Triple tests

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for reproduction biology PhD students

“Noninvasive” PND

- Maternal serum screening
  - in the second trimester
  - in the first trimester
  - combined screening

Screening based on Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Risk</th>
<th>Trisomy 21</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>15%</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>30%</td>
</tr>
</tbody>
</table>

Sensitivity

- 30%
Maternal serum analysis
(hormones and fetal products in maternal circulation)

**Advantages**
- No risk for the pregnancy
- May reduce the number of invasive tests requested
- Screening test which indicates at risk pregnancies
- Can be applied on a large scale (general population)

**Disadvantages**
- Gives only a risk of being affected; requires additional analyses
- Second trimester screening gives late results (later amniocentesis)
- Knowledge of physiological factors affecting markers still incomplete

**First trimester serum markers**
- Pregnancy-associated plasma protein A (PAPP-A)
- free beta-HCG
- Inhibin A,
- others

**Second trimester serum markers**
- Alpha-fetoprotein (AFP)
- Human chorionic gonadotropin (HCG)
- Unconjugated estriol (uE3)

*Not just for the diagnosis of malformations!*
*(useful for judging pregnancy “well-being”)*

**Down syndrome rates and false-positive rates**
(using triple test at different ages; risk cut-off 1/250)
(from Kennard & Wald, 1995 & 1996)

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Detection rate (%)</th>
<th>'positive' Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>45</td>
<td>5</td>
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<td>35</td>
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<td>40</td>
<td>89</td>
<td>41</td>
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<tr>
<td>45</td>
<td>99</td>
<td>79</td>
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</table>
Screening for neural tube defects and aneuploidy is an established part of prenatal care in many countries. Current protocols focus on the 2nd trimester and operate in 2 stages:

1. Primary screening test to identify women at high risk
2. Specific diagnostic test offered to confirm or exclude

- Neural tube defects (NTD)
  - screen using maternal serum AFP and then diagnose using ultrasound.

History of prenatal screening for Down syndrome:
- Early 1980s: screening based on maternal age only
- 1984: AFP added (low level, increased risk)
- 1988: triple test was created (AFP, hCG and uE3)
- Canada, USA, most European countries: triple screening was used (AFP, uE3, hCG)
- UK: double screening was mostly used (AFP, hCG)

NTDs \(-\text{~1/500}\) Leading cause of stillbirth, death in early infancy, handicap in surviving children

Result from a defective closure of neural tube
- Anencephaly
- Spina bifida
  - Anencephaly and spina bifida occur with equal frequency, constituting 90% of all NTD
  - 95% occur spontaneously (no family history)
  - However, if have 1 affected pregnancy, at 10 fold greater risk.
Incidence of NTDs has declined over the last 40 yrs

Periconceptional supplementation with folic acid
(at least 1 month before conception and continuing through the 1st trimester) decreases NTDs by ~ 75%.

Incidence of NTDs has declined over the last 40 yrs

AFP - alpha fetoprotein
fetal glycoprotein produced in the liver, secreted into the fetal circulation, excreted by the kidneys into the amnion

AFP levels normally increase until 10-14 weeks, then decrease steadily.

AFP - measured directly from amniotic fluid (AF AFP) or indirectly in maternal serum (MS AFP).

AFP levels are elevated among open NTDs

α-fetoprotein levels at 16-18 wks gestation

>97% of NTDs can be detected by AFP sampled directly from the amniotic fluid. Amniocentesis is not a screen, however, this observation is the basis for the maternal serum alpha fetoprotein screen.

MS AFP levels are elevated among open NTDs

If this were the only screen, 20% of NTD go undetected

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Combined use of MS AFP and ultrasound approach the accuracy of AF AFP

In many PND programs, first or second degree relatives of patients with NTDs may have an MS AFP assay at 16 weeks followed by detailed ultrasound at 18 weeks.

Factors affecting MS AFP levels
- Inaccurate gestational aging accounts for 40% of all high MSAPFs
- Race: African-Americans 10-15% higher than Caucasians, Hispanics
- Accuracy of assay
- Multiple pregnancy increased in proportion to the No. of fetuses
- Insulin dependent diabetes (DM1): 10% lower
- Smoking: lower levels
- Maternal weight: heavier women have diluted MS AFP due to greater blood volumes

PND of NTDs by ultrasound—anecephaly
- Detection of anencephaly is close to 100% by ultrasound.
- The nasion is the middle of the nasal frontal suture on the skull.
- In the anencephalic, there is no calvarium or brain compared to the normal fetus below.
- Sonography picks up over 90% of cases of open NTDs after 16-17 weeks.

