US when serum screen is abnormal

AND

New issues on the genetic horizon

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- No disclosures
Overview

1. Role of ultrasound with aneuploidy screen.
   • Serum screening
     • PAPP-A, hCG, uE3, APF, DIA
Overview

1. Role of ultrasound with aneuploidy screen.
   • Serum screening
     • PAPP-A, hCG, uE3, APF, DIA

2. Developments in prenatal screening
   • FISH (Fluorescent in-situ hybridization)
     • Molecular probes
   • QF-PCR (Quantitative fluorescent polymerase chain reaction)
   • aCGH (array Comparative Genomic Hybridization)
     • Microarray
   • NIPT (Non Invasive Prenatal Testing)
     • cffDNA in maternal plasma ....
History of screening and biomarkers

1960’s  Maternal age associated Down risk
        **HIGH** AFP = anencephaly [NTD] (Brock, *Lancet*)

1972  **LOW** AFP = T18 + Down (Merkatz, *AJOG*)

1984  Multiple biomarkers (PAPP-A, AFP, uE3, hCG, DIA...)

1990’s  Ultrasound  (Benacerraf, Nyberg, Nicholaides, ...)
        - Nuchal fold, EIF, pelviectasis, femur
        - NT (Nuchal Translucency)
        - Nasal bone, ductus venosus, tricuspid regurgitation, fronto-maxillary angle...

2000’s  NIPT  (Non-invasive prenatal testing) & more.
What do we screen for today?

- Standard of practice in Ontario is to offer screening for:
  1. Down syndrome
  2. Open neural tube defect (oNTD) [spina bifida, anencephaly]

- Will the screening tests detect other abnormalities?
  - Yes
    - Other aneuploidies (trisomy 18, trisomy 13…)
    - Many anomalies and syndromes
    - Pregnancies at risk for fetal and maternal well being.
What biomarkers are used today?

- **Fetal**
  - AFP (Alpha fetoprotein)
  - NT (Nuchal translucency)
  - Other US markers (Anomalies, NF, EIF, NB, femur, kidney, …)

- **Fetal + placental**
  - uE3 (unconjugated estriol)

- **Placental**
  - β-hCG (beta–human chorionic gonatotropin)
  - PAPP-A (Pregnancy Associated Plasma Protein – A)
  - hCG (Human chorionic gonadotropin)
  - DIA (Dimeric Inhibin A)

Fetus and placenta are a package. Biomarkers don’t only relate to the fetus!
Why these biomarkers?

- Best discrimination between: Normal - NTD - Down

- Are there other screening biomarkers?
  - Of course (>50 reported)

- Can the markers show anything else?
  - Absolutely, each biomarker has its own pathophysiology
  - Other aneuploidies and genetic conditions may show changes
  - May also identify women at risk for poor outcomes
    - PIH, IUGR, fetal demise, abruption, prematurity, accreta
  - Biomarker levels can guide ultrasound evaluation and clinical management.
Prenatal screening tests

INTEGRATED (IPS)

FTS

MSS
MMS
QUAD

18-20 week scan

free βhCG, PAPP-A, NT

AFP
(hCG, uE3, DIA)

10 12 14 16 18
Dilemma for me occurred when...

- Patient POSITIVE for both spina bifida and Down?
  - POSITIVE for spina bifida  
    - *ie.* High AFP
  - POSITIVE for Down  
    - *ie.* Low AFP
Digress to understand tests

- How are biomarkers reported?
- What is the initial risk?
- How is risk modified?
- Why is knowing gestation age important?
Screen positive spina bifida

Screen positive Down
What’s a MoM?
Why do we use MoMs and not means?

How to describe the average of a series of numbers?

1 2 3 4 10 30 1000

- "mean" is the arithmetic average
  \[(1+2+3+4+10+30+1000) / 7 = 150\]

- "median" is the middle number
  - useful to describe very **skewed** series.

- MoM = **M**ultiples of the **M**edian
  4 = 1 MoM, 8 = 2 MoM,….

- With MoM’s, the "normal" result is always 1 MoM
How is Down risk calculated? Start with initial Down risk for age

Initial risk = maternal age related risk of Down

<table>
<thead>
<tr>
<th>Age</th>
<th>Down Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1/1480</td>
</tr>
<tr>
<td>25</td>
<td>1/1350</td>
</tr>
<tr>
<td>30</td>
<td>1/940</td>
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<tr>
<td>35</td>
<td>1/353</td>
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<tr>
<td>40</td>
<td>1/85</td>
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<tr>
<td>45</td>
<td>1/30</td>
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</table>
How do we modify initial risk?

