IS NIPT THE GREATEST THING SINCE SLICED BREAD?

Ken Seethram MD, FRCSC, FACOG, Co-Director

Disclosures

- No financial interest in the current NIPT providers – we receive no research grants, educational grants, or other initiatives from any NIPT provider
- We draw and send plasma on patients for NIPT at PCRM
- We perform first trimester screening (non-insured service) in our centre
- Current research affiliations are with:
  - UBC Cell biology
  - Igenomix (non-invasive ERA microbiome study)
- Lastly, I have not affiliations with Wonder Bread, or Dempster’s Bakery of Canada
Objectives

01
Understand how **Non-Invasive Prenatal Testing** [NIPT] fits in with prenatal screening algorithms

02
Understand and differentiate current NIPT technologies

03
Review current advancements in NIPT

04
Review counseling considerations for prenatal screening and help you decide how to help your patients

Outline

1. History of prenatal screening and bread slicing
2. Prenatal Screening and the genetics behind it
3. NIPT and the Human Genome
4. Guideline based approaches to Screening
5. The future of prenatal screening and what to do today?
History of Prenatal Screening

**Dawn of Humans until 1930**
- nothing
- age
- 100% detection at birth
- 30%

**1930-1980’s**
- NT
- Quadruple Screening
- NT plus Quad
- NT plus PAPP-A/HCG
- NT plus PAPP-A/HCG & Nasal Bone & Ductus Venosus Flow

**1986-2012**
- 96% Detection Rate
- 3% FPR
- Anatomic review
- Placental screening
- 12 weeks

1928 – sliced bread
1943 – war ban on sliced bread

>2012
- NIPT
- Quantitative vs Qualitative

1928 – sliced bread
1943 – war ban on sliced bread
**Sliced bread saves time....**

One woman even wrote a letter to the New York Times admonishing the ban on sliced bread:

➤ “I should like to let you know how important sliced bread is to the morale and saneness of a household. My husband and four children are all in a rush during and after breakfast. Without ready-sliced bread I must do the slicing for toast—two pieces for each one—that’s ten. For their lunches I must cut by hand at least twenty slices, for two sandwiches apiece. Afterward I make my own toast. Twenty-two slices of bread to be cut in a hurry!”

➤ The government lifted its ban in March (2 months later, March 1943)

Mental Floss article, Kaitlyn Boettcher, July 7, 2013

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**NIPT saves time also....**

➤ By 2012, Prenatal screening had become very difficult

➤ What used to be a conversation about being 35 or older, or younger, and whether or not CVS/Amniocentesis is required, now became an extremely detailed, long conversation about the myriad of prenatal screening options, detection rates, risk, and counseling

➤ Suddenly, NIPT - “Here’s a risk-free blood test with over 99.9% accuracy”
So the question is…

is NIPT the best thing since sliced bread?

Outline

1 ✓ History of prenatal screening and bread slicing
2 Prenatal Screening and the genetics behind it
3 NIPT and the Human Genome
4 Guideline based approaches to Screening
5 The future of prenatal screening and what to do today?
What do we talk about when we talk about prenatal screening?

- Aneuploidy?
- Twins?
- Neural tube defect screening?
- Congenital anomaly screening?
- Pre-eclampsia screening? (which affects 3-6% of pregnancies)
- Preterm birth screening?

Although one focus is aneuploidy, keep in mind that aneuploidy is simply one thing that can affect a pregnancy adversely.

The biggest thing we are screening for is Trisomy 21 (also known as Down Syndrome)

- T21 is one of the most common aneuploidy to affect live-born children and has a background prevalence of 1:691, increasing with maternal age
- What causes T21 – hypo-methylation by the extra chromosome
- Prenatal diagnosis relies upon the procurement of fetal cells via amniocentesis (ACT) / trophoblast via chorionic villus sampling (CVS)
The biggest thing we are screening for is Trisomy 21 (also known as Down Syndrome)

- Back in the 1970-1980’s - if your age based risk exceeded the risk of diagnostic testing, then do the test, which was roughly age 35-38
- So the only screening tool we had – was age

Quick Diversion: What is the inherent risk of Amniocentesis or CVS?

