The Role of Ultrasound

Introduction
- Least effective primary screening for trisomy 21
- Detects 50 to 60 percent of affected fetuses
- Should not be used in isolation to confirm or exclude trisomy 21

Introduction
- Rate of aneuploidy directly related to number of anomalies identified
- Specific anomaly may provide clues to the underlying aneuploidy
- Major structural anomalies vs minor markers
  - Minor marker
    - Structural change that may be transient and when in isolation is thought to have little pathologic significance
    - May be seen more frequently in chromosomally abnormal fetuses
  - Examples
Major Ultrasound Findings and Associated Risks

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Aneuploidy Risk</th>
<th>Associated Aneuploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic Hygroma</td>
<td>60 to 75%</td>
<td>45X, 21, 18, XXY</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>3-8%</td>
<td>13, 21, Triplody</td>
</tr>
<tr>
<td>Cardiac Defects</td>
<td>5-30%</td>
<td>21, 18, 13, 22, 8, 9,</td>
</tr>
<tr>
<td>Complete AVSD</td>
<td>40-70%</td>
<td>21</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>30-49%</td>
<td>13, 18</td>
</tr>
<tr>
<td>Hydrops</td>
<td>30-80%</td>
<td>13, 21, 18, 45X</td>
</tr>
</tbody>
</table>

Cystic Hygroma

![Ultrasound Image]

Cystic Hygroma

![Ultrasound Image]
Hydrocephalus

The Normal Heart

Atrioventricular Septal Defect
The Normal Abdomen

Duodenal Atresia

Specific Minor Markers

- Thick Nuchal Fold
  - First sonographic marker associated with DS
  - Measured 15 - 20 wks
  - ≥ 6 mm
  - Identifies 40% of fetuses with DS
  - Single most specific and sensitive marker for DS
  - Confers highest risk of aneuploidy
Specific Minor Markers

- Echogenic Bowel
  - 0.2 to 1.4% scans
  - Normal variant
  - Fetal aneuploidy
  - IUOGR
  - Cystic fibrosis
  - Bleeding
  - Congenital infection

Renal Pyelectasis
- 0.3 to 4.5%
- > 4 mm AP diameter
- PPV 1 in 340
- AP diameter varies over time
- Association greatest when other anomalies seen
- Clinical relevance may lie in post-delivery concerns

Echogenic Intracardiac Focus
- 4% of scans
- Highest in Asians; lowest in Blacks
- Calcifications of papillary muscle of unknown etiology
- Can be single or multiple
- Most are in the left ventricle
- Original studies in high risk populations
- In low risk population does not increase risk significantly
- Carries lowest risk for aneuploidy
Specific Minor Markers

Choroid Plexus Cyst
- 1% incidence
- Can be single or multiple
- Associated with trisomy 18 (Not trisomy 21)
- High risk group – 1 in 128
- Low risk group – 1 in 189
- Recent studies show risk low when heart and hands appear normal >=18 weeks

Nasal Bone
- Most recent addition
- 1st trimester
  - 73% of fetuses with trisomy 21 vs 0.5% of normal fetuses
  - Unable to obtain in 6% of cases
- 2nd trimester controversies
  - Absence vs Presence vs Hypoplastic
  - Nasal bone length ratios
  - Interpretation varies by ethnic group
Specific Minor Markers

- Other markers
  - Clubfoot
  - Single umbilical artery
  - Dangling of the choroid plexus with normal atrial dimension
  - Ileac angle widening
  - Hypoplasia middle phalanx 5th digit
  - Clinodactyly
  - Delayed fusion of chorion/amnion
  - Sandal-gap toe

Statistics Review

- Positive predictive value
  - Chance that a positive test will end up in an affected fetus
- Negative predictive value
  - Chance that a fetus will be normal and is not affected
- Likelihood ratio (positive)
  - How much more likely is a fetus with a specific finding to have a chromosomal abnormality than one without the finding
- Likelihood ratio (negative)
  - How much more likely is absence of a finding in a normal fetus vs an affected fetus

Statistics and Prenatal Diagnosis

- Sensitivity - true positives
  - How well the test identifies fetuses with aneuploidy
- Specificity - true negatives
  - How well the test identifies fetuses with normal chromosomes
- Positive Predictive Value
  - Chance that a positive test will result in an affected fetus
- Negative Predictive Value
  - Chance that a negative test will result in a normal fetus
- Likelihood Ratio (positive)
  - How much more likely a fetus with a specific finding will have a chromosomal abnormality than one without the finding
- Likelihood Ratio (negative)
  - How much more likely is absence of a finding in a normal fetus vs an affected fetus
**Likelihood Ratios-Trisomy 21**

