Multi-Center Feasibility Study of a Neonatal IRT-PAP Screening Concept for Cystic Fibrosis

Participating Sites:

Wisconsin State Laboratory of Hygiene, USA
Gary Hoffman

New England Newborn Screening Program
Massachusetts State Lab Institute NBS Lab, USA
Anne Comeau, Roger Eaton

LABORATORIO DI RIFERIMENTO REGIONALE PER LO SCREENING NEONATALE, A.O. Istituti Clinici di Perfezionamento, Ospedale dei Bambini, “V. Buzzi”, Milano, ITALY
Paola Vigano, Carlo Corbetta

Universitätskinderklinik Dresden, GERMANY
Marina Stopsack
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OBJECTIVE

Evaluate the effectiveness of an IRT/PAP cystic fibrosis protocol against the more traditional IRT/IRT and IRT/CFTR protocols
Current Cystic Fibrosis Screening Protocols

Immunoreactive Trypsinogen (IRT)
Lacks CF Specificity

Two Tiered Approach
Repeat Newborn screen
Cutoffs at 99 percentile (1% false positives)
IRT decreases with time (variable with CF patients)
Establish 2nd specimen cutoff (difficult)

DNA analysis
3 – 5% are referred to mutation analysis
Expensive
Detects carriers
Consent
The New Kid on the Block
Pancreatitis Associated Protein (PAP)

PAP is elevated at birth in CF newborns

PAP elevation is not strictly specific to CF

PAP is a STRESS protein synthesized by diseased pancreas

PAP measurements alone have no advantage over IRT for CF screening.

A combination of PAP and IRT may improve CF screening

Study Design

Testing Protocol

IRT assay
- routine procedure at each site

PAP assay
- Developed by PerkinElmer life and Analytical Sciences / DynaBio
- On-site training

Precision study
- Six runs
- Calibrators in duplicate
- Controls (two levels)
- 10 results each

Study Period
- Approximately 4,700 specimens
- IRT and PAP analysis
Study Design
Data Collected

Specimen Analyzed:
18,080 Total
~ 4,700 specimens / site

IRT Results

PAP Results

Positive IRT results
CFTR mutation results

Sweat test results

Diagnosis
Principles of the DELFIA PAP assay

SPECIMEN ELUTION

1st incubation

2nd incubation

3rd incubation

Over night

1st incubation

2nd incubation

3rd incubation

Fluorescence measurement

Enhancement Solution

Eu-labelled Streptavidin

Biotinylated anti-PAP IgG

PAP molecule

Solid phase anti-PAP IgG

Eu-labelled Streptavidin

Fluorescence measurement

Enhancement Solution

Eu-labelled Streptavidin

Biotinylated anti-PAP IgG

PAP molecule

Solid phase anti-PAP IgG
## Data Summary

### IRT and PAP concentrations

#### IRT

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean</th>
<th>Median</th>
<th>95%</th>
<th>97.5%</th>
<th>99%</th>
<th>99.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madison - USA</td>
<td>29</td>
<td>25</td>
<td>60</td>
<td>72</td>
<td>90</td>
<td>108</td>
</tr>
<tr>
<td>Boston - USA</td>
<td>29</td>
<td>24</td>
<td>62</td>
<td>75</td>
<td>92</td>
<td>105</td>
</tr>
<tr>
<td>Milan - Italy</td>
<td>23</td>
<td>19</td>
<td>48</td>
<td>65</td>
<td>83</td>
<td>103</td>
</tr>
<tr>
<td>Dresden - Germany</td>
<td>22</td>
<td>19</td>
<td>43</td>
<td>52</td>
<td>65</td>
<td>82</td>
</tr>
</tbody>
</table>

#### PAP

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean</th>
<th>Median</th>
<th>95%</th>
<th>97.5%</th>
<th>99%</th>
<th>99.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madison - USA</td>
<td>0.6</td>
<td>0.4</td>
<td>1.5</td>
<td>2.3</td>
<td>3.7</td>
<td>5.4</td>
</tr>
<tr>
<td>Boston - USA</td>
<td>0.7</td>
<td>0.5</td>
<td>1.6</td>
<td>2.1</td>
<td>3.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Milan - Italy</td>
<td>1.5</td>
<td>1.2</td>
<td>3.7</td>
<td>4.6</td>
<td>6.4</td>
<td>10</td>
</tr>
<tr>
<td>Dresden - Germany</td>
<td>0.6</td>
<td>0.5</td>
<td>1.3</td>
<td>1.6</td>
<td>2.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Combined results of the Feasibility Study

