NON-INVASIVE TESTING IN PREGNANCY: UPDATE FOR THE PERINATAL PROVIDER

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OBJECTIVES

• Describe common screening tests for low-risk pregnancy by trimester.

• Discuss new methods to accomplish genetic screening as part of routine prenatal care.

• Identify abnormal screening results and subsequent follow-up care.
INITIAL PRENATAL ASSESSMENT

- CBC
- ABO, Rh, antibody screens
- VDRL/ RPR
- HbSAg
- Rubella status
- HIV
  - Texas requires initial visit and third trimester or labor HIV
- UA w/culture
- G/C-chlamydia probe
COMPLETE BLOOD COUNT (CBC)

- Can diagnose pre-existing anemia in early pregnancy
  - In pregnancy, anemia is Hgb less than 10
- Gives baseline to evaluate for physiologic drop at 28 weeks
- Elevated WBC can indicate infection
- May also lead to identification of thalassemia or sickle cell disease
- Platelet abnormalities can be diagnosed
BLOOD TYPE, RH, ANTIBODY SCREEN

• Possibility of ABO incompatibility with type O mother and type A or B baby
• Rh issues:
  • Rh negative mother does not have Rhesus factor
  • With Rh+ fetus, any mixing of fetal-maternal blood will result in antibodies made by mom
  • Rh+ is foreign tissue

• Type/Rh/Antibody – ex: A+/−
RH AND OTHER ANTIBODIES

• Rh issues
  • RhoGam prevents formation of maternal antibodies
  • Given at 28 weeks of pregnancy, for any invasive procedures, or SAB/TAB
  • Given whenever Rh negative mother has an Rh+ baby

• Besides Rh there are other rare antibodies
  • Lewis is not a threat to fetus
  • Duffy-b can cause hemolytic disease with hydrops
  • Kell (K or K1) can cause severe anemia, hydrops and death
  • Other, even less common, types exist
VDRL/RPR - SYPHILIS

- Venereal disease research laboratory/ rapid plasma reagin tests
- These tests screen for antibodies that can be produced with syphilis & other diseases
- Positive result requires further testing
  - FTA-ABS:
    - Fluorescent treponemal antibody absorption
    - not positive in 1st 3-4 wks of infection
  - Darkfield exam: shows actual spirochetes
- In pregnancy: causes stillbirth or congenital syphilis
- Texas law requires a repeat test at delivery
HEPATITIS B

- Mother is tested for surface antigen (HepBSag), a sign of chronic infection
  - Test required at first visit & at delivery by Texas law
- Fetus at risk when it comes in contact with maternal blood at birth
- 90% of + mothers are chronic carriers; 10% chronically infected
- Hep B + mom: infant care
  - No FSE or IUPC in labor if mom is Hep B +
  - Must bathe baby as soon as possible, before injections or percutaneous tests
  - Give Hep B vaccine + immunoglobulin (HBIG)
- Giving Hep B at birth to all newborns meant to protect against hidden maternal disease
RUBELLA

- Rubella titer is drawn
- Titer above level set by the lab shows immunity
- Immunity means no new rubella in pregnancy
- Rubella in pregnancy linked to fetal disease
  - Congenital rubella syndrome 1st trimester
    - Deafness, mental retardation, heart, eye, brain malformations, other malformations can occur
  - Rarely a problem after 20 weeks pregnancy
- Mothers who are rubella equivocal or non-immune will receive an MMR vaccination postpartum prior to dismissal – usually just before discharge home
  - Women should NOT become pregnant for 3 months after this vaccine, so immediate postpartum is a good time to vaccinate
HIV

• Anytime STD test is positive, offer HIV testing
• ELISA test (enzyme-linked immunosorbent assay) is for HIV antibodies
  • Less specific, so confirm with Western blot if + ELISA
  • Many times this is what is called “reflex” testing
• It takes from 2 weeks to 6 months for antibodies to be made with HIV (window)
REPORTABLE DISEASES

• Conditions that are contagious and can have serious public health consequences are reported to the state department of health.
• Each state has some variation.
• [http://www.dshs.state.tx.us/idcu/investigation/forms/101A.pdf](http://www.dshs.state.tx.us/idcu/investigation/forms/101A.pdf)
• Common STIs are reportable in Texas: HIV, Hepatitis, syphilis, chlamydia, gonorrhea.
• Herpes is not reportable.
URINE TESTING

