Combined screening at 11-13 wks
(age, fetal NT, serum ß-hCG & PAPP-A)

- High risk >1:10
  - Intermediate risk
    - cfDNA test
      - +ve
      - -ve
  - Low risk <1:1000
    - Nothing else
  - Invasive test

1 in 1000 98%
1 in 500 95%
1 in 100 85%
Committee Opinion

In cases of failed result women should be offered diagnostic testing because of an increased risk of aneuploidy. 2015

Singleton pregnancies (n=10,619)

- Normal: n=10,393
- T21: n=160
- T18: n=50
- T13: n=16

<table>
<thead>
<tr>
<th>Condition</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>YES</td>
</tr>
<tr>
<td>Trisomies 18/13</td>
<td>Yes</td>
</tr>
<tr>
<td>Sex chromosomes</td>
<td>No</td>
</tr>
<tr>
<td>Microdeletions</td>
<td>Not yet</td>
</tr>
</tbody>
</table>

Thank you
The message

We spent 25 years in establishing a first trimester screening
Don’t give it up for NIPT,
but focus on early anatomy assessment
and predicting pregnancy complications!

R. Chaoui
Give the patient the complete picture

NIPT

100% T21
100% T18/T13
Boy/Girl

Anencephaly
Ectopia cordis
Body stalk
„Hydrops“
Large Omphalocele
Large Gastrochisis
Megacystis
Alobar HPE
Molar Placenta

....
**IMPACT UPON PRACTICE**

- Large cytogenetic labs reporting volume drops of over 30%
- Many primary ob’s are no longer sending to MFM/genetics centers for counseling and testing.
- Relying on NIPS
- First MFM encounter is often for 20 week US

**PCOGS Papers**

Utilization of noninvasive prenatal testing: impact on referrals for diagnostic testing

John Williams III, MD; Steven Rad, MD; Sarah Beitzl; RDM; Dilar Ratnasi, MS; Vahshini Subramaniam, MGG; Sayeh Farivar, MS; Margaretta D. Pisarska, MD

- Referrals for genetic counseling declined 28%
- Referrals for patient who are NOT NIPT candidates declined 20%
- As a result, patients are getting worse care than before
SIMULTANEOUS TRENDS IN
PREGNATAL DIAGNOSIS & SCREENING

• Increasing expertise in ultrasound
  and visualization
• Increasing safety of diagnostic
  procedures
• Increasing numbers of genetic
  screening test possibilities
• Shift from cytogenetic techniques
to molecular ones
• Under appreciation of residual
  risks
Figure 4 Forest plot showing estimated procedure-related risk of miscarriage before 24 weeks’ gestation with 95% CIs derived from each of the controlled studies and weighted pooled summary estimate using a random-effects model and incidence-rate difference meta-analysis in women who underwent chorionic villus sampling.
THE REAL PLAYING FIELD

{not “alternative facts”}

CELL FREE FETAL DNA IS A SCREENING TEST.

FETAL CELLS IN MATERNAL BLOOD OR CERVICAL SWABS MIGHT BECOME A DIAGNOSTIC TEST.

CVS AND AMNIOCENTESIS SPECIMENS PROVIDE A DIAGNOSTIC TEST.
Regardless of the screening risk score

I will not quote a patient < 1/500 risk of something on karyotype

With microarray: now 1/100 or even higher

Everyone is “38+”
PARTICULARLY FOR YOUNGER WOMEN,
CONCENTRATING ON DS IGNORES THE 500lb GORILLA FOR THE
MOSQUITO IN THE CORNER

aCGH 10 x more likely than cffDNA to find abnormality of comparable severity
Accurate Description of DNA-Based Noninvasive Prenatal Screening

Table 1. True and False Positive Cases with Nonmosaic Karyotypes.

<table>
<thead>
<tr>
<th>Chromosomal Abnormality</th>
<th>True Positive Result (N = 238)</th>
<th>False Positive Result (N = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no./total no. (%)</td>
<td>no./total no. (%)</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>14/26 (54)</td>
<td>12/26 (46)</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>40/52 (77)</td>
<td>12/52 (23)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>161/177 (91)</td>
<td>16/177 (9)</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>8/21 (38)</td>
<td>13/21 (62)</td>
</tr>
<tr>
<td>XXX or XXY</td>
<td>15/17 (88)</td>
<td>2/17 (12)</td>
</tr>
<tr>
<td>XYY</td>
<td>0/1</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>n=1,051</td>
<td>DR</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.2%</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>n=389</td>
<td>96.3%</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>n=139</td>
<td>91.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trisomy 21</th>
<th>n=1,963</th>
<th>DR</th>
<th>FPR</th>
<th>LR +ve</th>
<th>LR -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>99.7%</td>
<td>0.04%</td>
<td>2493</td>
<td>333</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>n=563</td>
<td>97.9%</td>
<td>0.04%</td>
<td>2448</td>
<td>48</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>n=119</td>
<td>99.0%</td>
<td>0.04%</td>
<td>2475</td>
<td>100</td>
</tr>
</tbody>
</table>

Screening for trisomies
Cell free DNA in maternal blood

Thank you
Non-Invasive Prenatal Screening Using Cell-Free DNA in Maternal Plasma: Risk For a Fetal Chromosome Abnormality When There is a No-Call Due to Low Fetal Fraction

Trudy McKanna MS, Allison Ryan PhD
Natera, Inc.

Peter Benn, DSc*
University of Connecticut Health Center, USA

Background

• Low fetal fraction (FF) is thought to be associated with an increased risk for trisomy 18, trisomy 13, and digynic triploidy due to a small placenta.
• In 2015, Ryan, et al,¹ described a “Fetal Fraction Based Risk (FFBR)” algorithm that would potentially identify the “no-call” cases at highest risk for one of these cytogenetic abnormalities.
• The algorithm combines prior risk, gestational age, and expected FF for these abnormalities to estimate risk. High risk defined as >1%.

