Northwest Perinatal Center

Perinatal Progress

Recommendations for

Genetic Screening in Pregnancy

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Chromosome abnormalities occur in 1 in 150 live births, with an even higher prevalence earlier in pregnancy. Since aneuploidy occurs with high prevalence and great clinical significance, the American College of Obstetrics and Gynecology (ACOG), Society of Maternal Fetal Medicine (SMFM) and American College of Medical Genetics (ACMG) recommend all women should be offered aneuploidy screening or diagnostic testing regardless of maternal age. In addition, preconception or prenatal counseling should include carrier screening for relatively common conditions like cystic fibrosis and spinal muscular atrophy. The emergence of commercial screening tests and interest in personalized genetics have expanded knowledge of an individual's genetic risk. Without appropriate counseling, this can lead to confusion or be a lost opportunity. The purpose of this article is to review the current ACOG recommended genetic screening strategies. Our next issue will include a detailed discussion on carrier screening.

Aneuploidy screening options

Aneuploidy indicates the presence of an abnormal chromosome number through either the loss or addition of a chromosome with resultant large gains or losses in genetic material. This can alter gene expression and result in pregnancy miscarriage, neonatal demise or long term health problems, such as intellectual disability, seizures, structural abnormality and shortened life span. The risk to a woman of having a fetus with a chromosomal abnormality does not vary by race or ethnicity but does increase with age. A prior affected pregnancy or presence of fetal structural abnormality increases the risk for fetal aneuploidy.[1] Non-invasive options of aneuploidy screening include sequential screen, serum integrated screen, Quad screen and what has been known as non-invasive prenatal testing (NIPT).

Sequential screening

One strategy for first trimester aneuploidy screening is the sequential screen. This testing requires an ultrasound measurement of the nuchal translucency (NT) and maternal serum hormone levels. Between 11- 13 weeks, when the crown-rump length typically measures between 45-84 mm, an ultrasound is performed to measure the NT. If NT is obtained, the patient also has the serum hormones B-hCG and PAPP-A drawn, and both are sent to a laboratory for a preliminary risk assessment for Down syndrome and trisomy 18. An enlarged NT can increase concern for a broad range of fetal abnormalities, including aneuploidy, genetic conditions, neuromuscular disorders and structural abnormalities.

Other factors, such as maternal age, weight, race and fetal number are included in the test algorithm. In the second trimester, serum hormone level of hCG, alpha fetal protein (AFP), estriol and inhibin A are obtained. Sequential screening has a detection rate of 91-95% with a screen positive rate of 5% (Table 1).[1] Sequential screening is validated in twin gestations.

Sequential screening is the recommended screening test for low risk women because of its high detection rate of Down syndrome and ability to screen for a wide variety of other conditions.

Serum integrated screening

Serum integrated screening is an option for women when the NT cannot be obtained. This testing takes the two serum portions of sequential screening and calculates a screening result without the NT. These results are only reported if both blood samples are obtained. Serum integrated screening can be offered in twin gestations. Detection rates for Down syndrome with serum integrated screening are 88%.[1] (Table 1)

Cell free DNA screening

In 2013, aneuploidy screening saw a big change when cell free DNA screening (cfDNA) became commercially available. Though it is commonly referred to as "NIPT," this is a misnomer and it is important to recognize this is a screening and not a diagnostic test. Therefore, we will refer to it as "NIPS"—non-invasive prenatal screening. This blood test utilizes fragments of DNA from the placental trophoblast cells undergoing apoptosis. The DNA is present in