- For the last 40 years, it has been quoted at 1%

- 42,000 women who underwent ACT and 138,000 who did not, data collected >1999
- Attributable miscarriage risk: 0.11%
- 54,000 women who underwent CVS and 670,000 who did not, data collected >1999
- Attributable miscarriage risk: 0.22%

- 11,746 amniocenteses and 5,243 CVS
- 0.13% loss rate in amnio and 0.7% loss rate in CVS was no different than the loss rate in those without invasive procedures

Our previously agreed-upon 1% may not be so true
The risks of invasive testing and resources should govern the decision to do the test.

And probably the risks of testing are ~1:1000 rather than 1:100.

However....end of the day – nobody wants invasive testing.

**This is fundamentally why we do prenatal screening – to figure out who needs the invasive test, and who does not.**

What are the major chromosomes we’re looking for?........
Prenatal Screening - Genetics

- **Age**
- **Double screening > Triple marker screening > Quad Screening**
  - MSAFP for Neural tube defect screening in the early 80’s was then linked with hCG for T21 detection
  - 1991 (Cuckle and Wald) published in the BJOB – uE3, hCG for T21 detection
  - Combined this became triple marker screening
  - Modified in 1996 by the addition of dimeric Inhibin-A to become QUAD screen

Prenatal Screening - Genetics

- **Age**
- Double screening > Triple marker screening > Quad Screening
- **Combined First Trimester Screening (NT+NB+DV+PAPP-A+bHCG)**
  - BMJ 1992 – K. Nicolaides published on the use of beta-hCG, PAPP-A and a single ultrasound measurement at 12w called nuchal translucency (NT)
  - Modified since 1992 to include nasal bone, FHR, ductus venosus flow (hepatic vein flow) using color flow Doppler measurements
  - Accreditation provided through single site – Fetal Medicine Foundation, UK
  - **Detection rate: 96% with screen positive rate = 3%**
And then people got fancy and started mixing first and second trimester screening to try and improve performance while lowering costs

- **Combining PAPP-A with Quad (SIPS)**
- **Combining NT, PAPP-A with QUAD (IPS)**
  - DR 88% with screen positive rate of 3%

This is like mixing red and white wine – it feels like you’re doing the right thing, until later

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**By 2011 however, globally, the focus was on the first trimester:**

- NT
- Ductus Venosus
- Nasal Bone
- PAPP-A and bhCG

And then things got really complicated in 2011 with the introduction of NIPT

Actually things got complicated long before that.....
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The first two drafts of human genome were published by the Human Genome Sequencing consortium and Celera Genomics (Venter) 2001.
The HuRef Browser – online tool for browsing genome assemblies and studying individual human sequence variation
And our understanding of how our genome was assembled led to the application of new discoveries such as NIPT

Axelrod, 2009 The HuRef Browser: a web resource for individual human genomics

Non-Invasive Prenatal Testing or NIPT
Emerged in 2011, but first described in 1997
“cell-free ‘fetal’ DNA (cffDNA),” actually derived from the placenta, comprises about 4% of all free-DNA in the maternal blood
Can be detected as early as 4-5 weeks of gestation
Usually does not exceed 150 base pairs of length (very small fragments) but the entire fetal genome is represented
Keep in mind:
Our entire genome - 6.5B base pairs
Chromosome 1 has 249M base pairs
Chromosome 21 has 48M base pairs
So there’s a lot of math required to re-construct part or all of the fetal genome from these bits of DNA
And that’s what NIPT is:

An assembly of the fetal genome through fragments of the placental free DNA circulating in maternal blood

‘non-invasive prenatal testing’

