Prenatal Screening for Trisomy 21: Recent Advances and Guidelines

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Pregnancy Related Disorders:
Present Perspectives and Emerging Challenges
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Other:
- Patent Holder – uE3 in prenatal screening
1. Brief history of prenatal screening.
2. How is screening performance measured?
3. Performance of second trimester, first trimester, integrated, and sequential tests for Down syndrome.
5. Trisomy 18 and 13
6. A laboratory perspective on nuchal translucency (NT) measurement and quality assurance.
Prenatal Screening History Begins with Prenatal Screening for Open Neural Tube Defects using MSAFP

The birth prevalence of spina bifida and anencephaly in England and Wales between 1964 and 1975. (N Wald)

1970s Maternal Serum AFP screening
1980s acceptance in U.S. (ACOG liability alert)
1980s primary prevention with folic acid
2000s move to ultrasound screening?

Prenatal Screening History Begins with Prenatal Screening for Open Neural Tube Defects using MSAFP

The birth prevalence of spina bifida and anencephaly in England and Wales between 1964 and 1993. (N Wald)

Effect of primary prevention (folic acid) and prenatal screening (MSAFP)
Prenatal Screening for Aneuploidies:

- 2nd trim AFP (with Mat Age)
- 2nd trim Multiple markers (Double, Triple, Quad)
- 1st trim nuchal translucency (NT)
- 1st trim NT + PAPP-A + free βhCG

Integrated 1st and 2nd trim
Sequential 1st and 2nd trim
Fetal nucleic acids in maternal plasma?

Simple Description of Prenatal Screening

General pregnant population

Screening test
(non-invasive, inexpensive, accessible, equitable)

Screen Positive
("high risk")

Counsel on risks and benefits of diagnostic testing
(invasive, risky)

Screen Positive
("low risk")

No further action
How is screening performance measured?

**Screening Performance:**

*The challenge in screening is to have a test that has a high detection rate and low false positive rate.*

- **Detection Rate** (sensitivity)
  - percentage of *affecteds* called screen positive by the test
  - *The higher the better!*

- **False Positive Rate** (1 – specificity)
  - percentage of *unaffecteds* called screen positive by the test
  - *The lower the better!*
Performance of second trimester, first trimester, integrated, and sequential tests for Down syndrome

Screening in the Early 2\textsuperscript{nd} Trimester (15-20 weeks) with Multiple Serum Markers

\textit{Advantages:}
\begin{itemize}
  \item Simplest screening method (all serum at one time)
  \item Includes screening for open NTDs using MSAFP
  \item Will include a larger proportion of pregnant women
  \item Follow-up diagnostic test is amniocentesis
\end{itemize}
2nd Trimester Serum Markers in Down Syndrome Pregnancies

Data from FASTER

Prenatal Screening for Down Syndrome in the Early 2nd Trimester (15 - 20 weeks)

at 5% false positive rate

Detection Rate (%)
Screening in the Late 1st Trimester (11-13 weeks)

Advantages:

- Patient privacy
- Earlier diagnosis (if CVS is available)
- Greater availability of pregnancy termination
- Earlier, safer pregnancy termination

First Trimester Combined Test:

- NT ultrasound
- serum PAPP-A
- serum β-hCG

Data from FASTER.

This discussion of general population markers will not include:

- Nasal Bone
- Ductus venousus blood flow
The Integrated Test:
Two Stages to Reach a Single Risk Assessment

- Combination of the best markers measured at different times in pregnancy into a single test result.
- This will be more effective than current tests performed at any one time.

PAPP-A + quad markers = SERUM INTEGRATED
NT + PAPP-A + quad markers = FULL INTEGRATED

Integrate results into a single risk
Performance of the Integrated Test

at 5% false positive rate

- SURUSS
- FASTER

Detection Rate (%)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>30%</th>
<th>69%</th>
<th>79%</th>
<th>85%</th>
<th>85%</th>
<th>94%</th>
<th>85%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>triple</td>
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<tr>
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<tr>
<td>combined</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serum</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>full</td>
<td></td>
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</tr>
</tbody>
</table>

Performance of the Integrated Test

at 1% false positive rate

- SURUSS
- FASTER

Detection Rate (%)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>12%</th>
<th>50%</th>
<th>60%</th>
<th>66%</th>
<th>66%</th>
<th>72%</th>
<th>73%</th>
<th>85%</th>
<th>87%</th>
<th>87%</th>
<th>86%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>triple</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quad</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>combined</td>
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</tr>
<tr>
<td>serum</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>full</td>
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</tr>
</tbody>
</table>

85% DR at 1%
### Relative Screening Performance of 1st Trimester Combined and Integrated Tests

<table>
<thead>
<tr>
<th>If 1st Trimester Combined Test Performance is:</th>
<th>Then Full Integrated Test Performance will be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td>FPR</td>
</tr>
<tr>
<td>85%</td>
<td>5%</td>
</tr>
<tr>
<td>90%</td>
<td>5%</td>
</tr>
<tr>
<td>85%</td>
<td>1%</td>
</tr>
<tr>
<td>90%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Is there a way to have both:

- earlier screening and diagnosis – as in the 1st trimester test

  *and*

- much lower screen positive rate – as in the Integrated Test

**YES**

Sequential protocols
Sequential Screening: Towards 1st trimester combined or towards integrated, depending on the 1st trimester risk cut-off

![Graph showing detection rate vs. false positive rate for different screening protocols.]