Screening for ch. aneuploidy
- Most prominent risk factor for aneuploidy, specifically Down syndrome—increasing maternal age
  - Down syndrome risk by age:
    - 20 - 1/1400
    - 30 - 1/900
    - 35 - 1/385
    - 40 - 1/100
    - 45 - 1/25
  - However, maternal age alone is an inefficient method of screening: need biochemical markers

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Age of the mother

![Graph showing the risk of Down's syndrome with maternal age.](image)

<table>
<thead>
<tr>
<th>Age of the mother</th>
<th>Risk %</th>
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<tbody>
<tr>
<td>15</td>
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<td>20</td>
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<td>45</td>
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<td>50</td>
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Risk factors for Down's syndrome:
- Previous Down's pregnancy
- Advanced maternal age

Chromosome aneuploidy and MS AFP

![Graph showing chromosome aneuploidy.](image)

- MS AFP is lower in fetuses with Down syndrome
- Maternal age + MS AFP level; 40% detection rate

Maternal Serum Screening (MSS) (Triple screen)

Measures 3 blood markers and is available 15-20 weeks gestation to identify those at risk for:
- Down syndrome
- Trisomy 18
- NTD
Biomarkers used in maternal serum screening

- Accuracy is increased by measuring serum levels of multiple biomarkers
- 60-80% detection rate of Down syndrome and Edward's syndrome; 5% false positive rate

<table>
<thead>
<tr>
<th>Down syndrome</th>
<th>MSAFP uE3</th>
<th>HCG</th>
</tr>
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<tbody>
<tr>
<td>Trisomy 18</td>
<td>↓</td>
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</tbody>
</table>

MSS is just a screen

- Not a diagnostic tool
- MSS is considered a "screen positive" for Down syndrome
  - Use ultrasound to confirm the age of the fetus
  - Estimate risk to mother based on age
- Further counseling and amniocentesis should be offered to screen positive women
- A negative result does not guarantee a normal child

Biomarkers have also been identified for the first trimester

First Trimester Markers (measured at 7-14 wks):
- Human chorionic gonadotrophin, pregnancy-associated plasma protein A (PAPP-A)
- 55-80% detection rate, 5% false positive rate

<table>
<thead>
<tr>
<th>Increased DS risk</th>
<th>hCG</th>
<th>PAPP-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 18</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>↓</td>
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</tbody>
</table>

Combined ultrasound & biochemical screening in first trimester

1st trimester screening at 10-13 weeks:
- Maternal age
- Free β human chorionic gonadotrophin
- PAPP-A (pregnancy associated plasma protein A)
- Nuchal translucency (NT)

<table>
<thead>
<tr>
<th>DS:</th>
<th>90% detection rate; 5% false positive rate</th>
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<tbody>
<tr>
<td>ts 18:</td>
<td>86% detection rate; 0.5% false positive rate</td>
</tr>
<tr>
<td>ts 13:</td>
<td>90% detection rate; 0.5% false positive rate</td>
</tr>
</tbody>
</table>
**Pregnancy-associated plasma protein A, pappalysin**

This gene encodes a secreted metalloproteinase which cleaves insulin-like growth factor binding proteins (IGFBPs). It is thought to be involved in local proliferative processes such as wound healing and bone remodeling.

**Inhibin** down regulates FSH synthesis and inhibits FSH secretion

In females
Inhibin is produced in the gonads, pituitary gland, placenta and other organs.
In women, FSH stimulates the secretion of inhibin from the granulosa cells of the ovarian follicles in the ovaries. In turn, inhibin suppresses FSH.
Inhibin B reaches a peak in the early- to mid-follicular phase, and a second peak at ovulation.
Inhibin A reaches its peak in the mid-luteal phase.
Inhibin secretion is diminished by GnRH, and enhanced by insulin-like growth factor-1 (IGF-1).

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**Biochemical markers**

**First trimester**

![Pregnancy Associated Plasma Protein A](image)

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**What’s recent (or coming fast....)**

- First trimester integrated screening
  - Ultrasound (nuchal fold measurement)
  - Combined with Serum screening

Can detect up to 90% of trisomy 21....
  -- experience of operator
  -- do you then do CVS?

