replacing MSS with universal NIPT would result in an increase of 893 detections and a cost savings of approximately $170 million (Table 4).

We conducted one-way sensitivity analysis of the ICERs (Fig 5). Universal NIPT remained less costly than conventional MSS so long as the cost of NIPT was below $619. In the probabilistic sensitivity analysis, universal NIPT was more effective 100% of the time and less costly 91.1% of the time compared to MSS (Fig 6).

Government perspective

Indirect costs were not included in the government perspective. When indirect lifetime costs were excluded from the analysis, the contingent NIPT policy had detection rates of 87%, 82.1%, and 77.7%, for trisomy 21, 18, and 13, a false positive rate of 0.033%, and a failure rate

Table 4. Total cost, cases detected, incremental costs, incremental cases detected and incremental cost effectiveness ratio (ICER).

	Total cost	Cases detected	Incremental costs	Incremental cases detected	ICER
Societal perspective					
No screening	$3,347,297,152	0			Strictly dominated
MSS	$2,475,580,143	2,516			Strictly dominated
Contingent NIPT	$2,315,959,639	3,077			Strictly dominated
Universal NIPT	$2,305,749,493	3,409			
Government perspective					
No screening	$822,000,565	0			Strictly dominated
MSS	$711,465,188	2,516			Strictly dominated
Contingent NIPT	$693,996,197	2,817			
Universal NIPT	$814,224,159	3,409	$120,277,962	592	$203,088
Payer perspective					
No screening	$0	0			
MSS	$142,723,273	2,516			Dominated by extension
Contingent NIPT	$148,208,927	2,729	$148,208,927	213	$25,754
Universal NIPT	$327,675,783	3,409	$179,466,856	680	$263,922

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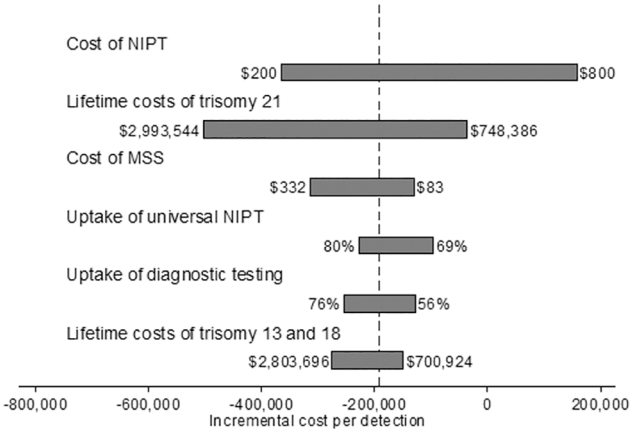

Fig 5. One-way sensitivity analysis of universal NIPT vs MSS from a societal perspective. Below are the one-way sensitivity analysis results of the ICER between universal NIPT and MSS. Universal NIPT is less costly than MSS as long as the cost of NIPT remains below $619.

doi:10.1371/journal.pone.0131402.g005

of 0.24%. Approximately 8.7% of women screened by contingent NIPT received NIPT after the initial screen.

Contingent NIPT dominated MSS when evaluated from the government perspective. Out of 1,000,000 pregnancies, replacing combined MSS with contingent NIPT would result in an increase of 301 detections and a cost savings of approximately $17.5 million (Table 4). Universal NIPT was more effective but also more costly than contingent NIPT. Universal NIPT would increase the number of cases detected by contingent NIPT by 592 and increase costs by $120 million, for an ICER of $203,088 per additional case detected. The one-way analysis shows that contingent NIPT screening dominated MSS unless the cost of NIPT exceeded $663 (Fig 7). In the probabilistic sensitivity analysis, contingent screening was more effective 100% of the time and less costly 87% of the time compared to MSS (Fig 8).

