Prenatal Screening for Fetal Defects

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Salt Lake City, UT
Disclosures

• Beckman Coulter, Inc.
  – Research support

• AFP, hCG, uE3, DIA, and PAPP-A tests are not FDA-cleared for aneuploidy screening.
Objectives

• *Describe* how biochemical and ultrasound markers are used to screen for open neural tube defects and determine aneuploidy risk

• *Explain* new molecular-based approaches for aneuploidy screening
## Screening for Which Defects?

<table>
<thead>
<tr>
<th>Method</th>
<th>Fetal defect</th>
<th>Incidence (live births, approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical screening only</td>
<td>Open neural tube defects (ONTD)</td>
<td>1 in 1,000</td>
</tr>
<tr>
<td>Biochemical &amp; DNA-based screening</td>
<td>Trisomy 21 (Down syndrome)</td>
<td>1 in 700</td>
</tr>
<tr>
<td>Biochemical &amp; DNA-based screening</td>
<td>Trisomy 18 (Edwards syndrome)</td>
<td>1 in 5,000</td>
</tr>
<tr>
<td>DNA-based screening only</td>
<td>Trisomy 13 (Patau syndrome)</td>
<td>1 in 16,000</td>
</tr>
</tbody>
</table>
## Biochemical Screening Test Choices

<table>
<thead>
<tr>
<th>Test Name</th>
<th>ONTD</th>
<th>DS</th>
<th>T18</th>
<th>Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP Only</td>
<td>✔</td>
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</tr>
<tr>
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<td>✔</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; &amp; 2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum Integrated</td>
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<td>1&lt;sup&gt;st&lt;/sup&gt; &amp; 2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sequential</td>
<td>✔</td>
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<td>✔</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; &amp; 2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
## Biochemical Screening Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-fetoprotein (AFP)</td>
<td>Fetus</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
<td>Placenta</td>
</tr>
<tr>
<td>Unconjugated estriol (uE3)</td>
<td>Fetus/Placenta</td>
</tr>
<tr>
<td>Dimeric inhibin A (DIA)</td>
<td>Placenta</td>
</tr>
<tr>
<td>Pregnancy-associated plasma protein A (PAPP-A)</td>
<td>Placenta</td>
</tr>
<tr>
<td>Nuchal translucency</td>
<td>Fetus</td>
</tr>
</tbody>
</table>
Nuchal Translucency (NT)

- The space that can be visualized between the fetal skin and the soft tissues covering the cervical spine
- Performed on fetuses at 10 – 14 wks gestation
  - Measurement requires specific training and extended practice
- Increased thickness strongly associated with fetal aneuploidy
  - Cardiac defects with over-perfusion of head and neck; abnormal lymphatics?
- Not specific for aneuploidies

www.fetalmedicine.com
Marker Concentrations by Gestational Age
Multiple of the Median (MoM)

• Ratio between the patient’s result and the median result appropriate for the gestational age of fetus

\[
\text{MoM} = \frac{\text{Patient's result}}{\text{Median result}}
\]

• Medians determined by the laboratory for each marker across all gestational ages required for a given test strategy
## Multiple of the Median (MoM)

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>Patient’s AFP concentration (ng/mL)</th>
<th>Median AFP concentration (ng/mL)</th>
<th>Ratio</th>
<th>MoM</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>15</td>
<td>30</td>
<td>15/30</td>
<td>0.5</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>30</td>
<td>30/30</td>
<td>1.0</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>40</td>
<td>30/40</td>
<td>0.75</td>
</tr>
</tbody>
</table>
SCREENING FOR OPEN NEURAL TUBE DEFECTS
Open NTD Screening with AFP

- Peaks in fetus at 9 weeks (~3x10^6 ng/mL) then steadily declines
- AF-AFP mirrors that of serum but concentration is ~100x lower
- MS-AFP detected at ~10 weeks (~10,000x lower)