Keep in mind – it’s called fetal DNA, but it’s not, it’s placental
Keep in mind – it’s called testing, but it’s screening
Keep in mind – it’s not non-invasive, but a blood test is pretty easy

Free DNA comes from apoptotic cells derived from:

• Maternal Circulation
  • Adipocytes
  • White Blood Cells

• Fetal
  • Placental cells (trophoblasts) in the maternal circulation

• Fetal Fraction – the percent ratio between placental:maternal DNA
Fragments of DNA in NIPT can then be compared against Venter’s Human Genome Library (HuRef) to essentially either sequence the genome from those fragments, or use targeted gene analysis to count signals.

**Counting** - For example, if you know a certain gene resides on Chromosome 13, and you have three copies of that gene assembled from the NIPT fragments, then you can diagnose Trisomy 13 simply by counting (ex. Harmony).

**Sequencing** - Or, if you can assemble the bits of DNA then sequence them together or compare single nucleotide polymorphisms (ex. Panorama).

That is the big difference between NIPT products – counting and sequencing.

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**How good is it?**

**As a screening tool, it is very very good**

Most studies report detection rates exceeding 99%, with false positive rates of 0.1%.

- Study, Zhang – (Whole Genome sequencing) in over 140,000 pregnancies:
  - The overall sensitivity of NIPT was
  - T21 99.17% (specificity of 99.95%)
  - T18 98.24% (specificity of 99.95%)
  - T13 100% (specificity of 99.96%)

Zhang, H, Ultrasound in Obstetrics and Gynecology January 2015
NIPT and the Human Genome

Screening for Trisomy 21: NEXT clinical trial

15,841 women

hCG + PAPP-A + NT

Cell-free DNA Screening

- False Positives
  - 854
  - 30
  - 8

- True Positives
  - 9
  - 38

- False Negatives
  - 0


Traditional Screening

NIPT

PPV: 4-5%
Maternal serum screening would require **265** women to undergo invasive testing to discover **9** true positives.¹

PPV: 91%
With NIPT, **10** women will undergo invasive testing to discover **9** true positives.²

NIPT and the Human Genome

➤ Bottom line – it’s a great screening tool
  ➤ It’s super for T21
  ➤ Performance and specificity for T13/T18 is less

Have you heard of fetal fraction?
  ➤ Early gestation, high BMI, collection issues, aneuploidy
  ➤ Can all cause a low proportion of placental DNA versus maternal
  ➤ This can drop the accuracy of results
  ➤ Minimum required: 4% for counting, 2.8% for SNP

➤ If low, you have to:
  ➤ Redraw
  ➤ Option of other screening methods
  ➤ Up to 30% of people on redraw will still get non-reporting
  ➤ So although it’s a great test, 3% of all patients will get non-reporting
NIPT and the Human Genome

What about microdeletions
- SNP sequencing is the only NIPT which can look for small deletions in the chromosomes
- Syndrome from microdeletions can be quite severe
- But those syndromes are RARE
- Also, positive predictive values are low (20% maximum for 22q11.2 deletion syndrome)

What about Twins
- Sequencing with Single nucleotide polymorphisms (SNPs) provides one thing: zygosity (if in doubt)

<table>
<thead>
<tr>
<th>Microdeletion syndrome</th>
<th>Incidence (at birth)</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11.2 deletion syndrome/ DiGeorge</td>
<td>1 in 4,000-2,000</td>
<td>heart defects, immune system problems, and mild-to moderate intellectual disability. They may also have kidney problems, feeding problems, and/or seizures.</td>
</tr>
<tr>
<td>1p36 deletion syndrome</td>
<td>1 in 10,000-5,000</td>
<td>weak muscle tone, heart and other birth defects, intellectual disabilities, and behavior problems. About half will have seizures.</td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td>1 in 12,000</td>
<td>delayed milestones (like sitting, crawling and walking), seizures, and problems with balance and walking. They also have severe intellectual disability and most do not develop speech.</td>
</tr>
<tr>
<td>Prader-Willi syndrome</td>
<td>1 in 25,000- 10,000</td>
<td>low muscle tone and problems with feeding and gaining weight. They also have intellectual disabilitly. As children and adults, they have rapid weight gain and often develop obesity related medical problems.</td>
</tr>
<tr>
<td>Cri-du-chat syndrome (5p-)</td>
<td>1 in 50,000- 20,000</td>
<td>low birth weight, small head size, and decreased muscle tone. Feeding and breathing difficulties are also common. They have moderate-to-severe intellectual disability.</td>
</tr>
</tbody>
</table>
## Risks