Payer perspective

When all lifetime costs were excluded from the analysis, the optimal risk cutoffs resulted in detection rates of 85.1%, 75.8%, and 63.3%, for trisomy 21, 18, and 13, respectively, a false

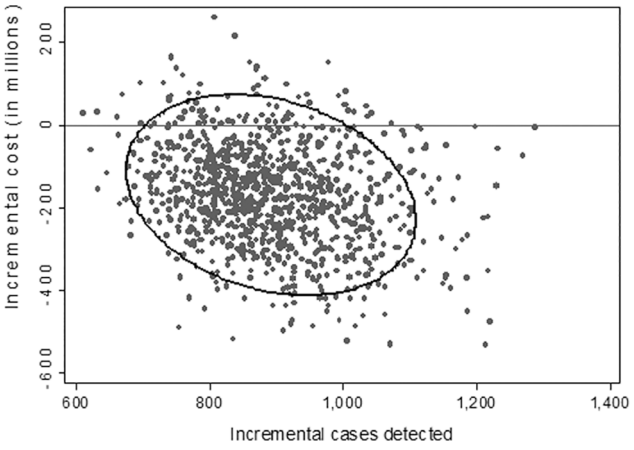

Fig 6. Scatter plot of probabilistic sensitivity analysis, universal NIPT vs MSS. The figure below plots the incremental cost and effectiveness results from 1,000 simulations. Compared to MSS, there is a 100% probability that universal NIPT is more effective and 91.8% probability that universal NIPT is less costly.

doi:10.1371/journal.pone.0131402.g006

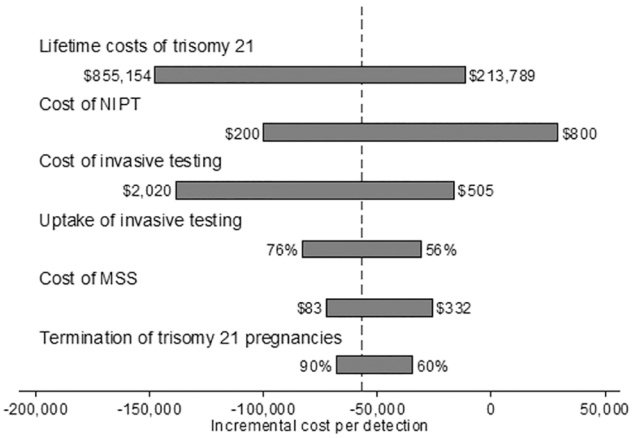

Fig 7. One-way sensitivity analysis of contingent NIPT vs MSS from a government perspective. Below are the one-way sensitivity analysis results of ICER between contingent NIPT and MSS. Contingent NIPT is less costly than MSS as long as the cost of NIPT remains below $663.

doi:10.1371/journal.pone.0131402.g007

positive rate of 0.026%, and a failure rate of 0.19%. Approximately 7% of women screened with the contingent NIPT received NIPT after the initial screen.

No screening is the least costly strategy, followed by MSS, contingent NIPT, and universal NIPT. Although contingent NIPT is more effective and more costly than MSS in terms of cost per additional detection, contingent NIPT is a more efficient strategy (Table 4). Compared to no screening, MSS would cost $56,726 per case detected; however, contingent NIPT would cost $54,309 for each detection. Therefore, contingent NIPT dominates MSS by extension.

Compared to contingent NIPT, universal NIPT would increase the number of cases detected by 680 and increase costs by $179 million, for an ICER of $263,922 per additional case detected. The one-way analysis shows contingent NIPT was less costly than MSS when (1) the cost of NIPT was below $293, (2) contingent NIPT uptake was below 72%, and (3) the cost of invasive screening was above $1,235 (Fig 9). In the probabilistic sensitivity analysis, contingent

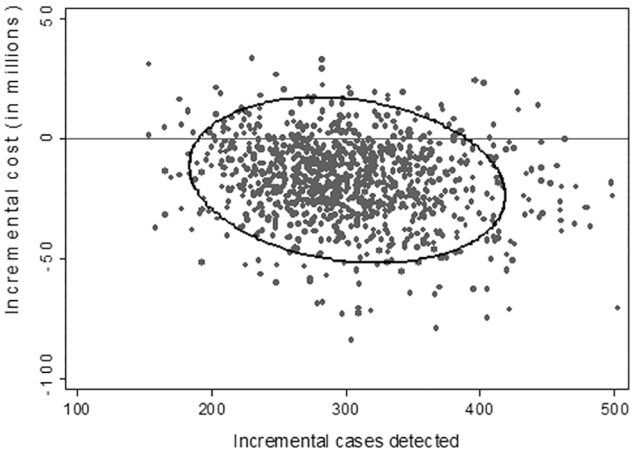

Fig 8. Scatter plot of probabilistic sensitivity analysis of contingent NIPT vs. MSS from a government perspective. The figure below plots the incremental cost and effectiveness results from 1,000 simulations. Compared to MSS, there is a 100% probability that contingent NIPT is more effective and a 87% probability that contingent NIPT is less costly.

