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Medical Policy Manual

Topic: Sequencing-based Tests to Determine Trisomy 21 from Maternal Plasma DNA

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Section: Genetic Testing

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

National guidelines recommend that all pregnant women be offered screening for fetal chromosomal abnormalities, the majority of which are aneuploidies (an abnormal number of chromosomes).[1] Fetal chromosomal abnormalities occur in approximately 1 in 160 live births. The trisomy syndromes are aneuploidies involving 3 copies of one chromosome. Trisomies 21(Down syndrome, T21), 18 (Edwards syndrome, T18) and 13 (Patau syndrome, T13) are the most common forms of fetal aneuploidy that survive to birth. The most important risk factor for Down syndrome is maternal age, with an approximate risk of 1/1500 in young women that increases to nearly 1/10 by age 48.[2]

There are numerous limitations to standard screening for these disorders using maternal serum and fetal ultrasound. Commercial non- invasive, sequencing-based testing of maternal serum for fetal trisomy 21, 18, and 13 has recently become available and has the potential to substantially alter the current approach to screening. The detection rate for various combinations of non- invasive testing ranges from 60-96% when the false-positive rate is set at 5%. When tests indicate a high risk of a trisomy syndrome, direct karyotyping of fetal tissue obtained by amniocentesis or chorionic villous sampling (CVS) is required to confirm that trisomy 21 or another trisomy is present. Both amniocentesis and CVS are invasive procedures and have an associated risk of miscarriage.

Sequenced-based testing of maternal serum for fetal trisomy 21, 18, and 13 may reduce unnecessary amniocentesis and CVS procedures and has the potential to improve outcomes. The test technology

involves detection of fetal cell-free DNA fragments present in the plasma of pregnant women. As early as 8 to 10 weeks of gestation, these fetal DNA fragments comprise 6 to 10% or more of the total cell-free DNA in a maternal plasma sample. The tests are unable to provide a result if fetal fraction is too low, that is, below about 4%. Fetal fraction can be affected by maternal and fetal characteristics. For example, fetal fraction was found to be lower at higher maternal weights and higher with increasing fetal crown-rump length.

Sequencing-based tests use 1 of 2 general approaches to analyzing cell-free DNA. The first category of tests uses quantitative or counting methods. The most widely used technique to date involves massively parallel sequencing (MPS; also known as next generation or "next-gen" sequencing) which can be used to design assays for prenatal diagnosis of chromosomal trisomy. DNA fragments are first amplified by polymerase chain reaction (PCR); during the sequencing process, the amplified fragments are spatially segregated and sequenced simultaneously in a massively parallel fashion. Sequenced fragments can be mapped to the reference human genome in order to obtain numbers of fragment counts per chromosome. The sequencing-derived percent of fragments from the chromosome of interest reflects the chromosomal representation of the maternal and fetal DNA fragments in the original maternal plasma sample. An additional technique to the first approach of testing is direct DNA analysis which analyzes specific cell-free DNA fragments across samples and requires approximately a tenth the number of cell-free DNA fragments as MPS. The digital analysis of selected regions (DANSR™) is an assay that uses direct DNA analysis.

The second general approach is single-nucleotide polymorphism (SNP)-based methods. These use targeted amplification and analysis of approximately 20,000 SNPs on selected chromosomes (e.g., 21, 18 and 13) in a single reaction. A statistical algorithm is used to determine the number of each type of chromosome.

In order to be clinically useful, the technology must be sensitive enough to detect a slight shift in DNA fragment counts among the small fetal fragment representation of a genome with a trisomic chromosome against a large euploid maternal background. Whether sequencing-based assays require confirmation by invasive procedures and karyotyping depends on assay performance. However, discrepancies between sequencing and invasive test results that may occur for biological reasons could make confirmation by invasive testing necessary at least in some cases, regardless of sequencing test performance characteristics.

Fetal sex determination

Sequencing-based testing of maternal serum for determination of fetal sex in the first trimester of pregnancy is possible. Fetal sex can also be determined non-invasively by ultrasonographic examination of the fetal genitalia. Studies have reported that the accuracy of ultrasound ranges from 68 to 78% at 11 weeks to 83 to 100% at 13 weeks.[3] Some laboratories offer screening for fetal sex determination, as an additional option to sequencing-based testing of maternal serum for aneuploidies.