- DiGeorge syndrome 22q11.2 deletion risk: **1:2000-4000**
- Risk of dying in a car: **1:5000**
- Risk of dying in pregnancy in Canada: **1:8800**
- Risk of having a child with autism/spectrum: **1:68**

**What’s my point?**
- Microdeletion syndromes are rare
- People read about these on the google and on the twitter, and they displace their baby concern with the concern for truly rare diseases

## NIPT and the Human Genome

**What are the major NIPTs available?**

- **Targeted (counting method) – example - Harmony©**
  - Where we look for gene loci unique to certain chromosomes (like 21) and compare that number with the reads from other chromosomes (like 1)
  - If you get more reads from the locus on 21 in a ratio of 1.5X versus 1, then you have trisomy 21
  - Benefits – lower costs

- **SNP based – example - Panorama©**
  - Where DNA sequence variations (normal in all of us) are compared between placental and maternal DNA
  - Variations of up to 1% are normal
  - This can then help segregate and compare maternal versus placental DNA
  - Benefits – microdeletions, zygosity, slightly lower fetal fraction threshold

- **Whole Genome sequencing – example NIFTYpro©**
  - All chromosome numeric abnormalities
  - 84 microdeletions
  - March 2018
NIPT

- So now y’all know:
  - What NIPT is
  - How it performs
  - The methods used
  - The variations like microdeletion assessment with SNP and Whole Genome sequencing

- So how does it fit into our prenatal screening world?
1. History of prenatal screening and bread slicing
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Guideline based approaches to screening

All women, regardless of maternal age, should be offered prenatal assessment for aneuploidy

<table>
<thead>
<tr>
<th>Organization</th>
<th>Policy</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>“[cfDNA] appears to be the most effective screening test for aneuploidy in high risk women”</td>
<td>2012 - 2013</td>
</tr>
</tbody>
</table>
NIPT Funding in BC

- has received a Positive Screen result from IPS, SIPS, or Quad;
- has had a previous trisomy 13, 18, or 21 pregnancy; or
- has a risk of Down syndrome greater than one in 300 based on results of screening and ultrasound marker(s) of aneuploidy (ie. Contingency model)

American College of ObGyns 2015

- any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risks status
- Conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population

International Society for Prenatal Diagnosis – all are acceptable:

- Offer NIPT to all
- Offer NIPT to high risk only
- Use a contingency approach (high, moderate, low risk)
- First trimester screening
- Quad screen if after 14w
- 2nd trimester scan
Guideline based approaches to screening

▷ So why not just do NIPT on everyone?
  ▷ Gives us no information about the baby – just about the placental DNA
  ▷ Tells us nothing about anatomic problems
  ▷ Tells us nothing about placental disorder risks (PIH, IUGR)

Why not do NIPT alone?

▷ Conventional screening, whether it’s FTS, or sequential integrated screening provides other information including fetal structures, multiples etc.
  ▷ Baer 2014 ACOG identified 9,051 FTS screen-positive and 30,928 screen-negative pregnancies
  ▷ FTS screen positive women were
    ▷ 1.7X more likely to be diagnosed with preeclampsia, placenta previa, or abruption
    ▷ 3.5X more likely to experience fetal loss before 20 weeks
    ▷ Women with positive results for more than one screened condition were at substantially greater risk (34-157X) relative risk of fetal and neonatal mortality
  ▷ PAPP-A screening with FTS – those under 10th percentile MoM have a 4.2X increased risk of several Pre-eclampsia

Why not do NIPT alone?