doi:10.1371/journal.pone.0131402.g008

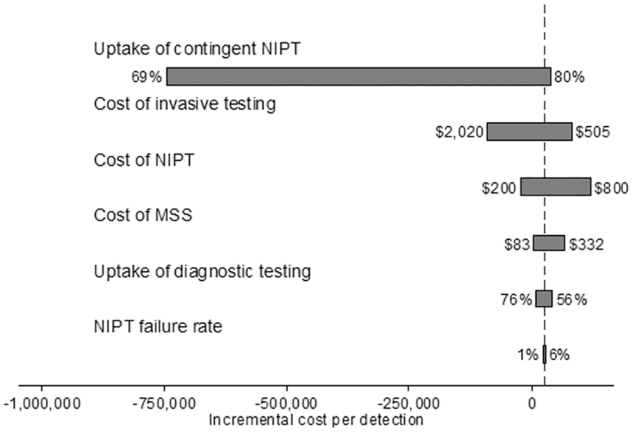

Fig 9. One-way sensitivity analysis of contingent NIPT vs MSS from a payer perspective. Below are the one-way sensitivity analysis results of ICER between contingent NIPT and MSS. Contingent NIPT is more costly than MSS as long as the cost of NIPT is above $293, contingent NIPT uptake is above 72%, and the cost of invasive screening is below $1,235.

doi:10.1371/journal.pone.0131402.g009

screening was more effective 100% of the time and more costly 73.2% of the time compared to MSS (Fig 10).

Discussion

We compared the cost effectiveness of four screening policies to identify fetal trisomies: (1) no screening, (2) conventional MSS, (3) universal NIPT screening, and (4) optimized contingent NIPT. We conducted our analysis from three different perspectives: societal, government, and payer.

We optimized the risk cutoff used to classify "high-risk" pregnancies in the primary stage of the contingent NIPT screen. We found that the optimal risk cutoff depended on the cost perspective. The optimal risk cutoff of the primary stage decreased when more downstream costs were included in the analysis. Lower risk cutoffs in the first stage increased the referral rate to

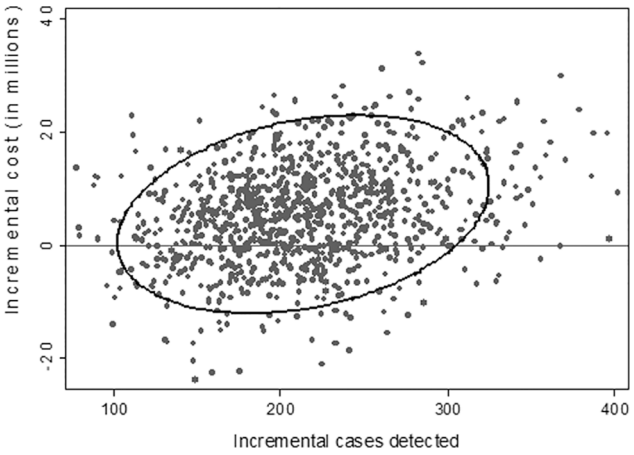

Fig 10. Scatter plot of probabilistic sensitivity analysis of contingent NIPT vs MSS from payer perspective. The figure below plots the incremental cost and effectiveness results from 1,000 simulations. Compared to MSS, there is a 100% probability that contingent NIPT is more effective but a 73.2% probability that contingent NIPT is more costly.

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the second stage (NIPT) which, in turn, increased the detection rate. The risk cutoffs were lowest when optimized for the societal perspective and highest when optimized for the payer perspective.

