The Rise and Fall of the Maternal Serum Screening Empire.
An Evolution of Testing
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Jim Waurin, MS, CG(ASCP)CM

Population Screening for Defects

- Screening for Open Neural Tube Defects
- Progressed to Screening for fetal aneuploidies.

In the beginning of serum screening...

- 1972 – David Brock publishes data on significance of high MS-AFP and ONTDs
- This set the tone for the use of MS-AFP as a screening tool for ONTDs.
**AFP during pregnancy**

<table>
<thead>
<tr>
<th>Gestational Age (weeks)</th>
<th>Birth</th>
<th>Fetal serum</th>
<th>Amniotic fluid</th>
<th>Maternal serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>20</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

**MS-AFP and Neural Tube Defects**

- Observation by Brock that an elevation in the maternal serum AFP gave the patient an increased risk for having a fetus with an open Neural Tube Defect.
- A screening program was started in the UK as they had a significantly high rate of ONTDs.

**MoM**

- In order to standardize all labs to one set of measurements, the Multiple of the Median (MoM) was established.
- The Median value is the value in the middle of an array of numbers.
- So the MoM value is how many times greater than, or less than 1.0 MoMs, is our patient value.
Measurement and Standardization

- Since the empiric amount of AFP changes during the course of the pregnancy, we needed to have some common unit of measurement.
- Thus begat...the MoM
- Example: If at 18 weeks, the Median value for AFP is 44.13 ng/ml. If a patient has a value of 88.26, her MoM would be 2.0.

AFP MoM values in NTDs

The United States Screening Program

- Pre-screening meetings held in DC in 1981
- Dr. Neil Holtzman was going to head a pilot program for several cities in the country
- There were many who objected saying it was just a "search and destroy" program.
- Dr. Brock pondered why things were so hard to do in this country
Chromosome Issues

- 1866 -- John Langdon Down made the first detailed description of affected individuals with unique characteristics.

Name changes...

- Originally referred to as "mongolism" due to facial features.
- Historical landmarks --
  - 1961 -- A delegation of scientists write a letter to the Lancet asking the name be changed to "Langdon-Down anomaly", or something similar.
  - 1965 -- A delegation from the Mongolian People's Republic asked WHO to change the name from "mongolism".
  - 1975 -- Classification of Nomenclature determines it should be called Down syndrome.

Maternal Age

- Observation made by Lionel Penrose in 1933 suggested a partial correlation between advanced maternal age and the risk of having a child with Down syndrome.
And 35 years became
Advanced Maternal Age
because...

IBM Hollerith Card, cir 1974

Advanced Maternal Age

- Epidemiological data were kept in "packets" of 5 year intervals.
  - e.g. 25-29, 30-34, 35-39, etc.
- You could look at the data and see the curve of Incidence of Down syndrome rise as the age of the mother rose.
- You could then determine who best to offer the invasive test of amniocentesis.
Term Risks for Age Related Chromosome Anomalies

Screening Rates

- Detection Rate (DR) – How many people with the disease are actually picked up with the test.
- False Positive Rate (FPR) – How many people are called “Screen Positive”, but in fact do not have the disease.

…and from the MSAFP screening data

- 1984 – Merkatz IR, publishers data on the significance of low MS-AFP and chromosome aneuploidies.
Down syndrome and maternal biochemistries

- So the questions had to be asked:
  - "If a low, or decreased, MS-AFP meant an increased risk of having a fetus with Down Syndrome, then who are we going to call 'at increased risk'? And of course, what are we going to call 'low'?"

- Answer:
  - "We're going to call women of Advanced Maternal Age, at increased risk."

This was because the only way to diagnose a fetus with DS was with an amniocentesis, and we recommended amnios to women of AMA.

Low MS-AFP

- "By selecting for amniocentesis women with serum AFP levels < 0.5 MoM at 14-20 weeks gestation...21% of pregnancies with Down Syndrome would be identified as well as 5% of unaffected pregnancies."

Cuckle, HS, Wald, NJ
The Lancet, April 28, 1984
Risk Calculation

- "Estimating a woman’s risk of having a pregnancy associated with Down syndrome using her age and serum alpha-fetoprotein"

Cuckle, HS, Walt, NJ, Thompson, SG
British Journal of Obstetrics and Gynaecology
May 1987

More Developments

- 1985 – ACOG issues the first of what will be several Professional Liability Alerts
  
  "It is now imperative that you investigate the availability of these tests in your area and familiarize yourself with the procedure, location, and mechanism of the follow-up tests to screen for neural tube defects." May 1985

Another development

- 1987 – Bogart MH, publishes data on significance of elevated hCG values and aneuploidies
Another risk calculation

- Maternal serum screening for Down syndrome in early pregnancy

Wald, Cuckle, Densem, et al.
British Medical Journal
8 October 1988

And finally...

- 1988 – Wald et al., publish data on significance of low uE3 values and aneuploidies.

Second trimester Medians in DS

- Graph showing median values of various markers (uE3, AFP, hCG) over gestational age for Down Syndrome (DS)
Second Trimester Screening

- Goal is to identify as many abnormal fetuses as possible, while...
- Keeping the false positive rate as low as possible.
- Keep the Detection Rate (DR) high, while minimizing the False Positive Rate (FPR).

