BIOCHEMICAL MARKERS AND GENETIC SCREENING

EFFECTIVENESS OF A SCREENING TEST

- Detection rate
  - How many affected pregnancies will be identified by the test
- False positive rate
  - Number of unaffected pregnancies that screen positive by the test
- Odds of being affected given a positive result (OAPR)
  - Allows comparison of the number of diagnostic procedures required to identify one affected pregnancy using each screening test

MATERNAL SERUM SCREENING QUAD SCREEN

- Geared to detection of Down syndrome
  - 95% trisomy 21
  - 3% translocations
  - 2% mosaic
  - Phenotype variable
- Trisomy 18
- Open neural tube defects
- Smith-Lemli-Opitz
QUAD SCREEN
FACTORS AFFECTING RESULTS

Gestational age
Maternal weight
Maternal race
Maternal insulin-dependant diabetes
Multiple fetal pregnancy
Family history of Down syndrome

Most accurate between 16 -18 weeks' gestation
Can be drawn from 15 – 22 6/7 weeks' gestation

QUAD SCREEN - SUMMARY

- Alpha-fetoprotein (AFP)
  - Protein produced by the fetal liver
  - Rises as pregnancy progresses
  - Decreased in trisomy 21

- Unconjugated Estriol (UE)
  - Produced by placenta, fetus liver, and
    input from maternal liver
  - Rises as pregnancy progresses
  - Decreased in trisomy 21

- Human Chorionic Gonadotrophin (hCG)
  - Produced by the placenta
  - Peaks at 10 weeks' gestation
  - Elevated in trisomy 21

- Inhibin-A
  - Hormone produced by the placenta
  - Addition to the "triple screen" resulted in
    the "quad screen"
  - Elevated in trisomy 21

SERUM ALPHA-FETOPROTEIN

High
- Normal variant
- Open neural tube defects
- Abdominal wall defects
- Blunting
- Fetal demise
- Kidney disorders
- Oligohydramnios
- Multihem gavage
- Under estimated fetal age
- Increase in 3rd trimester complications

Low
- Normal variant
- Chromosomal trisomies
- Gestational trophoblastic disease
- Fetal death
- Over estimated fetal age
OTHER CLINICAL IMPLICATIONS

- Second trimester
  - Elevated hCG or AFP, or dimeric inhibin A
  - Fetal death
  - Fetal growth restriction
  - Preeclampsia
  - Decreased MSAFP (less than 0.25 MOM) or estriol (less than 0.5 MOM)
    - Fetal death
    - Fetal growth restriction
    - Preeclampsia
    - Estriol less than 0.3 MOM
      - Genetic consultation for Smith-Lemli-Opitz

OTHER CLINICAL IMPLICATIONS

- Smith-Lemli-Opitz
  - Abnormality of cholesterol synthesis resulting in increased levels of 7-dehydrocholesterol
  - Due to absence of 7-dehydrocholesterol reductase
  - Results in elevated levels of 7-DHC in the amniotic fluid

PRENATAL GENETIC SCREENING

- Maternal serum screening
  - 1st trimester
  - 2nd trimester
  - Combinations
  - Circulating cell free fetal DNA

- Prenatal sonography
  - 1st trimester
  - Nuchal translucency
  - Nasal bone
  - 2nd trimester
  - Nuchal fold
  - Minor markers
  - Cardiac defects
THE 1ST TRIMESTER SCREEN

- Combination of free βhCG, PAPP-A, and maternal age
- PAPP-A - Pregnancy Associated Plasma Protein-A
  - Produced by the placental trophoblast
  - Detection rate - 60% with 5% false positive rate
  - Independent of other markers
  - Allows addition of other markers to alter the calculated risk
- Down syndrome detection rates - 73% to 84% (FPR of 5%)

NUCHAL FLUID ACCUMULATION

Potential Etiologies
- Aortic isthmic narrowing
- Other fetal cardiovascular defects
- Abnormalities in the extracellular matrix
- Abnormal or delayed development of the lymphatic system
- Specific etiology may vary with the underlying condition

Assessment Criteria
- 10 – 13 6/7 weeks’ gestation
- Crown-rump length 45 (38) and 84 mm
- Sagittal view of the fetus in a horizontal position with profile visible
- Neck in a neutral position
- Image fills 75% of the screen
- Widest part of translucency is measured
- Measurements taken with calipers placed inner border to inner border of the nuchal translucency at its widest point
NUCHAL TRANSLUCENCY