We found that the best screening policy depended on the economic perspective. When analyzed from a societal perspective, universal NIPT was both more effective and less costly than contingent NIPT. Universal NIPT was also less costly than MSS. Replacing MSS with universal NIPT would result in a 35.5% increase in total cases detected and 6.9% reduction in total costs.

From a government perspective, contingent NIPT dominated MSS and was the least costly strategy. Under this perspective, the optimized risk cutoffs would result in a 12% increase in the number of total cases detected and a 2.5% reduction in costs. In contrast to a societal perspective, the government perspective excluded the indirect costs from the analysis. When indirect costs are no longer included, universal NIPT is not a cost-effective alternative to MSS unless there is a substantial willingness to pay for the test. For universal NIPT to be a cost-effective replacement for MSS, there must be a willingness to pay of $203,088 per detection.

From a payer perspective, MSS is the least costly screening strategy. Although MSS was less costly, contingent NIPT dominated MSS by extension. The ICER between no screening and contingent NIPT was $54,309 per case detected; however, a comparison of no screening and MSS produces an ICER of $56,726 per case detected. Given that MSS is currently the standard of care, we can reasonably assume that there is at least a willingness to pay of $56,726 per case detected, making a contingent NIPT a cost-effective alternative to MSS from a payer perspective.

Contingent and universal NIPT produced fewer false positive results than MSS. MSS had a false positive rate of 5.6%, universal NIPT had a false positive rate of 0.4%, and all contingent NIPT polices had false positive rates below 0.1%. Thus, contingent NIPT can obtain higher detection rates than MSS with a relatively small increase (0.3%) in the overall false positive rate.

The first stage of contingent NIPT produces a higher level of false positive results than conventional MSS. Unlike conventional MSS, most false positives from the first stage of contingent screening are identified in the second stage so that the overall false positive rate is lower than MSS and only slightly lower than universal NIPT. The high false positive rate of first stage could be a problem if women were provided these results; however, we believe that contingent NIPT should be implemented as a testing system that produces a single test result. Positive results from the first stage would be reflexed to NIPT. Women would only be given the final result and would not be informed of the preliminary false positive result. This approach would spare women the anxiety associated with false positive results.

Previous studies have examined the cost effectiveness of changing the risk cutoff in the primary screen; however, none of these studies included the downstream costs of trisomy births that result from false negative results.[5–7] By including both the immediate costs of screening and the downstream costs of false negative results, we were able to optimize the decision limit. Therefore, our findings provide a new approach to setting the risk thresholds for contingent NIPT.

Our results are consistent with the results of three previous studies that found contingent NIPT to be a cost-effective alternative to conventional MSS.[5,8,9] However, our analysis used multiple perspectives and found risk cutoffs for each perspective that minimized the overall costs of contingent NIPT.

We are unaware of a standard protocol for failed NIPT. We assumed that pregnancies would be classified by conventional MSS in the event of NIPT failure. For contingent NIPT, we assumed that women with a risk greater than or equal to 1:270 on the initial serum screen would be offered invasive testing. Similarly, for universal NIPT we assumed that cases with

NIPT failure would be tested with MSS and those with risks greater or equal to 1:270 would be offered invasive testing. When NIPT fails, approximately 56%-83% are successful after another blood draw.[24,65–67] Because some cases are probably redrawn, our analysis probably underestimated the overall detection rate of contingent and universal NIPT. We conducted sensitivity analyses to examine the impact of our assumptions regarding NIPT failure. We found that our results were insensitive to the assumptions. Contingent NIPT was particularly insensitive to this change because only a subset of women initially screened received follow-up NIPT.

The unit cost of NIPT is uncertain. Prices for NIPT testing range from $500 to $2100; however, prices do not necessarily reflect costs. The relevant cost in a cost-effectiveness analysis is the *resource* cost, or the cost to perform the test. Although prices cannot be used directly, we used prices to estimate underlying costs. We reasoned that the producer with the lowest price is most likely making a profit and the variability in price most likely represents variation in profit margins rather than variation in costs. Thus, the lowest price most likely represents an upper bound on the cost. Further, if the variation in prices reflects variation in costs, the lowest cost producer will eventually dominate and is the most relevant cost for the analysis. For that reason, we used the lowest list price ($500) to estimate the cost of NIPT. We assumed a 20% profit margin and used a unit cost of $400 per test. This cost estimate is consistent with previous published results.[4,38] Our sensitivity analysis demonstrated that even at a cost of $800, contingent NIPT was less costly and more effective than conventional screening.