Table 1: Summary of genetic screening and diagnosis options

TEST	GESTATIONAL AGE FOR SCREENING	DETECTION RATE	SCREEN POSITIVE	BENEFITS	LIMITATIONS / RISKS
Sequential screening	• Step 1: 11-14 weeks • Step 2: 15-22 weeks (ideally 16-18)	• T 21: 95% • T 18: 90% • ONTD: 80%	5%	1. 1st trimester access to results 2. Early fetal anatomy assessment 3. Analyte correlation with placenta and adverse pregnancy outcomes	1. Ultrasound required 2. Stepwise approach with two samples needed
Serum Integrated screening	• 11-14 weeks then 15-22	• T 21: 88% • T 18: 90% • ONTD: 80%	5%	1. Best option for screening low risk women when NT can't be obtained 2. Screens for neural tube defects 3. Analyte correlation with placenta and adverse pregnancy outcomes	1. Two samples needed before results are reported 2. Does not include NT; therefore, no early evaluation of structural abnormality
Quad screen	• 15 0/7 - 22 6/7 weeks	• T 21: 80% • T 18: 75% • ONTD: 80%	5%	1. Easily available and low cost for second trimester screening 2. Screens for neural tube defects 3. High risk results may indicate other aneuploidy or adverse pregnancy risks	1. Does not include ultrasound 2. Possibly fewer options for patient since done later in gestation 3. Lower detection for Down syndrome
Cell-free fetal DNA screening	• > 10 weeks	• T 21: 99.3% • T 18: 97.4% • T 13: 91.6% • Sex chromosome aneuploidy (XX, XY): 91%	0.5%	1. High detection rate 2. Low false positive rate in women over 3 5 3. Available at most gestational ages	1. Limited to singleton gestation 2. High false positive rate in low risk women 3. May detect maternal conditions 4. Not validated in twins 5. Does not screen for all chromosome conditions
Chorionic villus sampling	• 11-14 weeks	• T 21: > 99% • All aneuploidies: > 99%	1%	1. First trimester diagnostic assessment for aneuploidy and genetic syndromes 2. Early prenatal diagnosis with known familial hereditary conditions	1. Pregnancy loss rate 1:100 2. Confined placental mosaicism 3. Maternal cell contamination
Amniocentesis	• 15-20 weeks	• T 21: > 99% • All aneuploidies: > 99%	0.2%	1. Recommended for fetal structural abnormalities or positive screening tests for comprehensive genetic assessment 2. Second trimester prenatal diagnosis with known familial genetic conditions	1. Pregnancy loss rate 1:1000

Adapted from ACOG Practice Bulletin 163 Screening for Fetal Aneuploidy, 2016 and SMFM, Prenatal aneuploidy screening with cfDNA, Am J Obstet Gynecol 2015..

the maternal blood and comprises 3-13% of the total cell free DNA in maternal blood (fetal fraction) and can be screened for aneuploidy.[1,2] Maternal blood is drawn after 10 weeks gestation and molecular testing analyzes DNA for common aneuploidies. Cell free DNA screening can be used to screen for Down syndrome, trisomy 13, 18 and sex chromosome aneuploidy (as well as fetal Rh status in Rh negative mothers).

Detection for Down syndrome is reported at over 98% but with lower detection of trisomy 13 and 18.[1,2] Around 3% of NIPS samples may not give a result or be considered a "no call." This may be related to technical failure or low fetal fraction. Reasons for low fetal fraction include maternal obesity, as well as an increased risk of fetal aneuploidy.[1,2,4] The former is related to dilution of the fetal cell free DNA while the later is likely due to smaller placental volume and thus decreased release. Ultrasound for fetal viability and

genetic counseling should be considered as next steps after a "no-call." Although NIPS has a higher sensitivity for aneuploidy, a positive result requires accurate counseling. The chance of an affected infant reflects the positive predictive value (PPV) and is dependent on the prevalence of disease in the patient population, which varies based upon the patient's age. Therefore, younger women have a lower PPV than women over age 35 and this may impact their decision for diagnostic testing.[2]

Additional limitations of NIPS include the possible detection of maternal genetic conditions, including mosaicism and malignancy. NIPS is not recommended in twin gestations or "vanishing twin" pregnancies.

Current recommendations favor use of NIPS screening in women over age 35 because they are at increased risk for the selected aneuploidies included in this screening test and this test has a low false positive rate. NIPS has a higher false positive rate in low risk women.

