Have We Done Our Last Amniocentesis?
Updates on cffDNA for Down Syndrome Screening

LOUISE WILKINS-HAUG, MD, PHD
DIVISION DIRECTOR, MATERNAL FETAL MEDICINE
DIRECTOR, CENTER FOR FETAL MEDICINE
BRIGHAM AND WOMEN'S HOSPITAL, BOSTON, MA
Invasive Testing at Brigham and Women’s Hospital Cytogenetics Lab 2005 - 2014
35 yo, NIPT + 21

40 yo, NIPT negative

20 yo, NIPT + 13

35 yo, NIPT negative
Objectives

1) Examine current guidelines for aneuploidy screening
   ◦ Compare serum screening to Non-Invasive Prenatal Testing (NIPT, cfDNA, ffDNA)

2) Compare NIPT in high risk and low risk populations

3) Understand “false positive” and “false negative”
   ◦ Positive predictive value and biologic discordance

4) Examine some clinical dilemmas
Screening for Down Syndrome - Maternal Age and A Priori Risk

Chance of having a live-born baby with down syndrome

Age of mother at delivery

Percentage chance
ACOG (and SMFM) Guidance

All women - offer screening and diagnostic testing for aneuploidy ideally in the first trimester (2007)

Options – serum screen vs cffDNA

- **2012 ACOG/SMFM** - Cell free fetal DNA (cffDNA)
  - Appropriate for high risk women, singletons, as screening for common aneuploidies

- **2015 SMFM update** – women’s autonomy respected if cffDNA requested by low risk women, pretesting counseling needed, routine screening remains the preferred option

- **2016 ACOG/SMFM** – cffDNA an aneuploidy screening method without delineation to maternal age

(ACOG and SMFM update, PB 163, May 2016)
Serum Screening for Down Syndrome (5% screen positive rate) – 1990-2000

(Malone, 2005; Cuckle, 2008)
Serum Screening for Down Syndrome (5 % screen positive rate) – 2000-2010

(Malone, 2005; Cuckle, 2008)
Noninvasive Prenatal Genetic Testing 2010 - 2017

Cells pass between mother and fetus
  ◦ Extracted, quantified and studied

Cell free nucleic acids in adult serum since 1947
  ◦ Fragments of DNA / RNA without cell membranes

Increased with cell turnover
  ◦ Inflammatory diseases (Lupus, glomerular nephritis, pancreatitis)
  ◦ Cancer
  ◦ Tissue injury (trauma, stroke, myocardial infarct)

(Desai and Cregel, 1963)
How does this apply to pregnancy?

Presence of fetal DNA in maternal plasma and serum


Fetus-derived Y sequences:
- 80% (24/30) maternal plasma
- 70% (21/30) maternal serum
- 17% (5/30) fetal cells
Characteristics of cffDNA

- Comes from the placenta
- cffDNA is 5-10% total cell free DNA in maternal circulation
- Present at 5-7 weeks, cleared within hours

<table>
<thead>
<tr>
<th>Levels not altered</th>
<th>Levels altered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>Gestational age</td>
</tr>
<tr>
<td>Race</td>
<td>BMI</td>
</tr>
<tr>
<td>Parity</td>
<td>Aneuploidy</td>
</tr>
<tr>
<td>Mode of conception</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Placental volume</td>
<td></td>
</tr>
</tbody>
</table>

(Bianchi, 2006, Pergament, 2014)
Distinguishing Fetal from Maternal Free Nucleic Acids

Fetal gene is different from mother’s gene
SRY – fetal sex
Father’s genes different from mother’s

Fetal gene is the same as mother’s gene

Aneuploidy
How to detect aneuploidy?

- Next Generation Sequencing (Massively parallel genomic sequencing)

- 10s of millions DNA fragments sequenced at same time
- First 36 bases are sequenced
- “Binned” by chromosome

(Chiu et al. PNAS 2008 Palomaki, 2011,)
Performance Estimates for High Risk Pregnancies

<table>
<thead>
<tr>
<th>Company</th>
<th>Detection Rate (%)</th>
<th>False Positive Rate (%)</th>
<th>Failure Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>T18</td>
<td>T13</td>
</tr>
<tr>
<td>Sequenom</td>
<td>99%</td>
<td>99%</td>
<td>92%</td>
</tr>
<tr>
<td>Verinata</td>
<td>&gt;99%</td>
<td>97%</td>
<td>87%</td>
</tr>
<tr>
<td>Ariosa</td>
<td>&gt;99%</td>
<td>98%</td>
<td>80%</td>
</tr>
<tr>
<td>Natera</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