- Initial risk \times \text{new info (LR)} = \text{New adjusted risk}

\text{Likelihood Ratio} = \frac{\text{Likelihood of test result from AFFECTED}}{\text{Likelihood of test result from NORMAL}}
Chance of result coming from abnormal is 2 (66%)
Chance of result coming from normal is 1 (33%)

\[ LR = \frac{2}{1} = 2 \]

New adjusted risk = initial risk x 2
\[ e.g. \text{If initial risk is 1:500, new risk is } 2 \times \frac{1}{500} = 1:250 \]
Multiple LR’s can be combined

- Risk LR’s can be multiplied to give new risk.

New risk = initial x LR$_1$ x LR$_2$ x LR$_3$ x...x LR$_n$ x LR modifiers*

e.g.

Down = age risk x LR$_{NT}$ x LR$_{PAPP-A}$ x LR$_{\beta-hCG}$ x LR modifiers*

*LR modifiers: smoking, weight, diabetes, history, ethnicity, fetal number..
Why is it important to know fetal age? Biomarker levels change with age!!

Change in biomarker median with gestational age

- B-hGC
- PAPP-A
- AFP
- uE3
- hGC
- DIA
Risk calculation volatility with dating error 16 – 21 wk.
(Set AFP, hCG, uE3 at 1 MoM(normal) for 17 weeks; vary gestational age)

Effect of Gestational Age Error on Calculated Risks

Important to confirm dates with ultrasound.
Back to my dilemma

- **NTD** risk is calculated from:
  - AFP only

- **Down** risk is calculated from:
  - AFP and uE3 + hGG (and NT, PAPP-A, β-hCG, DIA)

- As result
  - NTD screen is positive if AFP is high
  - Down screen positive depends on AFP + other biomarkers
Roles for ultrasound in all cases of biomarker abnormality

Confirm the obvious:
- Viability
- Dates
- Fetal number
- Anatomy

If other testing excludes NTD\(^1\) and Down\(^2\):
- Review screening results to direct and focus scan
- ‘unexplained’ biomarker results should be followed and have specialty counseling and management.

\(^1\)NTD test = ultrasound; \(^2\)Down test = CVS/amnio
In screen positive patients, another use for ultrasound

- Some patients do not want to avoid invasive testing (CVS/amniocentesis)

- Request US for further evidence before invasive tests

- Does risk decrease if no US markers are found?
  - Maybe?
  - LR of ‘no markers’ is 0.4 - 0.5 (i.e. Risk appears halved)

Smith-Bindman 2001 JAMA; Agathokleous...Nicolaides 2013 UOG
Some first trimester US findings which have been proposed as aneuploidy markers.

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**NT > 3.5 mm**

- Nasal bone
- 2 vessel cord
- Megacystis
- Heart rate
- Short maxilla
- Maxillary frontal angle
- Ductus venosus flow
- Others....

**Anomalies**

- Omphalocele
- Congenital heart disease
- Holoprosencephaly
- Partial mole
- Hygroma

Free on-line book:
Nuchal Translucency, Nasal Bone,

Nasal bone absent
- Euploid  1-3%
- Down    60%
- Trisomy 18  50%
- Trisomy 13  40%

*Borenstein. UOG 2008
Increased NT at 11-14 wks

- Cardiac defects / failure
- Intrathoracic compression
- Abnormal lymphatic system
- Neuro-muscular abnormalities
- Altered composition of dermis
First trimester markers

Echogenic intracardiac focus (EIF)\(^1\)
- Down LR x 2 - 6

Megacystis\(^2\)
- 7-15 mm – 25% aneuploidy
- >15 mm - 11% aneuploidy
  - obstructive uropathy.

Delayed amnion-chorion fusion
- Normal fusion by 14 wk.
- If delayed, risk of:
  - aneuploidy\(^3\)
  - adverse outcomes\(^4\)

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1 Dagklis 2008 *UOG*; 2 Liao 2003 *UOG* 3 Ulm 1999 *UOG*; 4 Bromley 1999, Ob Gyn
### Second trimester soft markers

**Aneuploidy (LR)^2**

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<thead>
<tr>
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<th>T18</th>
<th>Congenital/Anomaly Association³</th>
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<td>Nuchal fold (III, A)</td>
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<td>Echogenic bowel (II-2, A)</td>
<td>6</td>
<td>—</td>
<td>CF2%, infection 3%, GI 6%</td>
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<td>—</td>
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<td>—</td>
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<tr>
<td>Choroid plexus cyst (II-2, A)</td>
<td></td>
<td>7</td>
<td>—</td>
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<tr>
<td>Single umbilical artery (III, A)</td>
<td>—</td>
<td>—</td>
<td>Renal, cardiac</td>
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<tr>
<td>Enlarged cisterna magna (III, A)</td>
<td>—</td>
<td>—</td>
<td>OFD, MG, DiG</td>
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<tr>
<td>Renal pyelectasis (II-2, A)</td>
<td>—</td>
<td>—</td>
<td>Hydronephrosis; reflux</td>
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<td><strong>B. Comprehensive scan (calculation; detail)</strong></td>
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<tr>
<td>Clinodactyly (II-2, A)</td>
<td>5.6</td>
<td>—</td>
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<tr>
<td>Humerus (short) (II-2, A)</td>
<td>7.5</td>
<td>—</td>
<td>skeletal dysplasia; IUGR</td>
</tr>
<tr>
<td>Femur (short) (II-2, A)</td>
<td>2.7</td>
<td>—</td>
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<tr>
<td>Double泡 (I)</td>
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<tr>
<td>Coarctation (I, A)</td>
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<td></td>
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<tr>
<td>Polyhydramnios (III, A)</td>
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<tr>
<td>Polyglycemic twins</td>
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<tr>
<td>Twin-to-twin transfusion</td>
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In screen positive patients, what if diagnostic tests are normal?