Example of Sequential Screening

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Detection Rate (DR)</th>
<th>False Positive Rate (FPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1st trimester markers measured on all women at 10-13 wk. Risk cut-off set at ≥1:63</td>
<td>71.8%</td>
<td>1.5%</td>
</tr>
<tr>
<td>2</td>
<td>Screen negatives (98.5% of population) have quad markers measured at 15-18 wk. Risk cut-off set at ≥1:65</td>
<td>12.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>84.3%</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

Assumes 1:600 prevalence
Comparing Sequential Screening to 1st Trimester Combined Test and Integrated Test

<table>
<thead>
<tr>
<th>Test</th>
<th>FPR @ 84.3% DR</th>
<th>DR @ 2.0% FPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester Combined</td>
<td>6.0%</td>
<td>74.5%</td>
</tr>
<tr>
<td>Sequential example</td>
<td>2.0%</td>
<td>84.3%</td>
</tr>
<tr>
<td>Full Integrated</td>
<td>1.2%</td>
<td>87.4%</td>
</tr>
</tbody>
</table>

Sequential Integrated Screening at Women & Infants (Expected Performance)

Stage 1:
- 1st trimester markers
- PAPP-A and NT
- 1:25 risk cut-off
- 59% DR, 0.9% FPR

Stage 2:
- Quad markers
- Full Integrated Risk
- 1:110 risk cut-off
- 31% DR, 1.6% FPR

Sequential Integrated Test: 90% DR, 2.5% FPR
Compare to Integrated Test: 90% DR, 2.0% FPR
Compare to 1st Trimester Test: 90% DR, 8.0% FPR
Contingent Screening

Step 1: - 1st trim. NT + PAPP-A (+ β-hCG)
  - very high risk (≥1 in 25 or 1 in 50) called Screen Positive and offered CVS (<1% of all women screened)
  - very low risk (<1 in 2000) called Screen Negative and have no further testing (60-70% of all women screened)

Step 2: - all between 1 in 25 or 1 in 50 and 1 in 2000 go on to full integrated test (30-40% of all women screened)
  - high risk by integrated test (≥1 in 100) called Screen Positive and offered amniocentesis (1-2% of all women screened)

New Study:
Gekas J et al. Cost-effectiveness and accuracy of prenatal Down syndrome screening strategies: should the combined test continue to be used widely?
*Am J Obstet Gynecol* 2011;204:175.e1-8

- “Patients seen early in their pregnancy may benefit from…screening that combined 1st and 2nd trimester evaluations.”
- “Contingent strategy appears to be the most cost-effective.”
- “Cost-effectiveness analyses are only 1 element among many that need to be taken into account…”
### Prenatal Screening Guidelines: Practice Standards in EUROCAT Countries, Canada, and the United States

**Prenatal Screening for Down Syndrome in 14 EUROCAT Countries (Special Report 2010)**

<table>
<thead>
<tr>
<th>Nat’l Policy</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>YES</td>
<td>NT/serum</td>
</tr>
<tr>
<td>Croatia</td>
<td>NO</td>
<td>NT</td>
</tr>
<tr>
<td>Denmark</td>
<td>YES</td>
<td>combined</td>
</tr>
<tr>
<td>Finland</td>
<td>YES</td>
<td>combined</td>
</tr>
<tr>
<td>France</td>
<td>YES</td>
<td>combined</td>
</tr>
<tr>
<td>Ireland</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>YES</td>
<td>NT/combined</td>
</tr>
<tr>
<td>Malta</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>YES</td>
<td>NT/serum/combined</td>
</tr>
<tr>
<td>Spain (Catalonia)</td>
<td>YES</td>
<td>combined</td>
</tr>
<tr>
<td>Sweden</td>
<td>NO</td>
<td>combined (routine in parts of Sweden)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>YES</td>
<td>combined</td>
</tr>
<tr>
<td>UK</td>
<td>YES</td>
<td>combined triple/quad</td>
</tr>
</tbody>
</table>

EUROCAT: European Surveillance of Congenital Anomalies
1. “All pregnant women in Canada, regardless of age, should be offered… a prenatal screening test for the most common clinically significant fetal aneuploidies…”

2. “Maternal age screening is a poor minimum standard for prenatal screening for aneuploidy and should be removed as an indication of invasive testing.”

3. “In 2007, as a minimum standard, any prenatal screen …should have a 75% detection rate with no more than a 5% false positive rate for Down syndrome.”

SOGC Clinical Practice Guideline, No. 187, February 2007
Available Screening Options that Meet Minimum Standard (75% DR with a 5% FPR)

- First Trimester Screen
- Second Trimester Quad Screen
- Two Step Screens:
  - Contingent
  - Integrated
  - Serum Integrated
  - Sequential

SOGC Clinical Practice Guideline, No. 187, February 2007

ACOG 2007

1. “… all women should be offered aneuploidy screening before 20 weeks of gestation, regardless of maternal age.”