Microdeletion syndromes

Microdeletion syndromes are defined as a group of clinically recognizable disorders characterized by a small (< 5Mb) deletion of a chromosomal segment spanning multiple disease genes, each potentially contributing to the phenotype independently. The phenotype is defined as the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. Clinical implications of prenatal testing for microdeletions, such as 22q deletion syndrome (DiGeorge),

15q (Prader-Willi/Angelman syndromes), 5p (Cri-du-chat syndrome), and 1p (1p36 deletion syndrome) are not defined. Whether prenatal diagnosis is appropriate is uncertain given the inherent difficulty in accurately predicting the phenotype for the myriad of microdeletion syndromes. Though laboratories offer screening for microdeletion syndromes as an additional option, screening for these microdeletion syndromes is not currently the main intent of prenatal screening programs.

Regulatory Status

None of the commercially available sequencing assays for detection of trisomy 21, 18 and 13 or other chromosomal abnormalities has been submitted to or reviewed by the U.S. Food and Drug Administration (FDA). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service. Laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories offering LDTs must be licensed by CLIA for high-complexity testing.

The following commercial tests are available:

- The Harmony™ Prenatal Test from Ariosa Diagnostics (formerly Aria) tests for trisomies 21, 18, and 13. This test is available from Integrated Genetics, a division of LabCorp. (Uses directed DNA analysis, results reported as risk score.) There is an option to test for fetal sex chromosome analysis.
- MaterniT21™ Plus from Sequenom tests for common trisomies 21, 18 and 13 and several aneuploidies. (Uses MPS; reports results as positive or negative.) As part of the "Enhanced Sequencing Series" option, MaterniT21 Plus includes testing for chromosomes 22, 16, 22q deletion syndrome (DiGeorge), 15q (Prader-Willi/Angelman syndromes), 5p (Cri-du-chat syndrome), and 1p (1p36 deletion syndrome).
- The verifi® Prenatal Test from Verinata Health tests for trisomies 21, 18, 13, X, Y, monosomy X, and fetal sex chromosomes. (Uses MPS and calculates a normalized chromosomal value [NPS]; reports results as 1 of 3 categories: No Aneuploidy Detected, Aneuploidy Detected, or Aneuploidy Suspected.)
- The Panorama prenatal test from Natera tests for aneuploidy at 13, 18, 21, X, Y and triploidy. (Uses SNP technology; results reported as risk score.) Offers an add-on option for a microdeletion screen panel for microdeletion syndromes that combined, result in intellectual and sometimes physical disorders that are often severe, have equal risk across all maternal ages and occur in approximately 1 in 1,000 live births.

NOTE. For components of the available nucleic acid sequencing-based tests please see Appendix I for details.

MEDICAL POLICY CRITERIA

I. Aneuploidy Screening

A. Nucleic acid sequencing-based testing of maternal plasma for aneuploidies (Trisomy 13, 18, 21, Turner syndrome, Klinefelter syndrome, Jacob's syndrome) may be considered **medically necessary** in women with high-risk singleton pregnancies when one or more of the following criteria are met:

1. Maternal age 35 years or older at delivery;

2. Fetal ultrasonographic findings indicate increased risk of aneuploidy

3. History of previous pregnancy with a trisomy;

4. Standard serum screening test positive for aneuploidy; or

5. Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.

B. Nucleic acid sequencing-based testing of maternal plasma for aneuploidies (Trisomy 13, 18, 21, Turner syndrome, Klinefelter syndrome, Jacob's syndrome) in women who do not meet the above criteria is considered **investigational.**

II. Other Indications

A. Nucleic acid sequencing-based testing of maternal plasma for fetal sex determination is considered **not medically necessary**.

B. Nucleic acid sequencing-based testing of maternal plasma for all other indications, including but not limited to screening for microdeletion syndromes, is considered **investigational**.

POLICY GUIDELINES

This policy focuses on detection of trisomy 21, as it is the most common cause of human birth defects and provides the impetus for current maternal serum screening programs. Detection of trisomy 21 by DNA-based sequencing methods would likely be representative of the testing technology and interpretation for additional aneuploidies, including Trisomy 13, 18, Turner syndrome, Klinefelter syndrome, and Jacob's syndrome. The prevalence of other trisomy syndromes is much lower, however, than the prevalence of trisomy 21.

