Antenatal screening

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RPA Women and Babies
Perspective / conflicts of interest

Chair of RANZCOG NTUEMP
Contributor to RANZCOG / HGSA C-Obs 59

Trustee of Fetal Medicine Foundation
Trustee of ISUOG (Education chair)

Member of Subcommittee on prediction and prevention of NCDs in pregnancy at FIGO

Research / Education partnerships:
GE | Toshiba
Perkin Elmer | Roche | Natera
C-Obs 59
Prenatal screening and diagnostic testing for fetal chromosomal and genetic conditions.
August 2018

• Change of title
• Highlights recommendations for acceptable practice
• Identifies T1 / T2 screening tools more clearly
• Introduces elective diagnostic testing
• Promotes population based carrier screening
Current baseline strategy (cFTS)

- Maternal age (yrs)
- Serum free β-hCG (MoM)
- Serum PAPP-A (MoM)
- Crown-rump length (mm)
- NT (mm)

Detection rate [at 5% FPR]

+ Biochem + NT
cFTS at a population level

Danish study
Population based application
Public health system
>80% uptake
50% reduction in invasive testing
N = 95,645 including 225 T21

Detection rate: 89.3%
False positive rate: 3.7%
Positive predictive value: 5.6%
Genomic innovation: isolation of cell free fetal DNA

DNA extraction from plasma

Multiplex PCR to ‘enrich’ SNPs of interest
NIPT [cffDNA] for *common* trisomies

<table>
<thead>
<tr>
<th>Trisomy</th>
<th>Detection Rate</th>
<th>FPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>99.7% (CI: 99.1 – 99.9)</td>
<td>0.04%</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>97.9% (CI: 94.9 – 99.1)</td>
<td>0.04%</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>99.0% (CI: 65.8 – 99.9)</td>
<td>0.04%</td>
</tr>
<tr>
<td>45X</td>
<td>95.8% (CI: 70.3 – 99.5)</td>
<td>0.14%</td>
</tr>
</tbody>
</table>

Gil *et al*. Meta-analysis UOG 2017
Comparison of cFTS and NIPT

Standard screening AUC, 0.958
cfDNA testing AUC, 0.999

Sensitivity
False Positive Rate

- Standard screening
- cfDNA testing
Screening for T21: cffDNA – ‘no results’ discarded

Norton et al. NEJM 2015

Detection rate: 100%
False positive rate: <0.1%
Positive predictive value: 80.9%
NIPT – genotyping rather than phenotyping?

- placental mosaicism
- 'vanishing' twin
- maternal mosaicism
- maternal malignancy
- no cell free fetal DNA
- low cell free fetal DNA
- 'coding' complexity
Screening for T21: cffDNA – including the ‘no results’

- 192 (1.2%) low ff
- 83 (0.5%) no ff
- 213 (1.3%) variance
- 488 (3.0%) not reported

1 in 38 (2.7%) aneuploid including 3 T21

### Detection rate
- 100%
- 92.7%

### False positive rate
- <0.1%
- 3.1%

### Positive predictive value
- 80.9%
- 7.1%

Norton et al. NEJM 2015
cFTS at a population level

Danish study
Population based application
Public health system
>80% uptake
50% reduction in invasive testing
N = 95,645 including 225 T21

Detection rate: 89.3%
False positive rate: 3.7%
Positive predictive value: 5.6%
The range of aneuploidy in the era of aCGH for prenatal diagnosis

- 15% pathogenic CNVs
- 30% T21
- T18
- T13
- OthT
- 45X
- OthSex
- BalT
- UNbalT
- Trip
- Marker
- PathCNV
- PathVous
- NonPathVous

Wapner et al. NEJM 2012
Sex chromosome aneuploidy: Verinata data

Samples tested (n=18,161)

- No sex chromosome aneuploidy detected (n=17,957)
  - Sex chromosomes by noninvasive prenatal testing consistent with XX (n=8,721)
    - Concordant (karyotype): 52
    - Discordant (karyotype): 8
      - (4 full or mosaic MX)
    - Discordant (ultrasound): 10
    - No follow-up available: 8,651
  - Sex chromosomes by noninvasive prenatal testing consistent with XY (n=9,236)
    - Concordant (karyotype): 49
    - Discordant (karyotype): 35
      - No follow-up available: 18

- Sex chromosome aneuploidy detected (n=204)
  - MX (n=148)
    - Concordant (karyotype): 9
    - Discordant (karyotype): 45
      - No follow-up available: 18
  - XXX (n=38)
    - Concordant (karyotype): 1
    - Discordant (karyotype): 12
      - No follow-up available: 25
  - XXY (n=12)
    - Concordant (karyotype): 2
    - Discordant (karyotype): 0
      - No follow-up available: 10
  - XYY (n=6)
    - Concordant (karyotype): 1
    - Discordant (karyotype): 1
      - XXY
      - No follow-up available: 4

Approx 1%
PPV 20%!