Quad screening

In some cases, a woman may not present for prenatal care until the second trimester. The quadruple marker screen can be performed from approximately 15 0/7 weeks to 22 6/7 weeks of gestation (although some variation among labs exists). The test is ideally performed between 16-18 weeks. This test measures hCG, AFP, inhibin A and estriol, in addition to maternal demographic information and fetal number. Down syndrome detection rate is greater than 80% at a 5% screen positive rate (Table 1). This test also screens for open neural tube defects and other genetic conditions that are not screened for with NIPS. ***This is an appropriate and cost effective option for the low risk woman who elects screening in the second trimester.***

Genetic counseling and consideration of diagnostic testing is recommended for any positive aneuploidy screen result.

Applying screening strategies to clinical practice

Why is sequential screening the recommended option for low risk women? As mentioned above, sequential screening with serum markers and nuchal translucency is the most appropriate option for a pregnancy at low-risk for chromosome abnormality. This scenario generally applies to women under 35 years of age, without prior affected pregnancy or other significant family history. Women at higher risk for common aneuploidy (e.g., those over 35, prior affected pregnancy by common aneuploidy, Robertsonian translocation carriers) may benefit from screening with cfDNA. The rationale for this recommendation comes from a study that compared cfDNA and sequential screening for detection of chromosomal abnormalities. The study included women with a positive sequential screen result who elected diagnostic testing with amniocentesis to expected aneuploidy detection with cfDNA alone. Comparing cfDNA to sequential screening, they reported detection would be expected to be improved for Down syndrome (cfDNA 95.9% vs sequential screen 92.9%), unchanged for trisomy 13 or monosomy X, improved among other sex chromosome abnormalities and lower for trisomy 18 with cfDNA. Of all women in this cohort who had a fetus with a chromosome abnormality, 53% would have been detected by sequential screening and reflex amniocentesis but not detectable with cfDNA. In total, ***cfDNA would have detected 70% of all chromosome abnormalities in the cohort while sequential screen would have detected 81.6%.***[3]

SMFM has endorsed respecting patient autonomy and permitting a woman to choose the testing option that she prefers. Therefore, genetic counseling is strongly recommended to inform patients about testing options and ensure they are aware of the benefits and limitations of cfDNA screening.[2,3,4] (Table 1)

Is there a role for nuchal translucency ultrasound with cell free DNA screening? If cfDNA screening is the elected primary testing strategy, the residual risk of chromosome abnormality after a negative screen is 2.5%. Does addition of NT measurement reduce this residual risk? Yes and no. An increased NT measurement has been correlated with increased risk of aneuploidy and structural abnormalities. An enlarged NT is most useful for detection of trisomy 13, 18 and 21 because they are higher prevalence aneuploidies. An enlarged NT can also signal an increased risk for other rare genetic syndromes or structural abnormalities. In a high-risk cohort of women with negative cfDNA screening, obtaining a NT measurement in conjunction with cfDNA improved detection of chromosome abnormality by just 6%, while increasing the

CVS rate significantly from 2% to 22%. If the NT measurement is less than 3.0mm in the setting of negative cfDNA screening, the residual risk for chromosome abnormality is reduced to 1%.

Weighing the limited additive benefit vs. risk of additional invasive procedures, current guidance from ACOG and SMFM state that NT measurement for aneuploidy is not necessary if cfDNA is chosen. Therefore, if a woman has had a prior dating and viability ultrasound at 9 weeks and then elects cfDNA at 11 weeks, a repeat ultrasound is not indicated. If she elects cfDNA screening and an ultrasound has not been done, one should be done to confirm viability, fetal number and gestational age. As the availability of an anatomy survey in the 1st trimester increases, the ideal timing will be at 12 and 13 weeks. In these cases, the region of the NT should be viewed, if between 11-13 weeks, and if it appears enlarged, genetic counseling should be provided to inform the patient about invasive testing options.[4]