Our analysis assumed that CVS, rather than amniocentesis was used for diagnostic testing. Our model simulates screening at the 12th week. Amniocentesis is usually not performed until the 15th week. We assumed that most women would prefer rapid resolution of a positive screen result and, for that reason, would prefer CVS in the 12th week over amniocentesis at week 15. However, our results are insensitive to this assumption. The number of trisomy cases detected would remain the same while the total costs would be slightly lower due to the small difference in cost between CVS and amniocentesis. Therefore, our conclusions would remain unchanged even if we assumed that diagnostic testing were conducted with amniocentesis.

We conducted extensive sensitivity analysis and probabilistic sensitivity analysis. Our results were most sensitive to changes in the lifetime costs, the increase in uptake of NIPT policies relative to MSS uptake (i.e., ΔU_{cNIPT} and ΔU_{uNIPT}), uptake of MSS (U_{cMSS}), and the cost of NIPT. Because of the uncertainty regarding the lifetime cost estimates of trisomies 13 and 18, we varied these costs over a wide range in the one-way sensitivity analysis. We found that our results were insensitive to assumptions concerning the lifetime costs associated with trisomies 13 and 18.

Our study had the following limitations regarding the reliability of the lifetime cost estimates. First, the data used to estimate lifetime costs in our analysis is roughly two decades old.[29] Although we adjusted the initial estimates by accounting for inflation and changes in life expectancy, these adjustments do not take into consideration changes in treatment. For example, advances in care may increase the intensity of treatment, therefore increasing lifetime medical expenses.[68] Second, in the absence of lifetime cost estimates for trisomies 13 and 18, we made several simplifying assumptions in order to estimate these costs. Cost data on trisomies 13 and 18 are difficult to obtain. Therefore, we used the annual costs of Down syndrome provided by Waitzman et al. Although trisomies 13 and 18 have similar one-year survival rates, *[32,69–71]* we were unable to find survival estimates beyond one year for trisomy 13; therefore, we assumed that survival rates for trisomy 13 were the same as for trisomy 18 beyond year one.

Because of the uncertainty of the lifetime estimates, we conducted extensive sensitivity analysis on these costs (see Figs 5, 7, and 9). Our conclusions held even when lifetime costs were reduced by 50%, with the following exception. From a societal perspective, universal NIPT no longer dominated contingent NIPT once the lifetime costs of trisomy 21 were below $1.3

million or when the lifetime costs of trisomies 13 and 18 were below $704,000. However, contingent NIPT remained less costly than MSS over the full range of lifetime costs that was covered in the one-way analysis. This remained true even when the lifetime cost of trisomies 13 and 18 were excluded from the analysis, due to the low birth prevalence of these trisomies. Therefore, our conclusions are robust even when taking into account uncertainty surrounding the lifetime cost estimates, particularly those of trisomies 13 and 18.

For this reason, assumptions about trisomies 13 and 18 have relatively little impact on the analysis.

Our study also has several strengths. Our analysis included trisomies 13 and 18, which have only been included in one previous analysis.[8] Although other studies have investigated contingent policies, we identified the optimal risk cutoffs and were able to compare the cost effectiveness of an optimal contingent policy against the cost effectiveness of universal NIPT and MSS. Our analysis shows the potential cost savings of contingent NIPT. We included multiple cost perspectives. We analyzed cost effectiveness from a broad allocative standpoint (societal perspective), as well as from narrower perspectives that would be relevant to government and payer decision makers. Finally, our results were robust. We found that contingent NIPT was less costly than MSS over a wide range of costs and probabilities used in our analysis.

Conclusion

From a societal perspective, universal NIPT is a cost-effective alternative to MSS and contingent NIPT. When viewed from government or payer perspectives, contingent NIPT is cost-effective relative to MSS but is both less costly and less effective than universal NIPT. In these cases, the choice of policy depends on the willingness to pay for additional detections. Adopting universal NIPT would cost $203,088 for each additional case detected from a government perspective and $263,922 for each additional case detected from a payer perspective.

