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ACMG SYSTEMATIC EVIDENCE REVIEW

Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies

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ABSTRACT

Purpose: Noninvasive prenatal screening (NIPS) using cell-free DNA has been assimilated into prenatal care. Prior studies examined clinical validity and technical performance in high-risk populations. This systematic evidence review evaluates NIPS performance in a general-risk population.
Methods: Medline (PubMed) and Embase were used to identify studies examining detection of Down syndrome (T21), trisomy 18 (T18), trisomy 13 (T13), sex chromosome aneuploidies, rare autosomal trisomies, copy number variants, and maternal conditions, as well as studies assessing the psychological impact of NIPS and the rate of subsequent diagnostic testing. Random-effects meta-analyses were used to calculate pooled estimates of NIPS performance ($P < .05$). Heterogeneity was investigated through subgroup analyses. Risk of bias was assessed.
Results: A total of 87 studies met inclusion criteria. Diagnostic odds ratios were significant ($P < .0001$) for T21, T18, and T13 for singleton and twin pregnancies. NIPS was accurate (≥99.78%) in detecting sex chromosome aneuploidies. Performance for rare autosomal trisomies and copy number variants was variable. Use of NIPS reduced diagnostic tests by 31% to 79%. Conclusions regarding psychosocial outcomes could not be drawn owing to lack of data. Identification of maternal conditions was rare.
Conclusion: NIPS is a highly accurate screening method for T21, T18, and T13 in both singleton and twin pregnancies.

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Introduction

Since its introduction in 2011, noninvasive prenatal screening (NIPS) using cell-free DNA (cfDNA) for the detection of common fetal aneuploidies has been rapidly assimilated into prenatal care.[1] With a resolution similar to karyotyping[2] and regardless of the methodology used, cfDNA is the most sensitive and specific screening test for common chromosomal aneuploidies (chromosomes 13, 18, and 21).[3,4] Before its introduction into clinical use, no large-scale randomized control trials were performed to assess the clinical validity or clinical utility of this screening test. Subsequently, multiple studies have determined the sensitivity and specificity of this testing, focusing largely on high-risk patient populations with singleton pregnancies.[1,5-7]

Before the implementation of NIPS, screening for aneuploidy consisted mainly of multiple serum analytes with or without ultrasound to achieve a detection rate ranging from 80% to 95% for Down syndrome.[8] Although NIPS has a greater accuracy for aneuploidy detection, approximately 99% for Down syndrome at 10 weeks of gestation or greater,[4] detection rates vary slightly between laboratories owing to differences in methodologies and reporting methods.

When diagnostic testing is performed to evaluate a screen-positive high-risk result generated through NIPS, a subset of individuals will have discordant results, with varying false positive rates (FPRs) depending on the specific chromosome interrogated, the type of variant, and the prevalence of the condition. Although the intent of screening is to determine whether fetal aneuploidy is present, the specimen obtained contains predominantly maternal DNA, and the test often cannot distinguish between fetal and maternal chromosomal material. This may lead to unexpected maternal findings for which patients are unprepared, including the suggestion of maternal malignancy, a maternal submicroscopic duplication or deletion, or a maternal sex chromosome aneuploidy (SCA). Finally, all screening tests have false-positive (FP) and false-negative (FN) results but given the enhanced accuracy to detect the common trisomies, some health care providers and patients may inappropriately consider the test to be diagnostic.[9]

Current national guidelines from multiple organizations state that pregnant individuals should be made aware of both the accuracy and limitations of cfDNA screening for the detection of the common trisomies. The most recent American College of Medical Genetics and Genomics (ACMG) position statement states that "all women should be informed that NIPS is the most sensitive screening option for traditionally screened aneuploidies."[3] The American College of Obstetrics and Gynecology reinforces this statement.[8] Both organizations stress that NIPS is not equivalent to diagnostic testing.

Although initially NIPS was used to screen for the common trisomies and SCAs in singleton pregnancies, many laboratories have adapted this technology to screen twin gestations.[10] Furthermore, in some laboratories, the application has been expanded to screen for rare autosomal trisomies (RATs), as well as for both common and unique copy number variants (CNVs). However, the positive predictive values (PPVs) for these conditions are significantly lower than the PPVs for common aneuploidies and large-scale outcome studies have not been performed, nor has clinical utility of screening for these rarer conditions been established.

This systematic evidence review (SER) is designed to assess the clinical performance of NIPS in a general-risk population of both singleton and twin pregnancies. It also evaluates the use of NIPS with respect to the identification of CNVs, SCAs, RATs, and maternal conditions, its impact on the uptake of diagnostic testing, the economic implications of its use, as well as the psychological impact of this technology on the individuals undergoing prenatal screening for aneuploidy.

Materials and Methods

We performed an SER using best practices and report our methods and results in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist.[11] In 2020, ACMG convened an SER workgroup to develop the evidentiary basis for a clinical guideline. The SER workgroup comprised ACMG members, including a board-certified medical geneticist and maternal fetal medicine physician (N.C.R.), clinical directors of laboratory medicine (E.S.B., M.L.L.), a laboratory genetic counselor (D.L.), and methodologists (J.M., G.P.J., M.R.M.). Working group members had no conflicts of interest according to ACMG policy. The goal of the SER was to assess the use of NIPS in a population of general-risk individuals, ie, a population reflective of a range of risks that might be encountered in general obstetrical practice, including low-risk, intermediate-risk, and high-risk patients. To address this question, a separate guideline panel external to the authors and methodologist (M.R.M.) defined the population, intervention, comparator(s), outcomes, timing, and setting and developed a set of 10 key questions (KQ) and corresponding search queries (Supplemental Material).