Studies published to date report rare but occasional false positives. In these studies, the actual false positive test results were not always borderline; some were clearly above the assay cutoff value, and no processing or biological explanations for the false positive results were reported. Therefore, before testing, women should be counseled about the risk of a false positive test and that karyotyping via invasive prenatal diagnostic testing would be necessary to exclude the possibility of a false positive test.

SCIENTIFIC BACKGROUND

Literature Review

Assessment of a diagnostic technology such as maternal plasma DNA sequencing tests typically focuses on 3 parameters:

1. Analytic validity
2. Clinical validity (i.e., sensitivity and specificity) in appropriate populations of patients; and
3. Demonstration that the diagnostic information can be used to improve patient health outcomes (clinical utility).

The evidence on these three questions was addressed in a 2012 BlueCross BlueShield Association Technology Evaluation Center (TEC) Assessment.[4] The Assessment focused on detection of trisomy 21/Down syndrome because the majority of published data was concentrated on this trisomy, large numbers of cases were included in several publications, and all companies had published data regarding the detection of trisomy 21. The Assessment also reviewed the available data for detection of trisomy 18 and 13. The scope of the TEC Assessment was limited to the evaluation of tests that are available in the United States. Additional literature published after publication of the TEC Assessment is also addressed in the analysis below.

Analytic Validity

No studies were identified that provided direct evidence on analytic validity. Each of the commercially available tests uses massively parallel sequencing (MPS; also called next generation sequencing), a relatively new technology but not an entirely new concept for the clinical laboratory. Currently, there are no recognized standards for conducting clinical sequencing by MPS. On June 23, 2011, the U.S. Food and Drug Administration (FDA) held an exploratory, public meeting on the topic of MPS, in preparation for an eventual goal of developing "a transparent evidence-based regulatory pathway for evaluating medical devices/products based on NGS [next generation sequencing] that would assure safety and effectiveness of devices marketed for clinical diagnostics."[5] The discussion pointed out the differences among manufacturers' sequencing platforms and the diversity of applications, making it difficult to generate specific regulatory phases and metrics. It was suggested that "the process may need to be judged by the accuracy and fidelity of the final result." A consistent discussion trend was that validation be application- specific. Thus, technical performance may need to be more closely linked to intended use and population and may not be generalizable across all sequencing applications. Each of the companies currently offering a maternal plasma DNA sequencing test for fetal trisomy 21 has developed a specific procedure for its private, CLIA-licensed laboratory where all testing takes place.

Conclusions

Although all currently available commercial tests use MPS, actual performance and interpretive procedures vary considerably. Clinical sequencing in general is not standardized or regulated by the FDA or other regulatory agencies, and neither the routine quality control procedures used for each of these tests, nor the analytic performance metrics have been published.

Clinical Validity

- High-risk Women with Singleton Pregnancies

  To determine clinical validity, new tests must be compared against a gold standard. The comparison for maternal plasma DNA sequencing-based tests for trisomy 21 is karyotyping. Eight studies provided data on the sensitivity and specificity of the final, clinical nucleic acid sequencing-based assay of maternal plasma for trisomy 21 in singleton pregnancies.[6-15] Tests from 4 commercial sources were identified: 2 studies used the Sequenom test, 2 studies used the Verinata test, 4 studies used the Ariosa Diagnostics test, and 1 study used the Natera test. Seven studies were entirely prospective, and 2 retrospectively evaluated archived samples. Eight of 9 studies were industry-funded; in the non-industry-funded study, testing was provided gratis.

  The approach to analysis varied. Some studies analyzed samples from all enrolled women and others analyzed samples from all women with pregnancies known to have a trisomy syndrome and selected

controls (i.e., nested case-control analysis within a cohort). The studies evaluated the results of maternal fetal DNA testing in comparison to the gold standards of karyotyping or, in individual cases when a sample did not allow karyotyping, fluorescence in situ hybridization (FISH) for specific trisomies. All studies included testing for trisomy 21 and some additionally tested for trisomy 18 and/or trisomy 13. There were fewer cases of T18 and T13 per study compared to T21. Four studies had 50 or more cases of T21, and one study, Palomaki et al. 2011[7], had 212 cases.