Supporting Information

S1 Text. Explanation of Lifetime Cost Estimates.
(DOCX)

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Author Contributions

Conceived and designed the experiments: BSW RLS. Performed the experiments: BSW RLS. Analyzed the data: RLS BSW ERA BRJ DGG REN. Wrote the paper: BSW RLS REN ERA BRJ DGG.

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REVIEW

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

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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.

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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.[1] 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.[2] 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.[3] 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.[4,5] 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.[6]

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.[7–10] Already, more than half a million NIPTs have been performed worldwide in more than 61 countries.[11,12] 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.[13] Patients were equally as interested in NIPT for screening of trisomy 13 and 18 as they were for trisomy 21.[14,15] 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.[5,16–18] 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.[19] 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.[20] 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.[21] 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.[22] 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.[6]

EFFECT OF NIPT ON ALTERNATE SCREENING PROCEDURES

The introduction of NIPT in clinical practice was unprece dented 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.[23] 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.[24] 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.[25] 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%).[26] 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%.[9] 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.[27] 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%.[28] 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.[29] 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.[30] 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

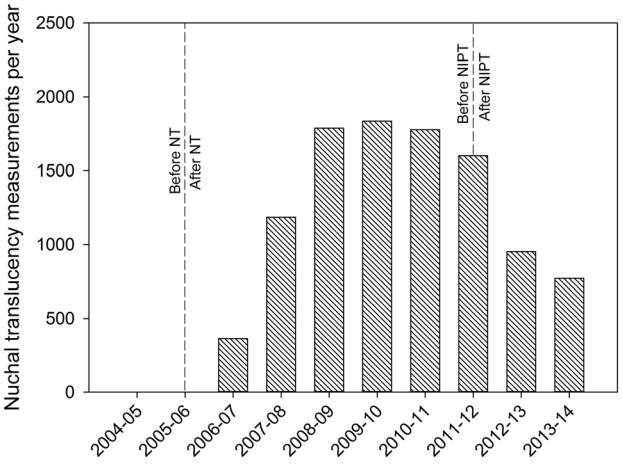

Figure 1 Yearly nuchal translucency measurements in a single referral center in the United States, including after introduction of NT in 2005 and noninvasive prenatal testing in 2012. NT, nuchal translucency; NIPT, noninvasive prenatal testing

Tab e 1 **Review of studies investigating the effect of N PT on diagnostic procedures**

