THE GENETIC SONOGRAM
Jude P. Crino, M.D.

SCREENING PRINCIPLES

EVALUATION OF TESTS
KEY MEASURES

- Sensitivity – proportion of people with the disease who test positive (aka detection rate)
- Specificity – proportion of people without the disease who test negative
- Positive Predictive Value – proportion of people with a positive test who have the disease
- Negative Predictive Value – proportion of people with a negative test who do not have the disease

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A (true positive)</td>
<td>B (false positive)</td>
</tr>
<tr>
<td>C (false negative)</td>
<td>D (true negative)</td>
</tr>
</tbody>
</table>

Sensitivity = A/(A+C)
Specificity = D/(B+D)
Positive Predictive Value = A/(A+B)
Negative Predictive Value = D/(C+D)

SCREENING PRINCIPLES
PREDICTIVE VALUE

- Predictive value varies with prevalence
  - with increasing prevalence:
    • positive predictive value increases
    • negative predictive value decreases
  - at low prevalence, positive predictive value will be low and negative predictive value will be high regardless of how good the test is

SCREENING PRINCIPLES
SENSITIVITY AND SPECIFICITY

- Sensitivity and specificity do not vary with prevalence
- Sensitivity varies with the threshold value (cutoff) for a positive test
- Specificity and positive predictive value vary with sensitivity
  - with increasing sensitivity, specificity and positive predictive value decrease
SCREENING PRINCIPLES

CUTOFF

<table>
<thead>
<tr>
<th>Test Result</th>
<th>Normal (%)</th>
<th>Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPR</td>
<td>1%</td>
<td>20%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

SCREENING PRINCIPLES
FALSE POSITIVE RATE

- False positive rate varies with sensitivity
  - with increasing sensitivity, false positive rate increases
- At low prevalence, false positive and screen positive rates are approximately equal
- To compare different screening tests for the same population, either the false positive rate or the sensitivity must be fixed

EXAMPLE

Second Trimester Triple Screen
DS Prevalence 0.125% (1/800)

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>-</td>
<td>300</td>
</tr>
</tbody>
</table>

Sensitivity 6/10 = 60%
Specificity 7900/7990 = 96%
Positive Predictive Value 6/306 = 2%
Negative Predictive Value 7900/7994 = ~100%
False Positive Rate 300/8000 = 3.8%

SCREENING PRINCIPLES
LIKELIHOOD RATIO

- Frequency of abnormal / frequency of normal at a given test result
- Used to calculate patient-specific risk based upon:
  - patient’s individual test result
  - population distribution of test results

CALCULATION OF PATIENT-SPECIFIC RISK

Risk (%) = L.R. * Risk for Trisomy 21

1: 600
1: 60
1: 3,700
1: 3,700
1: 100
1: 10
1: 1
1: 0.1
1: 0.1
1: 0.01
1: 0.01
1: 0.001
1: 0.001
1: 0.0001
1: 0.0001
FIRST TRIMESTER SCREENING

- First trimester combination of NT and serum biochemistry is a very effective screen for Down syndrome (and other aneuploidy) in the general population (DR ~85% @ 5% FPR)
- Down syndrome screening in singletons based on NT alone (DR ~75% @ 5% FPR) is less effective than NT plus biochemistry
- Use of additional sonographic markers with the combined screen improves test performance (increased sensitivity, lower screen positive rate)

FIRST AND SECOND TRIMESTER SCREENING

INTEGRATED SCREEN

- 1st and 2nd trimester risk estimates combined to give single risk
  - 2-step screen / 1 result
- Maternal age & gestational age included
- Serum integrated (1st trim PAPP-A + 2nd trim quad), DR 85-88% at 5% FPR
- Fully integrated (1st trim NT/PAPP-A + 2nd trim quad), DR 94-96% at 5% FPR

SEQUENTIAL SCREEN

- 2 step screen, 2 risk results
- 11-14 weeks NT + biochem
- Results provided, CVS if ↑ risk
- 15+ weeks quad screen
- False positive rates are additive

INDEPENDENT SEQUENTIAL SCREEN

- First Trimester Combined Screen
  - DR: 85%
  - FPR: 5-7%
- Second Trimester Quad Screen
  - DR: 70%
  - FPR: 5-7%