We initially searched Medline (PubMed) and Embase for relevant studies on July 30, 2020 and updated our search on March 26, 2021. The search strategy for Medline is presented in the Supplement. We further identified relevant studies cited by other studies or from meta-analyses. We updated our search query to account for additional synonyms used for NIPS and limited returns on the basis of publication date consistent with the original search. Results from the databases were managed in an Endnote (version 9.3.3; version 20) library that was used for deduplication. Deduplicated results were uploaded to Covidence for review and data extraction/quality assessment.

All stages of the review were performed independently by 2 reviewers. Conflicts were resolved through discussion between reviewers or adjudicated by a third reviewer. Titles and abstracts of search results were screened according to prespecified inclusion and exclusion criteria (Supplemental Material). Articles not excluded in the title/abstract screening were reviewed in their entirety for inclusion; rationale for exclusion was documented (Supplemental Material). Data extraction and risk of bias forms were created within Covidence for diagnostic accuracy and clinical utility studies; data extraction was completed in Microsoft Excel spreadsheets guided by the Consolidated Health Economic Evaluation Reporting Standards checklist.[12] Data extracted included study, population characteristics, details about NIPS and any comparators, and outcome(s). Data for true positives (TPs), true negatives (TNs), FPs, and FNs were extracted when provided or calculated by reviewers when there was sufficient confidence in the data reported. Risk of bias was assessed using the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I)[13] framework or the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)[14] for diagnostic accuracy studies.

Data analysis

Data exported from Covidence was cleaned in Microsoft Excel. Analysis was performed using R Studio (v.1.4.1717) (R Development Core Team), R (version 4.1.0) with the R packages "meta," "metafor," "mada," "diagmeta," and "ggplot2." An analysis plan was prespecified; random-effects meta-analyses were planned to obtain pooled point estimates and 95% CI for each of the diagnostic performance outcomes for KQ1 to KQ6. Only studies where the TPs, TNs, FPs, and/or FNs were provided or calculable with relative certainty from the data presented in the manuscript were included in meta-analyses. Studies reporting their performance without also providing the number of people in each category were not meta-analyzed and their results are reported separately. Quantitative analysis was deemed unlikely to be possible for KQ7 to KQ10 and results for those KQs were narratively synthesized. Anticipated heterogeneity was investigated through sensitivity analyses, with subgroups defined for country, year of publication, risk of bias assessment (low, moderate, high, critical), and size of population screened (<10,000, ≥10,000). Heterogeneity is reported as I^2. Publication bias was evaluated using the method described by Peters et al[15] weighted by inverse variance of average event probability and visualized with funnel plots. Results of the meta-analyses, including heterogeneity, are presented as forest plots and summarized in tables.

Results

We identified 770 articles from our literature searches and review of included studies from published meta-analyses and SERs. After deduplication, we screened 753 titles and abstracts and excluded 538 of those. We reviewed 215 studies in their entirety and determined 128 did not meet inclusion criteria (Supplemental Material). Of the 87 studies that ultimately met our inclusion criteria, 78 reported clinical outcomes and/or NIPS performance and 10 reported on economic outcomes (with 1 study reporting both). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart is presented in the Supplement. A summary of all included studies is presented in the Supplement.

Trisomy 21

A total of 35 studies reported at least 1 performance characteristic (ie, sensitivity, specificity, PPV, negative predictive value (NPV), or FPR) for trisomy 21 (T21) (Supplemental Material). Of these, 28 were included in meta-analyses and the remainder were narratively synthesized. Studies reporting a statistic for >1 outcome combined are reported separately. The number of studies in the meta-analyses depended upon the specific data presented in the included studies. The pooled performance characteristics are presented in Table 1, with accompanying forest plots in the Supplement.

Two additional studies[16,17] reported sensitivity without presenting the number of TPs and/or FNs (98.9%, 95% CI = 95.90%-99.90%; 100%, 95% CI = 92%-100%, respectively). Together with the results of the meta-analysis, sensitivity ranged from 95% to 100% in 19 studies with no evidence of important heterogeneity between studies. Two additional studies reported specificity[18,19] without presenting the number of TPs and/or FNs (100%, 95% CI = 99.5%-100%; 99.95% [no CI given], respectively). Together with the results of the meta-analysis, specificity ranged from 99.89% to 100% in 17 studies. Costa et al[18] and Kypri et al[17] similarly reported PPV without presenting the number of TPs and/or FPs (100%, 95% CI = 59.0%-100%; 100%, 95% CI = 92%-100%, respectively). The pooled estimate of NPV was 100% (95% CI = 99.99%-100%) from 14 studies included in our meta-analysis. One additional study reported NPV without presenting the number of TNs and/or FNs (99.996% [no CI given]).[19] Sensitivity, specificity, PPV, and NPV of NIPS for T21 in Belgium were reported as 98.91% (95% CI = 97.24%-99.58%), 99.98% (95% CI = 99.97%-99.99%), 92.39% (95% CI = 89.34%-94.61%), and 100% (95% CI = 99.99%-100.00%), respectively.[20] Together with the results of the meta-analysis, NPV ranged from 99.99% to 100% in 16 studies and there was no important heterogeneity (I^2 = 0%) observed between the studies included in the meta-analysis. In total, 14 studies contributed to the meta-analysis for FPR; the pooled estimate was 0.04% (95% CI = 0.02%-0.08%) with considerable heterogeneity (I^2 = 76%) (Table 1). A total of 7 additional studies[18,19,21-25] reported FPR without presenting the number of TNs and/or FPs (Supplemental Material).

