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Screening for Fetal Chromosomal Abnormalities

Prenatal testing for chromosomal abnormalities is designed to provide an accurate assessment of a patient's risk of carrying a fetus with a chromosomal disorder. A wide variety of prenatal screening and diagnostic tests are available; each offers varying levels of information and performance, and each has relative advantages and limitations. When considering screening test characteristics, no one test is superior in all circumstances, which results in the need for nuanced, patient-centered counseling from the obstetric care professional and complex decision making by the patient. Each patient should be counseled in each pregnancy about options for testing for fetal chromosomal abnormalities. It is important that obstetric care professionals be prepared to discuss not only the risk of fetal chromosomal abnormalities but also the relative benefits and limitations of the available screening and diagnostic tests. Testing for chromosomal abnormalities should be an informed patient choice based on provision of adequate and accurate information, the patient's clinical context, accessible health care resources, values, interests, and goals. All patients should be offered both screening and diagnostic tests, and all patients have the right to accept or decline testing after counseling.

The purpose of this Practice Bulletin is to provide current information regarding the available screening test options available for fetal chromosomal abnormalities and to review their benefits, performance characteristics, and limitations. For information regarding prenatal diagnostic testing for genetic disorders, refer to Practice Bulletin No. 162, Prenatal Diagnostic Testing for Genetic Disorders. *For additional information regarding counseling about genetic testing and communicating test results, refer to* Committee Opinion No. 693, Counseling About Genetic Testing and Communication of Genetic Test Results. *For information regarding carrier screening for genetic conditions, refer to* Committee Opinion No. 690, Carrier Screening in the Age of Genomic Medicine *and* Committee Opinion No. 691, Carrier Screening for Genetic Conditions. *This Practice Bulletin has been revised to further clarify methods of screening for fetal chromosomal abnormalities, including expanded information regarding the use of cell-free DNA in all patients regardless of maternal age or baseline risk, and to add guidance related to patient counseling.*

Background

A normal human cell contains 46 chromosomes. Chromosomal abnormalities can include absent or additional entire chromosomes, as well as deletions, duplications, and translocations of varying sizes. Aneuploidy is defined as having extra or missing whole chromosomes, and microdeletions and duplications refer to loss or gain of a small portion of a chromosome and are known as copy number variants. The advent of chromosomal microarray analysis (CMA), an array-based molecular cytogenetic technique that can be applied to DNA from chorionic villus sampling (CVS) or amniocentesis specimens, has enabled prenatal detection of submicroscopic chromosomal gains and losses that can have important clinical implications. Because each chromosome consists of hundreds of functional genes, the loss or gain of genetic material can substantially interrupt gene function. If large amounts of genetic material are disrupted, it can result in a nonviable pregnancy or a newborn with a life-limiting condition. In the case of the surviving newborn, there are a wide range of potential outcomes depending on the type of chromosomal abnormality such as structural anomalies, failure to thrive, intellectual disability, and shortened lifespan.

Although chromosomal abnormalities occur in approximately 1 in 150 live births (1), the prevalence of chromosomal abnormalities is greater earlier in gestation because aneuploidy accounts for a large proportion of early pregnancy loss. The incidence of fetal chromosomal abnormalities increases as a woman ages (Table 1) but can affect patients at any age and is not related to race or ethnicity.

Trisomy 21 (Down syndrome) is the most common autosomal chromosomal aneuploidy in liveborn infants, with a prevalence of approximately 1 in 700 live births (1, 2). Trisomy 18 (Edward syndrome) is the second most common autosomal trisomy at the time of birth, with a prevalence of about 1 in 3,000 live births (2–4). The prevalence of trisomy 13 (Patau syndrome) at birth is approximately 1 in 6,000. The most common sex chromosome aneuploidy is 47, XXY (Klinefelter syndrome) with a prevalence of 1 in 500 males. The only viable monosomy is 45, X (Turner syndrome), in which the birth prevalence is approximately 1 in 2,500 and which is unrelated to maternal age (5).

Factors associated with the likelihood of chromosomal abnormalities include increasing maternal age, a parental translocation or other chromosomal abnormality, having a previous pregnancy with a chromosomal abnormality, prenatal ultrasonographic abnormalities, or a screen positive test result. Although the risk of aneuploidy increases with advancing maternal age, most children with trisomy 21 are born to younger patients because a larger proportion of all children are born to younger patients. Unlike aneuploidies, copy number variants are independent of maternal age and occur in approximately 0.4% of pregnancies. Therefore, based on a systematic review, pregnancies in patients under 36 years of age have a higher risk for microarray abnormalities than for trisomy 21 (11) (Table 1).

Testing for chromosomal abnormalities should be an informed patient choice based on provision of adequate and accurate information, and the patient's clinical context, accessible health care resources, values, interests, and goals. Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (CVS or amniocentesis) options should be discussed and offered to all pregnant patients regardless of age or risk for chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing. Pretest and posttest counseling is essential (12). The purpose of pretest counseling is to inform pregnant patients about chromosomal disorders, provide information regarding their specific risk of carrying a fetus with a chromosomal abnormality, review their relevant personal and family history, and discuss the risks, limitations, and benefits of available testing options so that they can make an informed choice regarding screening or diagnostic testing. Patients who prefer comprehensive prenatal detection of as many chromosomal aberrations as possible should be offered diagnostic testing and CMA. If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously. When results return, both screen negative and screen positive results should be communicated in a timely fashion. In the setting of a screen negative or low risk test result, discussion should include the concept of residual risk, which is defined as the chance that an abnormality may still be present even if the test result is screen negative. It should also include consideration of the detection rate of each test, as well as the conditions targeted in screening. In the case of a result indicating an increased risk, counseling should provide information regarding the likelihood that the fetus has a particular condition (ie, the positive predictive value [PPV]) and the options for additional testing if desired to further clarify this risk.

Screening Tests

Single time point screening approaches include first-trimester screening (NT and serum analytes); second-trimester

Table 1. Chromosomal Abnormalities in Second-Trimester Pregnancies Based on Maternal Age at Term

	Trisomy 21	Trisomy 18	Trisomy 13	Sex Chromosome Aneuploidy (XXX, XY, XYY, 45, X)	Microarray or Rare Chromosomal Abnormality	All Chromosomal Abnormalities
Age 20	8 per 10,000 1 in 1,250	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	82 per 10,000 1 in 122
Age 25	10 per 10,000 1 in 1,000	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	84 per 10,000 1 in 119
Age 30	14 per 10,000 1 in 714	4 per 10,000 1 in 2,500	2 per 10,000 1 in 5,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	91 per 10,000 1 in 110
Age 35	34 per 10,000 1 in 294	9 per 10,000 1 in 1,111	4 per 10,000 1 in 2,500	35 per 10,000 1 in 285	37 per 10,000 1 in 270	119 per 10,000 1 in 84
Age 40	116 per 10,000 1 in 86	30 per 10,000 1 in 333	14 per 10,000 1 in 714	51 per 10,000 1 in 196	37 per 10,000 1 in 270	248 per 10,000 1 in 40

Data from:

Srebniak MI, Joosten M, Knapen MF, Arends LR, Polak M, van Veen S, et al. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2018;51:445–52.