Objectives

• Retrospectively review the outcomes in a cohort of 1,350 pregnancies who received a first sample “No-call” due to a low fetal fraction (or low confidence data) from Natera, Inc.
• Review reason for referral, cytogenetic findings, and pregnancy outcome.
• Evaluate the FFBR algorithm as an additional NIPS screening tool.

¹Ryan et al., 2015 14th World Congress in Fetal Medicine, C
<table>
<thead>
<tr>
<th>Condition</th>
<th>Observed, n (%)</th>
<th>Expected, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T13</td>
<td>3 (0.26)</td>
<td>1.15 (0.01)</td>
<td>0.069</td>
</tr>
<tr>
<td>T18</td>
<td>11 (0.96)</td>
<td>3.62 (0.32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T21</td>
<td>8 (0.70)</td>
<td>8.63 (0.75)</td>
<td>0.436</td>
</tr>
<tr>
<td>MX</td>
<td>2 (0.17)</td>
<td>3.72 (0.32)</td>
<td>0.197</td>
</tr>
<tr>
<td>Triploidy</td>
<td>21 (1.8)</td>
<td>0.21 (0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other chromosome abnormalities</td>
<td>3 (0.26)a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>All chromosome abnormalities</td>
<td>48 (4.2)b</td>
<td>17.34 (1.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spontaneous pregnancy loss</td>
<td>120 (10.5)c</td>
<td>80.8 (7.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any chromosome abnormalities and/or loss</td>
<td>147 (12.8)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a. One abnormal (unspecified), one dup(22q11.2), one XXX
Conclusions

• This study confirms that no-calls due to low FF do contain an excess of trisomy 18, trisomy 13, and triploid pregnancies.

• These pregnancies are at high risk for pregnancy loss (even without a proven cytogenetic abnormality).

• The FFBR algorithm can identify high-risk and low-risk sub-groups among “No-calls” due to low FF.

• Women in the high FFBR sub-group should be referred for immediate ultrasound and counseling.

• FFBR low risk women can be counseled that their NIPT no-call does not materially increase their risk.
H. Cuckle
Failure $\Rightarrow$ Detailed ultrasound

Failure $\Rightarrow$ Reflex serum markers

FF covariables
- Gestation
- Maternal weight
- Serum markers
- ART
- South Asian
- Heparin
False positive rate of screenings

- Compared with traditional serum-ultrasound screening (TSS), cfDNA tests have a much lower FPR for T21,18,13.
- The higher FPR of TSS was often considered a limitation.
- Distinct advantage with TSS due to NT’s ability to pick up additional chromosomal abnormalities (‘off-target’) in addition to the higher reflex invasive testing rate.
- AIM: present detection rates of all (target and off-target) fetal karyotype abnormalities at birth by different screening strategies including cfDNA test and TSS.
# Comparison of Different Screening Strategies for the Detection of the Overall Fetal Cytogenetic Abnormalities at Birth

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Screening strategy</th>
<th>First-tier test</th>
<th>Second-tier test</th>
<th>Third-tier test</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTS</td>
<td>Combined first trimester</td>
<td>Combined FTS</td>
<td>Karyotype if risk is $\geq 1/270$</td>
<td>//</td>
</tr>
<tr>
<td>CON</td>
<td>Contingent</td>
<td>Combined FTS</td>
<td>Karyotype or cfDNA-T for HR ($&gt;1/10$); CfDNA-T or nothing else for IR ($1/10-1/1000$); Nothing else for LR ($&lt;1/1000$)</td>
<td>Karyotype if cfDNA risk is $\geq 1/100$ or 'high risk'</td>
</tr>
<tr>
<td>SEQ</td>
<td>Sequential</td>
<td>Combined FTS</td>
<td>CfDNA-T for $\geq 1/270$; Nothing else for $&lt;1/270$</td>
<td>Karyotype if cfDNA risk is $\geq 1/100$ or 'high risk'</td>
</tr>
<tr>
<td>cfDNA-T</td>
<td>Universal cfDNA for T13,18,21</td>
<td>cfDNA test for T21,18,13</td>
<td>Karyotype if cfDNA risk is $\geq 1/100$ or 'high risk'</td>
<td>//</td>
</tr>
<tr>
<td>cfDNA-TXY</td>
<td>Universal cfDNA for T13,18,22, SCAs</td>
<td>cfDNA test for T21,18,13 and SCAs</td>
<td>Karyotype if cfDNA risk is $\geq 1/100$ or 'high risk'</td>
<td>//</td>
</tr>
<tr>
<td>QUAD</td>
<td>QUAD test</td>
<td>Second trimester (T18,21)</td>
<td>Karyotype if risk is $\geq 1/270$</td>
<td>//</td>
</tr>
<tr>
<td>INT</td>
<td>Integrated</td>
<td>Integrated test</td>
<td>Karyotype if risk is $\geq 1/270$</td>
<td>//</td>
</tr>
</tbody>
</table>

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Grati et al, manuscript in preparation
Additional sources for false-positive results

- Vanished twins; up to 0.5% of women screened?
- Maternal CNVs; particularly when evaluating smaller imbalances
- Maternal cancer ("dysplody"). Perhaps 0.008% of all women screened

Additional sources of false-negative results

- Low level mosaics (especially with low fetal fraction)
- Smaller partial imbalances (especially with low fetal fraction)
- True mosaicism in the fetus and/or not represented in cytotrophoblasts (>0.01%)