Non-Invasive Prenatal Testing

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The speaker has signed a disclosure form and indicated she has no significant financial interest or relationship with the companies or the manufacturer(s) of any commercial product and/or service that will be discussed as part of this presentation.

Session Summary

This new technology is non-invasive and detects fetal trisomies 21, 18, and 13 in pregnancies of 10 weeks or more. The test analyzes maternal blood and provides a risk assessment for pregnant women with singleton and naturally conceived or self-egg donor twin pregnancies.

Session Objectives

Upon completion of this presentation, the participant will be able to:

- define the difference between prenatal screening versus diagnostic testing;
- state the approximate sensitivity of CVS and amniocentesis for Down syndrome;
- state what type of fetal DNA is used in NIPT;
- know what percentage of cfDNA comes from the fetus in the maternal blood;
- state the week of gestation when cfDNA can be reliably detected and the half life of cfDNA post partum;
- list the test accuracy for conventional screening and the false positive rate;
- list the ACOG Genetics Committee Opinion on test accuracy for cfDNA and the false positive rate;
- know how many women would need an amniocentesis or CVS after conventional screening versus cfDNA;
- know what test to order to resolve the discrepancy if the NIPT was normal but the infant has features of Down syndrome.

References

Gene Tests: www.genetests.org
OMIM -Online Mendelian Inheritance in Man: www.omim.org/

Session Outline

See presentation handout on the following pages.
FANNP Non-Invasive Prenatal Testing

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Some slides courtesy of Verinata and Integrated Genetics

Glossary of Abbreviations

- NGS – Next Generation Sequencing
- MPS – Massively Parallel Sequencing
- NCV – Normalized Chromosome Value
- NIPT – Noninvasive Prenatal Testing
- HEBIC – Health Economic Budget Impact Calculator
- cfDNA – Cell Free DNA
- ART – Assisted Reproductive Technology
- SAFeR™ - Selective Algorithm for Fetal Results
- CPM – Confined Placental Mosaicism

Prenatal Prevalence of Chromosomal Abnormalities

Data adapted from Wellesley, D, et al., Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. Eur J of Hum Gen, 11 January 2012.

Screening Tests

- Definition – a test applied to an asymptomatic population in order to classify them with respect to their likelihood of having a specific condition

- The difference between screening and diagnostic tests:
  - Screening tests give a risk for a condition
    - MSAFP, Multiple Marker Screen, Ultrasound for Down Syndrome
  - Diagnostic tests give a definitive result as to the presence or absence of a condition
    - Amniocentesis, CVS, Ultrasound for spina bifida
Numerous Options with Variable Performance

- Modelled predicted performance
  Cuckle et al, Semin Perinatol, 2005

What Do Screening Results Tell Us Today?

- Screening results offer estimates of risk.
  — A ‘negative’ or low-risk result may be misinterpreted to mean the fetus is normal.
  — Positive screens, based on risk can lead to unnecessary invasive procedures.

Invasive Diagnostic Options

<table>
<thead>
<tr>
<th>Trimester - Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st – CVS</td>
<td>99.25%(^1)</td>
<td>98.65%(^1)</td>
</tr>
<tr>
<td>2nd - Amniocentesis</td>
<td>99.4%(^2)</td>
<td>99.5%(^2)</td>
</tr>
</tbody>
</table>

Even the gold standard is not 100% sensitive and specific.

Noninvasive Prenatal Testing (NIPT)

What is NIPT
Tests that utilize the presence ofcff-DNA in the maternal circulation to screen for the risk of fetal aneuploidy and other chromosomal aberrations.

Serum Screening – What is Needed to Measure?

Account for These Factors:
- Age
- History
- Multiple Gestation
- Ethnicity
- Smoking
- Diabetes
- Weight
- Gestational Age

Measuring with NIPT – Eliminate External Factors

Factors Needed:
- Age
- History
- Multiple Gestation
- Ethnicity
- Smoking
- Diabetes
- Weight
- Gestational Age
**What are the Goals of NIPT?**

- Reduce exposure of fetus to risk
- Reduce false positives
- Enable a high detection rate

*When data supports testing in all patients, instead of only high risk patients.

**Technology Behind NIPT**

**Two Sources of Fetal DNA in Maternal Blood**

- **Fetal cells (intact)**
  - 1 in a billion of total cell population
  - Requires fetal cell isolation via mechanical and/or biochemical means

- **Cell-free DNA (cfDNA)**
  - 2–20% of total cfDNA is fetal
  - Requires DNA isolation and counting
  - Counting method developed by Dr. Steven Quake, Stanford University

**What is “cell-free DNA”?**

- Released through cellular death (apoptosis)
  - Cleaves DNA into small fragments (150-200bp)
  - Released into bloodstream as cell-free DNA (cfDNA)

- Fetal cells also release cfDNA
  - Maternal blood contains both fetal and maternal cfDNA

