Non-Invasive Prenatal Diagnosis Options for Prenatal Care

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Objectives:

- Review current screening and diagnostic practices
- Understand prognostic value of new screening markers
- Appreciate pros/cons/limitations of new "diagnostic" technology and tests
- Discuss utilization of emerging options

Current Screening: FTS

- Core Elements:
  - 10w 4d → 13w 6d
  - Nuchal translucency measurement
  - Maternal blood sample
    - hCG
    - PAPP-A
- Detection Rates
  - 85-90% for Down syndrome
  - 90-95% for Trisomy 13/18
- False Positive Rate
  - 5%

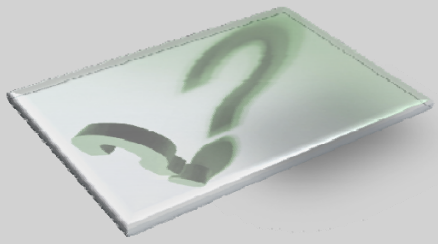
Current Screening: MSS

- Core Elements;
  - 15w 0d → 20w 6d
  - AFP, hCG, uE3, Inhibin/DIA
- Detection Rates:
  - 85-90% for Open Neural Tube Defects
  - 80-85% for Down syndrome
  - 60% for Trisomy 18
- False Positive Rate
  - 5%

Current Diagnostic Tests

- Chorionic Villus Sampling (CVS)
  - 11w0d-13w6d
  - Biopsy sample of the placenta/villi
  - Unique risk: Confined placental mosaicism
- Amniocentesis
  - 15w0d-23/24 wks
  - Withdrawal of amniotic fluid for whole free-floating fetal cells
- Additional studies available from sample:
  - Microarray, single-gene disorder, infections

So what's new



Emerging Strategies

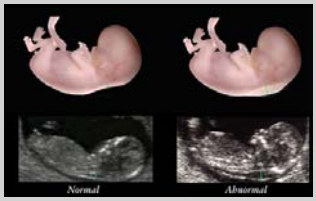
- First Trimester Ultrasound Markers
  - Nuchal Translucency (NT)
  - Nasal Bone (NB)
  - Tricuspid Regurgitation (TR)
  - Ductus Venosus Waveform (DVW)
  - Frontomaxillary Facial Angle (FMF angle)

Emerging Screening: NT

- Normal NT
  - @ 10wks GA:
    - Median NT = 0.7mm
    - 95th%ile NT = 2.1mm (at a CRL of 45mm)
  - @ 13wks GA:
    - Median NT = 1.5mm
    - 95th%ile NT = 2.7mm (at a CRL of 84mm)
  - 99th%ile NT = 3.5 mm (regardless of CRL)

Emerging Screening: NT

- Increased NT
  - Aneuploidy (T13, T18, DS, 45,X, Triploidy)
  - Structural anomalies (i.e. heart defects)
  - Poor pregnancy outcome



http://www.fetal.com/NT%20Screening/02%20NT%20Imaging.html

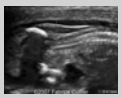
Emerging Screening: NT

- Increased NT
  - < 3.4mm = 7-12% aneuploid
  - 3.5mm – 4.4 mm = 20% aneuploid
  - 4.5mm - 8.4mm = 50% aneuploid
  - >8.4mm = 75% aneuploid

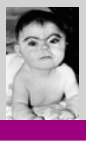
Emerging Screening: NT

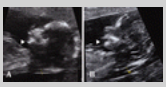
- Increased NT:
  - 15% of increased NT are other genetic syndromes or single gene disorders
    - Noonan syndrome
    - Cornelia de Lange
    - skeletal dysplasias, etc
  - 7% of increased NT are associated with other major structural malformations
    - In Euploid fetuses

Noonan Syndrome:



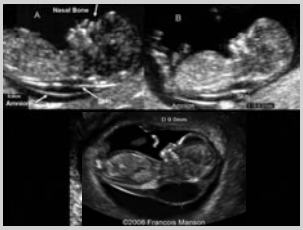
Cornelia de Lange:





Emerging Screening: NT

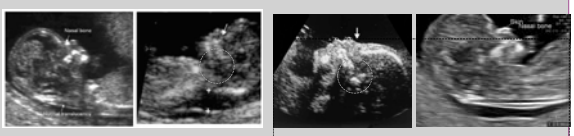
- NT vs. cystic hygroma
  - Definition of cystic hygroma
    - Septated space extending along the back
  - Prognostic value?



http://www.sciencedirect.com/science/article/pii/S0146056500000124

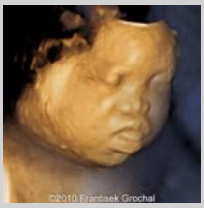
Emerging Screening: NB

- Nasal Bone
- 1st trimester = present vs. absent
  - i.e. look for the " = " sign
- Midsagittal plane: thin echogenic line within nasal bridge



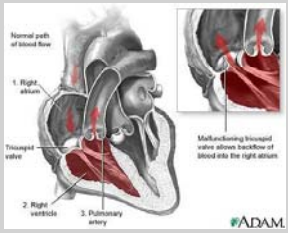
Emerging Screening: NB

- Prevalence in euploids?
  - 1-3%
- Prevalence in Turner syndrome?
  - 11%
- Prevalence in Trisomy 13?
  - 34%
- Prevalence in Trisomy 18?
  - 55%
- Prevalence in Down syndrome?
  - 65%



Emerging Screening: TR

- Tricuspid Regurgitation
- Correlated with:
  - Congenital heart
  - Intrinsic AV valve
  - Changes in the connective tissue



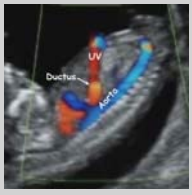
Emerging Screening: TR

- Prevalence in euploids?
  - 5.6%
- Prevalence in Turner?
  - 38%
- Prevalence in Trisomy 13?
  - 30%
- Prevalence in Trisomy 18?
  - 33%
- Prevalence in Down syndrome?
  - 56-67.5%

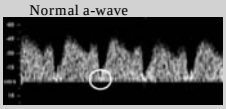
Emerging Screening: DVW

- Ductus Venosus Waveform
- Shunts blood from umbilical vein and joins inferior vena cava at the level of the right atrium
- Narrow diameter at isthmus → high-velocity, forward flow in all phases of cardiac cycle
  - Yields reversed a-wave on ultrasound
- Infers abnormal myocardium → i.e. DS?

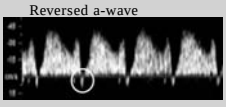
Emerging Screening: DVW



Normal a-wave

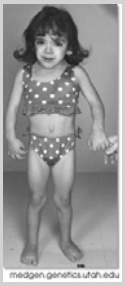


Reversed a-wave



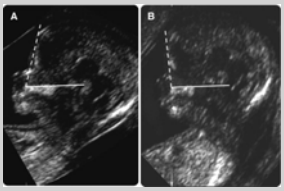
Emerging Screening: DVW

- Prevalence in euploids?
  - 3.7%
- Prevalence in Turner?
  - 75%
- Prevalence in Trisomy 13?
  - 55%
- Prevalence in Trisomy 18?
  - 58%
- Prevalence in Down syndrome?
  - 66%



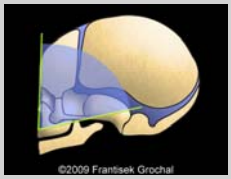
Emerging Screening: FMF Angle

- Frontomaxillary Facial Angle
  - Normal: 75°- 85°
- Increased FMF angle →abnormal development and/or displacement of maxilla in fetuses with DS



Emerging Screening: FMF Angle

- Prevalence in euploids?
  - 5%
- Prevalence in Turner?
  - unknown
- Prevalence in Trisomy 13?
  - 48%
- Prevalence in Trisomy 18?
  - 58%
- Prevalence in Down syndrome?
  - 45%

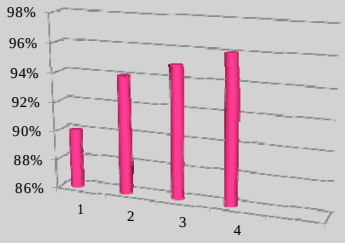


Emerging Screening: Limitations

- # of scans required to achieve competence in image acquisition and interpretation?
  - NB: 80 (range: 40-120)
  - FMF angle: 90 (range: 40-140)
- Sonographer experience/training
- Patient population
  - i.e. different populations will yield different prevalence rates/normal ranges

Emerging Screening: Strengths

- Projected **detection rate** @ 2% FPR:



Non-Invasive Prenatal Diagnosis/Screening

- Use of fetal cells/nucleic acids in maternal circulation to aid in prenatal diagnosis, screening and risk management.