Hook EB. Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 1981;58:282–5.

Gravholt CH, Juul S, Naeraa RW, Hansen J. Prenatal and postnatal prevalence of Turner's syndrome: a registry study. BMJ 1996;312:16–21.

Snijders RJ, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. Fetal Diagn Ther 1995;10:356–67.

Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999;13:167–70.

Forabosco A, Percesepe A, Santucci S. Incidence of non-age-dependent chromosomal abnormalities: a population-based study on 88965 amniocenteses. Eur J Hum Genet 2009;17:897–903.

Crider KS, Olney RS, Cragan JD. Trisomies 13 and 18: population prevalences, characteristics, and prenatal diagnosis, metropolitan Atlanta, 1994–2003. Am J Med Genet A 2008;146A:820–6.

Irving C, Richmond S, Wren C, Longster C, Embleton ND. Changes in fetal prevalence and outcome for trisomies 13 and 18: a population-based study over 23 years. J Matern Fetal Neonatal Med 2011;24:137–41.

triple, quadruple (quad), or penta screens; and cell-free DNA screening. Combined screening tests in which samples are obtained in the first and second trimesters include integrated, serum integrated, sequential, and contingent screening (Table 2).

Single Time Point Screening Approaches

Screening in Any Trimester

Cell-Free DNA Screening

Cell-free DNA screens for aneuploidies using the analysis of cell-free DNA fragments in the maternal circulation starting at about 9–10 weeks of pregnancy and, unlike analyte screening, can be sent until term. The fetal component of cell-free DNA is derived from placental trophoblasts that are released into the maternal circulation from cells undergoing programmed cell death. The fetal component is known as the fetal fraction; it comprises approximately 3–13% of the total cell-free DNA in maternal blood (13, 14). The quantity of cell-free DNA from the fetal component increases throughout gestation.

The quantity of the fetal fraction is affected by many factors, including but not limited to gestational age, maternal body mass index (BMI), maternal medication exposure, maternal race, aneuploidy status if present, fetal or maternal mosaicism, and singleton or multiple gestation (13–18). Depending on the laboratory, cell-free DNA screening can be performed as early as 9 weeks of gestation, although higher fetal fractions at 10 weeks and beyond are associated with lower rates of test failure.

Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing. Cell-free DNA is the only laboratory screening test to identify

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

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Cell-free DNA†	9–10 to term	99	2–4% Includes inability to obtain results, which is associated with increased risk†	1. Highest DR 2. Can be performed at any gestational age after 9–10 weeks 3. Lowest false-positive rate	Results may reflect underlying maternal aneuploidy or maternal disease	Several molecular methods
First trimester‡	10–13 6/7§	82–87‖	5	1. Early screening 2. Single time point test	Lower DR than tests with first and second trimester component NT required	NT+PAPP-A, free beta hCG, +/− AFP¶
Quad screen‡	15–22	81	5	1. Single time point test 2. No specialized US required	Lower DR than first trimester and first and second trimester combined tests	hCG, AFP, uE3, DIA
Integrated‡	10–13 6/7§, then 15–22	96	5	High DR	Two samples needed No first-trimester results NT required	NT+PAPP-A, then quad screen
Serum integrated‡	10–13 6/7§, then 15–22	88	5	1. DR compares favorably with first-trimester screening 2. No specialized US required	Two samples needed No first-trimester results	PAPP-A + quad screen
Sequential#: stepwise	10–13 6/7§, then 15–22	95	5	1. First-trimester results provided 2. Comparable performance to integrated, but FTS results provided	Two samples needed NT required	NT+ free beta hCG + PAPP-A, +/− AFP¶, then quad screen
Contingent screening**		88–94	5	First-trimester test result: Positive: diagnostic test or cell-free DNA offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed NT required	NT+hCG+ PAPP-A, +/− AFP¶, then quad screen

(*continued*)

Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Chromosomal Abnormalities (*continued*)

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Nuchal translucency alone#	10–13 6/7§	70	5	Allows individual fetus assessment in multifetal gestations Provides additional screening for fetal anomalies	Poor sensitivity and specificity in isolation NT required	US only

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

All patients should be offered second-trimester assessment for open fetal defects (by ultrasonography, with or without second-trimester serum AFP) and ultrasound screening for other fetal structural defects.

*A screen positive test result includes all positive test results: the true positives and false positives. For cell-free DNA, this includes the test failure rates given the association with increased risk of aneuploidy (see † below).

†Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017;50:302–14.

‡First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11 weeks, 12 weeks, and 13 weeks, respectively (Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. N Engl J Med 2005;353:2001–11.)

§Because of variations in growth and pregnancy dating, some fetuses at the lower and upper gestational age limits may fall outside the required crown–rump length range. Also, different laboratories use slightly different gestational age windows for their testing protocol.

||Use of free beta hCG in conjunction with nasal bone assessment increases the detection rate to 97% with a screen positive rate of 5% (Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. Prenat Diagn 2003;23:306–10.)

¶Testing of first trimester AFP depends on commercial lab used. First trimester AFP should not be used in lieu of second trimester AFP for open fetal defects screening.

#Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, et al. First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening. Cochrane Database of Systematic Reviews 2017, Issue 3. Art. No.: CD012600. DOI: 10.1002/14651858.CD012600.

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

fetal sex and sex chromosome aneuploidies; of note, the sex chromosome results for patients who have undergone organ transplantation will be affected by the sex of the organ donor and therefore sex chromosome testing is not recommended in this population. The most recent meta-analysis evaluating test performance for cell-free DNA screening (19) reports a greater than 99% detection rate for fetal trisomy 21, 98% detection rate for fetal trisomy 18, and 99% detection rate for fetal trisomy 13 with a combined false-positive rate of 0.13%; of note, these numbers were calculated for samples in which a result is returned. Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) are at increased risk for chromosomal abnormalities. Because trisomy 13 is a rare disorder, fewer affected cases are available to review, with reported detection rates varying from 40% to 100% in individual studies, with a false-positive rate between 0% and 0.25%. In this paper, the detection rate of sex chromosome aneuploidy could not be assessed because of the small study population (19). This meta-analysis included all women, although most were at higher risk for aneuploidy and sampling was not confined to the first trimester.