Should msAFP be collected in patients electing cfDNA? Women with fetuses with open neural tube defects (ONTD) typically have elevated AFP hormone. This is because neural tube defects and other lesions (omphalocele, gastroschisis, bladder extrophy, skin lesions, among others) can leak fetal AFP into the amniotic fluid which crosses over to the maternal circulation. AFP analysis is included in sequential and serum integrated screening since fetuses with Down syndrome can have low AFP levels. Maternal serum AFP (msAFP) can be ordered for ONTD risk assessment. It is ideally performed between 16-18 weeks. Interpretation of results is very dependent on gestational age since msAFP rises with gestation and even a 1-2 week discrepancy can lead to misleading results. An msAFP multiple of the median (MoM) greater than 2.0 has a high detection rate for anencephaly of 100% and between 85-92% for ONTD. The false positive rate ranges from 2-5%. Results are impacted by maternal weight, ethnicity and presence of diabetes.[5]

With the advancement of ultrasound technology, second trimester ultrasound screening is a reliable tool to evaluate for structural abnormalities with similar detection rate as described for msAFP screening[6] and lower false positive rates. ***Therefore, NWP does not recommend submitting an msAFP sample in women who have had NIPS, as a 20 week anatomy scan should identify these anomalies with high accuracy and low false positive rate.***

What is the role of second trimester anatomy ultrasound in genetic screening? All women should be offered an ultrasound exam for fetal evaluation of structural abnormalities in the second trimester. The sensitivity of ultrasound to detect structural abnormalities varies between 15-80 percent, with the highest rate of detection reported in tertiary-level centers.[6] A routine sonogram is generally performed women without additional pregnancy risk. A detailed exam is indicated based on specific history or exam, including (but not limited to):

- previous fetus or child with congenital, genetic or chromosome abnormality;
- known or suspected abnormality;
- fetus at increased risk for congenital, genetic or chromosome abnormality (e.g. advanced maternal age); or
- other maternal conditions or exposures that could affect the fetus.[7]

In women with advanced maternal age, a normal ultrasound can decrease the risk of aneuploidy by more than 80%. However, this is not true for low-risk women. Additionally, second trimester ultrasound should not be used with the primary goal of excluding the risk for Down syndrome because structural abnormality that leads to the diagnosis of fetal Down syndrome occurs in only half of fetuses with Down syndrome.[1]

Ultrasound soft markers can aid in the diagnosis of genetic disorders. Each marker has an associated risk for aneuploidy; in particular, Down syndrome. Identification of a soft marker on routine screening should prompt a detailed exam to further evaluate the fetus. If a soft marker is identified, correlation should then be made to prior aneuploidy screening results. If aneuploidy screening was not previously elected, the woman should be counseled about the implications of the soft marker and offered genetic evaluation or genetic counseling. A list of soft markers, their significance and management is included in Table 2.

Should women who are identified to have structural abnormalities be recommended cfDNA? Identification of a fetal structural abnormality significantly increases the risk of a chromosome abnormality. A negative cfDNA in

the setting of a structural abnormality will result in a residual risk of a chromosome abnormality of 1 in 15 (6.7%). Diagnostic testing with chromosome microarray is the preferred strategy to improve detection of chromosome abnormalities. Microarray will detect a genetic abnormality in 6-7 % of fetuses with a structural abnormality and a normal karyotype. In a National Institute of Child Health and Human Development-sponsored study utilizing microarray with clinically significant structural abnormalities, cfDNA would be expected to miss 32% of chromosome abnormalities detected on microarray.[4] ***Current guidelines recommend that women be offered diagnostic testing when a structural abnormality is found on ultrasound.***