Overall DR: 92%
FPR: 10-20%
**STEPWISE SEQUENTIAL SCREEN**

- **First Trimester Combined Screen**
  - CVS
  - Amnio
  - Integrated Screen
  - Detection Rate (DR): 93%
  - False Positive Rate (FPR): 2–4%

- **Overall**
  - Detection Rate (DR): 70%
  - False Positive Rate (FPR): 1–2%

**CONTINGENT SEQUENTIAL SCREEN**

- **First Trimester Combined Screen**
  - CVS
  - Amnio
  - Integrated Screen
  - Detection Rate (DR): 92%
  - False Positive Rate (FPR): 2–4%

- **Overall**
  - Detection Rate (DR): 70%
  - False Positive Rate (FPR): 1–2%

**DOWN SYNDROME SCREENING (FPR 5%)**

<table>
<thead>
<tr>
<th>Detection Rate (%)</th>
<th>NT Ultrasound</th>
<th>1st Trimester Blood Screen</th>
<th>2nd Trimester Blood Screen</th>
<th>Integrated Screen</th>
<th>Serum Integrated</th>
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<tbody>
<tr>
<td>1st Trimester</td>
<td>64–70</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2nd Trimester</td>
<td>82–87</td>
<td>69</td>
<td>81</td>
<td>94–96</td>
<td>85–88</td>
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</tbody>
</table>

**THE GENETIC SONOGRAM**

- Application of second trimester (14–24 w) sonography to adjust fetal aneuploidy risk
- Standardized, systematic approach
  - Complete anatomic survey
  - Markers of fetal aneuploidy
- Correlation with other risk factors
  - Maternal age, obstetric or family history, maternal serum testing results

**BASIC PRINCIPLES**

- Distinction between structural anomaly and a "marker"
- Ultrasound markers are "evolving"
- Predictive value varies with prevalence
- Most studies are in high risk patients
MARKERS OF FETAL ANEUPLOIDY

- Thickened nuchal fold
- Short femur/humerus
- Renal pelvis dilation
- Echogenic intracardiac focus
- Echogenic bowel
- Cerebral ventriculomegaly
- Absent or hypoplastic nasal bone
- Aberrant right subclavian artery

THICKENED NUCHAL FOLD

- Transverse, “off-axial” view through post fossa
  - include cisterna magna, cerebellum
- Outer occipital bone - outer skin edge
- Cutoff 5 or 6 mm

THICKENED NUCHAL FOLD

<table>
<thead>
<tr>
<th>BPD (mm)</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4</th>
<th>4.5</th>
<th>5</th>
<th>5.5</th>
<th>6</th>
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<tr>
<td>20</td>
<td>21.4</td>
<td>29.2</td>
<td>35.3</td>
<td>40.4</td>
<td>44.4</td>
<td>48.4</td>
<td>52.4</td>
<td>56.4</td>
<td>60.4</td>
<td>64.4</td>
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<tr>
<td>25</td>
<td>20.0</td>
<td>26.8</td>
<td>32.8</td>
<td>38.8</td>
<td>42.8</td>
<td>46.8</td>
<td>50.8</td>
<td>54.8</td>
<td>58.8</td>
<td>62.8</td>
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<tr>
<td>30</td>
<td>18.6</td>
<td>24.4</td>
<td>30.4</td>
<td>36.4</td>
<td>39.4</td>
<td>42.4</td>
<td>46.4</td>
<td>50.4</td>
<td>53.4</td>
<td>57.4</td>
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<tr>
<td>35</td>
<td>17.2</td>
<td>22.0</td>
<td>27.0</td>
<td>33.0</td>
<td>36.0</td>
<td>39.0</td>
<td>43.0</td>
<td>46.0</td>
<td>49.0</td>
<td>53.0</td>
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<tr>
<td>40</td>
<td>15.8</td>
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<td>25.6</td>
<td>31.6</td>
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<td>44.6</td>
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<tr>
<td>45</td>
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<td>19.2</td>
<td>24.2</td>
<td>30.2</td>
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<tr>
<td>50</td>
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<td>22.8</td>
<td>28.8</td>
<td>31.8</td>
<td>34.8</td>
<td>37.8</td>
<td>40.8</td>
<td>43.8</td>
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<tr>
<td>55</td>
<td>11.6</td>
<td>16.4</td>
<td>21.4</td>
<td>27.4</td>
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<td>46.4</td>
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<tr>
<td>60</td>
<td>10.2</td>
<td>14.1</td>
<td>19.1</td>
<td>25.1</td>
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<td>31.1</td>
<td>34.1</td>
<td>37.1</td>
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<td>44.1</td>
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<tr>
<td>65</td>
<td>8.8</td>
<td>12.6</td>
<td>17.6</td>
<td>23.6</td>
<td>26.6</td>
<td>29.6</td>
<td>32.6</td>
<td>35.6</td>
<td>38.6</td>
<td>42.6</td>
</tr>
</tbody>
</table>