Table 1 Performance of NIPS in a general-risk population for trisomy 21, trisomy 18, and trisomy 13 calculated in random-effects meta-analyses

Test Statistic	No. of Studies	Result (%) (95% CI)	I^2 (%)
Trisomy 21			
Sensitivity	17	98.80 (97.81-99.34)	0.0
Specificity	14	99.96 (99.92-99.98)	75.9
PPV	28	91.78 (88.43-94.23)	68.3
NPV	14	100 (99.99-100)	0.0
FPR	14	0.04 (0.02-0.08)	75.9
Accuracy	14	99.94 (99.91-99.96)	80.2
DOR[a]	14	110,000 (44,000-260,000); $P < .0001$	55.7
Trisomy 18			
Sensitivity	6	98.83 (95.45-99.71)	0.0
Specificity	7	99.93 (99.83-99.97)	94.9
PPV	17	65.77 (45.29-81.68)	88.5
NPV	7	100 (100-100)	0.0
FPR	7	0.07 (0.03-0.17)	75.9
Accuracy	6	99.91 (99.73-99.97)	95.7
DOR[a]	6	29,000 (4800-180,000); $P < .0001$	94.9
Trisomy 13			
Sensitivity	7	100 (0-100)	0.0
Specificity	8	99.96 (99.92-99.98)	81.5
PPV	18	37.23 (26.08-49.93)	71.9
NPV	8	100 (100-100)	0.0
FPR	8	0.04 (0.02-0.08)	81.5
Accuracy	8	99.95 (99.90-99.97)	82.2
DOR[a]	7	29,000 (8900-94,000); $P < .0001$	0

Results do not include studies without adequate data to include in meta-analyses.

DOR, diagnostic odds ratio; *FPR*, false positive rate; *NIPS*, noninvasive prenatal screening; *NPV*, negative predictive value; *PPV*, positive predictive value.

[a]Data presented as odds ratio.

The diagnostic odds ratio (DOR) could be assessed in 14 studies. The estimated odds ratio of the DOR in the random-effects meta-analysis was 108,000 (95% CI 44,000-265,000). The odds for someone receiving a positive NIPS result in patients who are TP for T21 is >100,000 times higher than the odds for a positive NIPS result in patients who are TNs for T21. This highly significant ($P < .0001$) result shows that the NIPS tests are highly accurate and is consistent with an overall NIPS accuracy of 99.94% for T21 (Table 1).

In sensitivity analyses, risk of bias, country, and populations of ≥10,000 individuals were inconsistently associated with reported higher performance (Supplement). Although some subgroups were significantly different from each other, many subgroups contained only a single study and differences were not clinically meaningful. Overall, performance statistics for NIPS to detect T21 in general- or mixed-risk populations were high.

Trisomy 18

A total of 21 studies contributed to our analysis of NIPS to detect trisomy 18 (T18), whereas 2 studies reported combined results for T18 and trisomy 13 (T13) and are presented separately. Summary results and forest plots from random-effects meta-analyses for T18 are presented in Table 1 and the Supplement, respectively. In addition to the meta-analyses, Chen et al[26] reported a PPV of 54.84% (no CI given) for T18 in their mixed-risk population of 42,910 individuals with singleton pregnancies; however, PPV specifically among individuals with no clinical indications was 0%. From a cohort of 10,975 low-risk individuals in China, 166 had an adverse pregnancy outcome. Follow up with ultrasound and additional diagnostic testing identified a T18 FN from NIPS drawn at 17^{+3} weeks gestational age in a 26 year old individual.[27] In the Belgian study, sensitivity, specificity, and NPV were each reported as >95%, whereas PPV was lower, at 84.62% (95% CI = 75.82%-90.61%).[20]

We observed considerable heterogeneity in our meta-analyses. Sensitivity analyses uncovered significant between-subgroup differences on the basis of country and year of publication; however, these differences were not clinically meaningful and for country, most subgroups contained a single study (Supplemental Material). Overall, sensitivity, specificity, NPV, and accuracy of NIPS to detect T18 was high and the FPR was low (0.07%), but PPV was substantially lower than the PPV of NIPS for T21 (Table 1).

T13

A summary of the performance characteristics of NIPS for detection of T13 reported by 19 studies and meta-analysis is presented in Table 1 with corresponding forest plots and sensitivity analyses in the Supplement.

Overall, we observed high sensitivity, specificity, accuracy, and DOR for T13 with low FPRs. PPV was low at 37%, which was lower than the PPV for T18 and substantially lower than the PPV for T21. Similar to the subgroup analyses performed for T21 and T18, performance may vary, although the data are insufficient to draw conclusions about any individual subgroup. One additional study reported specificity without presenting the number of TNs and/or FPs (99.94% [no CI given]).[28] In that study of 40,265 individuals who received NIPS, diagnostic testing confirmed 4 of 33 T13 positive results.[28] Chen et al[26] reported an overall PPV of 13.79% for T13; however, in the subset of their population with no clinical indications, PPV was 25.00%. In the large study of >150,000 singleton pregnancies from Belgium, sensitivity, specificity, and NPV of NIPS for T13 was very high (each >99%), whereas PPV was considerably lower in this general-risk population: 43.90% (95% CI = 33.67%-54.68%).[20]

Combined T21, T18, T13

Most studies reported NIPS performance separately for each trisomy; however, there were some that reported overall performance for multiple outcomes. Oneda et al[29] evaluated NIPS performance for T21/T18/T13 in both prospective and retrospective populations. In their prospective cohort, sensitivity was reported as 100% (95% CI = 91.96%-100%), specificity was 99.97% (95% CI = 99.81%-100%), PPV was 97.78% (95% CI = 86.11%-99.68%), and NPV was 100% (no CI). This resulted in test accuracy of 99.97% (95% CI = 99.81%-100%). In a Chinese population of 15,626 people, Yao et al[30] reported an overall PPV of 79.07% (95% CI = 68.69%-86.80%) for T21/T18/T13 with an FPR of 0.13% (95% CI = 0.08%-0.21%).[30]

Guy et al[16] reported combined sensitivity and PPV for T18 and T13 (90.4%, 95% CI = 80.0%-96.8%; 92.2%, 95% CI = 81.5%-96.9%, respectively). Together with the results of the meta-analyses, these data present a largely positive view of NIPS as a highly accurate screening method for T21, T18, and T13, although, variability in a number of factors influenced specific test metrics.

