Comparison of use of cutoffs to CLIR in screening for Pompe disease and Krabbe disease

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Krabbe/Pompe Screening Algorithm

Low IDUA or GAA activity (<20% daily mean/CLIR analysis)
Retest in duplicate using same assay and also with 6-plex LSDs

- \( \leq 10\% \) (GALC)
- \( \leq 12\% \) (GAA)
  - DNA testing
  - 1 or more variants*
    - Referral
  - 0 variants
    - Screen negative

- \( \geq 10.0 - 12.0\% \) (GALC)
- \( \geq 12 - 15\% \) (GAA)
  - Compare to other enzymes
    - Adjust if others low
  - Screen negative

- \( > 12\% \) (GALC) of daily mean
- \( > 15\% \) (GAA)
  - Screen negative
**Observation:** when GALC very low (<12%) or very high (e.g. >300%), the other enzymes follow

<table>
<thead>
<tr>
<th>Samples with:</th>
<th>% GALC</th>
<th>% GAA</th>
<th>% IDUA</th>
<th>% GLA</th>
<th>% GBA</th>
<th>% ASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GALC &lt;12%</td>
<td>8.3</td>
<td>60.9</td>
<td>73.2</td>
<td>48.8</td>
<td>64.3</td>
<td>95.2</td>
</tr>
<tr>
<td>GALC &gt;300%</td>
<td>464</td>
<td>130</td>
<td>116</td>
<td>309</td>
<td>136</td>
<td>86</td>
</tr>
</tbody>
</table>
Dried blood spot screening

**Markers:**
Can be present in serum, red cells, white cells or some combination

**Diagnostic tests:** target a specific component of the blood
Dried blood spot variables

Dried Blood Spot variables: not accounted for in calculating marker concentrations

1. Red cells (hematocrit): affects volume of blood in punch: affecting all calculated marker concentrations

2. White cells (leukocytes): contain lysosomes – for LSDs, the measured enzyme activity dependent on number of white cells

3. Exposure to heat, humidity in transport - affect enzyme activities
Variables in dried blood screening

**HCT(%)**

- 26-30 weeks
- 28wk
- 32wk
- Term (cord)
- 1-3d
- 2 week
- 1 month
- 2 month

**Total Leukocytes (x1000/mm³)**

- Birth
- 12hr
- 24hr
- 1wk
- 2wk
- 1mo
- 6mo

Data from The Harriet Lane Handbook

Li, Gelb et al, Clinical Chemistry, 2004
GALC versus birth weight and age: Marker Profile

Profile of GALC activity: vs. bwt and age

Plots from CLIR/ Mayo
Value of multi-marker approach

1. **Biochemical dependency** of markers with biochemical dependencies can be handled (phenylalanine and tyrosine)

2. **Physical effect** of hematocrit and blood filling circle:
   a. for many markers the concentrations will increase with increased hematocrit – simply more blood in 3 mm punch
   b. some marker concentrations will be lower, as less serum in high hematocrit sample punches.

3. **Biological variables**: Markers primarily present in white or red cells

**CLIR**: looks at markers and all possible ratios of markers that are evaluated in the screen. At simplest level, using ratios corrects for variables having a common affect on all markers (Enzymes).

**May also detect other relationships between markers**
# Live Screening Summary

<table>
<thead>
<tr>
<th></th>
<th>Krabbe</th>
<th>Pompe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Started</td>
<td>08-07-06</td>
<td>10-01-14</td>
</tr>
<tr>
<td>Samples Tested</td>
<td>~2,650,000</td>
<td>760,393</td>
</tr>
<tr>
<td>Referrals</td>
<td>485</td>
<td>109</td>
</tr>
<tr>
<td>Infantile cases</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Possible LOKD</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>PPV*</td>
<td>$26/485 = 5.4%$</td>
<td>$55/109 = 50.4%$</td>
</tr>
</tbody>
</table>

To date, none of the infants with a possible case have developed symptoms.
Reminder: Krabbe/Pompe Screening Algorithm

1. Screen enzymes (run CLIR)
   Low IDUA or GAA activity (<20% daily mean)
2. Retest in duplicate using same assay and also with 6-plex LSDs (CLIR analysis)