Bianchi et al. Obstet Gynecol 2015
Validation of a SNP-based process for detection of del22q11

- 1,351 SNPs
- Spanning 2.91Mb of 22q11.2
- Sequencing depth $3.2 \times 10^6$
- High-risk cases reflexed to sequencing depth of $6 \times 10^6$

**Table 3. Sensitivity and Specificity for the 22q11.2 Deletion**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90.0 (55.5–99.7)</td>
<td>99.74 (98.58–99.99)</td>
</tr>
</tbody>
</table>

Leonard et al. Natera
Data presented as abstract, Copenhagen 2017
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Prevalence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36</td>
<td>1 in 5,000</td>
<td>Severe intellectual disability: non-verbal, temper tantrums, self-harming, other behavior problems. Vision and/or hearing problems. Structural abnormalities of the brain, microcephaly, seizures, hypotonia, swallowing difficulties. Abnormalities of the skeleton, heart, gastrointestinal system, kidneys, or genitals. Deep-set eyes with straight eyebrows, mediolateral hypertelorism, a broad, flat nose, wide philtrum, a pointed chin low-set backward rotated abnormally shaped ears.</td>
</tr>
<tr>
<td>del4p</td>
<td>1 in 50,000</td>
<td>Wolf-Hirschhorn syndrome results in a characteristic facial appearance, delayed growth and development, intellectual disability, and seizures. Children have a broad, flat nasal bridge and a high forehead described as a “Greek warrior helmet”, widely spaced protruding eyes, a short philtrum, a downturned mouth, micrognathia, and poorly formed ears with small pits. Infants are typically growth restricted, microcephaly have failure to thrive and are hypotonic. They have mild to severe intellectual disability and are developmentally delayed. Socialisation skills are strong, while verbal communication and language skills tend to be weaker. Seizures may be resistant to treatment but tend to disappear with age. Associated structural anomalies include scoliosis and hydrocephalus, cleft lip/palate and abnormalities of the eyes, heart, genitourinary tract, and brain.</td>
</tr>
<tr>
<td>del5p</td>
<td>1 in 20,000</td>
<td>Cri-du-chat. Infants often have a high-pitched cry that sounds like that of a cat. Characterised by intellectual disability and delayed development, microcephaly, low birth weight, and hypotonia in infancy. Distinctive facial features include hypertelorism, low-set ears, and a small jaw. Some infants have a heart defect.</td>
</tr>
<tr>
<td>del8q</td>
<td>vary (v. rare)</td>
<td>Del8q is extremely rare and may result in a limited range of joint movement and pressure on nerves, blood vessels, the spinal cord, and tissue surrounding the exostoses. Affected individuals also have short stature and cone-shaped epiphyses of long bones. The characteristic appearance includes sparse scalp hair, a rounded nose, and a thin upper lip. Some people with this condition have loose skin in childhood, which typically resolves with age. Affected individuals may have some intellectual disability.</td>
</tr>
<tr>
<td>del11q</td>
<td>1 in 100,000</td>
<td>Langer-Giedion syndrome cause bone abnormalities (exostoses) and distinctive facial features. Additionally, affected infants have an increased likelihood of a platelet disorder (Paris-Trousseau syndrome), heart defects, feeding difficulties in infancy, short stature, frequent ear and sinus infections, and skeletal abnormalities. The disorder can also affect the digestive system, kidneys, and genitalia.</td>
</tr>
<tr>
<td>del15q (mat)</td>
<td>1 in 12,000</td>
<td>Jacobsen syndrome is very variable. Most affected individuals have delayed development, including the development of speech and motor skills, cognitive impairment and learning difficulties. Behavioural problems have been reported, including compulsive behavior, a short attention span and easy distractibility. Also associated with an increased likelihood of autism spectrum disorders. Characterized by distinctive facial features including small and low-set ears, hypertelorism, ptosis, epicanthal folds, a broad nasal bridge, downturned corners of the mouth, a thin upper lip, and a small lower jaw. Affected individuals are often microcephaly and have trigonocephaly. Pelvic disorder (Paris-Trousseau syndrome), heart defects, feeding difficulties in infancy, short stature, frequent ear and sinus infections, and skeletal abnormalities. The disorder can also affect the digestive system, kidneys, and genitalia.</td>
</tr>
<tr>
<td>Del15q (pat)</td>
<td>1 in 10,000</td>
<td>Angelman syndrome. Developmental delay, intellectual disability, severe speech impairment, and problems with movement and balance (ataxia). Resistant seizures, microcephaly.</td>
</tr>
<tr>
<td>Trisomy 16</td>
<td>vary (v. rare)</td>
<td>Prader-Willi: A complex genetic condition that affects many parts of the body. In infancy characterized by hypotonia, feeding difficulties, poor growth and delayed development. In childhood, affected individuals develop an insatiable appetite, which leads to chronic overeating ( hyperphagia) and obesity. MRI to moderate intellectual impairment and learning difficulties. Behavioural problems, sleep abnormalities. Distinctive facial features include a narrow forehead, almond-shaped eyes, and a triangular mouth. Short stature and small hands and feet. Puberty is delayed or incomplete, and most affected individuals are unable to have children (infertile).</td>
</tr>
<tr>
<td>Del22q11</td>
<td>1 in 4,000</td>
<td>22q11.2 deletion syndrome features vary widely, even among affected members of the same family. Structural abnormalities include cardiac anomalies, a cleft palate and distinctive facial features. Many children with 22q11.2 deletion syndrome have developmental delays, including delayed growth and speech development, and learning difficulties. Later in life, they are at an increased risk of developing mental illnesses such as schizophrenia, depression, anxiety, and bipolar disorder. Additionally, affected children are more likely than children without 22q11.2 deletion syndrome to have attention deficit hyperactivity disorder (ADHD) and developmental conditions such as autism spectrum disorders that affect communication and social interaction.</td>
</tr>
</tbody>
</table>