NIPS performance in multifetal gestations

In total, 11 studies reported at least 1 performance characteristic of NIPS to detect T21, T18, or T13 in multifetal gestations, 7 of which were included in meta-analyses. A summary of results from the random-effects meta-analyses are presented in Table 2 with corresponding forest plots in the Supplement.

In the limited number of studies reporting on use of NIPS for twin gestations, diagnostic performance to detect T21, T18, and T13 was generally high, with no/little observed heterogeneity. Apart from the studies included in the meta-analysis, 4 additional studies reported outcomes pertaining to NIPS use in twin gestations.[29,31-33] NIPS screen-positive results were identified in 11 twin and 1 triplet pregnancies, accounting for 2.7% of twin pregnancies, from a prospective mixed-risk cohort of 3053 individuals.[29] Diagnostic testing confirmed the results except for 1 individual, in which it was found in the placenta of 1 twin only and reported as an FP.[29] No FP results were observed in patients with confirmatory testing for T21, T18, or T13 in either monozygotic or dizygotic pregnancies.[33] In the same study, fetal sex confirmation and zygosity calls were found to be correct in all patients.[33]

In a study of singleton and multifetal pregnancies in China, fetal sex determination was concordant in 98.6% (95% CI = 92.19%-99.96%) of twins and 97.6% (95% CI = 91.76%-99.71%) of triplets.[30] Three cases of chromosomal aneuploidy were observed in twin pregnancies. A sample from a dichorionic diamniotic pregnancy with NIPS results suggesting T21 in both fetuses resulted in termination of pregnancy that was not confirmed on the products of conception in this report. A second dichorionic diamniotic pregnancy had NIPS results of suspected T21 in only 1 twin; this finding was confirmed through karyotype and a selective feticide was performed. A live birth was reported for the other twin. Trisomy 7 (T7) was suspected in 1 twin from a monochorionic diamniotic pregnancy, with normal NIPS findings for the other. Twin-to-twin transfusion syndrome was also present and resulted in fetal demise of the receipt twin at 25 weeks and a live birth of the donor twin at 28 weeks. Importantly, the T7 finding was not confirmed through diagnostic testing; the authors hypothesized that the T7 NIPS result was likely a mosaic artifact.[30]

A report from a commercial laboratory presented the results of 30,826 mixed-risk twin samples submitted between October 2011 and December 2017.[32] Of these, 635 had positive NIPS results: T21, n = 435; T18, n = 138; T13, n = 62. Despite the large numbers of positive NIPS results, confirmation of findings was communicated by the submitting physician for only 27, 13, and 10 samples, respectively. The authors further describe an "Enhanced Sequencing" option, selected by more than half of individuals, to screen for additional aneuploidies and microdeletion syndromes. Seven samples had a positive

Table 2 Diagnostic performance statistics of NIPS in twin gestations

Test Statistic	No. of Studies	Result (%) (95% CI)	I^2 (%)
Trisomy 21			
Sensitivity	7	98.18 (88.19-99.74)	0
Specificity	7	99.93 (99.78-99.98)	0
PPV	7	94.74 (84.91-98.29)	0
NPV	7	99.98 (99.83-100)	0
FPR	7	0.07 (0.02-0.22)	0
Accuracy	7	99.82 (99.61-99.92)	0
DOR[a]	7	6586.60 (1696.39-25573.83); $P < .0001$	0
Trisomy 18			
Sensitivity	5	90.00 (67.62-97.49)	0
Specificity	6	99.95 (99.80-99.99)	0
PPV	5	90.00 (67.62-97.49)	0
NPV	6	99.95 (99.80-99.99)	0
FPR	6	0.05 (0.01-0.20)	0
Accuracy	6	99.83 (99.61-99.92)	0
DOR[a]	5	3606.40 (710.38-18308.67)	0
Trisomy 13			
Sensitivity	4	80.00 (30.90-97.28)	0
Specificity	5	99.93 (99.41-99.99)	0
PPV	4	81.75 (1.82-99.91)	0
NPV	5	99.97 (99.82-100)	0
FPR	5	0.07 (0.01-0.59)	0
Accuracy	5	99.76 (99.39-99.91)	20.7
DOR[a]	4	1350.78 (206.12-8852.31)	0

Results do not include studies without adequate data to include in meta-analyses.

DOR, diagnostic odds ratio; *FPR*, false positive rate; *NIPS*, noninvasive prenatal screening; *NPV*, negative predictive value; *PPV*, positive predictive value.

[a]Data presented as odds ratio.

NIPS result for trisomy 16 and 6 samples received positive results for microdeletions. Four of the microdeletion results were reported to have diagnostic testing; 3 were TPs and 1 was FP. The other 2 cases were not confirmed diagnostically but were reported to be consistent clinically with the suspected microdeletion syndrome. All of the samples positive for microdeletions were in higher-risk samples (ie, ultrasound finding or other high risk). Of the 7 suspected cases of T16, 6 were reported as fetal (cotwin) demise after NIPS or as spontaneous abortion. Of these, 2 were reported to be FP after karyotyping was completed from amniocentesis.[32]

Overall, few studies have comprehensively evaluated the use of NIPS for twin gestations. The results from our meta-analyses show NIPS performance in this population are generally comparable to performance in singleton pregnancies for T21, T18, and T13. Results for other aneuploidies or microdeletions were less frequently reported and no firm conclusions can be drawn about the performance of NIPS for these outcomes. Very limited data is available on triplets or higher order multiple gestations.

SCAs

In total, 33 studies reported on identification of SCAs and 28 provided sufficient data to include in random-effects meta-analyses (Supplemental Material). We analyzed studies reporting on any SCA together (overall) and separately for the specific SCA (eg, XXX).