Combined data

- Sweat test positives
- Normal

IRT (ng/mL) vs. PAP (ng/mL)

- Dresden
- Milano
- Boston
- Madison

n = 18080
Combined results of the Feasibility Study

Combined data

- Sweat test positives
- Normal

Milano (157, 41.7)
Dresden

Sweat test positives
Normal

IRT (ng/mL)

PAP (ng/mL)

Milano
Boston
Madison

n = 18080
## Data Summary (Cont)

### Confirmed CF cases (n = 18 050)

<table>
<thead>
<tr>
<th>Site</th>
<th>IRT (ng/mL)</th>
<th>PAP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madison-USA</td>
<td>232</td>
<td>1.24</td>
</tr>
<tr>
<td>Boston- USA</td>
<td>240</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>189</td>
<td>2.56</td>
</tr>
<tr>
<td>Milan-Italy</td>
<td>157</td>
<td>41.6</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>4.82</td>
</tr>
<tr>
<td>Retrospective specimens</td>
<td>159</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>176</td>
<td>2.69</td>
</tr>
<tr>
<td>Dresden-Germany</td>
<td>355</td>
<td>5.47</td>
</tr>
</tbody>
</table>
## Data Summary (Cont)

### Positive rate

#### Current IRT referral

<table>
<thead>
<tr>
<th>IRT/IRT</th>
<th>~1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT/CFTR mutation</td>
<td>~5%</td>
</tr>
</tbody>
</table>

#### IRT and PAP Combination: (Hypothetical Cutoffs)

<table>
<thead>
<tr>
<th></th>
<th>Madison-USA</th>
<th>Boston-USA</th>
<th>Milan</th>
<th>Dresden</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT &gt;50 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAP &gt;1.8 ng/mL</td>
<td>0.57%</td>
<td>0.53%</td>
<td>1.92%</td>
<td>0.06%</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT &gt;100 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAP &gt;1.0 ng/mL</td>
<td>0.27%</td>
<td>0.31%</td>
<td>0.44%</td>
<td>0.09%</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT &gt;50 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAP &gt;1.8 ng/mL</td>
<td>OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRT &gt;100 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAP &gt;1.0 ng/mL</td>
<td>0.69%</td>
<td>0.79%</td>
<td>2.02%</td>
<td>0.13%</td>
</tr>
</tbody>
</table>
Combination of IRT and PAP
IRT/IRT Protocol

Newborns above threshold
IRT ~1%
PAP ~1%
PAP and IRT above threshold
Positive rate ~ 0.25%
### Combination of IRT and PAP

**IRT/CFTR Protocol**

<table>
<thead>
<tr>
<th>Positive rates Specimens referred for CFTR analysis</th>
<th>positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 95% IRT Percentile</td>
<td>4.95</td>
</tr>
<tr>
<td>2: IRT&gt;=50 &amp; PAP &gt;= 1.8</td>
<td>0.74</td>
</tr>
<tr>
<td>3: IRT &gt;= 100 &amp; PAP &gt;= 1.0</td>
<td>0.28</td>
</tr>
<tr>
<td>4: Either 2 OR 3</td>
<td>0.87</td>
</tr>
</tbody>
</table>
**Potential IRT/PAP Advantages**

**IRT/IRT Protocol**
- Decrease repeat specimens
- Reduce parental concerns/stress

**IRT/CFTR mutation**
- Decrease carrier identification
- Decrease mild forms of CF detection
- Decrease costs
- Eliminate out-sourcing needs
Study Conclusions
IRT and PAP Combination

Reduction in Positive Rate
IRT/IRT and IRT/CFTR protocols

Limitations
PAP assay not fully optimized
Small sample size
False negatives could not be addressed

The data collected warrants more study
Acknowledgements

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