Tietz, 4th ed, 2005
Open NTD Screening with AFP

- ONTD in direct contact with amniotic fluid
  - AF-AFP increases followed by MS-AFP
- Ideal screening time is 16-18 weeks
  - AFP MoM distributions of affected and unaffected are maximally different
  - Sufficient time for follow-up tests
- Can be done at 15-22 weeks
ONTD Screening Performance

- Some affected will be missed
- Some unaffected called abnormal
- MS-AFP interpretation based on AFP MoM (2.5 is common)
- 70-85% sensitive for open spina bifida; >95% for anencephaly
- Most positive screening tests are false-positive (2% PPV)
Other Causes of Abnormal NTD Screens

- Underestimation of GA (most common)
- Multifetal gestations
- Fetal demise
- Ventral wall defects
- Urinary tract abnormalities
When ONTD Screen Is Abnormal

• Perform targeted ultrasound
  – Confirm GA
  – Rule out multiple gestations or fetal demise
  – Observe fetal head and spine for defects

• If AFP MoM 2.5 – 2.9 then may repeat screen from a new specimen to sort out false-positive results
  – ~40% of false-positives become true-negatives
  – 2-3% increase in false-negatives

• Amniocentesis to obtain amniotic fluid
  - Measure AF-AFP
  - Qualitative detection of acetylcholinesterase (AChE)
Amniotic Fluid AFP and AChE

- AF-AFP
  - More powerful indicator of ONTD than MS-AFP

- AChE
  - Present in nerve tissue
  - Hydrolyzes acetylcholine
  - Not normally present in AF (pseudochoolinesterase is)
  - Electrophoretic detection is 98% sensitive and >99% specific for ONTD

- Evaluate for fetal blood when AChE positive
  - Contains both AFP and AChE
## Interpreting Abnormal ONTD Tests

<table>
<thead>
<tr>
<th>MS-AFP</th>
<th>AF-AFP</th>
<th>AF-AChE</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>Present</td>
<td>Very likely ONTD or ventral wall defect</td>
</tr>
<tr>
<td>↑</td>
<td>↑</td>
<td>Absent</td>
<td>Other fetal defect (ventral wall, demise, chromosome, urinary tract, cleft palate, nephrosis, others)</td>
</tr>
<tr>
<td>↑</td>
<td>N</td>
<td>Absent</td>
<td>Excludes nearly all cases of open structural defects</td>
</tr>
</tbody>
</table>
BIOCHEMICAL SCREENING FOR ANEUPLOIDIES
# Biochemical Screening Test Choices

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<tr>
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<th>hCG</th>
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Risk of Down Syndrome (2nd trimester)

Age equation from:

1 in 270
1. Determine the Pre-test odds (age-based)

2. Measure marker concentrations in maternal serum

3. Calculate MoM of each marker using GA-specific medians

4. Determine the likelihood ratio for each marker at the patient’s MoM

5. Multiply the pre-test odds by the likelihood ratios to determine the post-test odds
Likelihood Ratio

Probability of a given result in a person with an affected pregnancy divided by the probability of the same result in a person with an unaffected pregnancy

Determined by the heights of the Gaussian distributions in affected and unaffected pregnancies
Likelihood Ratio

\[ LR = \frac{\text{Prob of affected}}{\text{Prob of unaffected}} \]

\[ LR = \frac{0.6}{0.24} = 2.5 \]

If pre-test odds were 1 to 900 then new odds are 2.5x greater or 1 to 360
\[ LR = 2.5 \times 2.5 \times 13.0 \times 2.5 = 203 \]

Post-test Risk = 1 in 270 \times 203 = 1 in 1.3
Biochemical Screening

1. Determine the Pre-test odds (age-based)

2. Measure marker concentrations in maternal serum

3. Calculate MoM of each marker using GA-specific medians

4. Determine the likelihood ratio for each marker at the patient’s MoM

5. Multiply the pre-test odds by the likelihood ratios to determine the post-test odds

6. Interpret the post-test odds....what is abnormal?
Two philosophies among U.S. laboratories

1. Use 1 in 270 (DS risk for a 35 yo) regardless of test panel

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>Initial positive rate (Quad test)</th>
<th>Detection rate (Quad test)</th>
</tr>
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<tbody>
<tr>
<td>1 in 270</td>
<td>6.6%</td>
<td>86%</td>
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Selecting a Cutoff: What is Abnormal?