For screening of all SCAs, our meta-analyses found sensitivity, specificity, NPV, and high accuracy of NIPS; however, the PPV for SCAs was <50%, substantially lower than the PPV of NIPS for T21. When considering individual SCAs separately, we observed similar high-performance metrics for sensitivity, specificity, accuracy, NPV, and DOR, but PPVs ranged from 30% (45, X) to 74% (47, XXY; 47, XYY). The number of studies contributing to these analyses was generally small, although most studies reported sufficient data to include in meta-analyses for PPV. FPRs were similarly variable (Supplemental Material).

In addition to the 28 studies included in meta-analyses, 5 studies reported relevant SCA outcomes for NIPS.[24,27,29,34,35] DiNonno et al[34] described NIPS performance for common trisomies and SCAs from more than 1 million test results generated from 2014 to 2017, comparing PPVs obtained in individuals of advanced maternal age to those younger than 35 years. They found combined NIPS positive result rates for T18, T13, and 45, X declined over the 4-year period, commensurate with the uptake of NIPS by younger individuals without prior risk factors. Comparing results only for those with confirmation through ultrasound, pregnancy loss, or diagnostic testing, the PPV for 45, X in individuals aged <35 years was 92.0% (95% CI = 87.5%-94.9%) vs 88.5% (95% CI = 80.1%-93.6%) in individuals aged 35 years old or older.[34]

SCAs from a mixed-risk population from Germany was reported by Tekesin et al.[24] Among the 19 individuals with a suspected SCA, only 8 had confirmatory testing through either chorionic villus sampling ($n = 2$) or amniocentesis ($n = 6$). Of the 8, 6 were reported as normal, whereas the single case of XXY and 1 of the 6 cases of XXX were confirmed. Of the 11 individuals who did not receive confirmatory diagnostic testing, 1 of the 6 suspected cases of Turner syndrome was confirmed, 4 were reported as normal, and 6 did not undergo genetic testing.[24]

Snyder et al[35] presented the results from a retrospective analysis of 113,415 NIPS tests. The authors identified 36 suspected cases of a single autosomal trisomy (T21, T18, or T13) combined with an SCA. For T21 + SCA, 11 cases had clinical outcomes: 1 was fully concordant (T21, XXX), 8 were partially concordant (T21, 45, X), and 2 cases were completely discordant. Several suspected cases of T18 and T13 were also observed in this population in conjunction with a common trisomy. Full concordance was observed in a case of T18, XXY. However, all of the positive results were obtained from individuals with a high risk.

RATs

In total, 18 studies reported data pertaining to identification of RATs. Only 3 of these adequately reported data to enable determination of full test performance characteristics[19,26,36] (Supplemental Material). At a minimum, 17 of the included studies reported the numbers of TP and FP. For each rare chromosomal trisomy, at least 1 study reported a screen-positive result. However, in those with a positive result, those with no confirmatory testing and/or missing from follow up ranged from 0% to 100%. Consequently, quantitative analysis was performed for all RATs together and results pertaining to specific trisomies are narratively described (Supplemental Material).

CNVs

In total, 17 studies reported the ability of NIPS to detect CNVs (microdeletions or microduplications). The sample sizes in each study were relatively small and the sensitivities varied greatly. Tekesin et al[24] reported 7 cases that screened positive for DiGeorge syndrome (22q11.2 deletion), yet none were confirmed via diagnostic testing. Yin et al[37] confirmed TP CNVs in 10 of the 12 cases tested through amniocentesis, whereas in the study by Zheng et al,[36] none of the 3 CNVs were confirmed.

Three additional studies reported a relatively low number of samples with CNVs detected.[21,30,38] Taken together, they detected 14 CNVs, of which 5 were TP and 9 were FP. Reported overall sensitivity to detect CNVs ranged from 69.44%[29] to 80.56%.[39] When stratified by CNV size, in general, the sensitivity to detect larger CNVs was better than for detecting smaller CNVs. The sensitivity to detect CNVs larger than 5 megabases (Mb) was >90%, whereas for those

smaller than 5 Mb, it was 68.42%.[39] In the study by Ye et al,[40] the sensitivity to detect CNVs larger than 2 Mb (81.58%, 31/38) was higher than for detecting those smaller than 2 Mb (21.43%, 3/14).

In a study by Lin et al[27] with follow up of 10,975 negative NIPS results, there were 166 cases with adverse pregnancy outcome, of which 8 had diagnostic testing. Four cases of chromosome abnormalities were confirmed, including 2 results showing microdeletions/microduplications.

Liang et al[41] was able to stratify PPV on the basis of syndromes (n = 32), 93% (DiGeorge syndrome), 68% (22q11.22 microduplication), 75% (Prader-Willi/Angelman syndrome), and 50% (cri-du-chat syndrome). For the remaining genome-wide CNVs (n = 88), combined PPVs were 32% (CNVs ≥10 Mb) and 19% (CNVs <10 Mb). Chen et al[31] showed an overall PPV of 28.99% with the best sensitivity between 5 and 10 Mb in size (20.83% for ≤5 Mb, 50.00% for 5 to 10 Mb, 27.27% for >10 Mb) for CNVs. Schwartz et al[42] had the largest sample size of screen-positive CNV cases (N = 349) with an overall PPV of 9.2%.