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- Prospective studies of 200,868 pregnancies
- 871 with trisomies
- nuchal translucency identified 76.8% of tri(21), with false + of 4.2%
- With first trimester serum screen (hCG and PAPP-A), detection 87.0%, false + of 5.0%
- absent nasal bone will identify 69% (ethnic variation – false positives and negatives!)
Counseling difficulties linked to serum screening

- Time and timing (for counseling)
- Generation of anxiety
- False positives and false negatives
- Scientific unknowns
- Insurances and financial factors
- Disagreement within couples
- How far to go?

Assessment Areas

- Maternal age
- Family medical history (both sides)
- Current pregnancy/pre-pregnancy history
- Ethnic background (both sides)

Maternal Serum Screening

- Patient education points:
  - ‘This is only a screening test’
  - ‘The test is optional’
  - ‘A negative result does not guarantee a healthy baby’
  - ‘A positive result does not mean that the baby has a problem, BUT further testing (ultrasound & CVS or amniocentesis) would be offered’
  - Offered to all patients regardless of age - ‘there is a small risk in every pregnancy for these conditions’

2nd Trimester Serum Screening

- Timing: 15 to 20 weeks gestation
- Choices:
  - Triple screen
  - Quad screen
- Cost ~$200 in the USA
  - Insurance coverage varies
  - Triple covered by most, Quad by some
**1st Trimester Maternal Serum Screening**

- **Timing:**
  - 26-84 mm CRL (9 to 13+6 weeks gestation)
- **Analytes used (with maternal age):**
  - free Beta HCG
  - PAPP-A
- **Detection rates with 5% screen positive rate:**
  - Down syndrome: 68%
  - Trisomy 18: 90%
- **Costs:**
  - $100-200 for serum
  - $200 plus for NT U/S

**1st Tri Serum + NT**

- Serum results combined with nuchal translucency (NT) measurement *
  - *Measured by an NT-certified ultrasonographer
  - Best visualized at CRL = 45 - 84 mm (11-14 wks gestation)
  - Increased NT = increased risk for Down syndrome / other disorders
- **Detection rates with 5% screen positive rate:**
  - Down syndrome ~ 90%,
  - Trisomy 18 ~ >90%
  

**Increased NT**

- Increased NT measurement (>3.5 mm) associated with increased risk for:
  - Chromosome abnormalities
  - Major structural cardiac defects
  - NTDs, other structural anomalies, and specific genetic syndromes
  - SAB, IUFA, SGA and stillbirth
- If normal chromosomes and >NT, can offer:
  - 2nd trimester MSAFP screen
  - Fetal anomaly scan between 18-22 weeks
  - Fetal echocardiogram between 20-22 weeks

**Pros: 1st Trimester Serum + NT screen**

- Fingerstick dried blood sample easy to collect and send via prepaid FedEx envelope
  - Draw blood <11 wks if possible (more sensitive)
  - Results take about 1 week
- Results available at earlier gestation
  - Allows choice of CVS or amnio
- Higher detection rate than 2nd trimester screen
- More accurate for multiple gestations
  - Separate ultrasound/NT results on each fetus
Cons: 1st Trimester Serum + NT screen

- Requires NT measurement performed at a certified center
  - Often only available at perinatal centers
  - Often necessitates patient travel
- Does not screen for NTDs
  - Need to discuss 2nd trimester AFP screening with patients who have had 1st trimester screening
- May not be covered by insurance
Conclusion

Screening for fetal aneuploidies at 11 to 13 weeks.
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Abstract
Effective screening for major aneuploidies can be provided in the first trimester of pregnancy. Screening by a combination of fetal nuchal translucency and maternal serum free-β-human chorionic gonadotrophin and pregnancy-associated plasma protein-A can identify about 90% of fetuses with trisomy 21 and other major aneuploidies for a false-positive rate of 5%. Improvement in the performance of first-trimester screening can be achieved by firstly, inclusion in the ultrasound examination assessment of the nasal bone and flow in the ductus venosus, hepatic artery and across the tricuspid valve, and secondly, carrying out the biochemical test at 9 to 10 weeks and the ultrasound scan at 12 weeks.

Thank you for your kind attention