• Urine analysis
  • Test for RBCs, WBCs, urobilinogen, protein, glucose, nitrates, pH, ketone, specific gravity, bilirubin
    • RBCs, WBCs, nitrates, pH with UTI, vag infections
    • Protein: sometimes UTI or vag infection, pre-eclampsia
    • Glucose: possibly diabetes
    • Ketones, elevated specific gravity: dehydration
    • RBCs and WBCs: normal in labor (show)

• Urine culture
  • Many women have no s/s or altered s/s with UTI in pregnancy
  • UTI in pregnancy has been linked to preterm labor and birth
  • All women get a starting urine culture to identify asymptomatic bacteriuria
GONORRHEA & CHLAMYDIA

- Women often asymptomatic with gonorrhea
  - Linked to salpingitis, PPROM, preterm birth, chorioamnionitis, neonatal sepsis, IUGR in pg
  - Can cause opthalmia neonatorum/blindness in NB
  - Gonorrhea is reportable disease

- Women often asymptomatic with chlamydia
  - Linked to salpingitis, PID and infertility in men
  - Linked to PPROM, PTL, chorioamnionitis in pg
  - Neonatal pneumonia, conjunctivitis in NB
  - Chlamydia is reportable disease

- Cultures required by the state in early pregnancy
SICKLE CELL DISEASE/TRAIT

- Sickle cell affects 72,000 Americans, primarily those of African heritage, but also those of Arabian, Asian, Caribbean, Indian, Mediterranean, and South and Central American descent.

- Red blood cells become rigid, sticky and sickle-shaped and are more fragile.

- This results in periodic plugging of blood vessels, thereby preventing the delivery of oxygen to tissues and organs.
SICKLE CELL DISEASE/TRAIT

Why does sickle cell or thalassemia matter?
• With the disease, the pregnancy is higher risk
• With the trait, there is inheritance
  • Women with sickle cell trait are more prone to UTI in pregnancy
SUBSEQUENT LABS

- Targeted (Level II) ultrasound scan for women at high risk at 16-20 weeks
- Hbg/Hct ~ 28 weeks
- Glucose screening 26-28 weeks
- Antibody screen and RhoGAM for Rh negative women at 28 weeks
- Vaginal / rectal culture for Group B Strep ~35 weeks
DIABETES SCREENING

• Diabetes screening at 24-28 weeks
• One hour Glucose Tolerance Test (GTT):
  • 50 gm glucose load w/ one hour lab draw post dose; may draw fasting level pre-dose also
• Results >140-200 indicates need for 3 hour GTT
• 200 or > is diagnostic for gestational diabetes
• Repeat Hgb/Hct w/ this screen
GBS TESTING

• Group Beta Strep (GBS) swab of perineum and rectum ~ 35 weeks
  • Swab introitus, and through the anal sphincter for best sample
  • Mothers who are positive for GBS must be treated in labor
  • Must deliver at least 4 hours after first dose of antibiotic to ensure antibiotic in amniotic fluid
TESTS FOR FETAL WELL-BEING

- Kick counts
- NST
- BPP
- AFI
KICK COUNTS

- Evaluated by mother
  - Many different methods
  - In the third trimester all mothers should be monitoring their babies movement
KICK COUNTS

- Put a penny in a cup every time the baby moves in the morning; if 10 are not there by lunch, call clinic.
- Monitor movement for a 30 minute period
- Count 10 movements and chart how long it takes
- Mother should call clinic if eating or taking juice or sugar doesn’t “wake up” the baby
- Decreased fetal movement is often the first sign that a baby has a problem
INTIMATE PARTNER VIOLENCE

- Screen all women – at initial visit and once/trimester
- Always screen women alone
- Posters, business cards in bathrooms for hotlines
- Domestic violence may start in pregnancy or increase
- Up to 23% of women in prenatal care are battered
- Assess safety (i.e., weapons in home, type of previous assaults)
- Discuss exit plans with woman if possible
NST/BPP

- **Non-stress test:**
  - 20 min FHR tracing
  - \( \geq 2 \) accelerations = Reactive NST
  - If non-reactive:
    - Continue for additional 20 min, provide drink/snack
    - If still not reactive – complete BPP

- **Biophysical Profile:**
  - Components: fetal movement, fetal tone, fetal breathing, and AFI
  - Scored 0 (absent) or 2 (present)
AFI ASSESSMENT

• Amniotic Fluid Index measured via ultrasound
• Oligohydramnios: <5cm
• Polyhydramnios: single pocket >8cm
• Other assessments:
  • Fundal height measurement
  • Hydration status
  • Fetal anatomy assessment
  • Presence of fetal anomalies
ONGOING ASSESSMENT