A large study (N = 80,449) of NIPS for a panel of microdeletion syndromes (22q11.2 deletion, 1p36 deletion, cri-du-chat, Prader-Willi, Angelman) was reported from a laboratory sample after revision of their algorithm.[43] In >42,000 individuals screened for the full panel, in those without any abnormal ultrasound findings, PPV was 18.5% for 22q11.2 deletion, 50% for 1p36 deletion, 50% for cri-du-chat, 0% for Prader-Willi, and 10% for Angelman syndromes; however, there was incomplete follow up of positive NIPS results. For individuals with abnormal ultrasound findings identified before NIPS, PPVs were significantly higher: 100% for 22q11.2, 1p36 deletion, and cri-du-chat syndromes. The authors report that the revision to their algorithm both improved PPV and reduced FPRs for these microdeletion syndromes.[43]

Psychosocial outcomes

There is limited literature regarding psychosocial outcomes after NIPS. In a study of 40 participants who received positive NIPS results, a significant portion regretted their decision to have NIPS in light of the stress and additional medical interventions they experienced. However, this was a biased sampling of individuals who posted in online forums.[44] Eight participants expressed positive opinions, 20 had mixed feelings, and 12 had negative opinions.[44] In another study that assessed the effect of genetic counseling after positive NIPS results, 76% of participants accepted confirmatory diagnostic testing, whereas 24% elected not to proceed with follow-up diagnostic testing.[45] Given the minimal evidence, no conclusions can be drawn about the impact of NIPS on psychosocial outcomes.

Maternal conditions

We identified 14 studies that included outcomes for maternal conditions (Supplemental Material). Of these, 8 were specifically directed at reporting maternal outcomes, the others were reported as part of a larger NIPS study. One study[35] included cases that were published in another study.[46] The predominant reported results were maternal neoplasms (n = 5 studies) and maternal X chromosome abnormalities (n = 3 studies). Other outcomes included actionable maternal CNVs (n = 4 studies), Duchenne muscular dystrophy gene CNV identification (n =1), and various structural chromosomal abnormalities, such as mosaicism for an interstitial deletion and an unbalanced translocation. In a study describing the implementation of NIPS as a universal screening method in Belgium, reported maternal imbalances were found in 0.32% of NIPS results.[20] Another study similarly identified 9 clinically actionable CNVs in 3053 samples (0.29%).[29] In this study, 8 of 9 patients had symptoms of the identified disorders with 1 of 9 asymptomatic with a genetic diagnosis of Ehlers-Danlos syndrome.[29] Two confirmed maternal cases of 22q11.2 deletion were identified in a large laboratory study of NIPS from the United States for a panel of 5 microdeletion syndromes.[43] One additional maternal case was unconfirmed in the parent; however, the individual had learning disabilities and tetralogy of Fallot, which are both associated with 22q11.2 deletion syndrome.[43] Neoplasms were identified by noting unique gains and losses of multiple CNVs across chromosomes; neoplasms sometimes included uterine myomas and therefore did not consistently represent a malignancy. The Belgian population-level study reported maternal neoplasms were identified in 0.008% of NIPS results.[20] Although X chromosome anomalies were identified, including 2 interstitial X deletions,[47] 47, XXX,[46,48] and a mosaic 45, X/47, XXX complement, it is unclear if these findings had any effect on maternal health. Maternal outcomes were consistently a rare finding in NIPS and follow up with clinical outcomes was not reported.

Uptake of diagnostic testing

We identified 10 studies that included outcomes for uptake of diagnostic testing.[18,20,29,49-55] Some studies examined the rate of uptake of diagnostic testing in those screening positive on NIPS whereas others looked at the rate of uptake of diagnostic testing over time, comparing the period before NIPS was available with the period after NIPS was available.

Screening for chromosome 7 aneuploidy as part of "supplemental NIPT" in 31,250 patients found 35 at high risk.[50] Of those, 25 patients (71%) chose diagnostic testing and 2 pregnancies had CNVs involving part of chromosome 7.[36] A general screening of 2998 patients found 278 with high-risk results. Of those, 98.5% received diagnostic

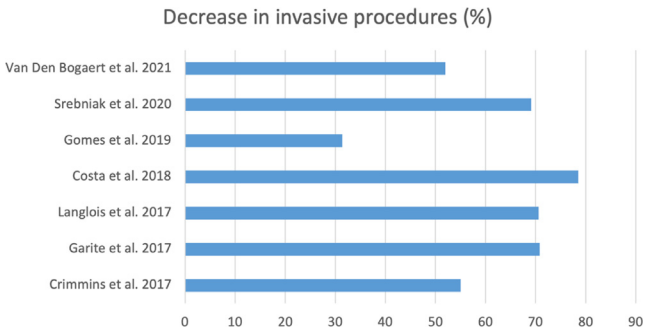

Figure 1 Percent reduction of diagnostic testing after noninvasive prenatal screening implementation.

testing, whereas only 4 patients did not.[29] Because neither of these studies looked at diagnostic testing over time, they are not included in Figure 1. In a South Korean medical center, the mean number of amniocenteses performed before NIPS was 8.8 per month that decreased to 4.1 per month after offering NIPS.[51] Because the raw data on total numbers or percentages of procedures was not provided, this study was not included in Figure 1.

One of these studies was limited to modeled data. In the model, if all participants received an amniocentesis after a "positive" result, there would be a 55% reduction in the rate of amniocentesis performed when initially screened with NIPS.[49] The total number of diagnostic procedures performed was reported to drop from 1176 in 2009 to 846 in 2015 and then 363 in 2018, likely due to the introduction and subsequent growing use of NIPS,[52] although the total number of patients screened was not provided. In another study, the rate of diagnostic testing dropped from 3.5% (before implementation of NIPS) to 2.4% (with the use of a contingent model incorporating NIPS), although, this was not statistically significant.[53] In the high-risk group, 83.3% (25/30) had a diagnostic test. In the intermediate-risk group only 12.2% (6/49) chose diagnostic testing, whereas 75.5% opted for NIPS (37/49). Costa et al[18] described that use of NIPS decreased the potential rate of diagnostic procedures from 8.2% with maternal serum screening (MSS) alone to 1.9% with a combination of NIPS and MSS. In this group of 789 patients, there were 15 diagnostic procedures performed, with potentially an additional 50 procedures in patients receiving a high-risk MSS, but a low-risk NIPS. In another study, they postulated that the rate of diagnostic testing could potentially be as high as 6.8% (79/1165) with traditional screening, whereas in their study, overall it was 2% (23/1165) with 1.2% (14/1151) of individuals with a negative NIPS result choosing diagnostic testing.[54] In the final study, Garite et al[55] found an overall 70.8% (calculated for this publication) decline in procedures (73% decrease in amniocenteses and 62% decrease in chorionic villus sampling) between the first 6 months of the control period and the last 6 months of the study period.