There are currently several laboratory methods to analyze cell-free DNA and the detection of fetal trisomies

is comparable between these techniques (20). Different technologies offer some subtle differences in the information reported. Of the methods, the single nucleotide polymorphism (SNP) method can identify triploidy (21). Laboratory reporting information, such as PPV and fetal fraction, is not standardized. Because of the effect of fetal fraction on test accuracy, a laboratory test that reports fetal fraction is preferred to fully evaluate the test results (22, 23).

Before cell-free DNA screening, a baseline sonogram may be useful, as some ultrasound findings detectable early in pregnancy may affect the timing of cell-free DNA testing, the appropriateness of performing cell-free DNA testing, or the ability to interpret cell-free DNA test results. These findings include an earlier than expected gestational age, confirmation of viability, number of fetuses, presence of a vanishing twin or empty gestational sac, or presence of a fetal anomaly. One retrospective study of high-risk patients found at least one of these factors in 16.1% of first-trimester ultrasound examinations (24). Patients with fetal anomalies should be offered genetic counseling and diagnostic testing instead of genetic screening. In a patient with both a vanishing twin and a viable intrauterine pregnancy, cell-free DNA screening is not advised because of the high risk for aneuploidy in the nonviable sac or embryo, which can lead to false-positive results (25).

Other Potential Chromosomal Abnormalities Identified by Cell-Free DNA

In addition to screening for the common aneuploidies, some laboratories offer testing for other aneuploidies such as trisomy 16 and trisomy 22, microdeletion testing, and genome-wide screening of large copy number changes (26–28). Nonmosaic fetal trisomy 16 or 22 is associated with a nonviable gestation. Mosaic trisomy 16 and 22 can be associated with fetal survival; however, screening is not recommended because the screening accuracy with regard to detection and the false-positive rate is not established.

Screening for a limited number of microdeletions with cell-free DNA is available; however, this testing has not been validated clinically and is not recommended. Although microdeletions are relatively common when considered in aggregate, cell-free DNA panels only include a few specific clinically significant microdeletions and these are very rare. Therefore, the PPV for these disorders is much lower than for common trisomies. If a microdeletion is identified through cell-free DNA screening, it should be confirmed by diagnostic testing, as most positive results will be false-positive results because of the low prevalence of these disorders. If the diagnostic test confirms a microdeletion, the patient should be referred to a health care professional with genetics expertise to discuss the diagnosis and implications and to develop a management plan. For women who wish to evaluate their pregnancy for submicroscopic chromosomal changes, prenatal diagnostic testing with CMA from CVS or amniocentesis is recommended (28). At this time, there is no genetic screening test available to comprehensively screen for all copy number variants.

Genome-wide cell-free DNA screening for large deletions or duplications is also offered by some laboratories. This testing evaluates the entire genome and is designed to detect abnormalities larger than those evaluated by cell-free DNA microdeletion screening. Screening for these ancillary disorders is not recommended because this testing has not been validated clinically and the screening accuracy with regard to detection and false-positive rate is not established.

First-Trimester Screening: Serum Analytes Plus Ultrasound

Typically performed when the crown–rump length measures between 38 and 45 mm and 84 mm (generally between approximately 10 and 14 weeks of gestation), first-trimester screening includes a NT measurement and measurement of serum analytes that can include serum β-human chorionic gonadotrophin (free or total human chorionic gonadotropin [hCG]) along with pregnancy-associated plasma protein A (PAPP-A), and alpha-fetoprotein (AFP) levels depending on the particular laboratory being used. A risk estimate for common trisomies (generally trisomies 13, 18, and 21) is calculated using these test results along with other maternal factors such as age, history of aneuploidy, weight, race, and number of fetuses.

The NT refers to the fluid-filled space on the dorsal aspect of the fetal neck. An enlarged NT (often defined as 3.0 mm or more or above the 99th percentile for the crown–rump length) is independently associated with fetal aneuploidy and structural malformations such as cardiac anomalies (29). The risk of adverse fetal outcome is proportional to the degree of NT enlargement. Meticulous technique in nuchal translucency imaging and measurement is essential for accurate risk assessment because under measurement by even 0.5 mm can reduce the test sensitivity by 18% (30). Independent credentialing and ongoing quality assurance of individuals performing these measurements is required to maintain screening performance.

First-trimester screening gives the potential for earlier diagnoses as well as the ability to screen for other structural, genetic, or placental disorders; like any other form of analyte screening, it also may identify other

aneuploidies (31). All patients should be offered a second-trimester ultrasound for fetal structural defects, since these may occur with or without fetal aneuploidy; ideally this is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein) (32).

Second-Trimester Screening

The quadruple marker screen ("quad" screen) can be performed from approximately 15 0/7 weeks to 22 6/7 weeks of gestation; the gestational age range for screening varies among laboratories. This serum test does not require specialized ultrasonography for NT measurement and gives information regarding the risk of open fetal defects in addition to risk assessment for trisomy 21 and 18. The quad screen involves the measurement of four maternal serum analytes—human chorionic gonadotropin (hCG), alpha-fetoprotein (AFP), dimeric inhibin A (DIA), and unconjugated estriol (uE3)—in combination with maternal factors such as age, weight, race, and the presence of pregestational diabetes to calculate a risk estimate. Second-trimester quad screening has a detection rate for trisomy 21 of 80% with a 5% false-positive rate (33) (Table 2). A few laboratories offer the penta screen, which adds hyperglycosylated hCG to the quad screen. Although there is some evidence from one limited retrospective trial that this test may improve second-trimester screening performance, its performance has not been evaluated rigorously in prospective studies (30). The triple marker screen measures serum hCG, AFP, and uE3, and provides a lower sensitivity for the detection of trisomy 21 (sensitivity of 69% at a 5% positive screening test result rate) than quad screen and first-trimester screening (33). The quad screen has been shown to be more effective and less costly than the triple screen in a cost-effectiveness analysis (34).

Combined First-Trimester and Second-Trimester Screening Tests

Combined first-trimester and second-trimester screening with either integrated, sequential, or contingent screening involving serum analytes, NT, or both measurements provides a higher detection rate for trisomy 21, 18, and 13 than one-step serum analyte screening. Depending on the test selected, there is variable timing of results available to the patient.