Two philosophies among U.S. laboratories

1. Use 1 in 270 (DS risk for a 35 yo) regardless of test panel

2. Use different cutoff for each test panel to lower the initial positive rate

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<td>1 in 150</td>
<td>4.1%</td>
<td>82%</td>
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</table>

Higher detection rate; more false-positives ➔ 1 in 270, 6.6%, 86%

Lower detection rate; fewer false-positives ➔ 1 in 150, 4.1%, 82%
## Selecting a Cutoff: What is Abnormal?

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>100,000 women screened</th>
<th>200 DS cases expected</th>
<th>(1 in 500 prevalence)</th>
</tr>
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<tbody>
<tr>
<td><strong>1 in 270 cutoff</strong></td>
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<td>Detection Rate</td>
</tr>
<tr>
<td>Detection Rate</td>
<td>86%</td>
<td></td>
<td>DS Cases Detected</td>
</tr>
<tr>
<td>Abnormal Screens</td>
<td>6,600</td>
<td></td>
<td>172</td>
</tr>
<tr>
<td>DS Cases Detected</td>
<td>172</td>
<td></td>
<td></td>
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<td>82%</td>
<td></td>
<td>DS Cases Detected</td>
</tr>
<tr>
<td>Abnormal Screens</td>
<td>4,100</td>
<td></td>
<td>164</td>
</tr>
<tr>
<td>DS Cases Detected</td>
<td>164</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ashwood ER, unpublished data (2012)
Selecting a Cutoff: What is Abnormal?

1 in 270 cutoff

Abnormal Screens: 6,600
DS Cases Detected: 172

1 in 150 cutoff

Abnormal Screens: 4,100
DS Cases Detected: 164

2,500 fewer abnormal screens
313 amniocenteses to detect 1 additional DS case
8 fewer DS cases detected

Ashwood ER, unpublished data (2012)
When Biochemical Screen is Abnormal: DO

• Targeted ultrasound
  – Confirm GA (overestimated gives DS pattern)
  – Evaluate fetus for anomalies consistent with aneuploidy

• Offer diagnostic testing (fetal chromosomes)
  – 1st trimester: CVS
  – 2nd trimester: amniocentesis
  – Fetal loss rates vary (0.5-1.0%) and lowest in institutions that perform the frequently
When Biochemical Screen is Abnormal: DON’T

• Do not re-test! Regression towards the mean
  – Repeated measurements at tails of distribution return results closer to the population mean
  – Repeat testing will increase false-negative screens

• Okay to repeat if sample collected at <11 weeks (1st tri) or <14 weeks (2nd tri)
Which Biochemical Screening Test is Best?

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<th>Test Name</th>
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</tbody>
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Which Biochemical Screening Test is Best?

First and second trimester antenatal screening for Down’s syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS)

N J Wald, C Rodeck, A K Hackshaw, J Walters, L Chitty, A M Mackinson

J Med Screen 2003; 10:56–104

First-Trimester or Second-Trimester Screening, or Both, for Down’s Syndrome

Which Biochemical Screening Test is Best?