NIPD/S Potential Tissues

- Intact nucleated cells
  - Lymphocytes, trophoblasts, nucleated RBCs
- Cell free fetal DNA (cffDNA)
- Cell free fetal messenger RNA

Intact Pros/Cons

	PRO	CON
Lymphocytes	Standard karyotyping	Persistant
Nucleated RBCs	Do not persist Abundance	No antibody

NIFTY study (2003): 74% detection (0.6-4% FP) for aneuploidy, 41% detection of males (11% FP).

Cell Free Fetal DNA (cffDNA)

- 1997, Lo et al, Y cffDNA detection
- Originates from placenta.
- 5% of total cfDNA in maternal plasma
  - Higher [ ] with advancing gestation
  - > 7 weeks reliable detection
- Short (<200bp) DNA fragments detectable
- Very short half-life
  - 15-20 mins
  - Undetectable hours after delivery
- Extraction is difficult

cffDNA Applications

- Aneuploidy
- Rh(D)
- Gender Determination
  - Sex-linked diseases
- Paternity
- Single gene disorders
  - Paternally inherited disease alleles

Down Syndrome/Aneuploidy Screening by NIPD/S

- NOT A DIAGNOSTIC TEST
  - NO KARYOTYPING
- Circulating ccfDNA extracted from maternal plasma.
- Tag sequences known to be on chromosome 21.
- Allows for frequency estimation (allelic ratio) of DNA (RNA) sequences from chromosome 21.
- Increased representation/increased sequence tag density = trisomy

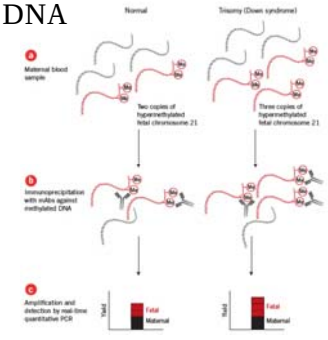
DNA sequences from fetal chromsome 21 are hypermethylated.

Use of Antibodies against methylated DNA

Amplification and Yield measurement by real time PCR.

In theory, would be most sensitive/specific.

DNA



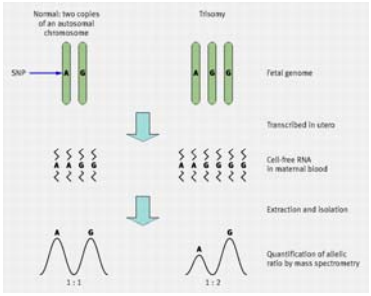
mRNA

SNPs on each chromosome #21.

SNPs also located on fetal **mRNA** transcript

These transcripts are only expressed in fetus/placenta

Allelic mass ratio obtained through mass spec



TRIAL COMPARISONS: cffDNA Aneuploidy

Sequenom	Verinata
Validation Study • 1696 Samples, blinded • Trisomy 21 only • 210/212 trisomy 21 samples correctly id'ed • 99.1% sensitivity (95% CI) • 99.9 specificity (95% CI)	Validation Study • 532 Samples • All abnormal karyotypes • 89/89 trisomy 21 samples correctly id'ed: 100% sensitivity (95%CI) • 35/36 trisomy 18 samples correctly id'ed: 97.2% sensitivity (89-96% CI) • 11/14 trisomy 13 samples correctly id'ed: 78.6% (49-99.2%)