Integrated Screening and Serum Integrated Screening

With integrated screening, the patient undergoes a first-trimester NT measurement and serum analyte screening followed by a second-trimester blood draw for additional analytes and receives a single test result in the second trimester. In locations where a n NT measurement by a certified ultrasonographer is unavailable, or if fetal position, maternal body habitus, or imaging properties preclude an accurate nuchal translucency measurement, serum integrated screening, which includes only the first-trimester and second-trimester serum analytes, also is an option. Serum integrated screening has a lower detection rate than integrated screening that includes an NT measurement, but a similar detection rate to first-trimester screening (Table 2). Limitations of integrated screening include the lack of results until the second-trimester sample and the potential that no result will be provided if the patient does not undergo the second blood draw. Reported rates of failure to obtain a second blood draw may be as high as 25% without a written reminder to complete the test (35). The benefit of integrated screening over single time point testing is the higher detection rate and lower false positive rate (Table 2).

Sequential and Contingent Screening

Sequential screening maintains a high detection rate using the combined first-trimester and second-trimester screening approach, providing some information in the first trimester to allow for earlier diagnostic testing and reproductive management options. Using stepwise sequential screening, the patient is given a risk estimate after completion of the first-trimester analytes and NT testing. If the first-trimester screening result indicates that the risk of aneuploidy is greater than the laboratory's positive screening cutoff, the patient is notified and offered additional testing. If patients have a lower risk than the cutoff level, they are informed that they have received a negative screening test result and analyte screening is planned in the second trimester to receive a final combined numerical risk. The sequential approach takes advantage of the higher detection rate achieved by incorporating the first trimester and second-trimester screening test results with only a marginal increase in the false-positive rate.

The contingent model classifies aneuploidy risk as high, intermediate, or low on the basis of the first-trimester screening test results. Patients at high risk are offered additional testing (diagnostic testing or cell-free DNA), and those below a defined low risk threshold are reassured and no further screening or testing is recommended. First-trimester and second-trimester results are used together to calculate a final risk of aneuploidy in patients at intermediate risk in the contingent screening model. Theoretically, the contingent approach should maintain high detection rates with low false-positive rates and reduce the number of second-trimester tests performed.

The use of multiple serum screening approaches performed independently (eg, a first-trimester screening

test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.

Ultrasonographic Screening

Although fetuses with trisomy 13 or trisomy 18 usually have major structural anomalies that are evident on ultrasound examination, the ultrasonographic identification of trisomy 21 is less consistent. First-trimester NT is the primary sonographic marker that is used in combination with serum analytes to determine aneuploidy risk, and sonographer certification and quality assurance is needed to standardize this method for screening. When NT alone is used to modify the age-related risk of trisomy 21, the detection rate is approximately 70% (36). A NT measurement alone does not add benefit in detecting aneuploidy when cell-free DNA screening has been performed in a singleton gestation (37). Nuchal translucency can be useful in multifetal gestations, in which serum screening methods are not as accurate, may be unavailable, and cannot provide information specific to each fetus.

The absence of a nasal bone or an absent or reversed ductus venosus Doppler waveform increases the risk for aneuploidy (36). As isolated sonographic markers these findings have limited utility: the absence of the nasal bone has a 49% sensitivity with a 1% false-positive rate and an abnormal ductus venosus waveform has a 67% sensitivity with a 5% false-positive rate for trisomy 21. Although these findings are reported to be useful as ancillary ultrasound methods to assess aneuploidy risk in the first trimester, the reported studies are limited by lack of standardization, small sample size of reported cohorts, and different patient populations.

With regard to screening for structural anomalies, all patients should be offered a second-trimester ultrasound for fetal structural defects, ideally performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein) (32). This ultrasound seeks to identify major structural abnormalities but may also identify ultrasonographic "soft markers" of aneuploidy. The major structural anomalies associated with chromosomal abnormalities include cardiac anomalies, neuroanatomic abnormalities, and other major structural abnormalities that generally have functional significance in addition to increasing the likelihood of a genetic condition. In contrast, "soft" ultrasonographic markers are nonspecific ultrasound findings that are generally not pathologic but are more common among fetuses with trisomy 21 (eg, echogenic intracardiac focus, thickened nuchal fold, renal pelvis dilation, or echogenic bowel) or trisomy 18 (choroid plexus cysts). Because soft markers for aneuploidy are most commonly identified in euploid fetuses, it is difficult to use these findings to distinguish between pregnancies affected or unaffected by aneuploidy. If a soft marker is identified on the fetal anatomic ultrasound survey, the patient's medical record should be reviewed to determine if aneuploidy screening has been performed previously; if not, it should be offered. If screening has been performed, the finding should be placed in context with those results. (For more information, see Clinical Question, What is the role of ultrasonography in screening for fetal chromosomal abnormalities?)

Clinical Considerations and Recommendations

▶ ***Who should be offered testing for chromosomal abnormalities?***

Screening (serum screening with or without NT ultrasound or cell-free DNA screening) and diagnostic testing (CVS or amniocentesis) for chromosomal abnormalities should be discussed and offered to all patients early in pregnancy regardless of maternal age or baseline risk. Historically testing was offered only to patients considered to be high risk because of maternal age or personal or family history. However, given the personal nature of prenatal testing decision making as well as the inefficiency of offering testing only to patients at high risk, the current recommendation is that all patients should be offered both screening and diagnostic testing options.

▶ ***What information should be included when counseling patients regarding the option of prenatal screening for chromosomal abnormalities?***

There is not one screening test that performs optimally in all clinical scenarios and all screening tests detect fewer abnormalities than diagnostic testing that include microarray analysis. Health care professionals should be knowledgeable about limitations and benefits of screening options for chromosomal abnormalities in pregnancy and provide balanced information to patients. Patients should be provided with general information about the disorders that are potentially detectable with prenatal screening for chromosomal abnormalities and the disorders that are not detectable through screening before making a decision to undergo the specific tests being offered.

Patients should be counseled regarding their specific risks based on their age (Table 1) and their genetic and family history. Younger patients should be counseled

that although cell-free DNA is a very accurate screening test for trisomy 21, they are at higher risk for having a fetus with microarray abnormalities which can be detected through diagnostic testing with microarray. Prenatal genetic testing should be based on individual values and preferences with pretest counseling to facilitate informed decision making. Counseling should be performed in a clear, objective, and nondirective fashion, allowing patients sufficient time to understand and make informed decisions regarding testing (12).

The choice of screening test is affected by many factors, including the number of fetuses, gestational age at presentation, the availability of a reliable NT measurement, screening test sensitivity and limitations, the cost of screening, and obstetric and family history. Prenatal genetic testing may be desired to obtain information before delivery or to inform a decision for pregnancy termination.