How does comprehensive chromosome screening (CCS) change genetic testing options for pregnancies conceived with artificial reproductive technology? Preimplantation genetic testing describes the process of testing an embryo prior to implantation. It can be done for a specific, known familial genetic condition or with the goal of comprehensive chromosome screening. It can be performed on the polar bodies from the oocyte and zygote, a single blastomere from a cleavage-stage embryo or a group of cells from the trophectoderm (future placenta) at the blastocyst stage. Since this test uses one or a few cells from the future embryo or placenta, errors are possible due to mosaicism. In general, this technology has a nearly 98% detection rate for chromosome abnormalities. In pregnancies where there is concern for a specific genetic disorder or a suspected structural abnormality, diagnostic testing with either CVS or amniocentesis is recommend-

Table 2: Second trimester soft markers of aneuploidy

SOFT MARKER	FINDING ON IMAGING	ANEUPLOIDY ASSOCIATION	MANAGEMENT
Echogenic intracardiac focus (EIF)	• Echogenic focus of tissue seen in either cardiac ventricle's in the four chamber view	• DS LR 1.4-4.8 • 4-7% euploid fetuses • Seen in 15-30% DS fetuses	1. Prior screening low risk- no further evaluation needed 2. Offer genetic screening for aneuploidy if not previously done
Choroid plexus cyst	• Cyst located within one or both of the choroid plexus	• Associated with Trisomy 18 however, in isolation does not significantly increase the risk for aneuploidy	1. Complete a detailed anatomy exam 2. Consider aneuploidy screening if not previously completed 3. No follow-up indicated if isolated, generally resolve
Renal pyelectasis	• Renal pelvis measurement ≥ 4mm before 20 weeks	• DS LR 1.5-1.6	1. In isolation, offer aneuploidy screening if not previously performed 2. Repeat 3rd trimester ultrasound to evaluate for urinary tract abnormalities
Echogenic bowel	• Fetal small bowel presenting as echogenic as fetal bone	• DS LR 5.5-6.7 • Associated with aneuploidy, intra-amniotic bleeding, cystic fibrosis, and congenital CMV	1. Further counseling, risk assessment 2. CMV serology, CF carrier screening, and aneuploidy screening. 3. 3rd trimester ultrasound follow-up
Thickened nuchal fold	• ≥6mm prior to 20 weeks gestation	• DS LR 11 – 18.6, 40-50 % sensitivity and › 99% specificity	1. Detailed anatomy survey 2. Consider aneuploidy screening or diagnostic testing 3. Offer consult with MFM/genetics
Ventriculomegaly	• Lateral ventricle measurement 10 -15 mm	• DS LR 25 Association with aneuploidy	1. Detailed anatomy survey 2. Consider diagnostic testing for aneuploidy and CMV 3. Offer consult with MFM/genetics
Short long bones Short femur Short humerus	• Measurement <2.5 % for gestational age • M/E <0.91 for femur, 0.9 for humerus	• DS LR 2.7 femur • DS LR 7.5 humerus • Associated with aneuploidy, IUGR, skeletal dysplasia	1. Detailed anatomy survey 2. Offer consult with MFM/genetics 3. 3rd trimester growth evaluation

Adapted from ACOG Practice Bulletin 163 Screening for Fetal Aneuploidy, 2016. DS, Down syndrome. LR, likelihood ratio. M/E, measured to expected.

ed.[8] For all other pregnancies, prenatal aneuploidy testing should be offered to all women pregnant with embryos chosen using CCS technology due to the risk of false negative, as well as the limitations of this technology. The choice of which test to perform is often a complex and detailed conversation and genetic counseling is recommended to review the available screening tests.

Conclusion

Patient counseling is invaluable before choosing a testing method. It allows the provider to learn about the patient's values and goals for testing, and may direct discussion toward diagnostic testing if more accuracy is desired. Alternatively, screening may be the preferred strategy in cases where the risk of invasive testing exceeds the patients comfort or would not affect their management of pregnancy. Some patients may opt out of testing all together. Understanding each test's detection rates, advantages and limitations should be shared for her decision making. Hopefully, what we've shared will help inform and expand the discussion between providers and patients, leading to better patient satisfaction through improved understanding—and we are always happy to assist.

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