TWO-TIER NEWBORN SCREENING FOR CYSTIC FIBROSIS

A Practical Perspective

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In this presentation, we cover:

- Rationale for newborn screening for cystic fibrosis (CF-NBS)
- New IRT/DNA protocol, and reasons for replacing the IRT/IRT protocol in Virginia
- Interpretation of results, and actions required
- Sweat testing, indeterminate values, and “CRMS”
- Informational resources about CF newborn screening
Newborn Screening in Virginia

Mandatory since 1965
- Centralized lab for dried blood spot testing
- Division of Consolidated Laboratory Services

Expanded NBS (28 conditions) since March 1, 2006
- Introduction of screening for CF (IRT/IRT)

Revised CF-NBS methodology since December 1, 2011
- Change to IRT/DNA protocol
Relative Incidence of Inherited Metabolic Disorders

- Cystic fibrosis ⇒ most common! (≈1 in 3,300)
- Hypothyroidism (≈1 in 4,000)
- MCAD deficiency (≈1 in 10,000)
- PKU (≈1 in 15,000)
- Galactosemia (classical) (≈1 in 60,000)
- Maple syrup urine disease (≈1 in 100,000)
- Homocystinuria (≈1 in 100,000)
Earliest clinical presentation is meconium ileus at birth: ~20% of patients

Excluding those patients with meconium ileus, the average age to diagnosis of CF-based on symptoms was **14 months**

Side note: Newborns with meconium ileus occasionally have false negative NBS by IRT (low IRT).

Diagnosis of CF Prior to the Introduction of NBS

Always test for CF in babies with meconium ileus!
- Prevent early malnutrition and vitamin deficiency (often present by 2 months of age)
- Reduce early pulmonary complications (atelectasis, pneumonia, establishment of airway infection)
- Improve long-term outcomes including growth, cognitive function, survival
- Prevent prolonged “diagnostic odyssey”
- Ability to offer genetic counseling to families regarding future risk

Why Should We Screen?
RCT and Epidemiologic Data (US, Europe, Australia, others)

- Nutritional benefit of CF-NBS strongly verified: not only at diagnosis, but persistent advantage
- Cognitive benefit seen in Wisconsin study
- Variable strength of evidence for long-term pulmonary function benefit of CF-NBS but trend for positive effect
- Survival benefit emerging
Patients with CF who were diagnosed by newborn screening have better linear growth than patients diagnosed after they developed clinical symptoms.

(Pancreatic insufficient patients only; meconium ileus patients excluded)
Complication rates in the year of diagnosis for CF infants <12 months old
(Diagnosis by NBS vs. symptoms vs. meconium ileus)

CF patients diagnosed by NBS have better pulmonary function in later childhood than those diagnosed after clinical symptoms or CF patients who had meconium ileus.
CF Diagnosis by NBS confers survival benefit (Data from New South Wales)

Adapted from: Dijk FN et al, Arch Dis Child 2011. Copyright © BMJ Publishing Group Ltd & Royal College of Paediatrics and Child Health. All rights reserved.
IRT: immunoreactive trypsinogen in dried blood spots
- Trypsinogen is an enzyme precursor made by pancreas
- Detectable in blood of normal and CF newborns
- IRT is elevated in CF newborns, even those with “pancreatic sufficiency” (approx. 10-15% of CF patients )
- Damaged pancreatic acinar cells “leak” this enzyme precursor into bloodstream
- Nonspecific elevation can occur with perinatal stress

DNA: CFTR mutations
- Over 1,500 known mutations
- ΔF 508 mutation = 69% of alleles
- Defined additional panel of 25-40 mutations can allow identification of 88-96% of CF alleles
Prior to December 2011, CF Newborn Screening in Virginia relied on IRT only, with repeat IRT on a new sample if the initial values were elevated.
Low sensitivity
- Sensitivity of 80% with standard IRT cutoffs

Need second sample after a “1st abnormal”
- Up to 20% of infants lost to follow-up in states without a mandatory second sample
- Timing of second sample is important as IRT declines naturally with age

IRT/IRT misses or delays diagnosis of CF in 50% of infants with CF

Issues with IRT/IRT Protocol for Newborn Screening
- Use IRT in dried blood spots as a “first tier” newborn screening test

- Those samples with an abnormal IRT are then studied for common CFTR mutations

- Samples with an elevated IRT and one or two identified CFTR mutations are “screen positive”

