Web Case Analysis: Non-Invasive Prenatal Testing

LEARNING OBJECTIVES:
A. Identify the various methods of non-invasive prenatal testing
B. Describe the clinical applications and interpret the results of common prenatal screening tests
C. Identify the indications for prenatal diagnosis by cytogenic or cytogenomic analysis
D. Describe the role of cfDNA in prenatal screening
E. Describe the genetics and biochemistry of cfDNA

PRETEST QUESTIONS

Use this word bank to answer questions 1-3. Each question may have more than one answer. Answers can be used once, more than once, or not at all.

- Prenatal ultrasound
- Maternal age
- Paternal age
- Amniocentesis
- Maternal serum screening
- Percutaneous umbilical blood sampling (PUBS)
- Cell free fetal DNA (cfDNA) sequencing
- Chorionic villous sampling (CVS)

1. Name the common primary screening measures used for prenatal testing:  
   a. Prenatal ultrasound
   b. Maternal age
   c. Paternal age
   d. Amniocentesis
   e. Maternal serum screening

2. Name the common secondary screening measures used for prenatal testing:  
   g. next generation sequencing using cfDNA

3. Name the common measures used for diagnosis during prenatal testing:  
   d. f, h. amniocentesis, percutaneous umbilical blood sampling, chorionic villous sampling

4. Which of the following are considered invasive testing measures:
   a. chorionic villus sampling
   b. amniocentesis
   c. next generation sequencing using cfDNA
   d. A and B
   e. A, B and C

5. Which of the following first trimester serum profiles are associated with Trisomy 21?
   a. maternal hCG is elevated and PAPP-A is reduced
   b. maternal hCG and PAPP-A are elevated
   c. maternal hCG is elevated, all other serum values are normal
   d. maternal hCG and serum estriol are elevated, PAPP-A is reduced
6. At what age do we become concerned for an increased risk of pregnancy associated aneuploidies?
   a. 30
   b. 35
   c. 40
   d. 45

CASE STUDY

Miranda is a 36 year old G3P1011 with a past medical history significant for sarcoidosis presenting to her primary care provider for new onset fatigue and nausea. Over the past three weeks, Miranda has been feeling increasingly exhausted despite getting adequate sleep and exercise. She denies any change in her daily routine. Additionally, for the past 4 days, she notes intermittent waves of nausea that have caused her to vomit on more than one occasion. She lives in a DC apartment with her healthy 7 year old son and boyfriend Mark of 2 years. She has no pets, recent sick contacts or other exposures. Earlier in the day, Miranda was giving a presentation at work when she suddenly became so nauseous she had to throw up in a trash bin next to the podium. This prompted a visit to her PCP. During the workup, it became clear that Miranda was pregnant.

Miranda and Mark were delighted to hear the news and both very excited about the new baby. However, they had some cause for concern and questions they needed to get answered. Miranda’s older sister lives in Ghana and recently gave birth to a baby diagnosed with trisomy 21. They want to know what this means in terms of their new pregnancy. What are the chances their baby will have a similar condition? Are their other conditions their child is at risk for? What are the best next steps? Miranda’s PCP referred them to an obstetrician to better address these questions and learn more about their options.
Non-Invasive Prenatal Testing (NIPT), sometimes referred to as prenatal screening, is a blanket term used to describe an array of low-risk screening measures used during pregnancy to identify those at risk for common aneuploidies or genetic disorders. Non-invasive prenatal testing is not diagnostic but rather a primary screening measure that can provide information about a pregnancy and help facilitate a discussion between a patient and her physician. By using a combination of maternal serum markers and ultrasound screening tests, NIPT generates likelihood ratios to assess the risk of a particular diagnosis. These ratios identify pregnancies at “high risk” and help guide whether additional work-up with more invasive testing procedures is warranted.

Serum screening tests are blood tests used to measure pregnancy associated anylates. There are various maternal serum markers used in NIPT, each of which are components of specific first and second trimester screening tests. Ultrasound examination is a routine screening measure utilized for NIPT and a safe and cost-effective method of medical imaging. Ultrasounds are able to detect congenital structural abnormalities, abnormalities in fetal growth, identify multiple gestations and estimate gestational age. Ultrasound machines generate high frequency sound waves that pass through body tissues and are then reflected back through a transducer to create a real-time image on a screen. Serum screening tests, ultrasound examination and maternal age are all used in conjunction with one another for non-invasive prenatal testing.

### MARKERS USED FOR NON-INVASIVE PREGNATAL TESTING

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<th>Marker</th>
<th>Description</th>
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| Serum AFP                      | Elevated levels associated with Trisomy 21.  
Decreased levels associated with neural tube and abdominal wall defects. |
| Serum Unconjugated Estriol     | Isolated decrease associated with uterine dysfunction  
Decreased Estriol in combination with normal ultrasound may indicate Smith-Lemli-Opitz Syndrome |
| Serum beta-hCG                 | Elevated levels associated with Trisomy 21 |
| Serum PAPP-A                   | Decreased levels associated with aneuploidies |
| Serum Inhibin A                | Elevated levels associated with Trisomy 21 |
| Ultrasound: Nuchal Translucency Measurement | Increased measurement (>3.5mm) associated with high risk for trisomy 21, cystic hygroma, and cardiac defects |
| Ultrasound: Nuchal Fold Measurement | Increased nuchal skin fold (>6 mm) increase the risk of trisomy 21 |
| Targeted Ultrasound           | Can detect many birth defects and subtle signs of aneuploidy  
Accurately detects anomalies for Trisomy 21 (50%) and Trisomy 13, 18 (80%) |
| Next Generation Sequencing (cfDNA) | can be measured in maternal serum and is used as a screening tool for trisomy 21, trisomy 18, trisomy 13 and sex chromosome aneuploidies |
**1st Trimester Combined Screen:** Serum beta-hCG, pregnancy-associated plasma protein-A (PAPP-A), ultrasound measurement of nuchal translucency, maternal age