<table>
<thead>
<tr>
<th>Finding</th>
<th>Likelihood ratio</th>
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<tbody>
<tr>
<td>Structural Defect</td>
<td>25</td>
</tr>
<tr>
<td>Nuchal Thickening</td>
<td>18.6</td>
</tr>
<tr>
<td>Echogenic Bowel</td>
<td>5.5</td>
</tr>
<tr>
<td>Short Humerus</td>
<td>2.5</td>
</tr>
<tr>
<td>Short Femur</td>
<td>2.2</td>
</tr>
<tr>
<td>Echogenic Intracardiac Focus</td>
<td>2</td>
</tr>
<tr>
<td>Renal Pyelectasis</td>
<td>1.6</td>
</tr>
<tr>
<td>Normal Ultrasound</td>
<td>0.4</td>
</tr>
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</table>

**Risk Modification** *(Example)*

- Maternal age 35 years = 1/270 risk for trisomy 21
- 1st trimester screen places risk at 1 in 450
- Ultrasound shows bilateral pyelectasis
  - Likelihood ratio of 1.6
  - 1/450 x 1.6 = 1 in 281
  - Still a low risk result

**Minor Marker Follow-up**

- Third trimester follow-up
  - Renal pyelectasis
  - Echogenic bowel
  - Short femur and/or humerus
- No follow-up necessary
  - Choroid plexus cyst
  - Echogenic focus
  - Detailed counseling
  - Hypoplastic or absent nasal bone
  - Echogenic bowel
  - Thickened nuchal skinfold
### High risk for Aneuploidy

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<td>High beta hCG and low PAPP A</td>
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<td>Low hCG, AFP, estradiol</td>
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<td>Congenital heart disease, CPC, agenesis of corpus callosum, dandy walker, NTD's (20%), IUGR (60-90%), Cleft lip/palate, skeletal anomalies like clenched hands or absent thumb, club feet, rocker bottom feet, single umbilical artery, horseshoe kidney (20%)</td>
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<td>Reduces hCG, PAPP A, and AFP</td>
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<td>VSD or hypoplastic left heart (50-80%), holoprosencephaly, enlarged cisterna magna, spina bifida, cleft lip/palate (40%), cyclopia, proboscis, polydactyly (70%), omphalocele, cryptorchidism</td>
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### Cell free DNA (cfDNA) Screening and Role of Ultrasound

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<td>Exon analysis of default and additional panels in setting of negative cfDNA screening</td>
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<td>Single umbilical artery</td>
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<tr>
<td>Renal agenesis</td>
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<td>NTD's</td>
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<td>IUGR (15%)</td>
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<th>cfDNA, cell free DNA screening</th>
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<td>cfDNA is based on the use of the test of choice for screening for Down syndrome in pregnant patients.</td>
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### Minor Markers for Aneuploidy

**Summary**

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Definitions

- A Screening Test
  - Offered to a large, low-risk group
  - Identifies smaller group who may benefit from further testing
  - Inexpensive
  - Low risk to the patient
  - Easy

- A Diagnostic Test
  - Offered to a high-risk group
  - Provides a decisive result
  - Expensive
  - Higher risk to the patient
  - More difficult to perform

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- Prenatal Diagnostic Testing for Genetic Disorders
- Determines whether a genetic disorder/condition present in the fetus
  - Amniocentesis
  - Chorionic villus sampling (CVS)
  - Fetal imaging with ultrasonography, echocardiography, or magnetic resonance imaging may be diagnostic

Objective
- Detect health problems that could affect the woman, fetus, or newborn
- Provide information to allow fully informed decision about pregnancy management
- Important that patients understand the benefits and limitations
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- Recommendations/conclusions based on good and consistent scientific evidence (Level A)
  - Early amniocentesis (before 14 weeks’ gestation) not recommended
  - Abnormal FISH not be considered diagnostic
  - Rate of procedure-related pregnancy loss attributable to a prenatal diagnostic procedure
    - 0.1–0.3% in procedures performed by experienced providers
  - Transmission of HIV with amniocentesis not increased in women treated with CART and viral load undetectable

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- Recommendations/conclusions based on consensus/expert opinion (Level C)
  - All pregnant women should be offered prenatal assessment for aneuploidy
  - Prenatal genetic testing cannot identify all abnormalities or problems in a fetus
  - Genetic testing should be discussed as early as possible in pregnancy