Every visit:
- BP, P
- Weight
- Urinanalysis for glucose, protein, s/s dehydration or infection
- Fetal Heart Rate by Doppler after 12 weeks
- After 20 weeks, fundal heights for fetal growth
- Evaluation for HA, visual changes, edema as s/s pre-eclampsia
- Surveillance for common discomforts of pregnancy
MATERNAL SERUM SCREENING OPTIONS
• Integrated Screening vs Sequential Screening
• Available Tests
• Follow-Up for high risk patients
FIRST TRIMESTER SCREEN

- This test is done between 10 and 13 6/7 weeks after a woman’s LMP.
- Can show if a baby is at increased risk for trisomy 13, 18 or 21
- Women who have the first-trimester screening test for Down syndrome should be screened for NTDs in the second trimester by checking MSAFP levels or having an ultrasound exam
FIRST TRIMESTER SCREEN

- Blood test for free-beta hCG (a specific form of hCG) and pregnancy-associated protein A (PAPP-A).
  - Levels of PAPP-A tend to be decreased, and hCG increased, with Down syndrome.
- Ultrasound exam for nuchal translucency
- Positive results should be followed up with either chorionic villi sampling or second trimester amniocentesis
- Combined test is more accurate for Down’s, so is helpful if woman has other risk factors

Source: http://www.marchofdimes.com/professionals/14332_1166.asp
NUCHAL TRANSLUCENCY

• refers to the normal subcutaneous fluid-filled space between the back of the fetal neck and the overlying skin.

• In the fetus, fluid collects behind the neck, much like it does in dependent ankle edema in later life.

• This fluid can represent the end point of several pathological processes, including heart failure.

• The more fluid that has accumulated, the greater the risk of abnormality
NUCHAL TRANSLUCENCY – U/S

Source: http://www.womenshealthsection.com/content/obsdu/obsdu001.php3
ULTRASOUND

• Used to measure fetal size
• Estimate due dates
• Specific exams for anomalies: Level 2 US
• Nuchal translucency
  • Normal translucency measures 3 mm (.3cm) or less
• Locate fetus, cord or placenta for diagnostic procedures
QUAD SCREEN

- At 15-19 weeks offer quad screen
  - Earlier is better in case of abnormalities
  - Alpha-fetoprotein (AFP) -- a protein produced by the fetus' liver
  - Unconjugated Estriol (UE) -- a protein produced in the placenta and in the fetus' liver
  - Human Chorionic Gonadotrophin (hCG) -- a hormone produced by the placenta
  - Inhibin-A -- a hormone produced by the placenta
- Without inhibin-A, called triple screen
- If first trimester screen done, only need MSAFP
### QUAD SCREEN RESULTS AT 15-18 WEEKS

<table>
<thead>
<tr>
<th>Quad Screen</th>
<th>NTD</th>
<th>Tri 21</th>
<th>Tri 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Unconjugated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estriol</td>
<td></td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Beta hCG</td>
<td>↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Inhibin A</td>
<td>↑</td>
<td></td>
<td>↓</td>
</tr>
</tbody>
</table>

[www.mayomedicallaboratories.com](http://www.mayomedicallaboratories.com)
AMNIOCENTESIS

• Used to obtain amniotic fluid and fetal cells for karyotyping or other tests
• Follow up for suspected genetic anomalies
• Near term-fluid is used for fetal lung maturity (FLM)

CHORIONIC VILLUS SAMPLING

- Priority if the woman will want to terminate the pregnancy
- Amniocentesis cannot be done until 15-16 weeks of pregnancy
- Chorionic villi sampling (CVS) is an option
- Can be done from 10+1 to 12+6 weeks

## Preventing Early-Onset GBS Disease
### CDC, 2010 Guidelines

<table>
<thead>
<tr>
<th>Indications</th>
<th>Non-indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous infant with invasive GBS disease</td>
<td>GBS colonization in previous pg</td>
</tr>
<tr>
<td>GBS bacteriuria during any trimester of the current pregnancy</td>
<td>GBS bacteriuria during previous pregnancy</td>
</tr>
<tr>
<td>Positive GBS screening culture during current pregnancy (except elective C/S prior to ROM, labor)</td>
<td>Cesarean delivery performed before onset of labor on women without ROM</td>
</tr>
<tr>
<td>Unknown GBS status at the onset of labor &amp; any of the following:</td>
<td>Negative vaginal and rectal GBS screening culture in late gestation during the current pregnancy, regardless of intrapartum risk factors</td>
</tr>
<tr>
<td>● Delivery at &lt;37 weeks’ gestation</td>
<td></td>
</tr>
<tr>
<td>● Ruptured membranes (ROM) &gt;18 hours</td>
<td></td>
</tr>
<tr>
<td>● Intrapartum temp &gt;100.4°F (&gt;38.0°C)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. Recommended regimens for intrapartum antibiotic prophylaxis for prevention of early-onset GBS disease*

Patient allergic to penicillin?