- Nuchal translucency greater than or equal to 3.0 mm considered screen positive
- NT measurement of 3.0 mm - minimal benefit in waiting for serum screening results
- No benefit for NT of 4.0 mm or greater
- Serum screening is not necessary
- Genetic consultation should be offered
- Fetal echocardiogram should be considered
- Thicker the nuchal translucency, greater the risk of chromosomal abnormality
- Greater the nuchal translucency, greater the risk of adverse pregnancy outcome
TYPES OF SCREENING

- Integrated screening
  - First- and second-trimester combined test
  - Patient receiving a single risk assessment after second test
  - Can be performed with or without nuchal translucency
  - High rates of non-adherence (25% without reminder)
  - Highest detection rate

- Independent sequential screening
  - Obtain 2 individual results from 1st trimester then 2nd trimester testing without incorporating information from 1st trimester test
  - Has unacceptably high false-positive rate
  - NOT RECOMMENDED

- Step-wise sequential screening
  - First-trimester screen and prenatal diagnosis if calculated risk above a specific cutoff
  - If patient screens below risk cutoff, she is offered second-trimester screening and receives a combined result

- Contingent screening
  - Groups women into a high, low, or intermediate risk categories after first-trimester screen with management dependent on first-trimester risk assessment
  - Most cost effective

CURRENT ANALYTE SCREENS
PRENATAL GENETIC SCREENING

- Following 1st trimester screening
  - MSAFP in second trimester
    - Drawn as a single order
    - Not part of a Quad screen
- Ultrasound
- Both

SCREENING FOR OPEN NEURAL TUBE DEFECTS

- First trimester
  - PAPP A less than 0.4 MOM and/or low HCG less than 0.5 MOM
  - Spontaneous fetal and neonatal loss
  - Fetal growth restriction
  - Preeclampsia
  - Placental abruption
  - Preterm delivery
CELL FREE DNA SCREENING (AKA NIPT, OFFDNA SCREENING, PANORAMA, MATERNIT 21, CLARITY, UNITY, ETC.)

- Short fragments of DNA found in maternal blood from placental apoptosis
- Cell-free DNA fragments from both mother and fetus
- Maternal blood obtained after 10 weeks’ gestation
- Relative amount of free DNA assessed in relation to maternal controls
- Determines chance fetus has trisomy 21, 18 or 13 based on relative amount of DNA from chromosomes 21, 18 and 13
- Can also assess abnormalities of the X and Y chromosomes
- Results usually available within 10 to 14 days

CIRCULATING CELL FREE DNA

- What can be screened
  - T21, T18, T13
  - Sex chromosome aneuploidy including Monosomy X, Klinefelter Syndrome, XYY, Triple X syndrome
  - Microdeletion Syndromes
    - 22q deletion Syndrome (1 in 4000 regardless of age), 1p36 deletion, Prader-Willi/Angelman Syndrome, Cri-du-chat, Wolf-Hirschhorn)
  - Some platforms scan the entire genome for deletions or duplications greater than 7 Mb
  - Some platforms screen for common select single gene disorders
  - Newer technology can offer some capability to screen for identified familial mutations or common variants for conditions like CF
CELL FREE DNA
ACOG PRACTICE BULLETIN - MAY 2016 NUMBER 163

- Cell-free DNA as a screening test
- More effective with higher fetal fractions
- Low fetal fraction
  - Sampling before 10 weeks' gestation
  - High maternal body mass index
- Fetal aneuploidy
  - 6% fail to obtain a result - 22% aneuploid
- Non-reportable should be offered consultation and invasive diagnostic testing
- Should not be used as a substitute for diagnostic test
- Capabilities
  - Screen for a variety of fetal conditions
  - Determine fetal gender
  - Identify presence of Rh-positive fetus
  - Detect some paternal abnormal dominant conditions
- Positive predictive value
  - 95% for Down syndrome,
  - 64% for trisomy 18
  - 44% for trisomy 13
  - 39% for sex chromosome aneuploidy
- Positive screen may be placental mosaicism, resulting in maternal malignancy or maternal aneuploidy

CIRCULATING CELL FREE DNA – UNUSUAL RESULTS

- Triploidy
  - Consideration for unrecognized twin pregnancy or presence of vanishing twin
- Atypical Finding
  - Atypical finding may be referring to placental or fetal origin
  - Variation of normal, mosaicism, microdeletion or duplication of placental or maternal origin
  - Recommendation for continued ultrasound monitoring, diagnostic testing, or postnatal testing
- High risk due to low fetal fraction/insufficient fetal DNA
  - Low fetal fraction may result from early gestation age, normal variation, maternal BMI, rarely a chromosome difference
  - May indicate high risk for T13,18, and triploidy in some women
  - Consideration for re-analysis or diagnostic testing