Down Syndrome Detection Rates

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age alone</td>
<td>20%</td>
</tr>
<tr>
<td>Age, AFP</td>
<td>42%</td>
</tr>
<tr>
<td>Age, AFP, hCG</td>
<td>67%</td>
</tr>
<tr>
<td>Age, AFP, hCG, uE3</td>
<td>72%</td>
</tr>
<tr>
<td>Age, AFP, hCG, uE3, Inh-A</td>
<td>80%</td>
</tr>
<tr>
<td>Penta Screen</td>
<td>83%</td>
</tr>
</tbody>
</table>

Point being however:

- That all these are Second Trimester Screening tests.
First Trimester Screening

First Trimester Markers

- Free-β hCG
  - Or hCG, or h-hCG
- Pregnancy Associated Plasma Protein – A (PAPP-A)
- Nuchal Translucency (NT)
- Nasal Bone (NB)

FTS Advantages

- Earlier Detection of Aneuploidies
- Detection Rates as high as second trimester Quad Screen (~84% for Down’s)
- False Positive Rate also equal to second trimester
FTS Disadvantages

- No NTD Detection
  - Patient needs to come back in ST for AFP
- Needs an earlier, perhaps more dangerous diagnostic test.
- NT (and maybe CVS) not available everywhere
  - A barrier to Standard of Care perhaps.

A Major Screening Study

- First-trimester or second-trimester screening, or both, for Down Syndrome. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium.

Malone F et al.
New England Jour of Med
2005;353:2001-11

Screening Options

- First Trimester only
- Second Trimester only
- First Trimester combined with Second
- First Trimester then “maybe” Second
- If First Trimester, then with/without NT
Choice, choices, choices

• First Trimester Screen (FTS)
  • hCG (or h-hCG)
  • PAPP-A
  • NT (can be done with or without)

• Detection Rates:
  • Down Syn. – 83% (w/NT) 74% (w/o NT)
  • Trisomy 18 – 75% (w/NT) 68% (w/o NT)

More choices...

• Second Trimester Screening (STS)
  • MSS Quad Test: AFP, hCG, uE3, Inhibin-A
  • MSS AFP Only (if an FTS was performed)

• Detection Rates
  • Down Syn. – 80%
  • Trisomy 18 – 73%
  • NTdQ (w/ AFP only)
  • Anencephaly – 95%
  • Open Spina Bifida – 55-80%

Sidebar...

• In 1983 we had 1 screening option
• In 2015, our requisition lists 14.
Now, let’s combine them...

- Integrated – Uses both the FTS and STS to adjust a woman’s age-related risk of having a child with DS.
- The results are reported only AFTER both screening tests are completed.
- The DR for Down syndrome using this is 95%
  - If you include NT
  - 87% if no NT
  - Trisomy 18 DR – 90%

Or…we could do them...

- Sequentially – Uses FTS and STS, but adds an ‘if-then’ statement:
  - IF the FTS is negative, i.e. DS risk is <1:50, then report without risks and do STS
  - IF the FTS is positive, i.e. DS risk >1:50, then suggest diagnostic test.
  - IF combined Second Trimester risk is >1:270, then suggest diagnostic test.
  - IF combined Second Trimester risk is <1:270, then she’s done.

Sequential Detection Rates

- Down Syndrome – 95% w/NT
  - (No data without NT)
- Trisomy 18 – 90% w/NT
  - 90% w/o NT
Other Risk Cut-offs

- Trisomy 18 –
  - No increased risk: < 1:100 in any test
  - Increased risk: > 1:100 in any test

- NTD Risk
  - AFP MoM < 2.5 MoM (or whatever the particular lab uses.)

ACOG Practice Bulletin

- Screening for Fetal Chromosomal Abnormalities

- January 2007

Points of the Bulletin

- "Ideally, all women should be offered aneuploidy screening before 20 weeks of gestation, regardless of maternal age."

- "The choice of screening test depends on many factors, including gestational age at time of first visit, availability of nuchal translucency measurement, risks of invasive diagnostics procedures, options for earlier termination..."
Other MSS Associations

Even with Normal Fetal Chromosomes and no NTD, it was found that an abnormal MSS could be associated with:

- Low Birth Weight
- Intrauterine Growth Retardation
- Pre-eclampsia and fetal loss
- Pre-term labor

MSS Findings

- High AFP (not NTD)
  - Low birth weight, IUGR, Preterm labor, Pre-eclampsia and fetal loss
- High hCG
  - Low birth weight, IUGR, Preterm labor, Pre-eclampsia and fetal loss
- Low uE3
  - Low birth weight, IUGR, fetal loss, SLO or STS in fetus
- High Inhibin A
  - Low birth weight, IUGR, Preterm labor, Pre-eclampsia and fetal loss, hypertension

The New Era

Non Invasive Prenatal Screening - NIPS
An Initial Paper

DNA Sequencing of maternal plasma to detect Down syndrome: An international clinical validation study.

Palomaki, GE, et al.
Genet Med.
2011: 13(11): 913-20

NIPS is intended for

• Patients in a High Risk Category:
  • Advanced maternal age
  • Abnormal MSS
  • Abnormal ultrasound
  • Contributing family history

But it’s getting used for...

In many cases, all patients.
A current publication

Is maternal plasma DNA testing impacting serum-based screening for aneuploidy in the United States?

Palomaki GE, et al.
Genetics in Med
February 2015

Points of the paper

- Sample numbers based on CAP Proficiency test data 2011 – 2014
- Matched lab data from 2011 to 2014
- Overall, second trimester testing decreased by 3%, first trimester increased by 126%.
- The majority of labs (59%) found an overall decline in numbers of samples tested.
- That decline had a 12% average in all testing methods.

Data supports a decline in screening AMA patients
More data on the decline of usage

One lab’s experience

Maternal Serum Screen Volume

So what have we learned?

- A screening test can change over time.
- They can come to mean something else than why they were originally designed.
- No matter how good, abnormal screening tests always need to be followed by a diagnostic test.
Remember:

They are ALL screening tests!