- Diagnostic tests are:
  - Down – CVS or amniocentesis.
  - Spina bifida – detailed ultrasound (af-AFP, af-AChE<sup>1</sup>).

- If these are normal, look at the serum testing results to determine what to do next

- The biomarkers have their own pathophysiology that can be evaluated.

*AChE = acetyl cholinesterase
MS-AFP (Maternal Serum – AFP)

- Fetal origin (fetal liver)
  - Normally some leaks from fetus to mother
    - Through fetal skin to amniotic fluid
    - Through placenta

- Excess AFP implies excessive leak

- Maternal origin (rare)
  - tumors (liver, germ cell)
Elevated AFP – not just spina bifida. Rather ‘leaky’ fetus and/or ‘sick’ placenta

‘Leaky’ fetus
- Open NTD
- Gastroschisis/Omphalocele
- Hydrops, edema
- Oligo-, polyhydramios
- Teratomas
- Hemangioma
- Tracheo Esophageal Fistula
- Congenital nephrosis
- Amniotic band
- Many others....

‘Sick’ placenta
- Poor placental development
- Feto-maternal bleed
- Growth restriction (IUGR)
- Hypertension
- Premature labor
- Placenta accreta
- Placental tumors – chorio.

Detailed complete anatomical scan!

Look at serum screen report for placental markers!!!
PAPP-A↓, hCG ↑, DIA ↑, AFP ↑
Placental markers

- At what biomarker level should increased pregnancy surveillance be considered?

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<th>Marker</th>
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<td>PAPP-A</td>
<td>&lt; 0.35</td>
</tr>
<tr>
<td>DIA</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>hCG</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>AFP</td>
<td>&gt; 2</td>
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http://www.mountsinai.on.ca/care/placenta-clinic
Placental function testing (> 22 wk)

**Umbilical Artery Doppler**

**Biochemistry**
- PAPP-A, AFP, hCG, Inhibin (DIA)

**Morphology**
- The Appearance of the Placenta
  - Long, thin, and without areas of damage

**Uterine Artery Doppler**
- Mother’s Blood Flow to the Placenta
  - High blood flow to nourish the baby

http://www.mountsinai.on.ca/care/placenta-clinic
Placental morphology with insufficiency

- Short, thick placenta
- Uterine Artery notch
- Placental infarcts
- Absent EDF

MSH Placenta Clinic: www.mtsinai.on.ca/care/placenta-clinic; Alkazaleh 2004 UOG; Whittle 2006 Clin Genetics
Summary of screening

- Not just NTD and Down syndrome
- Use screening report to guide next steps

The ‘new’ genetics
Old genetics tools

Until recently one main test:
Amniocentesis/CVS and karyotype
This remains the **STANDARD**
Some new tools in the geneticist’s toolbox

FISH (Fluorescent in-situ hybridization)
QF-PCR (Quantitative fluorescent polymerase chain reaction)
aCGH (array Comparative Genomic hybridization - microarray)
NIPT (Non-invasive prenatal testing - cffDNA)
Cell-free fetal DNA in maternal plasma

- Detectable from 4 weeks
- Around 4% - 10% of total circulating cell free DNA
- Proportion increases with gestation
- Originates from trophoblast (placenta)
- Cleared from circulation within 30 minutes of delivery

Fetal DNA fragments in maternal plasma can be analyzed for genetic abnormality

Courtesy: Dr. Lyn Chitty
cffDNA testing in UK

Maternal venipuncture. No risk to fetus.

ffDNA testing now accounts for a large proportion of all molecular prenatal diagnostic tests in the UK (excl aneuploidy screening)

Courtesy: Dr. Lyn Chitty
Cost of T21 sequencing-based tests

NIPD - Down screening by venipuncture.
99+ % accuracy.

Costs offset by decreased invasive test numbers and costs!?
Take home messages

• NTD and Down screening is standard of practice
• Screening can also identify:
  • many other fetal problems
  • placental and maternal problems
• Useful to have screening report to guide US
• US examination should be comprehensive
  • Fetus
  • Placenta
• Genetic testing evolving rapidly.
Thank you!

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