**Pre-test counselling**
Using cfDNA as a diagnostic test

Normal process of risk assessment
e.g. Combined first trimester screening (cFTS)

Low risk women
cFTS Risk <1 in 300

High risk women
cFTS Risk ≥1 in 300

choice of

‘negative’

No further testing

‘positive’

Invasive test

NIPT

Advantage: only 5% of women with a high risk cFTS result need have NIPT
Disadvantage: ‘atypical’ chromosomal abnormalities detected by CVS will be missed by NIPT not taking advantage of sensitivity of NIPT
All women offered cFTS as primary test
Risks interpreted in three rather than two groups

Low risk women
- cFTS Risk <1 in 1000
  (estimate 86.5% of women)
- NIPT ‘negative’
  (estimate 98%)
  → No further testing
  (a total of 99.2% of women)

Intermediate risk women
- cFTS Risk <1 in 50 to ≥1 in 1000
  (estimate 13% of women)
- NIPT ‘negative’
  (estimate 98%)

High risk women
- cFTS Risk ≥1 in 50
  (estimate 1.0% of women)
- NIPT ‘positive’
  (estimate 2%)
  → Invasive test
  (a total of 0.8% of women)

Advantage:
Only 15-20% of women with a high risk cFTS result need have NIPT
‘atypical’ chromosomal abnormalities will still be detected in those at highest risk.
All women offered NIPT and first trimester ultrasound: NIPT sample taken at 10 weeks; US scan performed and NIPT results reviewed at 12 weeks.

**Review results of NIPT:**

- **NIPT ‘negative’**
  (96.5%)

- **NIPT ‘positive’**
  (0.5%)

- **NIPT ‘no result’**
  (3.0%)

**Perform US scan**

- **US scan normal and NIPT ‘negative’**
  - No further testing

- **Structural abnormality on US and /or NIPT ‘positive’**
  - Invasive test

- **NIPT ‘no result’**
  - NT + Biochemistry → cFTS risk
    - High risk (≥1 in 300)
    - Low risk (<1 in 300)
  - No further testing

**Advantage:**
Fetuses with structural anomalies still assessed for atypical chromosomal abnormalities

**Disadvantage:**
Expensive
<table>
<thead>
<tr>
<th>Fetus A</th>
<th>GA (EDD)</th>
<th>U/S GA</th>
<th>U/S EDD</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12w2d</td>
<td>13w1d</td>
<td>10/03/2019</td>
<td>1.74mm</td>
</tr>
<tr>
<td></td>
<td>EDD</td>
<td>U/S EDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16/03/2019</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the graphs below you can see the distribution of your measurements plotted against gestational age and maternal weight.