The sensitivity and specificity estimates of testing for trisomy 21 in singleton pregnancies were uniformly high. The sensitivity ranged from 99.1% to 100%, and the specificity ranged from 99.7% to 100%.

Conclusions

Data from the available published studies consistently reported a very high sensitivity and specificity of maternal plasma DNA sequencing-based tests for detecting trisomy 21 in high-risk women with singleton pregnancies. Thus, there is sufficient evidence that the tests are accurate when used in this population.

- Average-Risk Pregnancies

Two studies have evaluated sequencing-based tests available in the U.S. for detecting trisomy 21 in average-risk singleton pregnancies. The studies were conducted by the same research group in the U.K. and both used the Ariosa (Harmony) test, which provides risk-scores rather than a positive versus negative result. The first study, by Nicolaides and colleagues did a preliminary analysis of the accuracy of cell-free DNA testing in a general population sample.[11] The authors evaluated archived samples from 2,049 women attending their routine first pregnancy visit at 11–14 weeks' gestation. Karyotyping results were available for only a small percentage of women in the study; for the rest of the enrollees, ploidy was imputed by phenotype at birth obtained from medical records. This study was judged to have a high risk of bias due to a high number of exclusions from analysis. Twenty-eight pregnancies ending in stillbirth or miscarriage were excluded for lack of karyotype; while unavoidable, these exclusions likely affect the case detection rate. Cases were verified primarily by phenotype at birth from medical records. Results were available for 1949 of 2049 cases (95%). In the remaining 5%, either the fetal fraction was too low or the assay failed. Overall, using the risk cutoff for the Harmony test, the trisomy detection rate was 100% (i.e., 10 of 10 cases identified) and there was a false-positive rate of 0.1%. The risk score was over 99% in all of the 8 cases of trisomy and both cases of trisomy 18. In the 1939 known or presumed euploid cases, risk scores for trisomy 21 and trisomy 18 were <0.1% in 1939 (99.9%).

Gill and colleagues prospectively studied 1005 pregnant women.[16] The authors evaluated a testing strategy that included analysis of serum markers (i.e., pregnancy-associated plasma protein-A [PAPP-A] and free beta-human chorionic gonadotropin [b-hCG]) and cell-free DNA at 10 weeks and ultrasound markers (i.e., nuchal translucency and presence or absence of fetal nasal bone) at 12 weeks. Parents were counseled primarily on the finding of the Harmony test if it indicated either a high- or low-risk of trisomy. If no results were available on the Harmony tests, parents were counseled based on combined first-trimester serum marker and ultrasound findings. Risk scores from cell-free DNA testing were available for 984 cases (98%), 27 of these required a second round of sampling. Risk scores were >99% for trisomy 21 in 11 cases and for trisomy 18 in 5 cases. In 1 case, the risk score for trisomy 13 was 34%. Sixteen of the 17 women with a high-risk score for aneuploidy underwent CVS and the suspected abnormality was confirmed in 15 of the 16 cases.

There was 1 case with a high risk-score for trisomy 21 and a negative CVS; at the time the article was written, the woman was still pregnant so the presence or absence of trisomy 21 could not be confirmed.

Conclusions

The data is limited on the diagnostic accuracy of cell-free DNA testing of women with average-risk singleton pregnancies. Two studies have been published—both are from the same research group in the U.K. and use the same sequencing-based test. The studies identified a small number of trisomies and did not confirm negative or positive findings in all cases. Thus, the evidence on accuracy of sequencing-based tests is less definitive for women with average-risk pregnancies than it is for women with high-risk pregnancies.

- Twin And Multiple Pregnancies

Detection of trisomy 21 in twin pregnancies was systematically evaluated in only one study, published in 2012 by Canick and colleagues; the study used the Sequenom test.[17] All 7 cases of twin pregnancies with Down syndrome were correctly classified. Five of these were discordant, where one twin had T21 aneuploidy and the other did not; 2 were concordant where both twins had T21 aneuploidy.