Current Clinical Offerings: Trisomy 21

- MaterniT21: Sequenom Center for Molecular Medicine (2011)
  - Sequenom Validation study ongoing, also beginning other trisomy studies
  - Insured patients, OOP max at $235, uninsured $1900-2000
  - In reality, cost of test is about $2500
  - Only patients with elevated a priori risk for Down syndrome; Trisomy 21 only test now
- Verifi: Verinata Laboratories (2012)
  - Green Journal (May 2012)
  - Only patients with elevated a prior risks for aneuploidy.
  - Will offer all aneuploidy
  - Cost is not announced, but stating no OOP up-front costs to patient.
- Used as early at 10 weeks gestation
- 20cc blood draw
- TOT: 10-12 business days
- Provider ordered only

NIPD for Fetal Rh Status

- Routine use in Europe
  - Only one that is altering obstetric management
  - Population screening efficacy?
- Potential Benefits/Cost Reductions
  - Avoid genotyping of Father
  - Avoid repeated MCA doppler studies
  - Unclear maternal ab titer values
  - Mothers who may oppose vaccination/injection
  - Reduction of Rh_0D Ig use

Fetal Rh NIPD/S

3 exons of Rh (D) gene on chromosome #1 are studied.

SRY (Y chromosome) also studied.

Pregnancies with Rh + male fetuses higher risk for sensitization

Sensitivity: 97.2% (95% CI)

Specificity: 96.9% (95% CI)

Obvious Limitations/Concerns

- Trisomies Only
  - Translocations
  - Mosaicism?
- Will take time to validate other aneuploidies
- Screening versus Diagnostic
- INSURANCE? Yeah right!

- No FDA regulation
- Increase demands on our clinics
- INFORMED CONSENT!!!
- Ethical dilemmas

References:

- http://www.fetalmedicine.com/fmf/training-certification/certificates-of-competence/11-13-week-scan/measurement-of-the-facial-angle/
- http://www.femicare.org/?run=content&id=2194
- http://www.fetalmedicine.com/fmf/2007_9.pdf
- Borenstein, M., Persico, N., et al. Frontomaxillary facial angle in screening for trisomy 21 at 11+0 to 13+6 weeks. *Ultrasound Obstet Gynecol.* 2008 Jul; 32(1):5-11.
- Cicero, S., Rembouskos, G., et al. Likelihood ratio for trisomy 21 fetuses with absent nasal bone at the 11-14 week scan. *Ultrasound Obstet Gynecol.* 2004 Mar;23(3): 218-23.
- Cicero, S., Bindra, R., et al. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. *Prenat Diagn.* 2003 Apr;23(4): 306-10.
- Cleary-Goldman, J., Morgan, MA, et al. Screening for Down syndrome: practice patterns and knowledge of obstetricians and gynecologists. *Obstet Gynecol.* 2006 Jan;107(1):11-7.
- Falcon, O., Faiola, S., et al. Fetal tricuspid regurgitation at the 11+0 to 13+6 week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound Obstet Gynecol.* 2006 Jun;27(6): 609-12.
- Kagan, KO., Cicero, S. et al. Fetal nasal bone in screening for trisomies 21, 18, and 13 and Turner syndrome at 11-13 weeks of gestation. *Ultrasound Obstet Gynecol.* 2009 Mar;33(3):259-64.
- Maiz, N., Valencia, C. et al. Screening for adverse pregnancy outcome by ductus venosus doppler at 11-13+6 weeks of gestation.*Obstet Gynecol.* 2008 Sep;112(3);598-605.
- Malone, FD., Ball, RH., et al. First-trimester nasal bone evaluation for aneuploidy in the general population.*Obstet Gynecol.* 2004 Dec; 104(6):1222-8.
- Maymon, R., Herman, A. The clinical evaluation and pregnancy outcome of euploid fetuses with increased nuchal translucency. *Clin Genet.* 2004 Nov;66(5):426-36.
- Sonek, J., Nicolaides, K., Additional first-trimester ultrasound markers. *Clin Lab Med.* 2010. Sep;30(3):573-92
- Yang, X., Chen, M., et al. Learning curve in measurement of fetal frontomaxillary facial angle at 11-13 weeks of gestation. *Ultrasound Obstet Gynecol.* 2010 May;35(5):530-534.