- ≤ 10% (GALC) ≤ 12% (GAA)
  - DNA testing
    - 1 or more variants*
      - Referral
    - 0 variants
      - Screen negative

- ≥ 10.0 -12.0% (GALC) ≥ 12-15% (GAA)
  - Compare to other enzymes
    - Adjust if others low
  - Screen negative

- > 12% (GALC) of daily mean >15% (GAA)
  - Screen negative

*1 or more variants
Sample flow using CLIR

Initial screen by 3-Plex (Krabbe/Pompe/X-ALD)

- Export data to .csv
- Upload to CLIR (LOINC)

Scores for 3Plex SCT (tool runner)

- Score = 0
- Score = FP
- Score > 0

3Plex DSP (tool runner)

Score = Informative (Low GAA or GALC)

Retest screen by 6-Plex (Krabbe/Pompe/Fabry/Niemann Pick AB/MPS1/Gaucher)

- Export data to .csv

Scores for 7Plex SCT (tool runner)

- Score = 0
- Score > 0

7Plex DSP (tool runner)

Score = Krabbe/Pompe or Indeterminate

2TT (Molecular)

Positive

Negative

Positive Screen

Negative Screen
Limitations of Study

• Retrospective data:
  - Ran all samples through a 3 marker tool (GALC, GAA, C26-LPC)
  - did not run six-plex enzyme tool on all samples that tested low for GALC and GAA.

• We tested many, but not all important positive samples (limited sample quantities)

• Affects how we look at numbers: had to project numbers based on results from a subset of samples that had full testing
## CLIR: Retrospective Case Analysis

<table>
<thead>
<tr>
<th>Disease</th>
<th># Positives tested</th>
<th># False Positives</th>
<th>#infantile cases</th>
<th># Possible Late onsets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krabbe</td>
<td>131</td>
<td>84</td>
<td>6 of 6</td>
<td>13 of 14*</td>
</tr>
<tr>
<td>Pompe</td>
<td>39</td>
<td>8</td>
<td>2 of 2</td>
<td>14 of 14</td>
</tr>
</tbody>
</table>

- All true Krabbe cases detected
- Case definitions are still very important
- In CLIR, can see location specific controls
## CLIR Results compared to Cutoffs

<table>
<thead>
<tr>
<th>Date</th>
<th>NY4 3-Plex</th>
<th>CLIR Retest Two enzymes*</th>
<th>NY (retest)</th>
<th># of Spec Run with 7-Plex tool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Cases</td>
<td>Krabbe</td>
<td>Pompe</td>
</tr>
<tr>
<td>June 2015- Aug 2017</td>
<td>586,763</td>
<td>555</td>
<td>298</td>
<td>5,026</td>
</tr>
<tr>
<td>Retest rate</td>
<td>0.09%</td>
<td>0.05%</td>
<td>0.86%</td>
<td>0.12%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease</th>
<th>CLIR RT*</th>
<th>CLIR second tier 6 enzymes*</th>
<th>NY Cutoffs second tier</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krabbe</td>
<td>555</td>
<td>113</td>
<td>248</td>
<td>-45%</td>
</tr>
<tr>
<td>Pompe</td>
<td>298</td>
<td>183</td>
<td>111</td>
<td>+165%</td>
</tr>
<tr>
<td>Pompe (hybrid)</td>
<td>111 NY (retest)</td>
<td>68</td>
<td>111</td>
<td>-61%</td>
</tr>
</tbody>
</table>

* Projected number –based on reduction rate of subset data (33%)
Objectives of Study

- Can CLIR be easily added to lab work flow ✓
- Compare performance of cutoffs versus CLIR ✓
- Reduce number of required retests ✓
- Reduce number of required second tier tests ✓
  - Big reduction for Krabbe
  - Pompe can use some work/currently “hybrid” approach works better – tool will be re-evaluated
- Reduced false positives, especially for Krabbe with no false negatives ✓
Next Steps

• Continue with prospective study
• Adjust tool to lower number of Pompe retest versus “hybrid” approach
• Evaluate MPS I and other LSDs with CLIR
Questions

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