Conclusions

For women with multiple pregnancies, there is insufficient evidence to draw conclusions about the diagnostic accuracy of these tests for detecting trisomy 21 or any other aneuploidy.+

- Fetal sex determination

The current standard of care for fetal sex determination is ultrasound.

Three reviews report on the use of cell-free fetal DNA for fetal sex determination. Davaney and colleagues conducted a systematic review and meta-analysis to determine if noninvasive prenatal determination of fetal sex using cell-free fetal DNA provides an alternative to invasive techniques for some heritable disorders. From 57 selected studies, 80 data sets (representing 3524 male-bearing pregnancies and 3017 female-bearing pregnancies) were analyzed. Authors reported that despite interstudy variability, performance was high using maternal blood. Sensitivity and specificity for detection of Y chromosome sequences was greatest using RTQ-PCR after 20 weeks' gestation. Tests using urine and tests performed before 7 weeks' gestation were unreliable.

Wright et al. conducted a review and meta-analysis of the published literature to evaluate the use of cell-free fetal DNA for prenatal determination of fetal sex.[18,19] The authors reviewed 90 studies, incorporating 9,965 pregnancies and 10,587 fetal sex results. Overall mean sensitivity was 96.6% (95% credible interval 95.2% to 97.7%) and mean specificity was 98.9% (95% CI = 98.1% to 99.4%). The authors identified one limitation of their study as the inability to properly evaluate the proportion of inconclusive or uncertain results, which is known to be problematic with this technique and may vary with gestational age. Further, literature-based reviews are at risk of publication bias due to the suppression of unwanted findings. The authors concluded that fetal sex can be determined with a high level of accuracy by analyzing cell-free fetal DNA.

Colmant and others performed a review of the published literature evaluating the use of cell-free fetal DNA and ultrasound for prenatal determination of fetal sex during the first trimester of pregnancy.[20] The authors identified 16 reports of the determination of fetal sex in maternal blood and 13 reports of the determination by ultrasound. Authors determined a sensitivity and specificity of nearly 100% from 8 weeks of gestation for cell-free fetal DNA and from 13 weeks of gestation for ultrasound respectively. Authors concluded that fetal sex can be determined with a high level of accuracy by analyzing cell-free fetal DNA and at an earlier gestation than ultrasound.

Conclusions

While there is high diagnostic accuracy on the use of cell-free fetal DNA for fetal sex determination, evidence does not demonstrate how the use of nucleic acid sequencing-based testing for fetal sex determination is more beneficial than fetal ultrasound, the current clinical standard for fetal sex identification.

- Microdeletion syndromes

A single study was identified that addressed the use of use of cell-free fetal DNA for the screening or diagnosis of microdeletion syndromes. Jensen and others examined subchromosomal copy number variants through the sequencing of cell-free fetal DNA.[21] The authors examined a clinically relevant genomic region, chromosome 22q11.2, the location of a series of well-characterized deletion anomalies that cause 22q11.2 deletion syndrome. The authors sequenced cell-free fetal DNA isolated from maternal plasma samples obtained from 2 patients with confirmed 22q11.2 deletion syndrome and from 14 women at low risk for fetal chromosomal abnormalities. The median fetal DNA contribution for all samples was 18%, with the affected samples containing 17%-18% fetal DNA. Using a technique similar to that used for sequencing-based fetal aneuploidy detection from maternal plasma, the authors detected a statistically significant loss of representation of a portion of chromosome 22q11.2 in both of the affected fetal samples. The authors concluded that noninvasive prenatal diagnosis of subchromosomal fetal genomic anomalies is feasible with next-generation sequencing.

Conclusions

This single study on a very limited number of patients is insufficient to reach conclusions regarding the use of cell-free fetal DNA for screening or diagnosis of microdeletion syndromes.

Clinical Utility

No comparative studies were evaluated that compared health outcomes in patients managed using the maternal plasma DNA tests compared to standard screening tests.