As a part of pretest counseling, a family history should be reviewed to include any history of birth defects, children with intellectual disabilities, a genetic diagnosis in the family, or multiple miscarriages, as this information may inform testing decisions. Pretest counseling should include a brief description of possible screening tests, the conditions that are and are not being screened for, the accuracy of the tests, and the time frame for the return of results. The obstetric care professional should include the current gestational age, maternal age, BMI, any known fetal findings, whether this is a single or multiple gestation and history of aneuploidy as part of the decision-making process.

▶ *What information should be discussed with patients considering serum analyte versus cell-free DNA screening?*

A patient's baseline risk for chromosomal abnormalities should not limit testing options; serum screening with or without NT ultrasound or cell-free DNA screening and diagnostic testing (CVS or amniocentesis) should be discussed and offered to all patients regardless of maternal age or risk for chromosomal abnormality. Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies (trisomies 21, 13, and 18) and can be performed any time after 9-10 weeks of gestation. Although the sensitivity (detection rate) for fetal trisomies with cell-free DNA is the same regardless of the population being tested, the lower prevalence of fetal trisomies in younger patients results in a lower likelihood of an affected pregnancy (a lower PPV) in the setting of a positive result compared to those at higher baseline risk (See Clinical Question, How should aneuploidy screening test results be interpreted and communicated?). Prior to testing, counseling should include the possibility of incidental findings affecting the patient, including medical conditions such as her own chromosomal aneuploidy, mosaicism, or malignancy. If fetal sex determination is elected, the risk of maternal and fetal sex chromosome aneuploidy should be discussed as a potential finding.

Serum analyte screening also screens for fetal trisomies in both the first and second trimester, but also indirectly identifies other chromosomal abnormalities, and with second-trimester screening, provides a risk for open fetal defects. One study compared a statewide database of patients who had true positive first-trimester or sequential screens for aneuploidy and calculated the percent of positive results that would have been identified by cell-free DNA screening (38). Although the detection rate with cell-free DNA screening was modeled and not actual, sequential screening identified more chromosomal abnormalities including other aneuploidies, mosaicism, or large deletions or duplications than cell-free DNA screening. Of note, diagnosis of these aneuploidies requires diagnostic testing as the follow-up to an abnormal serum screen; if cell-free DNA testing is used as follow-up screening, these abnormalities would not be detected.

Screening performance of each approach depends on the criteria being utilized and how no-call results are categorized. In a series of 15,841 patients for which cell-free DNA results could be obtained, when cell-free DNA screening for trisomy 21 was compared with first-trimester screening (NT and serum analytes) in a general population (mean maternal age 30.7 years), cell-free DNA screening had a lower false-positive rate (0.06% cell-free DNA versus 5.4% for serum screening) and a higher PPV (80.9% versus 3.4%) (39). A prospective randomized trial of cell-free DNA versus first-trimester screening in 1,366 patients with a mean age of 33.9 and a normal ultrasound examination at 11 to 13 weeks gestational age (NT less than 3.5 mm and no identified fetal defects) found that first-trimester screening had a 2.5% false-positive rate and cell-free DNA had a 1.5% no-call rate; they concluded that cell-free DNA in this context reduces the false-positive rate (40). In a recent retrospective study of 66,166 patients undergoing screening or diagnostic testing in 2015 in Victoria, Australia, the sensitivity of first-trimester screening for detection of trisomy 21, 13, and 18 was 89.6% with a screen positive rate of 2.9%, and the sensitivity of cell-free DNA for the same conditions was 100% with a screen positive rate of 2.4% when no-call results were included as positive (41). There was no statistically significant difference in the rate of any major chromosomal abnormality detected on prenatal or postnatal diagnostic testing after a low risk screening result (1 in 1,188 or 8.4 per 10,000 for first-

trimester screening and 1 in 762 or 13.1 per 10,000 for cell-free DNA, $P=.13$) (41).

If a patient chooses screening for aneuploidy, only one screening approach should be used. Analyte screening and cell-free DNA screening should not be sent concurrently as this strategy is not cost-effective and simultaneous, seemingly discordant results can be more distressing to patients than screen positive analyte results followed by reassuring cell-free DNA screening (42, 43).

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

In addition to pretest counseling to facilitate informed shared decision making regarding testing strategy, posttest counseling is important to disclose both screen positive and screen negative test results, review options for additional testing as indicated or desired, and to discuss the concept of residual risk (12).

Screen Positive Results

All laboratory-based screening tests provide improved aneuploidy screening performance over maternal age and ultrasound examination alone but are not diagnostic tests. When a screen positive test result is obtained, patients should be counseled regarding their revised risk of carrying a fetus with a chromosomal abnormality. Information regarding the characteristics of the condition should be reviewed to aid decision making. Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results. Because of inherent limitations, screening test results should not be used as the sole basis on which to make critical clinical decisions.

Although all methods of cell-free DNA screening have high detection rates in all age groups, the PPV, or the chance that a screen positive test is a true positive result, is affected by the population prevalence and the type of disorder studied. The individual risk for trisomy 21 is lower in younger women (Table 1) and this lower prevalence increases the likelihood that a positive screening test result is a false positive. The PPV for trisomy 21 at 10 weeks of gestation for patients at different maternal ages is illustrated in Table 3. Because the prevalence of trisomies 18 and 13 is much lower than trisomy 21, their PPV is less. The PPV can be calculated individually for each patient and discussed. Some, but not all, laboratories report the PPV as part of the results. Online calculators are available to help determine the chance that a positive cell-free DNA result will be confirmed and can be helpful for providing more accurate counseling for an individual patient: https://www.med.unc.edu/mfm/nips-calc/ from the University of North Carolina and https://www.perinatalquality.org/vendors/nsgc/nipt/, the NIPT/Cell-Free DNA Screening Predictive Value Calculator from the National Society of Genetic Counselors (NSGC) and Perinatal Quality Foundation (PQF).

In patients with a screen positive analyte or cell-free DNA screening result in the setting of abnormal fetal sonographic findings, the concern for a chromosomal abnormality is increased but not confirmed. Confirmatory testing with CVS or amniocentesis is recommended both to confirm the diagnosis and to determine if the

Table 3. The Effect of Maternal Age on the Positive Predictive Value of Cell-Free DNA Screening for Trisomy 21, 18, and 13 at 10 Weeks Gestation*

	Maternal Age	Age Related Risk†	Positive Predictive Value‡
Trisomy 21	20	1:804 or 12 per 10,000	38–80%
	35	1:187 or 53 per 10,000	73–95%
	40	1:51 or 196 per 10,000	91–99%
Trisomy 18	20	1:1,993 or 5 per 10,000	11–41%
	35	1:465 or 22 per 10,000	34–75%
	40	1:126 or 79 per 10,000	66–92%
Trisomy 13	20	1:6,347 or 1.6 per 10,000	5–13%
	35	1:1,481 or 7 per 10,000	17–40%
	40	1:401 or 24 per 10,000	43–71%

*Sensitivity and specificity approximately 99%

†Age related risk of aneuploidy per 10,000 pregnancies at 10 weeks gestation based on maternal age at term

‡Percent varies by laboratory

Adapted from University of North Carolina at Chapel Hill. Positive predictive value of cell free DNA calculator. Available at: https://www.med.unc.edu/mfm/nips-calc. Retrieved February 24, 2020.

aneuploidy is a trisomy or secondary to a translocation. This is important as a translocation may be inherited from either parent and may affect siblings or future offspring.