* 1 NT value greater than 4mm shown as 4mm
Additional ultrasound markers: risk of major cardiac defect

\[
e^{-6.924+0.833\delta NT + 2.039DV(\text{rev}) + 2.841TR} \\
(1 + e^{-6.924+0.833\delta NT + 2.039DV(\text{rev}) + 2.841TR})
\]
Outcomes by Chorionicity: from the 11-13 +6 week scan

Prevalence 2% (467 / 24,959)

<table>
<thead>
<tr>
<th>Condition</th>
<th>DC</th>
<th>MC</th>
<th>MC Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MISCARRIAGE</td>
<td></td>
<td></td>
<td>x6</td>
</tr>
<tr>
<td>12-24 wks</td>
<td>1.8%</td>
<td>12.2%</td>
<td></td>
</tr>
<tr>
<td>PERINATAL DEATH</td>
<td></td>
<td></td>
<td>x2</td>
</tr>
<tr>
<td>&gt;24 wks</td>
<td>1.6%</td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td></td>
<td></td>
<td>x2</td>
</tr>
<tr>
<td>Total fetuses</td>
<td>12%</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>PRETERM DELIVERY</td>
<td></td>
<td></td>
<td>x2</td>
</tr>
<tr>
<td>Gestation &lt;32 wks</td>
<td>5.5%</td>
<td>9.2%</td>
<td></td>
</tr>
</tbody>
</table>

Sebire et al. UOG 1996
New biochemical markers: PLGF

- Afro-Caribbean ≤10 w, 11 w, 12 w, ≥13 w
- South Asian
- East Asian
- Age 20 y, 30 y, 40 y
- Weight 50 kg, 70 kg, 90 kg
- Nulliparity
- Conception by IVF
- Diabetes mellitus
- Smoking ≤10 w, 11 w, 12 w, ≥13 w

Serum placental growth factor (MoM) vs. Gestation (wks)

<table>
<thead>
<tr>
<th>Marker</th>
<th>DR</th>
<th>FPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free β-hCG, PAPP-A</td>
<td>62%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Free β-hCG, PAPP-A, PLGF</td>
<td>69%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Biochemistry, fetal NT</td>
<td>89%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Biochemistry, fetal NT, PLGF</td>
<td>92%</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

Dr. FPR
The median MoM of your measurements is 0.959

Your measurements are within 0.1 standard deviation from the expected. This indicates that your measurements are satisfactory.

Please continue to audit your results at regular intervals.

In the graphs below you can see the distribution of your measurements plotted against gestational age and maternal weight.
Screening for pre-eclampsia

3rd ranked direct maternal death
15% of maternal deaths worldwide
60,000 maternal deaths / year

15% of NNU admission <32 weeks
500,000 infant deaths / year

AIHW 2016; ANZNN Report 2016
Do we have a reasonable understanding of the aetiology of disease?

Stage 1
- Placental dysfunction
  - Placental ischaemia
  - Oxidative stress

Stage 2
- Vascular compromise
  - Endothelial activation
- End organ damage
  - Systemic vascular dysfunction

- Hypertension
- Proteinuria
- Liver dysfunction
- Coagulopathy
- Cerebral oedema

First trimester screening for PET: a multiple logistic regression model

Poon et al. 2009
Prevention of preeclampsia

Prevention rate (%)

- <32w: 11%
- <34w: 18%
- <37w: 38%
- ≥37w: 95%

Incremental Cost Effectiveness Ratio

<table>
<thead>
<tr>
<th></th>
<th>Total Cost</th>
<th>Cases PET avoided</th>
<th>ICER / PET avoided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine care</td>
<td>65,563,471</td>
<td>137.929</td>
<td></td>
</tr>
<tr>
<td>ASPRE care</td>
<td>64,790,309</td>
<td>110.956</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>-773,162</td>
<td>26.973</td>
<td>-28,665</td>
</tr>
</tbody>
</table>
Use of sFlt: PIGF ratio in T3 (PET)

NPV  99.3%
Sensitivity  80.0%
Specificity  78.3%

PROGNOSIS Study: Zeisler et al. NEJM 2016
Use of sFlt: PIGF ratio in T3 (PET)

Cost saving:
Euro 670 / GBP344 per patient
Euro 30 Million / yr population

MacDonald et al. 2018
In the graph below you can see the distribution of your measurements plotted against gestational age, along with the expected normal range.

On the top right hand corner of the graph is the evaluation of your measurements in terms of bias, spread and trend:

- If the indicator is green, your measurements are satisfactory
- If the indicator is orange, your measurements are within acceptable limits, but they need to improve
- If the indicator is red, your measurements are outside acceptable limits

The blue horizontal line on the graph indicates the normal median and the red lines indicate the 5th and 95th centiles. The numbers and percentages on the right of each line indicate the distribution of your measurements above the median and 95th centile and below the median and 5th centile of the normal range.