Candidates for Prenatal Diagnostic Testing

- Previous fetus or child with autosomal trisomy or sex chromosome aneuploidy
  - Recurrence risk
    - 1.6 to 8.2 x the maternal age related risk of trisomy
    - Unclear level of increase with 47, XXX; 47 XXY
    - Not increased with 45,X
  - Structural anomalies identified by ultrasound
    - Increased risk for aneuploidy, copy number variants (deletions, duplications), and genetic syndromes
Diagnostic Prenatal Tests

- Amniocentesis
- Chorionic villus sampling (CVS)
- Percutaneous umbilical blood sampling
- Preimplantation genetics
- Fetal skin sampling
- Fetal tissue biopsy
- Fetoscopy

Diagnostic Methods

**Traditional Amniocentesis**
- Introduced in the 1950’s
- 15-20 weeks’ gestation
- 0.1 to 0.3% fetal loss rate
- 1 % leakage of fluid
- 0.1 % intra-amniotic infection
- Unclear risk - vertical transmission of maternal HIV, Hepatitis B and C
- 99 % accurate
- Results 10 to 14 days

**Early amniocentesis**
- 10 to 14 weeks’ gestation
- 2 to 5 % fetal loss rate
- 3.5 % leakage of fluid
- 1.3 % risk of clubfoot
- Should not be done prior to 13 weeks’ gestation
Diagnostic Methods

- **Chorionic villus sampling**
  - Sampling of chorion frondosum
  - Introduced 1968
  - 10-12 weeks' gestation
  - 1.1 to 1.3 % fetal loss rate
  - Limb reduction defects if < 10 weeks’ gestation
  - Transcervical contraindicated with a vaginal infection and maternal blood group sensitization
  - Risk – 0.5 %

- **Percutaneous umbilical cord sampling (Cordocentesis)**
  - Introduced 1973
  - Varying gestational ages
  - 1.4 to 2 % fetal loss rate
  - 6.6 % risk of bradycardia
  - Cord hematomas
  - Bleeding from puncture site
  - Infection
  - Maternal – fetal bleed

- **Preimplantation genetics (PID)**
  - Implies IVF
  - 8 cell stage
  - Blastomere day 3
  - Set apart 1-2 cells (embryobiopsy)
  - Tested by FISH
  - Implant embryos with favorable test results
Table 1. Tests Available for Prenatal Genetic Diagnosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Turnaround Time</th>
<th>Conditions Detected</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional karyotype</td>
<td>7-14 days</td>
<td>Chromosomal aneuploidy, structural abnormalities</td>
<td>Traditional method for diagnosis of chromosomal abnormalities</td>
</tr>
<tr>
<td>FISH</td>
<td>24-48 hours</td>
<td>Rapid assessment of genetic abnormalities (13, 18, 21, X,  Y, 6, and 18)</td>
<td>FISH with dual labeling of each chromosome allows for rapid diagnosis of chromosomal abnormalities. Results should be confirmed on cultured cells or fresh tissue prior to taking any action.</td>
</tr>
<tr>
<td>Microarray</td>
<td>7-14 days</td>
<td>Microdeletions and duplications</td>
<td>Can be used to detect genetic abnormalities only if already suspected.</td>
</tr>
<tr>
<td>Chromosomal microarray</td>
<td>5-7 days (batch testing)</td>
<td>Copy number variants, balanced rearrangements</td>
<td>Whole genome array for copy number variants, balanced rearrangements, and balanced rearrangements are recommended. These tests are useful in identifying genetic patterns.</td>
</tr>
<tr>
<td>Preimplantation genetic diagnosis</td>
<td>1-2 days</td>
<td>Genetic disorders in which testing has been indicated</td>
<td>Non-invasive approach. Genetic disorders in which testing has been indicated.</td>
</tr>
<tr>
<td>Molecular DNA testing</td>
<td>1-2 days (batch with dual labeling of each chromosome required)</td>
<td>Genetic disorders are affected in a family or in newborns and in prenatal diagnosis.</td>
<td>Positive or negative based on family history.</td>
</tr>
</tbody>
</table>

**Miscellaneous**

- Genetic diagnosis for fetal demise
  - Conventional karyotype
    - Can only be obtained with living tissue
    - Higher culture failure rate
  - Microarray
    - Does not require viable cells
    - Preferred test
    - Cannot be contaminated with maternal tissue or blood
Mosaicism

- Can occur with chorionic villus sampling
- Amniocentesis normal 90 percent of time
- Confined placental mosaicism carries an increased risk for 3rd trimester fetal growth restriction
- Uniparental disomy (both chromosomes from one parent) testing indicated as follow-up to confined placental mosaicism
  - Trisomy rescue