The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities. Given that the residual risk of a chromosomal abnormality after an abnormal traditional screening test followed by a normal cell-free DNA screening test result has been reported to be about 2%, patients should be informed of the residual risk of a chromosomal abnormality not detectable by cell-free DNA (44).

If diagnostic testing after a positive screen is declined, management of the pregnancy should be based on the sonographic features identified and the patient's preferences. Depending on the chromosomal condition and the PPV, a fetal cardiac ultrasound may be indicated. Serial ultrasound examinations may be helpful to inform multidisciplinary discussions to develop a delivery and neonatal care plan. A newborn examination and karyotype or microarray may be suggested at the time of delivery.

Although false-positive cell-free DNA results are less common in comparison to other screening methods, these can occur. In some cases, this is because of biologic factors or laboratory methods (45). Biological mechanisms that can cause false-positive results include mosaicism, in which there are both normal and abnormal cells in the fetus, placenta, or patient; a duplicated chromosomal region; a vanishing twin, or an underlying maternal condition such as malignancy. By directly and specifically testing the fetal chromosomal complement, a diagnostic test can determine whether a cell-free DNA test result is indicative of a fetal abnormality. Counseling patients with the finding of placental or fetal chromosomal mosaicism is complex, and referral for genetic counseling may be especially useful in these cases (46).

Screen Negative Results and Residual Risk

Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings become evident such as fetal anomalies identified on ultrasound examination (46).

The false-negative rate, or the chance that a fetus is affected with a common trisomy but has a low-risk cell-free DNA result, is low. Possible explanations for a false-negative test result include a sample labeling error, a low fetal fraction, or the possibility of a mosaicism that is present at low levels in the placenta (47).

Interpretation of Cell-Free DNA Test Failures and Low Fetal Fraction

The fetal fraction is the proportion of total cell-free DNA that is fetal in origin. The fetal component of cell-free DNA screening is derived from the placental trophoblast. The accuracy of cell-free DNA screening is affected by both biologic and technical factors that depend on the fetal fraction. A low fetal fraction can cause cell-free DNA test failure. Because test results are usually reported as either screen positive or negative, the discrimination of aneuploid and euploid pregnancies improves with increasing fetal fraction.

Accurate cell-free DNA screening requires a minimum fetal fraction, most commonly estimated at about 2–4% (13, 48). The median fetal fraction obtained between 10 and 14 weeks of pregnancy is around 10% (13). In patients who weigh more than 250 pounds (113 kg), 10% may have a fetal fraction of less than 4% (49). Because of the effect of fetal fraction on test accuracy, a laboratory test that reports fetal fraction is preferred. The American College of Medical Genetics and Genomics recommends that all laboratories should include a clearly visible fetal fraction on cell-free DNA test reports (22, 23, 50).

Cell-free DNA test failures may occur because of the complex laboratory processing procedures, early gestational age (less than 9–10 weeks), the types of laboratory methods, and the presence of a genetic condition, particularly trisomy 13 or 18 and are also seen more frequently in patients with high BMI, increasing maternal age, certain racial backgrounds (seen more frequently in Black women and South Asian women in comparison to white women), and IVF pregnancies, (45) as well as maternal drug exposure (low-molecular-weight heparin) (51).

Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling, and be offered comprehensive ultrasound evaluation and diagnostic testing. One large study of over 16,000 patients with a 3% rate of a failed test showed the prevalence of aneuploidy in this group to be 2.7% versus 0.4% in the overall cohort (39);

other studies using other screening platforms have also demonstrated a higher risk of aneuploidy in the setting of a failed test (18, 52). Although trisomy 21 pregnancies do not appear to have a higher no-call rate, pregnancies affected with trisomies 13 and 18 have lower fetal fractions and a higher rate of test failures (16). Sex chromosome aneuploidies also have higher no-call rates than trisomies (19). Although repeat screening may be considered in the setting of a sample drawn at an early gestational age or a specific concern regarding sample characteristics, because repeat sampling delays a diagnostic test, it is not advised if screening results are consistent with sonographic anomalies, or if a patient is at a gestational age at which the delay may compromise their reproductive options. The success of repeat sampling after a test failure in a general screening population is 75–80%, although it is substantially lower in patients with a high BMI (17, 53).

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

Information regarding gestational age, viability, the number of fetuses, evaluation for a vanishing twin or empty gestational sac, and the presence of an obvious fetal anomaly will affect counseling regarding the risks, benefits, and limitations of testing options.

First-Trimester Ultrasound

Nuchal translucency is the primary ultrasound marker that is used to assess for risk of chromosomal abnormalities in the first trimester (Table 4). An increased NT measurement increases the risk of genetic syndromes and anomalies, such as congenital heart defects, abdominal wall defects, and diaphragmatic hernia, even with normal chromosomes on diagnostic testing (54). The finding of an increased NT extending along the length of the fetus in which septations are clearly visible is referred to as a cystic hygroma. In a retrospective cohort of 944 fetuses with a cystic hygroma in the first trimester, a karyotype abnormality occurred in 55% of fetuses (most commonly trisomy 21, monosomy X, and trisomy 18) and a major congenital anomaly occurred in 29% of fetuses with a normal karyotype (cardiac anomalies were the most common form of major congenital anomaly, followed by urinary, central nervous system, and body wall anomalies). Perinatal loss occurred in 39% of fetuses not electively terminated. Overall, an abnormal outcome occurred in 87% of fetuses (55).

If an enlarged NT or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions as well as a comprehensive ultrasound evaluation including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities (32). Given the high risk of congenital heart disease in these fetuses, referral for fetal cardiac ultrasonography may be beneficial (56).