- **Integrated**: 17%
- **Serum Integrated**: 7%
- **Combined**: 3%
- **Quad**: 3%
- **Triple**: 2%

**PPVs at 85% DR**

*J Med Screen* 2003;10:56-104
DNA-BASED SCREENING FOR ANEUPLOIDIES
Cell Free Fetal DNA in Maternal Blood

- Reported by Lo, et al. in 1997
- Derived primarily from the placenta and represents ~10% of total DNA circulating in maternal blood
- Screening tests that identify molecular pathology of aneuploidies
### Commercially Available DNA-based Screening Tests

<table>
<thead>
<tr>
<th>Company</th>
<th>Location</th>
<th>Product</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ariosa Diagnostics, Inc.</td>
<td>San Jose, CA</td>
<td>Harmony™ Prenatal Test</td>
<td>Targeted SNPs</td>
</tr>
<tr>
<td>Natera, Inc.</td>
<td>San Carlos, CA</td>
<td>Parental Support™</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>Sequenom Center for Molecular Medicine</td>
<td>San Diego, CA</td>
<td>MaterniT21™ Plus</td>
<td>MPSS</td>
</tr>
<tr>
<td>Verinata Health, Inc.</td>
<td>Redwood City, CA</td>
<td>Verifi® Prenatal Test</td>
<td>MPSS</td>
</tr>
</tbody>
</table>

MPSS: Massively parallel shotgun sequencing

- Methods may differ but goal is the same
  - Identify extra copies of a specific chromosome
Massively Parallel Shotgun Sequencing

- 1st 36 bases of each DNA fragment sequenced and mapped to a specific chromosome
- Number of unique sequences are counted and expressed as percentage of all unique sequences (% chrN)
- Z-scores for each chromosome calculated and evaluated against a cutoff Z-score of +3
MPSS Clinical Performance (T21)

1,683 women; 8.1-21.5 weeks

98.6% sensitive (209/212)
99.8% specific (1468/1471)

209 true positives
3 false positives
1468 true negatives
3 False negatives

MPSS Clinical Performance (T18 & 13)

100% sensitive (59/59)
99.7% specific (1683/1688)

92.3% sensitive (12/13)
99.1% specific (1672/1688)

Genet Med 2012;14:296-305
# Clinical Performance of Commercially Available DNA-based Screening Tests

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
<th>Detection Rate (%)</th>
<th>False-positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ariosa Diagnostics, Inc.</td>
<td>Harmony™ Prenatal Test</td>
<td>100 100 NA &lt;0.1</td>
<td></td>
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<td>100 100 100 0</td>
<td></td>
</tr>
<tr>
<td>Sequenom Center for Molecular Medicine</td>
<td>MaterniT21™ Plus</td>
<td>99 100 92 0.3-1.0</td>
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</tr>
<tr>
<td>Verinata Health, Inc.</td>
<td>Verifi® Prenatal Test</td>
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AJOG 2012;206:319.e1-319.e9  
Genet Med 2012;14:296-305  
Obstet Gynecol 2012;119:890-901  
Prenat Diagn 2012;32:1233-1241
If DNA-based testing is so good, should it be the primary screening test?
Clinical Scenario: General Population Screening

• Offer DNA-based testing to all pregnant women as the primary screening test

• Consider:
  – 100,000 women from the general pregnant population
  – T21 prevalence of 1 in 500
## Biochemical vs. DNA-based Test as 1° Screen

<table>
<thead>
<tr>
<th></th>
<th>Quad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number screened</td>
<td>100,000</td>
</tr>
<tr>
<td>T21 prevalence</td>
<td>1 in 500 (N=200)</td>
</tr>
<tr>
<td>Detection rate (%)</td>
<td>80</td>
</tr>
<tr>
<td>False positive rate (%)</td>
<td>5</td>
</tr>
<tr>
<td>T21 identified (N)</td>
<td>160 (out of 200)</td>
</tr>
<tr>
<td>False-positives (N)</td>
<td>4,990 (out of 99,800)</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>3.1</td>
</tr>
<tr>
<td>Odds</td>
<td>1 to 31</td>
</tr>
</tbody>
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## Biochemical vs. DNA-based Test as 1° Screen