- no
  - Penicillin G, 5 million units IV initial dose, then 2.5 to 3 million units† every 4 hours until delivery
  - or
  - Ampicillin, 2g IV initial dose, then 1 g IV every 4 hours until delivery

- yes
  - Patient with a history of any of the following after receiving penicillin or a cephalosporin?§
    - Anaphylaxis
    - Angioedema
    - Respiratory distress
    - Urticaria
  
  - no
    - Cefazolin, 2g IV initial dose, then 1 g IV every 8 hours until delivery
  
  - yes
    - Isolate sensitive to clindamycin¶ and erythromycin**?
      
      - no
        - Vancomycin, 1 g IV every 12 hours until delivery
      
      - yes
        - Clindamycin, 900 mg IV every 8 hours until delivery
BIOPHYSICAL PROFILE

Combination of NST and ultrasound exam

• US looks for
  • Fetal breathing
  • Fetal movements
  • Fetal tone
  • Amniotic fluid index (AFI)
BIOPHYSICAL PROFILE
SCORING

► 2 points for reactive NST
  • 2 points each for
    • Fetal breathing
    • Fetal movement
    • Fetal tone
    • Amniotic fluid volume
  • Can be done in 4-8 minutes or up to 30" (breathing)
ASSESSMENT OF BPP SCORE

• Score of 8 or higher with normal fluid: risk of asphyxia rare or normal fetus.
• Score of 8 with low AFI: Induce
• Score of 6; low AFI: induce
• Score of 6; normal fluid but >36 weeks, cervix favorable: induce
• If repeat < or = 6, induce
• If repeat > 6, continue to monitor
AMNIOTIC FLUID INDEX (AFI)

• Ultrasound assessment of amniotic fluid
  • Each quadrant of the uterus is assessed for the largest pocket of fluid in cm- total is AFI
• Normal range for AFI is 5-25
• <5 is oligohydramnios
  • Think about poor placental flow, poor urine output of fetus, maternal dehydration or poor diet.
• >25 is polyhydramnios
  • Think about diabetes (elevated maternal glucose leads to polyuria in fetus)
  • Consider GI or renal problems-can’t swallow or process fluid
GENETIC AND CARRIER TESTING
SCREENING VS. DIAGNOSTIC

- Maternal Serum Screening Test
- Invasive Diagnostic Test
TESTING BY WEEK

• Not Pregnant
• 2-13 Weeks
• 14-21 Weeks
• 22+ Weeks
PRECONCEPTION TESTING

• Carrier Testing
  • Ashkenazi Jewish
  • Cystic Fibrosis (CF)
  • Fragile X
  • Spinal Muscular Atrophy (SMA)
FIRST TRIMESTER TESTING

- Carrier Testing
  - Ashkenzi Jewish
  - CF
  - Fragile X
  - SMA
- Chorionic Villus Sampling (CVS)
- First Screen® - maternal serum screening for T21 and T18
- Harmony Prenatal Test – serum screening for T13, T18, T21 – measures the relative amount of chromosomes in maternal blood
FIRST TRIMESTER

• Integrated Screen $^{SM}$ – MSS that combines 1st & 2nd trimester results to screen for T13, T18, T21, & ONTD. (2-part)

• Sequential Screen – MSS for T21, T18, and ONTD. (2-part)

• Serum Integrated Screen $^{SM}$ - MSS that combines results from 1st & 2nd trimester results to screen for T18, T21, ONTD. (2-part)

• Ultrasound
SECOND TRIMESTER
(14-21 WEEKS)

- Amniocentesis
- Carrier Testing (CF, Ashkenazi Jewish, SMA, Fragile X)
- MSS Tests (Harmony, Integrated Screen, Sequential Screen, Serum Integrated Screen)
- Afp4® Screening – MSS that measures proteins to screen for T21, T18, and ONTD.
- Ultrasound
SECOND TRIMESTER
(22+ WEEKS)

- Amniocentesis
- Carrier Testing
- Ultrasound
NON-INVASIVE PRENATAL TESTING
HOW TESTING HAPPENS?