**Fetal cfDNA Can Be Measured**

- Fetal cfDNA much more common than intact fetal cells
- Reliably detected after 7 weeks gestational age
- Short half life (16 min), undetectable after 2 hours postpartum

**How is this addressed by NIPT today?**

- 2 ways to sequence using cfDNA
  - Massively Parallel Sequencing
  - Targeted Sequencing

- 3 ways to analyze
  - Normalized Chromosome Value (NCV)
  - Z-Score
  - Estimate risk using combination of sequence data and other factors
Massively Parallel Sequencing (MPS)
Method of Analysis for MPS

- cfDNA
- DNA Sequencing
- Alignment
- CGATTTAAT... CGATTTAAT...
- > 5,000,000 “counts” per blood sample

Precise Molecular Counting Can Detect Fetal Aneuploidy
Example: 20% fetal fraction
- Fetus with trisomy contributes ~50% more cfDNA from the trisomic chromosome
- Millions of “counts” allows detection of relative cfDNA increase
- No need to distinguish fetal from maternal cfDNA

Fetal Trisomy Detection With cfDNA
- Each bar represents thousands of cfDNA fragments
- The overabundance of chromosome 21 cfDNA fragments in trisomy 21, although small, can be measured with DNA sequencing

Invasive Procedures Needed Because of False Positives
- Conventional Maternal Serum Screen and Ultrasound
  - False positive rate is 5%
  - 50 of 1000 women screened will need invasive procedure like CVS or amniocentesis
- NIPT Non Invasive Prenatal Testing (cfDNA)
  - False positive rate is <0.5%
  - 5 of 1000 women screened with need invasive procedure like CVS or amniocentesis

Clinical Data
MELISSA Study

Study Design, Demographics
- 2,882 samples collected
- 534 selected for analysis
  - Including all abnormal karyotypes (N = 221)
  - Gestational age: 10 – 23 weeks
  - BMI (kg/m²): 17 – 59
  - Includes 38 ART pregnancies
  - Diverse (27.3% non-white)

MPS Performance (Autosomes)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Classified</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>100 (99)</td>
<td>95.9 – 100</td>
<td>100 (94/99)</td>
<td>99.9 – 100</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>97 (85)</td>
<td>85.5 – 99.9</td>
<td>100 (46/60)</td>
<td>99.9 – 100</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>78 (40)</td>
<td>49.2 – 95.3</td>
<td>100 (485/485)</td>
<td>99.9 – 100</td>
<td></td>
</tr>
</tbody>
</table>

Other Abnormal Karyotypes

MELISSA Study Demonstrated Other Aneuploidy Detection
- Mosaicism
  - 4 / 4 detected (T21, T18)
- Robertsonian Translocations
  - 3 / 3 detected (T21, T18)
- Rare autosomal aneuploidies
  - Detected T16, T20
- Sex aneuploidies
  - Monosomy X, XXX, XXX, XYY

Clinical Recommendations

Invasive procedures are suggested to confirm a positive or unclassifiable NIPT result.

Test results should always be used in the context of all available clinical findings.

It is recommended that the healthcare provider determine the utilization of the test, including the need for genetic counseling.

Available Tests

- Harmony (Ariosa)
- Panorama (Natera)
- Verifi (Verinata)
- MaterniT21 Plus (Sequonom)
**Product Profile**

<table>
<thead>
<tr>
<th>Product Profile</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomes Analyzed</td>
<td>21, 18, 13 Monosomy X Option</td>
</tr>
<tr>
<td>Blood draw requirement</td>
<td>1 blood tube (7-10mL)</td>
</tr>
<tr>
<td>Patient Eligibility</td>
<td>21, 18, 13: High risk pregnancies Monosomy X: Cystic hygroma</td>
</tr>
<tr>
<td>Gestational Age</td>
<td>10 or more weeks estimated gestational age</td>
</tr>
<tr>
<td>Sample collection</td>
<td>On-site collection kits, ambient shipping</td>
</tr>
<tr>
<td>Turn-around time</td>
<td>8-10 business days</td>
</tr>
<tr>
<td>Clinical Support</td>
<td>Full-time genetic counselors on staff for provider phone support</td>
</tr>
</tbody>
</table>

**verifi® prenatal test – Test Report**

**Test Report**

- Abnormal results are flagged at top to alert the clinician
- Abnormal results are highlighted in red
- Comments included to provide additional guidance
- Test claims restated as reference

**ACOG Genetics Committee Opinion**

**ACOG - Noninvasive Prenatal Testing for Fetal Aneuploidy**

Patients at increased risk of aneuploidy can be offered testing with cell free fetal DNA. This technology can be expected to identify approximately 98% of cases of Down syndrome with a false positive rate of less than 0.5%

**Parents tell you they have a Trisomy 21 diagnosis by NIPT – what to do?**

- If they did not have amniocentesis to confirm:
  - Order chromosomes
- If they did have an amniocentesis and it confirmed the NIPT:
  - Do not need to repeat postnatal chromosomes
- If they had NIPT that was negative but infant has features of major trisomy:
  - Order chromosomes