1 Failed; uninformative; ‘no call’ (e.g., low fetal fraction, failed QC, failed sequencing).

(J Canick, 2013)
## Screening for Down Syndrome in Women > 35

<table>
<thead>
<tr>
<th></th>
<th>2nd trim quad</th>
<th>1st trim combined</th>
<th>Integrated</th>
<th>DNA testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DR</strong></td>
<td>80%</td>
<td>90%</td>
<td>95%</td>
<td>≥ 98%</td>
</tr>
<tr>
<td><strong>Screen Positive Rate</strong></td>
<td>5%</td>
<td>15%</td>
<td>2%</td>
<td>0.2%</td>
</tr>
<tr>
<td><strong>Chance of true positive</strong></td>
<td>2%</td>
<td>2-3%</td>
<td>4%</td>
<td>80-99 %</td>
</tr>
<tr>
<td><strong>Failure Rate</strong></td>
<td>&lt;&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>0.3 – 3%</td>
</tr>
<tr>
<td><strong>Complexity</strong></td>
<td>1 Blood draw</td>
<td>US and 1 blood draw</td>
<td>US and 2 blood draws</td>
<td>1 blood draw</td>
</tr>
</tbody>
</table>
Unanswered Questions with NIPT

#1 Should it be used in low risk women?
   - Advanced screen?
   - Primary screen?

#2 What do discordant results mean?
   - “False positives”
   - “False negatives”

#3 What should be done with a “no call” result?

#4 Should it be expanded beyond the common aneuploidies?
#1 As an Advanced Screen In Women < 35 Years Old?

- **2,800,000 women (5,000 trisomy 21 fetuses)**
  - Combined 1st trimester screening
    - 140,000 positive
      - cff DNA
        - 3,750 trisomy 21 (75% detection)
          - Loss of 1,050 normal fetuses
        - 3675 trisomy 21 (73.5% detection)
          - Loss of 10 normal fetuses
    - 1250 trisomy 21 (25% missed)
As A Primary Screen in All Women?

Fetal fractions, sensitivities and specificity are independent of maternal age

Positive predictive value dependent on a priori risk

- Ballpark estimates

<table>
<thead>
<tr>
<th>Indication</th>
<th>Positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 35 years old</td>
<td>80%</td>
</tr>
<tr>
<td>&lt; 35 years old</td>
<td>50%</td>
</tr>
<tr>
<td>US + serum screening</td>
<td>2-4%</td>
</tr>
</tbody>
</table>
Positive Predictive Value, Maternal Age and Specific Aneuploidy

<table>
<thead>
<tr>
<th></th>
<th>45 yo</th>
<th>35 yo</th>
<th>20 yo</th>
<th>45 yo</th>
<th>35 yo</th>
<th>20 yo</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks, trisomy 21</td>
<td>100%</td>
<td>80%</td>
<td>60%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks, trisomy 18</td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>80%</td>
<td>60%</td>
</tr>
</tbody>
</table>
Is there a “Hidden” Value of Serum Screen?

<table>
<thead>
<tr>
<th>Study</th>
<th>Serum screen positive</th>
<th>% with abnormal karyotype</th>
<th>Detectable by NIPT</th>
<th>Significance of nondetected by NIPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton, 2014</td>
<td>5.2%</td>
<td>11.0%</td>
<td>77 - 83%</td>
<td>Not all with abnormal phenotypes</td>
</tr>
<tr>
<td>Peterson, 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Karyotype Anomaly Detection by Serum Screen and Missed by cfDNA – Risk and “Cost”? 

Risk – abnormal karyotype not detected by cffDNA but found by serum screen – 2%

- Lower if exclude US anomalies, karyotype changes without abnormal phenotype

“Cost” - invasive studies for all positive serum screen

- Predominantly pregnancies with normal chromosomes
# 2 What About the Discordant Results? “False Positives” (0.1%) 

“ffDNA” positive
- Increased chromosome specific DNA but not its origin

Possible origins
- Placenta
- “Vanishing twin
- Maternal
Confined Placental Mosaicism

1-2% of all pregnancies at 10 – 12 wks
Normal fetal karyotype with normal and aneuploid cell lines in the placenta
Adverse pregnancy outcomes
◦ IUGR
◦ Uniparental disomy

Multiple case reports, overall prevalence?

(Futch, 2012, Hall, 2013, Pan 2013)
“Vanishing twins”

1-2% of singletons originate as twins

Discordancy with vanishing twins
- 2 of 3 of discordant trisomy 13 cases occurred in setting of an early twin demise

NIPT studies
- 0.43% of cases with two paternal haplotypes
- Second twin ffDNA detected >8 weeks after demise

(Futch, 2013; McAdoo, 2014)
Maternal Cancer - “Widespread genomic imbalance” NIPT

37yo, NIPT + 13 and -18; amniocentesis, neonate, placental biopsies normal – all normal
  ◦ Postpartum pelvic pain - small cell carcinoma of the vagina
  ◦ Majority of cancer cells with trisomy 13

Recent report of 12 cases with various malignancies

One study – 0.03% of tests > 2 aneuploidies
  ◦ affected fetuses - 4, normal fetuses – 14
  ◦ 5/14 women with a cancer diagnosis

(Osborne, 2013; Bianchi, 2015, Snyder, 2016)
Maternal Aneuploidies

Sex chromosome aneuploidies:
- 44 yo with an IVF conception
  - NIPT - abnormal X chromosome ratios, newborn karyotype normal