• cfDNA – cell-free DNA
• Fetal Fraction
  • SNP vs. Counting Method

• SNP Technology
### Comparison of Performance in Published Clinical Trials*

<table>
<thead>
<tr>
<th>Sensitivity False Positive Rate</th>
<th>Sequenom MaterniT21™ 1,2,3</th>
<th>Verinata Verifi™4,5</th>
<th>Ariosa Harmony™ 6,7,8</th>
<th>Natera Panorama™ 9,10,11,12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21 (Down Syndrome)</td>
<td>99.1% 0.2%</td>
<td>&gt;99.9% 0.2%</td>
<td>&gt;99% 0.1%</td>
<td>&gt;99% (83/83) 0%</td>
</tr>
<tr>
<td>Trisomy 18 (Edwards Syndrome)</td>
<td>&gt;99.9% 0.3%</td>
<td>97.3% 0.4%</td>
<td>98% 0.1%</td>
<td>&gt;99% (27/27) &lt;0.1%</td>
</tr>
<tr>
<td>Trisomy 13 (Patau Syndrome)</td>
<td>91.7% 0.9%</td>
<td>87.5% 0.1%</td>
<td>80% 0.05%</td>
<td>&gt;99% (13/13) 0%</td>
</tr>
<tr>
<td>Monosomy X (Turner Syndrome)</td>
<td>94.7% 0.5%</td>
<td>95.0% 1.0%</td>
<td>96.7% unreported</td>
<td>91.7% (11/12) &lt;0.1%</td>
</tr>
<tr>
<td>Sex Chromosome Trisomies</td>
<td>&gt;99.9%</td>
<td>67-100%</td>
<td>67-100%</td>
<td>&gt;99% (5/5)</td>
</tr>
<tr>
<td>Female</td>
<td>97.9% 0.5%</td>
<td>97.6% 0.8%</td>
<td>&gt;99% unreported</td>
<td>&gt;99% (469/469) 0%</td>
</tr>
<tr>
<td>Male</td>
<td>99.4% 2.1%</td>
<td>99.1% 1.1%</td>
<td>&gt;99% unreported</td>
<td>&gt;99% (533/533) 0%</td>
</tr>
<tr>
<td>Triploidy</td>
<td>Unable to detect</td>
<td>Unable to detect</td>
<td>Unable to detect</td>
<td>&gt;99% (8/8)</td>
</tr>
</tbody>
</table>

*Note: data on Panorama excludes 4 known mosaic cases: two Monosomy X, one T13, and one T18. Both cases of Monosomy X were called positive, the T18 was called negative and the T13 was no called. False positives and false negatives can occur on all chromosomes due to maternal, fetal and/or placental mosaicism or other causes.
HOW TESTING HAPPENS?

• Simple blood test from mom.
• Results as early as 9 weeks.
• Takes about 10 days to get results.
• Access to/contact from Genetic Counselors with high risk results.
Results suggest high risk of Trisomy 21. Follow-up counseling and testing is recommended.

### RESULTS

<table>
<thead>
<tr>
<th>Condition tested</th>
<th>Age-based risk</th>
<th>Panorama risk score</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>2/100 (2.1%)</td>
<td>&gt;99/100 (&gt;99%)</td>
<td>High Risk</td>
<td>Follow-up counseling and testing recommended</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>1/100 (1.1%)</td>
<td>&lt;1/10,000 (&lt;0.01%)</td>
<td>Low Risk</td>
<td>none</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>1/302 (0.33%)</td>
<td>&lt;1/10,000 (&lt;0.01%)</td>
<td>Low Risk</td>
<td>none</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>1/255 (0.39%)</td>
<td>&lt;1/10,000 (&lt;0.01%)</td>
<td>Low Risk</td>
<td>none</td>
</tr>
<tr>
<td>Triploidy/Vanishing twin</td>
<td></td>
<td></td>
<td>Low Risk</td>
<td>none</td>
</tr>
</tbody>
</table>

*Excludes cases with evidence of fetal and/or placental mosaicism. *Based on maternal age and gestational age where applicable. *Based on a priori risk and test results.

**SEX OF FETUS: Female**

Fetal fraction: 6.1%
FOLLOW UP FOR HIGH RISK PATIENTS

• Positive Carrier Screening:
  • Testing father of baby for carrier status
  • If both positive, IVF pregnancy with preimplantation testing, donor egg or sperm, or adoption

• High Risk Chromosomal Screening:
  • CVS
  • Amniocentesis
  • Cytogenetics/FISH/etc