Although a significant majority of patients who receive a high-risk result do choose to pursue diagnostic testing, overall, it appears that the total number of patients choosing diagnostic testing has decreased over time ranging from a 31% to 79% decrease (see Figure 1) depending on the study. The findings from the Belgian population study comparing 2013, before NIPS, uptake of diagnostic testing to 2018, after universal NIPS, found a 52% reduction, which was larger than would be expected on the basis of the incidence of T21 alone.[20] This choice of whether to pursue diagnostic testing may vary based on the specific aneuploidy, availability of genetic counseling, and personal values and decision-making, however, the data were not available to assess this level of granularity.

Economic impact

Of the 10 studies that reported outcomes pertaining to the cost-effectiveness of NIPS performed in a general-risk population, only 1 was done with the societal perspective with a time horizon of the maternal lifespan, in a theoretical cohort of 4 million individuals in the United States.[56] In this study, the authors compared NIPS to detect T13/T18/T21 with NIPS for the common trisomies and 5 microdeletion syndromes. If the cost to report the microdeletions added $47 or less to the cost of NIPS for the main trisomies, NIPS plus microdeletion screening increased quality-adjusted life years by 977, decreased overall costs by $90.9 million per year, and would result in fewer neonatal deaths and second trimester miscarriages.[56] The remaining studies compared NIPS, either as a universal screening method or as a contingent method presented after some initial risk evaluation. Notably, these studies were nearly all performed from a public payer perspective and limited the time horizon to the testing duration or length of pregnancy only (Supplemental Material).

Test failure

Although not an original KQ for this SER, the guideline panel requested information regarding test failure rates, given their known association with aneuploidy. Unfortunately, this was not reported in a standard manner across studies. Some reported only the overall failure (or no-call) rate without mention of redraws, whereas others included their redraw failure (or success) rate, with some even more granular, separating out failures from the first test compared with failures from the second. Estimated failure/no-call rate of NIPS was 0.85% (95% CI = 0.58%-1.23%) in 31 studies (Supplemental Material). Although heterogeneity was considerable (I^2 = 99%), no subgroup analyses were performed owing to the inconsistency and variability of the studies. Overall, NIPS failure rate appears relatively infrequent; however, this metric may be subject to considerable publication bias.

Change in birth rates

We identified a single study that reported on a change in birth rates after implementation of universal NIPS. Belgium,

which was the first country to implement universal access and reimbursement of NIPS as a first-tier prenatal screening test, compared the rate of trisomy 21 live births from 2014 to those in 2018. The rate decreased from 0.06% of all live births to 0.04% during the time period in question, a decline that the authors could not explain through population-level changes responsible for a concurrent rise in trisomy 21 miscarriages. They posit that the reduction may result from pregnancy termination combined with the improved FPRs for NIPS, as compared with first trimester combined screening.[20]

Risk of bias assessment

We observed no evidence of publication bias across most outcomes, although there was suspicion of publication bias for test failure rate. Risk of bias for individual studies reporting the clinical or diagnostic performance outcomes uncovered serious risk of bias for confounding and missing data (ROBINS-I) and patient selection and flow and timing (QUADAS2) domains (Supplemental Material). Risk of bias was assessed across 20 domains identified in the Consolidated Health Economic Evaluation Reporting Standards checklist[12] and Drummond criteria.[57] Most compared NIPS with at least 1 option without NIPS. Except for the Avram et al[56] study, none reported a discount rate or a time horizon beyond the duration of pregnancy. An overall risk of bias was not calculated for the economic studies; however, few domains received a high risk of bias judgment for more than a single study. Unreported and under-reported data was a significant concern (Supplemental Material).

Discussion

This assessment validates that NIPS with cfDNA is the most sensitive and specific screening test for fetal Down syndrome, T13, and T18 in both singleton and twin pregnancies. In contrast to conventional serum analyte screening, it can identify maternal conditions, such as aneuploidies and malignancies. Although rare, maternal aneuploidy findings are only possible with cfDNA screening. Other outcomes, such as RATs and CNVs (predominantly deletions) in both fetus and mother can be identified. However, the clinical utility of these findings is limited, given the rarity of these events and the lack of systematic follow up of clinical outcomes.

Several recent reviews and meta-analyses have been published on NIPS.[4,58-62] Compared with traditional screening, the 2019 health technology assessment by Health Quality Ontario determined that NIPS was effective in a general or average-risk population to screen for T21, T18, and T13.[58] Our results similarly show the high performance of NIPS to screen for the common trisomies in a general population. Of the studies that used meta-analysis of NIPS to screen for SCAs, we observed that several included high-risk population studies in their analyses and their results may not be as generalizable to an average-risk population. Despite this difference, we observed relatively consistent results with our meta-analyses for SCAs to these published studies, supporting our conclusion that NIPS is also effective and accurate for SCA screening.

Our SER and meta-analysis present several strengths and limitations. Building on existing evidence, we limited our literature search for several KQs to obtain the most recent data. We considered the utility of NIPS beyond diagnostic performance by including the uptake of diagnostic tests, the impact on individuals' psychosocial status, and the identification of maternal conditions. The large number of studies included in our SER is a considerable strength.