RENAAL PELVIS DILATION

- Transverse image of renal pelvis
- A-P measurement of pelvic diameter
- Cutoff 3, 4 or 5 mm

SHORT FEMUR/HUMERUS

- Expected values based on BPD in normal controls
- Standard measurement of bone length
- Measured to expected ratio
  - cutoffs 0.89-0.93

ECHOCOGENIC INTRACARDIAC FOCUS

- Calcified papillary muscle
- Discrete, bright focus within ventricle
- Usually left, may be right or bilateral
- Technique may affect prevalence
**ECHOGENIC BOWEL**

- Grading system for echogenicity
- Risk increases with brightness
- Sens for DS 12-13% at 1.4% FP
- Infection, CF, swallowed blood
- 50-75% normal

**CEREBRAL VENTRICULOMEGALY**

- Transverse, transventricular plane
- Internal diameter of distal atrium measured perpendicular to ventricular cavity at glomus of choroid plexus
- Cutoff 10 mm

**ABSENT/HYPOPLASTIC NASAL BONE**

- Fetal profile in mid-sagittal plane
  - Nose, lips, chin, palate
- Length of nasal bone measured
- Cutoff varies between studies

**ABERRANT RIGHT SUBCLAVIAN ARTERY**

**MULTIPLE MARKER SCREENING Risk Assessment Models**

- Any marker present
- Index Scoring System
- Application of likelihood ratios
  - Combining positive LR of any identified marker, risk reduction only if no marker identified (AAURA)
  - Combining positive LR of any identified marker and negative LR of absent markers

**INDEX SCORING SYSTEM**

<table>
<thead>
<tr>
<th>FINDING</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major anomaly</td>
<td>2</td>
</tr>
<tr>
<td>Nuchal fold ≥ 6mm</td>
<td>2</td>
</tr>
<tr>
<td>Short femur (M:E ≤ 0.91)</td>
<td>1</td>
</tr>
<tr>
<td>Short humerus (M:E ≤ 0.90)</td>
<td>1</td>
</tr>
<tr>
<td>Renal pelvis dilation ≥ 4mm</td>
<td>1</td>
</tr>
<tr>
<td>Hyperechoic bowel</td>
<td>1</td>
</tr>
<tr>
<td>Echogenic intracardiac focus</td>
<td>1</td>
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INDEX SCORING SYSTEM

<table>
<thead>
<tr>
<th>MATERNAL AGE</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 35</td>
<td>0</td>
</tr>
<tr>
<td>≥ 35 and &lt; 40</td>
<td>1</td>
</tr>
<tr>
<td>≥ 40</td>
<td>2</td>
</tr>
</tbody>
</table>

AAURA

Age
Adjusted
Ultrasound
Risk
Assessment

MA RISK X EGA RISK X LR

LR = likelihood ratio (sens/false pos)

Assessment of Risk

Gestational age

AAURA

FINDING | LIKELIHOOD RATIO DS
--------|---------------------
Structural defect | 25
Nuchal fold > 5mm | 18.6
Echogenic bowel | 5.5
Short humerus (M:E ≤ 0.89) | 2.5

FINDING | LIKELIHOOD RATIO DS
--------|---------------------
Short femur (M:E ≤ 0.91) | 2.2
Echogenic intracardiac focus | 2
Renal pelvis dilation > 3mm | 1.6
Normal ultrasound scan | 0.4
Age Adjusted Ultrasound Risk Assessment (AAURA)
FOR DOWN SYNDROME