2. “… patients seen early in pregnancy should be offered aneuploidy screening that combines first- and second-trimester testing (integrated or sequential).”

3. “The screening strategy chosen will depend on availability of CVS and of personnel trained in NT measurement …

ACOG Practice Bulletin, No. 77, January 2007
4. “When CVS is not available, … offer integrated screening to patients who present in the first trimester … to take advantage of the improved detection rate and low false-positive rate.”

5. “If NT measurement is not available or cannot be obtained …, offer serum integrated screening to patients who present early and second-trimester screening to those who present later.”

ACOG Practice Bulletin, No. 77, January 2007
Trisomy 18: Marker Levels
GE Palomaki 2011, analysis of published literature

<table>
<thead>
<tr>
<th>1st trim. marker</th>
<th>Median MoM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>2.17</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>0.20</td>
</tr>
<tr>
<td>Free β-hCG</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2nd trim. marker</th>
<th>Median MoM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>0.66</td>
</tr>
<tr>
<td>uE3</td>
<td>0.36</td>
</tr>
<tr>
<td>hCG</td>
<td>0.39</td>
</tr>
<tr>
<td>inhA</td>
<td>0.88</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Trisomy 18: Screening Performance
GE Palomaki 2011, unpublished

<table>
<thead>
<tr>
<th>Modeled Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
</tr>
<tr>
<td>2nd trim. triple</td>
</tr>
<tr>
<td>1st trim. combined</td>
</tr>
<tr>
<td>Serum integrated</td>
</tr>
<tr>
<td>Full integrated</td>
</tr>
</tbody>
</table>

Actual Performance
Based on clinical trial data, the observed screen positive rate in practice will be higher than the modeling suggests.
Trisomy 13

- 2nd trimester markers are not informative.
- 1st trimester markers are informative.
  - The pattern is similar to that found in trisomy 18.
  - But, published data on trisomy 13 marker levels are biased and too small to estimate their levels with accuracy.
- It is reasonable to assume that the majority of trisomy 13 cases will be identified as part of current trisomy 18 screening protocols that include 1st trimester markers.

The Laboratory’s Role in Nuchal Translucency (NT) Measurement:

Applying Serum Marker Quality Assurance Measures
### Prenatal Screening Markers

**Quality monitoring**

Marker parameters that are monitored:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of change with increasing gestation</td>
<td>may go up or down at a constant rate</td>
</tr>
<tr>
<td>Median</td>
<td>calculated MoM values should be stable at 1.0</td>
</tr>
<tr>
<td>SD of the distribution</td>
<td>calculated SD of the log MoM values expected to remain steady</td>
</tr>
</tbody>
</table>

### 1st Trimester Nuchal Translucency (NT)

**Quality monitoring**

NT parameters that are monitored:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of increase with CRL</td>
<td>log-linear over 10.3 - 13.6 weeks should go up by ~ 20% per week</td>
</tr>
<tr>
<td>Median</td>
<td>calculated MoM values should be stable at 1.0 MoM</td>
</tr>
<tr>
<td>SD of the distribution</td>
<td>calculated SD of the log MoM values expected to be about 0.1</td>
</tr>
</tbody>
</table>
Maternal Serum AFP Increases with Increasing Gestation

Nuchal Translucency Increases with Increasing Gestation

Schuchter et al, Prenat Diagn 1998

The Distribution of AFP and NT MoM in Unaffected Pregnancies

SD of log MoM = 0.15

SD of log MoM = 0.10
The Distribution of hCG and NT MoM in Unaffected Pregnancies

Why is NT such a good marker?

NT: 0.11 SD
50% DR
1% FPR

hCG: 0.24 SD
50% DR
8% FPR
Published Literature:
Variation in NT median measurement

Range of NT measurements (in MoM) between hospitals

Inter-operator variation at one hospital


Published Literature:
Variation in NT median measurement

Schielen PC et al., Prenat Diagn 2006;26:711-8

"Laboratories should routinely monitor the quality of nuchal translucency measurements… When possible, instituting sonographer-specific medians and providing individualized feedback about performance and numbers of women tested offer the potential to yield more consistent and improved performance."

NT monitoring: when to make changes

- Use objective criteria as guide
- Partially subjective process
  - Look for trends
  - Sample volume must be considered
  - What to do with very small volume sonographers?
- Sonographer feedback has been minimally useful
Conclusions: *Prenatal Screening for Down Syndrome*

- In the primary care and general laboratory setting, a detection rate of 90-95% at a 2-5% false positive rate can be routinely achieved.

- If the laboratory provides a risk assessment that includes nuchal translucency ultrasound as a marker, it has an obligation to maintain and review that marker’s screening performance.

- Guidelines published by professional societies and government agencies generally reflect these goals.

---------Women & Infants Hospital - Brown University---------

Geralyn Lambert-Messerlian  Jim Haddow  Glenn Palomaki  George Knight

Primary collaborators over many years

University of London

Nick Wald