SUMMARY OF DETECTION RATES TRISOMY 21
SCREENING IN MULTIFETAL GESTATIONS

- Affected by number of fetuses and zygosity
- No method of aneuploidy screening is as accurate as in singleton gestation
- Nuchal translucency measurements allow each fetus to be screened independently
- Single enlarged nuchal translucency in monozygotic twins may be early sign of twin to twin transfusion syndrome
- Cell free DNA is acceptable in twin gestation

SUMMARY- 2020

- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies
- Cell-free DNA screening can be performed in twin pregnancies
- Detection rate 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
- Preimplantation genetic testing is not uniformly accurate
- Prenatal screening and prenatal diagnosis should be offered to all patients regardless of previous preimplantation genetic testing

Prenatal genetic screening (with or without nuchal translucency ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling or amniocentesis) should be discussed/offered to all pregnant women regardless of maternal age or risk of chromosomal abnormality.

Cell-free DNA is most sensitive and specific screening test for common fetal aneuploidies - not equivalent to diagnostic testing.

All patients should have a 2nd trimester ultrasound for fetal structural defects - ideally between 18 and 22 weeks of gestation.

Patients with a positive screen should undergo genetic counseling, detailed ultrasound, and diagnostic testing if chosen.

Patients with a negative screen should be told this substantially decreases risk of the targeted aneuploidy but does not ensure an unaffected fetus.

If patients have a negative screen, they may choose diagnostic testing later in pregnancy, if additional findings become evident such as fetal anomalies.

Patients with cell-free DNA results not reportable or uninterpretable (a no-call test result) should be informed of the nature of the uninterpretable result and offered genetic counseling and detailed ultrasound and diagnostic testing.

Enlarged nuchal translucency or anomaly identified on ultrasound - should be offered genetic counseling and diagnostic testing, detailed ultrasound at 18–22 weeks to assess for structural abnormalities.
MICROARRAY ANALYSIS

- "Lab on a chip"
- Identifies chromosomal abnormalities too small to be detected by conventional karyotyping
  - Deletions
  - Duplications
- Fetal DNA obtained from chorionic villus sampling or amniocentesis
- Potential for complex results and clinically uncertain findings
  - Can result in patient anxiety

MicroArray Analysis

Microdeletions are genomic imbalances detected by microarray but not karyotype.

Microarray Analysis

- Genetic changes identified by microarray not associated with maternal age
- Recommended for evaluation of a fetus with one or more major structural abnormalities - Replaces standard karyotype
- Routine genetic amniocentesis - fetal karyotyping or chromosomal microarray acceptable
- Recommended in evaluation of fetal demise or stillbirth
- Pretest and posttest genetic counseling and informed consent imperative
- Routine use of whole-genome or whole-exome sequencing for prenatal diagnosis not recommended outside clinical trials

ACOG Committee Opinion Number 682, December 2016
EXPANDED CARRIER SCREENING

- Panels which simultaneously screen for multiple genetic conditions
- Most are autosomal recessive
- Most are rare
- Some are X-linked or autosomal dominant
- Cost effective
- More optimally completed preconception
- Can be tailored to particular ethnic groups
- 4 to greater than 300 genetic conditions can be screened

Acceptable strategy for pre-pregnancy and prenatal carrier screening
- If requested - test should be made available following counseling
- Criteria for disorders carrier screening panel
  - Carrier frequency of 1 in 100 or greater
  - Well-defined phenotypes
  - Detrimental effect on quality of life
  - Cause cognitive or physical impairment
  - Require surgical or medical intervention
  - Have an onset early in life
  - Prenatal diagnosis that leads to opportunities for antenatal intervention to improve outcomes
- Carrier screening panels should not include conditions associated with a disease of adult onset.

ACOG Committee Opinion 690 - (March 2017)

- Spinal Muscular Atrophy (SMA-1 and SMA-2)
- Cystic Fibrosis
- CBC with red blood cell indices should be completed
- Hemoglobin electrophoresis should be completed
- Risk based on ethnicity (African, Mediterranean, Middle Eastern, Southeast Asian, or West Indian descent)
- Low mean corpuscular hemoglobin or low MCV

ACOG COMMITTEE OPINION 691 (MARCH 2017)
### ACOG COMMITTEE OPINION 691
(MARCH 2017)

**Fragile X Syndrome**
- Screening recommended for family history or intellectual disability suggestive of Fragile X
- Unexplained ovarian insufficiency/failure or elevated FSH before age 40 years
- DNA-based molecular analysis preferred method in determining FMR1 triplet repeats