<table>
<thead>
<tr>
<th></th>
<th>Quad</th>
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<td>T21 prevalence</td>
<td>1 in 500 (N=200)</td>
<td>1 in 500 (N=200)</td>
</tr>
<tr>
<td>Detection rate (%)</td>
<td>80</td>
<td>99</td>
</tr>
<tr>
<td>False positive rate (%)</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>T21 identified (N)</td>
<td>160 (out of 200)</td>
<td>198 (out of 200)</td>
</tr>
<tr>
<td>False-positives (N)</td>
<td>4,990 (out of 99,800)</td>
<td>200 (out of 99,800)</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>3.1</td>
<td>49.7%</td>
</tr>
<tr>
<td>Odds</td>
<td>1 to 31</td>
<td>1 to 1</td>
</tr>
</tbody>
</table>
Which Approach is Best?

Biochemical Screening
- Normal
- Abnormal
- Stop
- Diagnostic testing

DNA-based screening
- Normal
- Abnormal
- Stop
- Diagnostic testing
DNA-based Test as 1° Screen Dilemmas

• All published studies have been performed in “high-risk” populations
  – Advanced maternal age
  – Prior affected pregnancy
  – High NT
  – Abnormal biochemical screening test

• Practical considerations
  – Limited availability
  – Longer TAT compared to biochemical screening
  – High costs (> $1,000)
  – Lack of insurance coverage
Which Approach is Best?

Biochemical Screening

- Normal
- Abnormal
- Stop
- Diagnostic testing

DNA-based screening

- Normal
- Abnormal
- Stop
- Diagnostic testing

DNA-BASED TEST AS A SECONDARY SCREEN
Quad First then DNA

100,000 women screened
1 in 500 T21 prevalence

Quad Test
80% DR (160/200) 5% FPR (4,990/99,800)

DNA-based Test for Quad positives
99% DR (158 /160) 0.2% FPR (10/4,990)

PPV 94% 16 to 1 odds

Diagnostic test for screen positives
Is this likely to change over time and with more evidence?

YES
Noninvasive Prenatal Testing for Fetal Aneuploidy

Box 1. Indications for Considering the Use of Cell Free Fetal DNA

- Maternal age 35 years or older at delivery
- Fetal ultrasonographic findings indicating an increased risk of aneuploidy
- History of a prior pregnancy with a trisomy
- Positive test result for aneuploidy, including first trimester, sequential, or integrated screen, or a quadruple screen.
- Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.
Summary

• Classic prenatal screening combines biochemical and US markers to identify pregnant women at increased risk for having a baby with an open neural tube defect, Down syndrome, or trisomy 18

• More conservative cutoffs decrease the number of abnormal biochemical screening tests and results in fewer unnecessary diagnostic tests

• DNA-based screening tests have excellent aneuploidy detection rates and can enhance the value of biochemical testing
Self-Assessment Questions

1. What is the AFP MoM at 20 weeks of gestation (median 40 ng/mL) in a woman with a serum AFP concentration of 120 ng/mL?
   A. 0.3
   B. 0.5
   C. 2.0
   D. 3.0

2. Changes to the Down syndrome risk cutoff has the most dramatic effect on what parameter?
   A. Detection rate
   B. Number of abnormal screen results
   C. Percentage of normal screen results
   D. Biomarker MoM

3. A primary advantage of DNA-based aneuploidy screening tests compared to biochemical screening tests is:
   A. They can be performed in the first trimester
   B. They do not rely on the NT measurement
   C. They are widely available
   D. They have a higher detection rate
Marker Dependent LR

- Determine the “marker dependent” likelihood ratio by calculating $H_{DS}$ and $H_{Unaffected}$

$$H = \frac{1}{\Pi \sigma (2\pi)^{p/2} \cdot \det(R)^{1/2}} \exp \left[ -\frac{Z^T R^{-1} Z}{2} \right]$$

- $LR = H_{DS}$ divided by $H_{unaffected}$