Second-Trimester Ultrasound

Independent of screening or diagnostic testing, all patients should be offered a second-trimester sonogram to assess for structural abnormalities (32). Fetuses with trisomy 18 and 13 are likely to have major structural anomalies. In contrast, only about 27% of fetuses with trisomy 21 have a recognizable major structural abnormality by ultrasound examination in the second trimester (57). Soft sonographic markers may also be identified, and these markers have different degrees of association with trisomy 21 and cannot be used in isolation to diagnose or exclude the diagnosis of trisomy 21. The risk of aneuploidy associated with each marker should be considered individually within the complete clinical context (Table 4). The presence of particular or multiple soft ultrasonographic markers for aneuploidy may warrant detailed fetal anatomic ultrasound examination to exclude other abnormalities and a review or offering of initial or additional screening and diagnostic testing for fetal chromosomal abnormalities. In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding, and cell-free DNA, quad screen testing, or amniocentesis should be offered. If aneuploidy testing is performed and the result is low risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended (58, 59).

▶ ***How does screening for chromosomal abnormalities differ in twin gestations?***

No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations. Further, there are no data available for serum screening for higher-order multiple gestations such as triplets and quadruplets. Analysis of the risks and benefits of screening or diagnostic testing in patients carrying multiple fetuses is complex, given the lower effectiveness of screening and how the prenatal

Table 4. Management of Ultrasonographic Markers for Aneuploidy

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
First trimester: enlarged nuchal translucency	Certified US measurement ≥3.0 mm or above the 99th percentile for the CRL	Aneuploidy risk increases with size of NT Also associated with other structural anomalies and genetic disorders	Genetic counseling. Offer diagnostic testing. Comprehensive US evaluation including a detailed US at 18–22 weeks. Fetal cardiac US may be considered if the NT is 3.0–3.4 and is recommended if the NT is 3.5 or greater.
First trimester: cystic hygroma	Large single or multilocular fluid-filled cavities, in the nuchal region and can extend the length of the fetus	About 50% are aneuploid	Genetic counseling. Offer diagnostic testing. Comprehensive US evaluation including a detailed US at 18–22 weeks and fetal cardiac US.
Second trimester: thickened nuchal fold	≥6 mm from outer edge of the occipital bone to outer skin in the midline at 15–20 weeks	Associated with Trisomy 21	Detailed anatomic survey. Genetic counseling. Aneuploidy testing should be offered if not previously performed.
Second trimester: absent or hypoplastic nasal bone	Nonvisualization of the nasal bone or nasal hypoplasia based on multiples of the median (MoM) or percentiles or the biparietal diameter/nasal bone length (BPD/NBL) ratio	Varies by race/ethnicity Absent in 30–40 percent fetuses with Trisomy 21 and 0.3 to 0.7 percent of euploid fetuses Hypoplastic in about 50–60 percent of fetuses with Trisomy 21 and 6 to 7 percent of euploid fetuses	Detailed anatomic survey. Genetic counseling. Aneuploidy testing should be offered if not previously performed.
Second trimester: pyelectasis	Renal pelvis measuring ≥4 mm in anteroposterior diameter up to 20 weeks of gestation	Associated with Trisomy 21	If isolated finding, aneuploidy testing should be offered if not previously performed. Repeat US in third trimester to assess need for postnatal imaging.
Second trimester: echogenic bowel	Fetal small bowel as echogenic as bone	Associated with Trisomy 21, intra-amniotic bleeding, CF, CMV, and FGR	Detailed anatomic survey. Genetic counseling. Offer CMV, CF, and aneuploidy testing. Consider follow up US for fetal growth because of the association with FGR.
Second trimester: mild to moderate ventriculomegaly*	Lateral ventricular atrial measurement measures between 10–15 mm	Associated with Trisomy 21, infection.	Detailed anatomic survey. Genetic counseling. Offer diagnostic testing for genetic conditions and CMV. Consider fetal MRI. Repeat US in third trimester.

(*continued*)

Table 4. Management of Ultrasonographic Markers for Aneuploidy (*continued*)

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
Second trimester: short femur length	Measurement <2.5 percentile for gestational age	Can be associated with aneuploidy, FGR, skeletal dysplasia, or other genetic diagnosis	Aneuploidy testing should be offered if not previously performed. Consider repeat US in third trimester for fetal growth.
Second trimester: echogenic intracardiac foci	Echogenic tissue in one or both ventricles of the heart seen on standard four-chamber view	Seen in 15–30% of fetuses with trisomy 21 and 4–7% euploid fetuses	If isolated finding, aneuploidy testing should be offered if not done previously. Describe finding as not clinically significant or as a normal variant with normal screening.
Second trimester: choroid plexus cysts	Discrete small cyst(s) in one or both choroid plexus(es)	Seen as an isolated finding in 1–2% of the normal population. Associated with trisomy 18 when seen in combination with other anomalies.	If isolated finding, aneuploidy testing should be offered if not previously performed. Describe finding as not clinically significant or as a normal variant with normal screening.

Abbreviations: CF, cystic fibrosis; cfDNA, cell-free DNA; CMV, cytomegalovirus; CRL, crown–rump length; CVS, chorionic villus sampling; FGR, fetal growth restriction; MRI, magnetic resonance imaging; NT, nuchal translucency; US, ultrasound.

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identification of a single aneuploid fetus might affect the pregnancy management.

Presumably, monozygotic twins have the same genetic information in both fetuses reflecting a single test result, although monozygotic twins discordant for karyotype have been described (60, 61). In a dizygotic twin pregnancy, a screen positive test infers that at least one of two fetuses would be aneuploid. This assumes that monozygotic pregnancies have equivalent trisomy 21 risk per pregnancy relative to maternal age-matched singletons and dizygotic pregnancies have twice the risk of at least one affected fetus. However, the observed incidence of trisomy 21 has been reported to be lower than expected for monozygotic, dizygotic, and all twin pregnancies, most notably among monozygotic pregnancies and with increasing maternal age (62).

First-trimester, quad, and sequential or integrated screening are options available to screen twin gestations, although few data on test performance are available from prospective studies. Second-trimester serum screening of

twin gestations can identify approximately 60% of fetuses affected with trisomy 21 at a 5% screen positive rate (63). A recent meta-analysis suggests that first-trimester combined screening in twins has a detection rate of 89% with a false-positive rate of 5.4%, which is similar to singleton gestations (64).

Although serum screening evaluates the pregnancy as a whole, the NT measurement directly evaluates the individual fetus. The distribution of NT measurements does not differ significantly between singletons and twins, and standard cutoffs used in singleton gestations can be used (65). One study reviewed individual first-trimester screening in twin gestations and generated individual risks for each fetus with NT and first-trimester screening. At a 1:300 risk cutoff, the detection rate was 75% with a 9% positive screening rate for trisomy 21 (66).

