IRT Cut-off Levels Related to Age of Sampling

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Background

- Swiss CF-NBS protocol:
  - IRT/DNA/(2\textsuperscript{nd} IRT)
- 2\textsuperscript{nd} IRT collected at various ages

Aim of the study:

Cut-off to be used for 2\textsuperscript{nd} IRT
Swiss CF-NBS algorithm

Screening

- Children with meconium ileus
  - always both tests

IRT measurement on the 4th day of life (Guthrie card)

- ≥ 50 ng/ml
  - No further tests

- < 50 ng/ml
  - No further tests

Screening for 7 CF mutations

- 1 or 2 CF mutations
  - No CF mutation
    - IRT from 1st test ≥ 60 ng/ml
      - Yes
      - No further tests

- ≥ 50 ng/ml
  - Referral to CF centre
  - Further IRT measurement for screening lab

- < 50 ng/ml
  - No further tests

Diagnostic

- Referral to CF centre
  - Sweat test
    - positive, borderline, unclear
    - Additional diagnostic tests: DNA analysis, pancreas elastase, etc.
    - normal
      - No further tests
Neonatal Screening

- **72 – 96 hrs Blood sampling**
  - **1st IRT measurement**
    - 99.3% negative
    - 0.70% positive
  - DNA screening
    - 10.3% with 1 or 2 CF mutations
      - 11.3% of positives referred to CF centre
      - 53.3% without CF diagnosis
      - 46.7% with CF diagnosis
    - 31.8% Ø CF Mutation and IRT <60ng/ml
      - Recall rate=0.48%
    - 57.9% Ø CF Mutation and IRT >60ng/ml
      - Recall rate for 2nd heel prick sample = 0.41%
    - 2nd IRT measurement
      - 98.3% negative IRT<50ng/ml
      - 1.7% 2nd IRT positive (IRT>50ng/ml)

Data Description

• 867 second IRT sampling
  – 1\textsuperscript{st} IRT >60 ng/ml / No “Swiss” mutations
• 55% girls / 45% boys
• GA range: 36 – 43 weeks (at birth)
• BW range: 2200 – 5120 g
• Age range: 11 – 55 days (2\textsuperscript{nd} sample)
• IRT range: 7.6 – 49.8 ng/ml (2\textsuperscript{nd} sample)
# Data Description

N = 867 ($♀ = 477$ $♂ = 390$)

<table>
<thead>
<tr>
<th>NBS Sample</th>
<th>Girls</th>
<th>Boys</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT ng/ml</td>
<td>83.6 (59 – 357)</td>
<td>89.0 (60 – 376)</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control sample</th>
<th>Girls</th>
<th>Boys</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age days</td>
<td>20.4</td>
<td>22.3</td>
<td>ns</td>
</tr>
<tr>
<td>BW g (birth)</td>
<td>3244</td>
<td>3441</td>
<td>0.0004</td>
</tr>
<tr>
<td>GA weeks (birth)</td>
<td>39.6</td>
<td>39.6</td>
<td>ns</td>
</tr>
<tr>
<td>IRT ng/ml</td>
<td>24.2</td>
<td>22.2</td>
<td>ns</td>
</tr>
</tbody>
</table>
Grouped results

- Results were grouped according age at control sampling:
  - Group A: 11 – 21 days
  - Group B: 22 – 28 days
  - Group C: 29 – 35 days
  - Group D: => 36 days
## Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>99.9 percentile</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25.23</td>
<td>8.99</td>
<td>49.8</td>
<td>426</td>
</tr>
<tr>
<td>B</td>
<td>22.79</td>
<td>8.78</td>
<td>49.0</td>
<td>261</td>
</tr>
<tr>
<td>C</td>
<td>19.24</td>
<td>7.80</td>
<td>37.2</td>
<td>111</td>
</tr>
<tr>
<td>D</td>
<td>17.74</td>
<td>5.91</td>
<td>29.6</td>
<td>44</td>
</tr>
</tbody>
</table>

### 1way ANOVA

**Tabular results**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Table Analyzed</td>
<td>Grouped</td>
</tr>
<tr>
<td>Kruskal-Wallis test</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exact or approximate P value?</td>
<td>Gaussian Approximation</td>
</tr>
<tr>
<td>P value summary</td>
<td>***</td>
</tr>
<tr>
<td>Do the medians vary signif. (P &lt; 0.05)</td>
<td>Yes</td>
</tr>
<tr>
<td>Number of groups</td>
<td>4</td>
</tr>
<tr>
<td>Kruskal-Wallis statistic</td>
<td>61.60</td>
</tr>
</tbody>
</table>
Means and percentiles

IRT [ng/ml]

- D11 - 21
- D22 - 28
- D29 - 35
- D=36

- Means (SD)
- 99.9 p
- 99 p
2\textsuperscript{nd} IRT values of Screening Positive cases in relation to the 99.9 Percentile cut-off
SCR positive 2 CFTR
2nd Sample

IRT [ng/ml] vs Days

- CF
- CutOff

W1282X / R347H
SCR positive
1 CFTR
2nd Sample

IRT [ng/ml]

Days

CF
No CF
CutOff

F508del /
SCR positive 1/2 CFTR 2nd Sample

IRT [ng/ml]

Days

F508del / R347H
Conclusions

• IRT levels in the first two months of life decrease significantly.

• Using a fixed cut-off IRT value for classification of results of 2\textsuperscript{nd} sampling specimen without considering the age at sampling, might lead to misinterpretation.

• Use of the data presented here, can help in avoiding this problem.
Conclusions

• A 2\textsuperscript{nd} second IRT sample, taken at the time when sweat testing failed or is not possible, can already help in substantiating a suspicion of CF.
Children’s Hospital Zürich

Swiss Newborn Screening Lab

THANK YOU
Neugeborenen Screening
Dépistage Néonatal
Screening Neonatale
Screening dal Novnaschì