As part of the 2012 TEC Assessment[4], a decision model was constructed to model health outcomes of sequencing-based testing for trisomy 21 compared to standard testing. The primary health outcomes of interest included the number of cases of aneuploidy correctly identified, the number of cases missed, the number of invasive procedures potentially avoided (i.e., with a more sensitive test), and the number of miscarriages potentially avoided as a result of fewer invasive procedures. The results were calculated for a high-risk population of women age 35 years or more (estimated antenatal prevalence of T21: 0.95%), and an average risk population including women of all ages electing an initial screen (estimated antenatal prevalence of T21: 0.25%). For women testing positive on initial screen and offered an

invasive, confirmatory procedure, it was assumed that 60% would accept amniocentesis or CVS. Sensitivities and specificities for both standard and sequencing-based screening tests were varied to represent the range of possible values; estimates were taken from published studies whenever possible.

According to the model results, sequencing-based testing improved outcomes for both high-risk and average risk women. As an example, assuming there are 4.25 million births in the U.S. per year[22] and two-thirds of the population of average risk pregnant women (2.8 million) accepted screening, the following outcomes would occur for the 3 screening strategies under consideration:,

- Standard screening

  Of the 2.8 million screened with the stepwise sequential screen, 87,780 would have an invasive procedure (assuming 60% uptake after a positive screening test and a recommendation for confirmation), 448 would have a miscarriage, and 3,976 of 4,200 (94.7%) trisomy 21/Down syndrome cases would be detected.

- Sequencing as an alternative to standard screening

  If sequencing-based testing were used instead of standard screening, the number of invasive procedures would be reduced to 7,504 and the number of miscarriages reduced to 28, while the cases of Down syndrome detected would increase to 4,144 of 4,200 (97.6%of total), using conservative estimates.

- Sequencing following standard screening

  Another testing strategy would be to add sequencing- based testing only after a positive standard screen. In this scenario, invasive procedures would be further decreased to 4,116, miscarriages would remain at 28, but fewer Down syndrome cases would be detected (3,948 of 4,200, 94.0% of total). Thus, while this strategy has the lowest rate of miscarriages and invasive procedures, it detects fewer cases than sequencing-based testing alone.

At least two decision models have also been presented in industry-funded publications, each using a different commercially available test and published estimates of sensitivity and specificity. Findings of both these models are similar to the TEC Assessment model in that detection of T21 is increased and miscarriage rates are decreased using sequencing-based testing compared to standard screening. Both of the studies specifically model use of sequencing-based tests offered to women who have had a positive standard screening test.

Garfield and Armstrong published a study modeling use of the Verinata test.[23] In the model, women were eligible for screening following a positive first-trimester or second-trimester screening test or following a second-trimester ultrasound. The model assumed that 71% of women at average risk and 80% of women at high risk would choose the test. In a theoretical population of 100,000 pregnancies, the detection rate of T21 increased from 148 with standard testing to 170 with Verify testing. In addition, the number of miscarriages associated with invasive testing (assumed to be 0.5% for amniocentesis and 1% with CVS) was reduced from 60 to 20.

Palomaki and colleagues modeled use of the Sequenom sequencing-based test offered to women after a positive screening test, with invasive testing offered only in the case of a positive sequencing-based test.[6] As in the TEC Assessment, they assumed 4.25 million births in the U.S. per year, with two-thirds

of these receiving standard screening. The model assumed a 99% detection rate, 0.5% false-positive rate, and 0.9% failure rate for sequencing-based testing. Compared to the highest performing standard screening test, the addition of sequencing-based screening would increase the Down syndrome detection rate from 4,450 to 4,702 and decrease the number of miscarriages associated with invasive testing from 350 to 34.

It is important to note that all of the above models include confirmatory invasive testing for positive screening tests. Sequencing-based testing without confirmatory testing carries the risk of misidentifying normal pregnancies as positive for trisomy. Due to the small but finite false-positive rate, together with the low baseline prevalence of trisomy in all populations, a substantial percent of positive results on sequencing tests could be false-positive results.

In 2013, Ohno and Caughey published a decision model comparing use of sequencing-based tests in high-risk women with confirmatory testing (i.e. as a screening test) and without confirmatory testing (i.e., as a diagnostic test).[24] Results of the model concluded that using sequencing-based tests with a confirmatory test resulted in fewer losses of normal pregnancies compared to sequencing-based tests used without a confirmatory test. The model made their estimates using the total population of 520,000 high-risk women presenting for first-trimester care each year in the U.S. Sequencing-based tests used with confirmatory testing resulted in 1441 elective terminations (all with Down syndrome). Without confirmatory testing, sequencing-based tests resulted in 3873 elective terminations, 1449 with Down syndrome and 2424 without Down syndrome. There were 29 procedure-related pregnancies losses when confirmatory tests were used.