Cell-free DNA screening can be performed in twin gestations. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13. Twin fetuses in a single pregnancy each contribute different amounts of cell-free DNA into the maternal circulation. It is possible that an aneuploid fetus would contribute less fetal DNA, therefore masking the aneuploid test result. Recent studies have suggested that sensitivity for trisomy 21 with cell-free DNA in twin pregnancies may be similar to singletons when a test result is returned; however, there is a higher rate of test failure (45, 67). In one study examining the reasons for test failure in singletons and multiple gestations, the test failure rate at the time of the first draw was 3.4% in singletons, 4.9% in monochorionic twins, and 11.3% in dichorionic twins (45). All reports give one test result for a twin pregnancy, although one laboratory method which uses SNP analysis reports zygosity as well as individual fetal fractions.

In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.

▶ ***What is the role of aneuploidy screening in patients who have undergone previous preimplantation genetic testing?***

Preimplantation genetic testing (PGT) yields genetic information for women undergoing assisted reproductive technologies. Preimplantation genetic testing for single gene (Mendelian) disorders (PGT-M) and structural rearrangements (PGT-SR) are available to test embryos to identify a specific X-linked, mitochondrial, single gene, or chromosomal disorder or to undergo HLA typing before embryo transfer into the uterus (68, 69). In contrast, preimplantation testing for aneuploidy (PGT-A) is designed to identify euploid embryos before transfer, with the intent to improve live birth rates and clinical outcomes (69). At present, the American Society of Reproductive Medicine states that there is insufficient evidence to support the use of PGT-A for the purpose of improving IVF success rates (70).

Data regarding aneuploidy screening for women who have undergone previous preimplantation genetic testing are lacking. In theory, for a patient with normal preimplantation genetic testing, the pretest risk for aneuploidy in pregnancy should be lower and might be used in conjunction with age and other factors to determine pretest risk (71). However, the role of preimplantation genetic testing in determining the pretest risk and need for aneuploidy screening has not been adequately studied. Additionally, false-negative test results can occur (72). Therefore, because preimplantation genetic testing (PGT-M, PGT-SR, and PGT-A) is not uniformly accurate, prenatal screening and prenatal diagnosis should be offered to all patients regardless of previous preimplantation genetic testing (73).

▶ ***What additional or incidental information may be obtained from tests intended to screen for chromosomal abnormalities?***

Fetal and Obstetric Complications Associated With Abnormal Screening Results

False-positive cell-free DNA test results occur because of confined placental mosaicism, which can be associated with an increased risk of fetal growth restriction. High or low fetal fraction has been associated with adverse pregnancy outcomes in some studies (74–76). Serum analyte screening can identify pregnancies at risk for certain adverse pregnancy outcomes in patients with abnormal analyte levels and normal appearing fetuses. The likelihood of an adverse pregnancy outcome increases with increasing number of abnormal marker levels in the same screening test and with more extreme analyte values (77). In the first trimester, maternal serum levels of PAPP-A below the 5th percentile are independently associated with obstetric complications, such as spontaneous fetal and neonatal loss, fetal growth restriction, preeclampsia, placental abruption, and preterm delivery (78), although the PPV of this marker alone is poor (79). In the second trimester, elevated hCG, AFP, and DIA levels in pregnancies without structural anomalies are associated with

an increased risk of fetal death, fetal growth restriction, and preeclampsia (80, 81). Although many of the associations between maternal serum markers and adverse obstetric outcomes are statistically significant, the sensitivity and PPVs for the individual outcomes are too low for them to be recommended as screening tests for adverse pregnancy outcomes (77, 82). If these findings are identified in the testing performed for fetal aneuploidy, follow-up ultrasound examination for growth or antenatal testing may be considered.

Maternal Conditions Associated With Abnormal Cell-Free DNA Results

When a screen positive cell-free DNA result differs from the fetal karyotype, the etiology may include maternal mosaicism, such as mosaic maternal 45, X, or in rare instances, it can occur secondary to a maternal malignancy. Malignancy in pregnancy, defined as cancer identified either in pregnancy or up to 1 year postpartum, complicates about 1:1,000 pregnancies (83, 84). If a single monosomy (other than 45, X) or if more than one aneuploidy is detected in a cell-free DNA result, the incidence of malignancy is increased (85, 86). In patients with multiple aneuploidies identified by cell-free DNA screening, the incidence of occult malignancies was reported in one study to be 18% (86) although follow-up data from this study are incomplete. Of the reported cases in this series, the majority of malignancies have been hematologic but other types of cancer, such as anal and colorectal malignancies, were also identified. If unusual or multiple aneuploidies are noted, a family history should be obtained for familial cancer syndromes and a physical examination for lymphadenopathy, breast, and thyroid masses should be performed. A review of the patient's complete blood count, complete metabolic profile, Pap test, and fecal occult blood testing followed by oncology consultation and imaging studies should be considered (87). Given the rarity of this presentation, no guidelines are available at this time. Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation.

Summary of Recommendations

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

- Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant women regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.
- If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously.
- Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing.
- All patients should be offered a second-trimester ultrasound for fetal structural defects, since these may occur with or without fetal aneuploidy; ideally this is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein).
- Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.
- Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings become evident such as fetal anomalies identified on ultrasound examination.
- Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.
- If an enlarged nuchal translucency or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions as well as a comprehensive ultrasound evaluation including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities.

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

- The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding and cell-free DNA, quad screen testing, or amniocentesis should be offered. If aneuploidy testing is performed and is low-risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended.
- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations.
- Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13.
- Because preimplantation genetic testing is not uniformly accurate, prenatal screening and prenatal diagnosis should be offered to all patients regardless of previous preimplantation genetic testing.

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

- The use of multiple serum screening approaches performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.
- Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation.

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The MEDLINE database, the Cochrane Library, and the American College of Obstetricians and Gynecologists' own internal resources and documents were used to conduct a literature search to locate relevant articles published between January 2000–February 2020. The search was restricted to articles published in the English language. Priority was given to articles reporting results of original research, although review articles and commentaries also were consulted. Abstracts of research presented at symposia and scientific conferences were not considered adequate for inclusion in this document. Guidelines published by organizations or institutions such as the National Institutes of Health and the American College of Obstetricians and Gynecologists were reviewed, and additional studies were located by reviewing bibliographies of identified articles. When reliable research was not available, expert opinions from obstetrician–gynecologists were used.

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

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

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

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

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

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

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The information is designed to aid practitioners in making decisions about appropriate obstetric and gynecologic care. These guidelines should not be construed as dictating an exclusive course of treatment or procedure. Variations in practice may be warranted based on the needs of the individual patient, resources, and limitations unique to the institution or type of practice.

Conflicts of Interest: Drs. Rose, Kaimal, Dugoff, and Norton have disclosed no conflicts of interest related to this topic.