Study	Year	Country	Methodo ogy	Study size	Principa re ated findings
Garfie d *et al* [29]	2012	Un ted States	Mu t-stage trans t on probab ty mode	Theoret ca 100 000 pregnancy cohort	mp ement ng N PT as an ntermed ate test fo ow ng pos t ve screen wou d resu t n a 72% decrease n nvas ve procedures and a 66% reduct on n procedure-re ated m scarr ages
Chetty *et al* [33]	2013	Un ted States	Retrospect ve cohort study	398 screen-pos t ve pat ents over 12-month per od	Proport on of women undergo ng nvas ve d agnost c test ng decreased from 47% to 39% after N PT ntroduct on
Song *et al* [28]	2013	Un ted States	Dec s on-ana yt c mode	Theoret ca 4 m on pregnancy cohort	mp ement ng N PT n h gh-r sk women wou d resu t n >95% decrease n nvas ve procedures and >99% decrease n eup o d feta oss
Lar on *et al* [30]	2014a	Un ted States	Retrospect ve rev ew of prospect ve y co ected database	15 418 tests over 9-year per od	N PT ntroduct on resu ted n a 48% decrease n FTS 69% decrease n CVS and 47% decrease n amn ocentes s from peak years
Wa erste n *et al* [26]	2014	Un ted States	Prospect ve study	163 pat ents undergo ng genet c counse ng over 18-month per od	nvas ve test ng decreased from 19% to 13% of screen-pos t ve pat ents n s m ar per od before N PT ntroduct on
Pett t *et al* [34]	2014	Un ted States	Retrospect ve cohort study	206 pat ents undergo ng N PT over 8-month per od	Rate of nvas ve procedures per tota number of pat ent v s ts decreased to 4 1% from 5 9% n s m ar per od before N PT ntroduct on
Fr e *et al* [35]	2014	Un ted States	Retrospect ve rev ew of prospect ve y co ected database	792 pat ents undergo ng genet c counse ng over 8-month per od	N PT ntroduct on decreased second tr mester nvas ve procedures from 35% to 18% and decreased first tr mester FTS from 89% to 59% of a v s ts
P att *et al* [37]	2014	Un ted States	Mu t center retrospect ve study	1477 pat ents across 6 s tes over 24-month per od	6 of 6 centers reported a decrease n amn ocentes s (from −23% to −50%) and 4 of 6 reported a decrease n CVS rates (from −14% to −66%)
Lar on *et al* [9]	2014b	Un ted States	Retrospect ve rev ew of prospect ve y co ected database	9287 tests over 51-month per od	N PT ntroduct on resu ted n a 49% decrease n FTS 77% decrease n CVS and 53% decrease n amn ocentes s from pre-N PT base ne per od
Wax *et al* [36]	2014	Un ted States	Retrospect ve cohort study	2510 pat ents cons dered h gh r sk for feta aneup o dy	N PT ntroduct on decreased amn ocentes s and CVS procedures by 49% and 17% respect ve y wh e ncreas ng genet c counse ng use by 23%
Wa d *et al* [40]	2013	UK	Hypothet ca cont ngent screen ng mode	—	N PT fo ow ng a pos t ve first stage of the ntegrated screen wou d resu t n 3 per 1000 women undergo ng amn ocentes s w th 2 of 3 d agnosed w th tr somy 21
Okun *et al* [38]	2014	Canada	8 hypothet ca screen ng a gor thms	—	Cont ngent N PT screen ng wou d decrease amn ocentes s procedures by 50–91% depend ng on a gor thm
O Leary *et al* [42]	2013	Austra a	Dec s on-ana yt c mode	Theoret ca 32 478 pregnancy cohort based on Austra a popu at on	N PT fo ow ng a pos t ve first tr mester screen wou d resu t n an 88% decrease n the number of nvas ve d agnost c tests and procedure-re ated feta osses n the h gh-r sk pat ent popu at on
Manego d-Brauer *et al* [43]	2014	Sw tzer and	Retrospect ve study	951 pat ents present ng for FTS over 18-month per od	N PT ntroduct on decreased nvas ve test ng to 3 1% from 8 8% n pre-N PT base ne per od
Neyt *et al* [39]	2014	Be g um	Mu t-stage trans t on probab ty mode	Theoret ca 129 199 pregnancy cohort based on Be g um popu at on	N PT as a first or second ne screen wou d resu t n a decrease n the number of procedure-re ated m scarr ages from 76 w th current screen ng to 26 and 34 respect ve y
Morr s *et al* [41]	2015	UK	Dec s on-ana yt c mode	Theoret ca 10 000 pat ents undergo ng screen ng	N PT as first- ne screen ng wou d decrease nvas ve d agnost c test ng by 86% from current screen ng parad gms
G *et al* [44]	2015	UK	Prospect ve study	6651 pat ents who presented for FTS	N PT ntroduct on decreased nvas ve procedures n the h gh-r sk (r sk > 1 100) pat ent popu at on from 54% to 40%
Chan *et al* [45]	2015	Ch na	Retrospect ve study	1251 pat ents w th pos t ve screen for tr somy 21 over 28-month per od	N PT ntroduct on decreased nvas ve test ng to 67% from 92% n pre-N PT base ne per od

N PT non nvas ve prenata test ng FTS comb ned first tr mester screen CVS chor on c v us samp ng