There are no studies that demonstrated the use of nucleic acid sequencing-based testing for fetal sex determination were more beneficial than fetal ultrasound, the current clinical standard of fetal sex identification. Studies that compare cell-free DNA analysis to ultrasound are necessary, in order to make valid conclusions about the benefits of this technology.

In addition, there are no clinical utility studies that determined the use of cell-free DNA for the screening or diagnosis of microdeletion syndromes. It is unclear how these testing results may change the clinical management or improve health outcomes for patients.

Conclusions

There is no published direct evidence that managing patients using sequencing-based testing improves health outcomes compared to standard screening. Modeling studies using published estimates of diagnostic accuracy (most of which include women with high-risk pregnancies) and other parameters predict that sequencing-based testing as an alternative to standard screening will lead to an increase in the number of Down syndrome cases detected and a large decrease in the number of invasive tests and associated miscarriages.

Clinical Practice Guidelines and Position Statements

American College of Obstetricians and Gynecologists (ACOG)

In November 2012, ACOG released a committee opinion on noninvasive testing for fetal aneuploidy.[25] The Committee Opinion, which did not include an explicit review of the literature, was issued jointly with the Society for Maternal-Fetal Medicine Publications Committee. ACOG recommended the

following:

- "Cell free fetal DNA should not be part of routine prenatal laboratory assessments, but should be an informed patient choice after pretest counseling.
- Cell free fetal DNA testing should not be offered to low-risk women or women with multiple gestations because it has not been adequately evaluated in these groups.
- Pretest counseling should include a review that although the cell free fetal DNA test is not a diagnostic test, it has high sensitivity and specificity. The test will only screen for the common trisomies and, at the present time, gives no other genetic information about the pregnancy.
- A family history should be obtained before use of this test to determine if the patient should be offered other forms of screening or prenatal diagnosis for familial genetic disease.
- If a fetal structural anomaly is identified on ultrasound examination, invasive prenatal diagnosis should be offered.
- A negative cell free fetal DNA test does not ensure an unaffected pregnancy.
- A patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results.
- Cell free fetal DNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with CVS or amniocentesis, which remain an option for women."

The International Society for Prenatal Diagnosis (ISPD)

In 2013, the ISPD published a position statement regarding prenatal diagnosis of chromosomal abnormalities. The statement included the following discussion of maternal cell-free DNA screening:[26]

- "..Although rapid progress has been made in the development and validation of this technology, demonstration that in actual clinical practice, the testing is sufficiently accurate, has low failure rates, and can be provided in a timely fashion, has not been provided. Therefore, at the present time, the following caveats need to be considered…"
- "Reliable noninvasive maternal cfDNA (cell-free) aneuploidy screening methods have only been reported for trisomies 21 and 18…."
- "There are insufficient data available to judge whether any specific cfDNA screening method is most effective."
- "The tests should not be considered to be fully diagnostic and therefore are not a replacement for amniocentesis and CVS…"
- "Analytic validity trials have been mostly focused on patients who are at high risk on the basis of maternal age or other screening tests. Efficacy in low-risk populations has not yet been fully demonstrated…"

The National Society of Genetic Counselors (NSGC)

In 2013, the NSGC published a position statement regarding noninvasive prenatal testing of cell-free DNA in maternal plasma.[27] The NSGC supports non-invasive cell-free DNA testing as an option in women who want testing for aneuploidy. The document states that the test has been validated primarily in pregnancies considered to be at increased risk of aneuploidy and the organization does not support routine first-tier screening in low-risk populations. In addition, the document states that test results should not be considered diagnostic and abnormal findings should be confirmed through conventional diagnostic procedures such as CVS and amniocentesis.

American College of Medical Genetics and Genomics (ACMG)

In 2013, the ACMG published a statement on non-invasive prenatal screening for fetal aneuploidy that addresses challenges in incorporating non-invasive testing into clinical practice.[28] Limitations identified by the organization include that chromosomal abnormalities such as unbalanced translocations, deletions and duplications, single-gene mutations and neural tube defects cannot be detected by the new tests. Moreover, it currently takes longer to obtain test results than with maternal serum analytes. The ACMG also stated that pretest and posttest counseling should be performed by trained individuals.