Nevertheless, there are some limitations to our study. First, although we revised our search query to account for the variety of definitions which describes NIPS in the literature, it is possible we did not identify all relevant studies. Second, despite prespecifying an analysis plan to address expected heterogeneity, there may be other variables that we did not include in our sensitivity analyses that contribute to the variation observed between studies. Third, we included studies in our meta-analyses for which the reviewers were confident in the data reported. It is possible that this confidence was misplaced, particularly for TNs, causing us to inappropriately include studies in our quantitative analyses. Furthermore, our meta-analyses did not use the bivariate model, as detailed in Reitsma et al.[63] Although there was sparse data for many of the reported studies, we re-evaluated our analyses (data not shown) and determined that the difference between our results and the bivariate model were small (eg, T21 $\text{sensitivity}_{\text{bivariate}} = 97.6\%$ [95% CI = 96.0%-98.6%] compared with reported results [98.8%, 95% CI = 97.8%-99.3%]), although the area under the curve remained consistent regardless of the model (area under the $\text{curve}_{\text{T21}} = 99\%$). Finally, although our research questions were developed to compare NIPS with conventional serum analyte screening, we did not identify any studies reporting direct comparisons that met our inclusion criteria.

Limitations of the included studies themselves were numerous. It was often difficult to distinguish between low- and high-risk cohorts in individual studies. Information on the complete ascertainment of cases is lacking, given that there is a lack of complete follow up to identify TNs and FNs through diagnostic testing or postnatally, although these numbers are expected to be small. Studies mostly relied on local providers to evaluate fetal outcomes through physical assessment or a chart review performed to determine the newborn phenotype that may introduce error. A few studies used more objective means of obtaining this data, such as national databases. A systematic follow up of individuals with low-risk NIPS results would provide a more accurate picture of the TNs and were unavailable for review. Furthermore, the laboratory techniques used, including sequencing methods, or cutoffs for test failures or screen positives are not standardized, may differ more owing to the applications in other

countries, and the details were inconsistently reported. These failures can be due to a variety of factors. Some may have issues with the specimen itself such as inadequate sample volume or coagulation and were therefore unable to complete the sequencing process. Others may successfully complete sequencing but have no result available after an issue with analysis. This can be due to a variety of reasons, including low fetal fraction, with minimum requirements varying between laboratories and some using a method to further amplify the fetal fraction.[64] A redraw can be recommended, in which a new blood specimen is collected. In general, increased gestational age (over 20 weeks) correlates with increased fetal fraction, so collection of a specimen later in pregnancy may overcome the issue of low fetal fraction, although this would reduce the clinical utility of screening. Other issues include sample contamination, high sequence homology between maternal and fetal, or other quality control metrics.

There was limited literature available to evaluate the psychosocial outcome of individuals undergoing NIPS. Although multiple studies were identified that surveyed attitudes toward NIPS, very few were available in which NIPS was actually performed, patients received results, and then were assessed for levels of anxiety, stress, and/or regret in a systematic manner. Additional studies with a systematic evaluation approach on a large cohort is needed to better understand the psychosocial impact of NIPS, which may further elucidate the uptake (or lack thereof) of NIPS in the general population. Moreover, the psychosocial reception of NIPS may also be affected by the cost for patients and payer coverage. Economic analyses based in the United States from the patient perspective are lacking; evidence from national health care systems such as Belgium, Canada, and the Netherlands suggest most pregnant individuals find NIPS as a primary screening method for fetal chromosomal aneuploidies acceptable and have not identified significant negative impact of NIPS on psychosocial outcomes.

As described in this SER, the performance of NIPS is significantly poorer when targeting RATs and CNVs than when looking for the common trisomies. This is likely because of the rarity of RATs and the insufficient data available to properly develop a method that can distinguish between clinically relevant RATs found in the fetus vs confined placental mosaicism. In addition, the NIPS technologies were originally designed to detect the common trisomies, and not to identify small CNVs. Deletions are more difficult to identify in the background of a normal maternal karyotype than are trisomies. Large collaborative studies may be needed to generate a sufficient cohort to develop a singular method with adequate sensitivity and specificity for findings other than common trisomies. Additional outcome studies are needed to understand the unique clinical value of NIPS, specifically for SCAs, RATs, and CNVs when compared with other approaches.

Comparisons between studies are difficult, because there is no standardized testing method, fetal fraction cutoffs and calculation methods vary, and there are different initial gestational ages for testing. Further delineation of sensitivity and specificity of NIPS methodologies by independent researchers is needed to determine the best modality and to improve the diagnostic utility. Ideally, studies would include a comprehensive ascertainment of clinical outcomes to calculate the TN rate. This information would help to develop best practice guidelines and improve patient care. Despite the large number of studies included in our analysis, we identified few that considered the psychosocial impact of NIPS, particularly in light of additional information (eg, maternal conditions) that would not be captured using traditional screening techniques.

Conclusion

Worldwide, and across all laboratory platforms, NIPS using cfDNA is the most effective screening test for the autosomal T21, T18, and T13 in singleton and twin gestations, with both high detection and low FPRs. Although less accurate for SCAs, RATs, and CNVs, it is the only laboratory-based prenatal screen that can identify these at all. The incidental identification of maternal conditions is rare and makes for potentially difficult patient counseling. Finally, no conclusions can be drawn with respect to the potential psychosocial effects of this test on the screened population. Despite its accuracy, NIPS using cfDNA is a screening test for which confirmation of a screen-positive test with a diagnostic procedure remains indicated.

Conflict of Interest

N.C.R. is a consultant for The Jackson Laboratories and the ObG Project. E.S.B. and M.L.L. serve as directors in, and D.L. is employed by, clinical laboratories that perform a breadth of genetic and genomic analyses on a fee-for-service basis. All other authors declare no conflicts of interest.

Additional Information

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