- So although placental DNA is important for aneuploidy detection, there are many other aspects to prenatal screening which are excluded from detection.
- Which is why we are moving to combining early pregnancy screening with NIPT.

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The future of Prenatal Screening

Advanced screening test

- First Trimester Combined Screen
  - Low risk Result
  - High Risk Result
    - Offer NIPT as an advanced screen
    - Diagnostic Testing

No Further Testing

→ Negative?

- Reduction in invasive testing rates
- UK National Screening Committee recommends introduction of this model into NHS


The future of Prenatal Screening

Fetal Medicine Foundation

- Blood draw 10w
  - Serum for PAPP-A/fb-hCG
  - Plasma for NIPT

- Combined FTS
  - Integrate results
    - Normal FTS/NIPT
    - Abnormal FTS or NIPT
    - Abnormal NT beyond 3.5mm
    - Abnormal DV

  - ACT/CVS
    - ACT/CVS with array
  - Fetal echo 22w
The future of Prenatal Screening

| First Trimester Screening | Multiple markers  
| History and Mean arterial pressure  
| Placental Growth Factor |
| NIPT | Genomic information |
| Second Trimester | Ultrasound and medical screening |
| By 20w | We will be able to triage those at very low risk, and those at high risk – concentrating prenatal care on the people who need it the most: anomalies, PIH risk, Preterm birth risk, etc |

- Prenatal screening should always be presented and discussed as an available option, not as standard of care or required
- There is no right or wrong choice
- Many expectant parents have a difficult time deciding whether or not to have a prenatal screening test
- The benefits and limitations of prenatal screening should be discussed in reference to the patients current feelings, beliefs and wishes.... And resources
Regardless of the patient’s age....

- Wants to screen but not out of pocket
  - MSP covered serum screening with NT if over age 35
  - CVS/Amnio if screen positive or meets criteria

- Wants to screen and okay to pay for non-insured services
  - First Trimester Screening with or without NIPT
  - CVS/Amnio if screen positive

- Wants to screen and wants everything possible
  - First Trimester Screening with NIPT – Sequencing
  - CVS/Amnio if screen positive

NIPT is a superior screening test for common aneuploidies but has limitations that are important to understand
- It is quick, and easy, but it is not the greatest thing since sliced bread
- The marketing power, and ease of results tempts patients and practitioners
- But it does so at the costs of ignoring the pregnancy as a whole
- FTS compliments NIPT and overcomes some of its limitations
- Pre-test counselling and informed decision making is KEY
- The future of prenatal screening will not be focusing on aneuploidy, but rather focusing on healthy outcomes for mother and baby

The End
### NIPT and the Human Genome

<table>
<thead>
<tr>
<th></th>
<th>Detection Rate</th>
<th>False Positive Rate</th>
<th>In BC ~ 1/608 births is T21 (N=74)</th>
<th>Missed cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPT</td>
<td>&gt; 99%</td>
<td>&lt; 0.1%</td>
<td>74 / 74</td>
<td>0</td>
</tr>
<tr>
<td>FTS</td>
<td>96%</td>
<td>&lt; 3%</td>
<td>71 / 74</td>
<td>3</td>
</tr>
<tr>
<td>IPS (&gt;35)</td>
<td>81%</td>
<td>3%</td>
<td>60 / 74</td>
<td>14</td>
</tr>
<tr>
<td>SIPS (&lt;35)</td>
<td>71%</td>
<td>3%</td>
<td>53 / 74</td>
<td>21</td>
</tr>
</tbody>
</table>

Vital Statistics Canada. 45,000 births per annum BC, of which 25,000 have screening.