Summary

Non-invasive sequencing-based testing of maternal serum for fetal trisomy syndromes has recently become available and has the potential to substantially alter the current approach to screening. The test technology involves detection of fetal cell-free DNA fragments present in the plasma of pregnant women.

High Risk Singleton Pregnancies

Direct evidence of clinical utility is not available; however, published studies from all three commercially available tests have consistently demonstrated very high sensitivity and specificity for detecting Down syndrome (trisomy 21) in high-risk singleton pregnancies. Modeling of comparative outcomes based on the published data on test performance, published estimates of standard screening performance, patient uptake of confirmatory testing, and miscarriage rates associated with invasive procedures suggest improved outcomes with sequencing based testing, e.g., increased rate of Down syndrome detection, and reduced number of invasive procedures and procedure-related miscarriages. In the modeling, the negative predictive value of testing approached 100% across the range of aneuploidy risk, while the positive predictive value varied widely according to baseline risk. The variable positive predictive value highlights the possibility of a false-positive finding and thus testing using karyotyping is necessary to confirm a positive result.

Based on the available evidence, nucleic acid sequencing-based testing for aneuploidies (Trisomy 13, 18, 21, Turner syndrome, Klinefelter syndrome, Jacob's syndrome) may be considered medically necessary in women with high-risk singleton pregnancies who meet specific criteria.

Average Risk and Multiple Pregnancies

While data from 8 studies consistently reported a very high sensitivity and specificity of maternal plasma DNA sequencing-based tests for detecting trisomy 21 in high-risk women with singleton pregnancies, only one of these studies included women at average-risk of trisomy 21. Thus, the evidence on women with average-risk pregnancies is insufficient. For women with multiple pregnancies, there is insufficient evidence to draw conclusions about the diagnostic accuracy of these tests for detecting trisomy 21. Therefore, nucleic acid sequencing-based testing for trisomy 21 is considered investigational in women with average-risk, twin, or multiple pregnancies.

Other indications

Current evidence does not demonstrate the use of nucleic acid sequencing-based testing for fetal sex determination is more beneficial than fetal ultrasound, the current clinical standard for determining fetal

sex. Therefore, nucleic acid sequencing-based testing is considered not medically necessary for fetal sex determination.

Only one small feasibility study addressed the use of use of cell-free fetal DNA for the screening or diagnosis of microdeletion syndromes. There are no studies of clinical utility, so it is not known how nucleic acid sequencing-based testing for the screening or diagnosis of microdeletion syndromes can alter clinical management and improve health outcomes. Therefore, nucleic acid sequencing-based testing is considered investigational for the screening or diagnosis of microdeletion syndromes.

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CROSS REFERENCES

Evaluating the Utility of Genetic Panels , Genetic Testing Policy No. 64

CODES	NUMBER	DESCRIPTION
CPT	81479	Unlisted molecular pathology procedure
	81507	Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy
	81599	Unlisted multianalyte assay with algorithmic analysis
	~~0005M~~	~~Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy~~ (Deleted 1/1/2014)
HCPCS	None	

APPENDIX I		
Test Name	**Core components**	**Add-on Options**
Harmony™ Prenatal Test (Ariosa Diagnostics)	Trisomies 21, 18, and 13 (may be medically necessary if criteria are met)	Fetal sex X/Y (not medically necessary)
MaterniT21™ Plus (Sequenom)	Trisomies 21, 18 and 13, sex aneuploidies, monosomy X (may be medically necessary if criteria are met)	Fetal sex X/Y (not medically necessary), microdeletions (investigational).
verifi® Prenatal Test (Verinata Health)	Trisomies 21, 18, 13, sex aneuploidies, monosomy X (may be medically necessary if criteria are met)	Fetal sex X/Y (not medically necessary)
Panorama (Natera)	Trisomies 13, 18, 21, sex aneuploidies, monosomy X (may be medically necessary if criteria are met)	Fetal sex X/Y (not medically necessary); microdeletions (investigational)