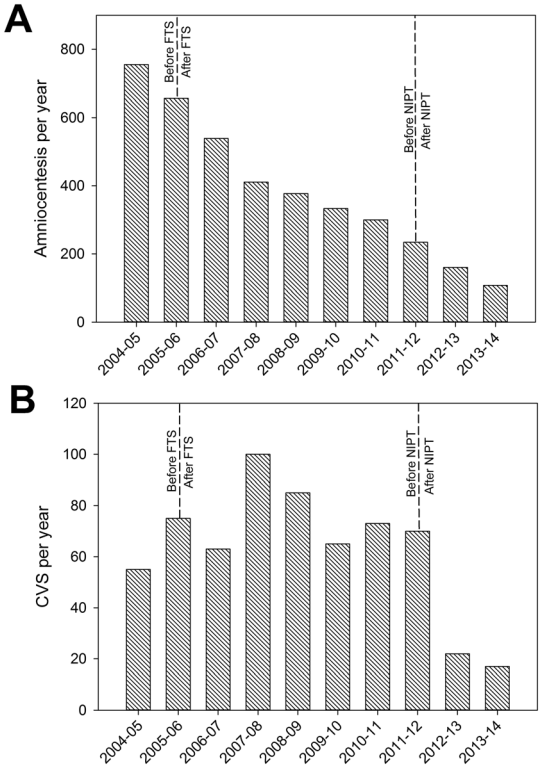

Figure 2 Yearly genetic amniocenteses (A) and CVS (B) procedures in a single referral center in the United States, including after introduction of the combined first trimester screen in 2006 and noninvasive prenatal testing in 2012. CVS, chorionic villus sampling; FTS, combined first trimester screen; NIPT, noninvasive prenatal testing

assessment.[31,32] 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.[33] 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.[34] 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.[35] 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.[26] 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; $P < 0.0001$).[36] 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.[37]

International studies using hypothetical models or clinical data report similar decreases in procedural volume. A Canadian population based study investigating the hypothetical perfor mance 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.[38] The group also reported that amniocenteses 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.[39] 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.[40] 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.[41] 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.[42]

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.[43] 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.[44] 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.[45] 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.[46] Furthermore, as genetic counseling increases patient knowledge regarding NIPT, diagnostic testing is likely to further decrease.[47]

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).[36] 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.

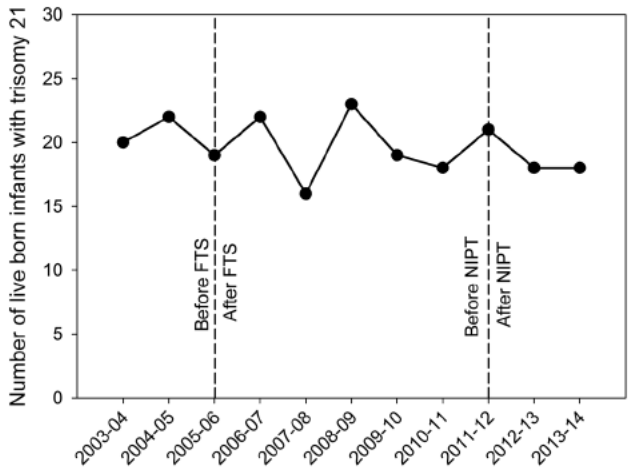

Figure 3 Yearly number of live born infants with trisomy 21 between 2003 and 2014 in the Hampton Roads, Virginia region, including after introduction of the combined first trimester screen in 2006 and noninvasive prenatal testing in 2012. FTS, combined first trimester screen; NIPT, noninvasive prenatal testing

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)

	N (%)
No or late prenatal care	5 (4)
No trisomy 21 screening	29 (24)
Positive screen but no diagnostic testing	28 (23)
Confirmed trisomy 21 diagnosis but continued pregnancy	45 (37)
Screen failures[a]	15 (12)

[a]Twelve of 15 screen failures involved quadruple maternal serum screens, and three of 15 involved combined first trimester screens.

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.[48] 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.[25]

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.[49,50] 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.[51] 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.[52,53] As there are significant ethical

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 amni ocenteses, 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 proce dures 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.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Noninvasive prenatal testing has been shown to have high sensitivity and specificity for fetal aneuploidy, decreasing the need for invasive testing and procedure-related fetal losses.

WHAT DOES THIS STUDY ADD?

- We describe institutional and global experience with NIPT uptake and its effect on invasive diagnostic procedures.
- The significant decrease in procedural volume creates unique challenges for training and maintenance of physician proficiency with invasive techniques.

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