- Those “screen positive” patients are referred for sweat chloride testing to confirm or rule out CF
Comparison of Common CF-NBS Protocols

Adapted from Kloosterboer et al, 2009

**Advantages**
- No detection of carriers
- No need to have genetic counseling services

**Disadvantages**
- Need for 2nd specimen
- Potential for incomplete f/u
- Burden on PCPs and NICUs, and NBS program
- Delay in collection of repeat sample blurs value of measurement due to naturally declining IRT

**Advantages**
- All on one specimen
- Potentially faster turnaround, earlier dx
- Better sensitivity

**Disadvantages**
- Need for genetic counseling services
- Higher cost
<table>
<thead>
<tr>
<th>Screening Algorithm</th>
<th>% Sensitivity (excluding infants with meconium ileus)</th>
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</thead>
<tbody>
<tr>
<td>IRT&gt;105 ng/ml; Post-2 week IRT&gt;70 ng/ml</td>
<td>80.2%</td>
</tr>
<tr>
<td>IRT&gt;daily top 4%; DNA: DF508 only</td>
<td>93.1%</td>
</tr>
<tr>
<td>IRT&gt;daily top 4%; DNA: 25 mutation panel</td>
<td>96.2%</td>
</tr>
</tbody>
</table>

Adapted from Kloosterboer et al, 2009: 
Data from 660,443 Wisconsin newborns 1994-2004
IRT/DNA Newborn Screening Algorithm

Bloodspot IRT at 24-48h of age

Elevated (≥ top 4% of the day)

Reflex: DNA for CF ~45-mutation panel (from same sample)

No mutations

Report as “negative screen”

1 mutation "Possible CF"

Sweat chloride

< 30

Carrier counseling

> 30

2 mutations "Probable CF"

CF consult/referral
- How many NBS samples will have “elevated” IRT? By definition, about 4% (for cutoff at 96th percentile)

- Of those samples with elevated IRT, how many will be normal on “reflex” DNA testing (i.e., No Mutations)? About 94%

- Of those patients with elevated IRT and ... One Mutation, how many will actually have CF? About 3% ... thus the need for the sweat test

- Two Mutations, how many will have CF? Almost all
• Sample mix-up at birth hospital or state lab
  wrong baby, wrong label, or misidentified sample

• 2 different CF mutations are present,
  but are in cis (both mutations are in one CFTR gene), and are
  balanced by a normal CFTR gene on the other chromosome

• Presence of at least one identified CF mutation of
  potentially subclinical severity: R117H
  R117H is 4th most common CF-associated allele (1.3%)
  “PolyT variant” in Intron 8 affects pathogenicity (5T > 7T/9T)
  Variable clinical manifestations

How could a “2 mutation result” not represent CF?
Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

**In the newborn nursery (or out-of-hospital birth)**

1. NBS specimen is not obtained
2. NBS specimen *quality is unacceptable*
3. NBS specimen *labeling error* in the neonatal nursery

Adapted from Rock MJ et al, 2011
Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

In the centralized testing laboratory

4. NBS specimen mix-up in the laboratory
5. Initial immunoreactive trypsinogen (IRT) cutoff level is inappropriate
6. Infant’s IRT level is below the cutoff (biologic false negative)
7. In IRT/IRT method, a second specimen is not obtained and there is no follow-up
8. In IRT/IRT method, the second IRT result is not above the cutoff value
9. In IRT/DNA method, uncommon mutation(s) is/are present and not identified
10. Lab errors (e.g., errors measuring IRT, or DNA mutation analysis)
11. Clerical/central error in recording and reporting the newborn screen result to the primary care provider

Adapted from Rock MJ et al, 2011
Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

**Follow-up**

12. *Miscommunication* of newborn screen result between primary care provider and family (e.g., sweat test not performed)

13. *Error in measurement* of sweat chloride

14. *Inappropriate cutoff value* of sweat chloride

Adapted from Rock MJ et al, 2011
Distribution of IRT values: CF patients in relation to normal newborn population (MoM = multiple of the median for IRT value)

- No DF508
- DF508 HET
- DF508 HOM

99th centile

“Biologic False Negative” (patients with CF but IRT in normal range)