**2nd Trimester Triple Screen:** serum alpha-fetoprotein (AFP), unconjugated estriol, and β-hCG, maternal age

**2nd Trimester Quad Screen:** alpha fetoprotein (AFP), unconjugated estriol, inhibin A and beta-hCG, maternal age

**Nuchal translucency (NT):** Ultrasound measurement of the translucent space at the back of the fetal neck

**Integrated Test:** First trimester NT measurement and serum PAPP-A levels combined with results of second trimester quad screen and maternal age. Results given to patient during second trimester.

**Sequential Screen:** First trimester NT measurement, serum PPAP-A and serum beta-hCG results are combined with second trimester quad screen and maternal age. Preliminary positive results obtained during the first trimester are offered to patients before second trimester screening takes place.

**Contingent Test:** Women are offered first-trimester screening results only if the reported risk is less than 0.005%. For any risk above the threshold value, results are held until both first and second trimester screening tests can be combined.

The three common indications for prenatal diagnosis by cytogenic or cytogenomic analysis are: maternal age of 35 or greater, abnormal findings on ultrasound, and a maternal serum profile suggestive of an increased risk for fetal aneuploidy. A patient meeting any of these criteria should be appropriately counseled and further worked up if so desired. Amniocentesis (15-17 weeks) and chorionic villous sampling (10-12 weeks) are common invasive testing measures used for prenatal diagnosis. Fetal blood sampling (PUBS) is another method of diagnosis but is more commonly used to follow up on abnormal amniocentesis results rather than as a primary diagnostic measure. Each of the above invasive procedures are associated with a risk of miscarriage, but in many cases the benefit of a definitive diagnosis outweighs this small but significant risk. The field of clinical genomics is constantly evolving to develop newer, safer, more cost-effective and accurate methods of analysis to inform patient diagnosis and care. The use of cell free fetal DNA (cfDNA) represents a relatively recent advance in the evaluation of prenatal genetic disorders. One major advantage of this secondary screening measure is that it necessitates a simple maternal blood sample, which can be obtained at minimal risk to the pregnancy.

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**GENETICS AND BIOCHEMISTRY OF NEXT GENERATION NIPT USING cfDNA**

Circulating cfDNA is derived from maternal serum and contains components of both maternal and fetal DNA. cfDNA is highly fragmented, with each fragment measuring approximately 50-200 BPs. The fraction of fetal DNA must be at least 4% of the total sample in order to achieve adequate results. Fetal fraction increases with gestational age and an adequate sample can typically be derived from maternal serum at ≥10 weeks. A low fetal fraction of DNA will result in either an inconclusive or false negative test result and can be attributed to early gestational age, maternal obesity, or poor sampling techniques.

There are various approaches to NIPT using cfDNA. Massively parallel shotgun sequencing involves random sequencing of cfDNA fragments from all chromosomes. This method requires a large sample (approximately 10 million) of cfDNA fragments in order to achieve reliable results. Targeted sequencing utilizes a modified mechanism that selectively amplifies genomic areas of interest. This mechanism requires a much smaller sample (approximately 1 million) of cfDNA fragments and allows for focused analysis of clinically important chromosomes (21, 18, 13, X and Y). Another method of cfDNA analysis involves comparing maternal single nucleotide polymorphisms (SNPs) on the chromosome of interest to the corresponding genotypes in the cfDNA sample. An aneuploid fetus will result in a shift in the pattern of SNPs due to the chromosomal anomaly.

*Ok, so what exactly are we using cfDNA for?*
cfDNA is used clinically as a secondary screening test for Trisomy 21, Trisomy 18, Trisomy 13 and sex chromosome aneuploidies. Screening performance is measured using detection rates (DR) and false positive rates (FPR) and varies by genetic disorder. Its efficacy as a screening test is directly related to the fraction of fetal DNA in the serum sample.

**Why is fetal fraction so important if we aren’t going to bother separating the maternal and fetal components of the cDNA?**

To help understand this concept, consider a fetus with Trisomy 21. Due to the extra copy of chromosome 21 the fetus in question has 50% more genetic material compared to its healthy counterpart. This results in a chromosome specific increase in fetal cfDNA, which is reflected by an increase in the overall cfDNA sample. If you consider that reliable test results depend on the ability to detect this increase, it becomes clear why an adequate fetal fraction is critical.

**POST-TEST FOLLOWUP ALGORITHM FOR NEXT GENERATION SEQUENCING USING NIPT USING cfDNA**

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Primary screening test indicates a patient is at high risk for an aneuploid pregnancy
- maternal age ≥ 35
- abnormal serum test
- abnormal ultrasound results

Is the patient ≥10 weeks gestation?
- yes
  - Collect a serum sample for secondary screening using cfDNA

  - Screen Positive
    - Offer invasive prenatal testing as a diagnostic test
    - Provide genetic counseling
  - Screen Negative
    - No further testing necessary
  - No Result
    - Provide counseling to discuss each of 3 options:
      - repeat cfDNA test
      - standard serum marker and ultrasound testing
      - invasive diagnostic testing

- no
  - Wait until the patient is ≥ 10 weeks gestation
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REFERENCES


7 Marion, DW. Prenatal screening for common aneuploidies using cell f-free DNA). In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on July 14, 2016.)