**Individuals of Eastern and Central European Jewish Descent**
- When only one partner is of Ashkenazi Jewish descent, that individual should be offered screening first
- If positive carrier status, other partner should be offered screening

**Tay–Sachs Disease**
- Screening for Ashkenazi Jewish, French–Canadian, or Cajun descent; or family history

### ACOG GUIDELINES: CARRIER SCREENING
**ACOG COMMITTEE OPINION NO. 690 AND 691**

- All patients who are considering pregnancy or are already pregnant, regardless of screening strategy and ethnicity, should be offered carrier screening for cystic fibrosis and spinal muscular atrophy; a complete blood count and screening for thalassemias and hemoglobinopathies.
- Other recommendations for fragile X and expanded carrier screening depending on the family history

### PREIMPLANTATION GENETIC
- Implies IVF
- 8 cell stage
  - Blastomere day 3
- Set apart 1-2 cells (embryo biopsy)
- Tested by FISH
- Implant embryos with favorable test results
- Should still be offered aneuploidy screening/diagnosis during pregnancy
NEXT GENERATION SEQUENCING

WHOLE GENOME
Obtaining the complete sequence of all 3 million base pairs of DNA of an individual

WHOLE EXOM
Obtaining complete sequence of the protein coding regions of DNA - 2%.

Not recommended by the ACOG

ETHICS OF PRENATAL SCREENING
ACOG COMMITTEES ON ETHICS AND GENETICS

Identify patients who are candidates for genetic testing and maintain competence in the face of increasing genetic knowledge.
Recognize geneticists and genetic counselors are important part of health care team and should consult and refer as needed.
Discuss with patients importance of genetic information for their kindred.
Be aware that genetic information has potential to lead to discrimination in workplace and affect individual’s insurability.

Physicians have an obligation that includes a mandate to prevent discrimination.
Advocacy for legislation to ban genetic discrimination.

SMFM RECOMMENDATIONS
Society for Maternal-Fetal Medicine Consult Series
Recommendations (March 2017)
### Table 1: Management of Chromosome Abnormalities in Pregnancy

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Advantage</th>
<th>Disadvantages</th>
<th>Method</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Trimester Screening</td>
<td>Moderate risk ofmiscarriage</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>1-13 weeks</td>
</tr>
<tr>
<td>Cell-Free DNA Test</td>
<td>Low risk ofmiscarriage</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>9-13 weeks</td>
</tr>
<tr>
<td>Genetic Counseling</td>
<td>None</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>Anytime before or during pregnancy</td>
</tr>
<tr>
<td>CVS (Chorionic Villus Sampling)</td>
<td>High risk ofmiscarriage</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>10-13 weeks</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>High risk ofmiscarriage</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>15-20 weeks (26 weeks max)</td>
</tr>
<tr>
<td>Genetic Microarray</td>
<td>Moderate risk ofmiscarriage</td>
<td>May not detect all abnormalities</td>
<td>Blood test/mother</td>
<td>Anytime before or during pregnancy</td>
</tr>
</tbody>
</table>

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### Genetic Testing Options in Pregnancy

- **First Trimester Screening**
  - Risk assessment for Edwards, Patau, and Down
  - Moderate risk of miscarriage
  - May not detect all abnormalities
  - Blood test/mother
  - 1-13 weeks

- **Cell-Free DNA Test**
  - Low risk of miscarriage
  - May not detect all abnormalities
  - Blood test/mother
  - 9-13 weeks

- **Genetic Counseling**
  - None
  - May not detect all abnormalities
  - Blood test/mother
  - Anytime before or during pregnancy

- **CVS (Chorionic Villus Sampling)**
  - High risk of miscarriage
  - May not detect all abnormalities
  - Blood test/mother
  - 10-13 weeks

- **Amniocentesis**
  - High risk ofmiscarriage
  - May not detect all abnormalities
  - Blood test/mother
  - 15-20 weeks (26 weeks max)

- **Genetic Microarray**
  - Moderate risk ofmiscarriage
  - May not detect all abnormalities
  - Blood test/mother
  - Anytime before or during pregnancy
ACOG PRACTICE BULLETIN
MAY 2016 NUMBER 163

- Minor markers
  - Nonspecific physical characteristics more common in fetuses with Down syndrome than those without
  - Genu valgum in unaffected fetuses as well
  - Increased nuchal skinfold
  - Highest risk of aneuploidy
  - Isolated echogenic intracardiac focus
  - Limited role of fetal anemia
- Isolated presence of minor marker
- Analyte screening or cell free DNA should be offered