Massie, J et al. Arch Dis Child 2006; 91:222-225
Newborn Screening Is Now the Main Pathway to CF Diagnosis
(2010 National CF Registry Data)
- Higher sensitivity due to lower initial IRT cut-off
- No need for second NBS sample
  *DNA testing done on the initial sample if the IRT is elevated*
- Shorter time to diagnosis and treatment
  - 2.3 weeks (IRT/DNA) to diagnosis vs. 4 weeks (IRT/IRT)
  - 5.9 weeks (IRT/DNA) to initial CF center visit vs. 7.7 weeks (IRT/IRT)
- Clarifies borderline sweat test results
  - Up to 10% of infants with CF will have borderline sweat chloride results
  - 17% of those infants will have 2 mutations on more extensive mutation testing
### 4 Possible Results

| “Normal Screen” | IRT value in the top 4% but <170 ng/ml, and No Mutations identified on screening using the mutation panel
| Screen normal; no further action required |
| “Low Risk of CF” | IRT >170 ng/ml, but No Mutations identified
| No sweat test required: not a failed screen
| Likely to include infants with severe neonatal problems
| ***Discuss with CF consultant if no obvious reason for high IRT |
| “Possible CF” | IRT top 4%, and One Mutation identified
| Needs sweat test (accredited CF Center) to confirm CF diagnosis (vs carrier)
| Needs genetic counseling |
| “Probable CF” | IRT top 4%, and Two Mutations identified
| Contact CF center without delay
| CF center will arrange sweat test plus clinical visit within a few days |
ANATOMY OF A VIRGINIA CF-NBS REPORT

Sample identifiers

Reminder that IRT was elevated and thus CF mutation screen was done

Key results

Interpretation and recommendation

CF Center contact info

State NBS program contact information

List of mutations in test panel
December 1, 2011 – March 9, 2012
n=1,103 samples analyzed for CF mutations following IRT above cutoff

<table>
<thead>
<tr>
<th>DNA Results</th>
<th>Virginia number/percent</th>
<th>Wisconsin percent*</th>
</tr>
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<tbody>
<tr>
<td>0 Mutations</td>
<td>1,033 = 93.7%</td>
<td>93.9%</td>
</tr>
<tr>
<td>1 Mutation</td>
<td>66 = 6.0%</td>
<td>4.6%</td>
</tr>
<tr>
<td>2 Mutations</td>
<td>4 = 0.36%</td>
<td>0.35%</td>
</tr>
</tbody>
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1. Stimulation of sweat production

2. Collection of sweat

3. Analysis of chloride concentration of sweat
### Sweat Chloride Results for Infants

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Age &lt;6 months</th>
<th>Age &gt; 6 months</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt; 30</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>Intermediate</td>
<td>30-59</td>
<td>40-59</td>
</tr>
<tr>
<td>Abnormal</td>
<td>≥ 60</td>
<td>≥ 60</td>
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Babies with a positive NBS and intermediate sweat test value should be followed at a CF Center.

Tentative diagnosis for those infants with a positive NBS result and an intermediate sweat chloride result is “CRMS” or “CFTR-related metabolic syndrome”. With serial sweat testing and clinical follow-up, these infants may ultimately be reclassified as either “normal” or as having CF.
If one or two CF mutations are found:

- Explanation of “carrier” vs CF
- Explanation of need for additional mutation testing (if indicated)
- Genetic risk for CF within the family
- Options regarding testing of family members

Who should provide this information?
Genetic counselor, CF provider, primary care physician

Availability and accessibility may be problematic
An important goal of newborn screening is early diagnosis of CF and initiation of specialized CF care (pre-symptomatic, if possible)
Cystic Fibrosis Foundation
www.cff.org

National Human Genome Research Institute
www.genome.gov/10001213

The “Gene Reviews”
www.ncbi.nlm.nih.gov/books/NBK1250/

Virginia Newborn Screening Program website
www.vahealth.org/VNSP
IRT/DNA protocols offer better CF detection than IRT/IRT, but newborn screening will still miss a small number of cases. Order sweat testing if there are symptoms concerning for CF.

Be sure to follow up NBS results promptly, even if they suggest that a carrier situation is most likely. Avoid delays in follow-up.

Genetic counseling should be offered if any CF mutations are found.

Sweat testing should preferably occur at a CF center.

Call your state NBS program or CF center for advice when needed, and consult the cff.org website for additional info or parent-oriented materials.