- 25 yo, normal height, intellect and fertility
  - NIPT positive for triple X, amniocentesis normal
  - Maternal karyotype 47, XXX

8.6% of NIPT positive for sex aneuploidy have maternal sex chromosome mosaicism

(Nicolaides, 2013; Lau, 2013; Wang, 2014)
Discordant Results - False Negatives

- Liveborn trisomy 13 and 18 fetuses have mosaicism (euploid and aneuploidy) in their placentas
- Case reports as source of a false negative for trisomy 18

(Kalousek, 1989; Pergament, 2014)
#3 What should be done with “no call” results? (2-8% of reports)

Low fetal fraction = higher false negatives

1) Early gestational age
   ◦ 60% get a result on redraw

2) Women with higher BMI have lower fetal fraction
   ◦ 20% in women > 250
   ◦ 50% in women > 350

3) Aneuploidy (13, 18, 21, triploidy)
   ◦ Increased aneuploidy rate with low fetal fraction / no results
   ◦ As high as 20% (1 in 5 are chromosomally abnormal)

(Ashoor, 2013; Pergament, 2014, Williams, 2014)
Should it be expanded beyond the common aneuploidies?

Micro deletions and duplications
- 1/100 neonates but widespread across genome
- 5 most common are 1/1000, most are 22q deletion
- Emerging technology, not currently supported by oversight societies

Whole Fetal exome
- Technically possible at 7 Mb level
- Extends analyses to other aneuploidies
- Emerging technology

(ACOG and SMFM statement, May 2016)
35 yo with positive NIPT for 21

Prenatal care 1st trimester, counseled about screening and diagnostic options
  ◦ Choose NIPT – positive for 21
  ◦ PPV – 83%

Is the continuation rate influenced by how result obtained
  ◦ NIPT 42%
  ◦ Amniocentesis 33%
  ◦ CVS 8%
20 yo with NIPT positive for trisomy 13

Ultrasounds normal but never complete survey (BMI = 40)
  - 80-90% of fetuses with trisomy 13 have an ultrasound detected anomaly

Declined invasive testing
Had “arranged for palliative care team”
Positive predictive value = 16 %
Could her placenta have CPM for trisomy 13?
40 yo, NIPT “negative” for 21,18,13, X,Y aneuploidy

NIPT at 11 weeks – negative, NL normal

Early onset IUGR, polyhydramnios

Newborn karyotype reveals 47,XY, +18

- NPV by age = < 1%
- Altered by US findings

Remember NIPT is screening test with false negatives

- Focus of screening is for trisomy 21
- False negatives often not discussed in the enthusiasm of the higher detection and PPV rates

True false negative or possible CPM?
35 yo, NIPT “negative” for 21,18,13, X,Y aneuploidy

At 20 wks of pregnancy delayed growth, possible VSD, and club foot

Counseled and chose NIPT for aneuploidies to exclude severe conditions (+13, + 18)
  ◦ Result = negative

Microarray reveals “Cri du Chat” (46,XX,5p-syndrome)
What is the Role of cffDNA?

1) Appropriate first line for advanced maternal age for trisomy 21 screening.
   ◦ Singletons, not twins
   ◦ Not validated for microdeletions
   ◦ Does not replace diagnostic study for ultrasound anomalies

(ACOG and SMFM joint statement, May 2016)
What is the Role of cfDNA in women < 35 years old?

Pros
- High detection, very low false-positives
- Single blood test any time past 10 weeks
- Provides a noninvasive risk assessment

Cons
- Calculation of patient’s positive predictive value essential
- Traditional serum screen identifies additional karyotype anomalies
- Cost efficacy remains to be established

(Norton, 2016)
What is the Role of cffDNA?

2) A screen, not a diagnostic test
   ◦ A sensitive screen for major aneuploidies
   ◦ But these account for only 80% of the abnormal karyotypes
   ◦ No result at particularly higher risk

3) The paradigm for screening for trisomy 21 has changed, evaluation for ultrasound anomalies should not be confused with Down syndrome screening
1) NIPT/Cell Free DNA Screening Performance Calculator (ACOG endorsed)
   ◦ www.perinatalquality.org

2) “Free Webinar: Prenatal Cell-Free DNA Screening”
   ◦ http://cfweb.acog.org/obpractice/webinars

3) “Prenatal Cell-Free DNA Screening: Q&A for Healthcare Providers”
   ◦ http://nsgc.org/page/non-invasive-prenatal-testing-healthcare-providers (ACOG endorsed)

4) “Abnormal Prenatal Cell-Free DNA Screening Results: What Do They Mean?”
   ◦ http://nsgc.org/page/abnormal-non-invasive-prenatal-testing-results (ACOG endorsed)

5) Resources for Women
   ◦ “Cell-free DNA Prenatal Screening Test“ (http://www.acog.org/Patients/FAQs/Cell-free-DNA-Prenatal-Screening-Test-Infographic)
   ◦ “Prenatal Cell-Free DNA Screening” -FAQ for patients (http://nasgc.org) (ACOG endorsed)
Thank You For Your Attention