Name Jane Doe by David A. Nyberg, MD

Instructions:  Fill in yellow spaces

Date 08/13/1992

LMP 04/14/1992

ASSUMPTIONS

LMP age 17.3

Risk Factor Scoring Inde

Question POSITIVE

THRESHOLD if positive if positive

Biparietal diameter (BPD) 4 (in mm)  (as isolated finding)

Femur Length (FL) 2.3 Short FL? NO 2.27 1.5 1

Humerus length (HL) 2.4 Short HL? NO 2.18 5 1

Nuchal fold (mm) 4 Nuchal thickness? NO 5 11 2

Renal pelvis (mm) 2 Pyelectasis? NO 3 1.5 1

Echogenic bowel (yes/no) no Echogenic bowel? NO 6.7 1

Echogenic intracardiac focus (yes/no) no Echogenic chorda tendi NO 1.8 1

Major anomaly (cardiac etc) (yes/no) no Major defect? NO 25 2

(may list here) Age factor

Recommend Total Benacerraf Score:

Apriori risk based on Age (1:__) 342 Amniocentesis?

Apriori risk based on biochemistry 500

(Ultrasound Risk (1: __) 833 NO

(provided courtesy of David A. Nyberg, MD

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ROLE OF GENETIC SONOGRAM AFTER PREVIOUS SCREENING

• May increase detection rate and/or decrease screen positive rate for patients with borderline results
• Does not have the power to change screen positive to negative if a priori risk sufficiently high
• Negative sonographic result may be falsely reassuring

Gynecology and Obstetrics

ROLE OF GENETIC SONOGRAM AFTER PREVIOUS SCREENING

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sequential</th>
<th>Nonsequential</th>
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<tbody>
<tr>
<td>DR, mN (%)</td>
<td>15.17 (6.82)</td>
<td>14.17 (6.87)</td>
</tr>
<tr>
<td>FPR, mN (%)</td>
<td>290.62 (6.4)</td>
<td>290.62 (6.4)</td>
</tr>
<tr>
<td>FPP, %</td>
<td>3.7</td>
<td>5.0</td>
</tr>
<tr>
<td>OAIR, 1A</td>
<td>25.9</td>
<td>38.8</td>
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<tr>
<td>AUC</td>
<td>0.994</td>
<td>0.963</td>
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J Ultrasound Med 2013;32:1607

ROLE OF GENETIC SONOGRAM AFTER PREVIOUS SCREENING

Table 1: Deflection Rate for a 3% False-Positive Rate With Standard Screening Policies and With Risk Modified by Genetic Sonogram Result

<table>
<thead>
<tr>
<th>Policy</th>
<th>Standard</th>
<th>After Sonogram</th>
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<tbody>
<tr>
<td>Combined</td>
<td>81</td>
<td>146*</td>
</tr>
<tr>
<td>Quadruple</td>
<td>81</td>
<td>146</td>
</tr>
<tr>
<td>Triple</td>
<td>70</td>
<td>146</td>
</tr>
<tr>
<td>Nuchal</td>
<td>97</td>
<td>146</td>
</tr>
<tr>
<td>Contingent</td>
<td>97</td>
<td>146</td>
</tr>
<tr>
<td>Second trimester</td>
<td>97</td>
<td>146</td>
</tr>
<tr>
<td>Genetic sonogram*</td>
<td>97</td>
<td>146</td>
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</tbody>
</table>

Data are %.

*Not interpreting the test would derange in sensitivity.
Replacing the second-trimester quadratic model with the sonogram.

Gynecology and Obstetrics

AJOG 2005;114:1889
THE GENETIC SONOGRAM

Limitations and unresolved issues

- Some markers are not independent
  - Femur and humerus lengths, nasal bone in first and second trimesters
- Incidence of some markers varies with ethnicity
  - Echogenic intracardiac focus, nasal bone
- Various cutoffs used in different studies
- Counseling for isolated minor marker in low risk patient

OTHER SONOGRAPHIC MARKERS

- Choroid plexus cyst
- Abnormal skull shape
- Wide iliac wing angle
- Hypoplastic 5th mid phalanx/clinodactyly 5th finger
- Sandal gap
- Single umbilical artery
- Chorioamniotic separation

"The theory that a greater number of fetuses with trisomy 21 could be identified in the low risk patient group, combined with the fear of missing an affected fetus if every minor marker is not reported, has fostered over-diagnosis and excessive counseling."

- Beryl Benacerraf, 2000

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