Baby Power: Improving Congenital Adrenal Hyperplasia Screening Performance with Neonatal Characteristics

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Current CAH screening workflow

DBS Specimen → Tier 1 Testing 17-OHP

- Positive
- Negative

Indeterminate → Tier 2 Testing 17-OHP, RCA, 21DC, 11DC

- Positive
- Negative
# Genetic Disease Screening Program

## CAH current cutoffs (17-OHP) and overall screening performance stratified by birth weight, 2018

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>Tier 1</th>
<th>Tier 2</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Screen Positive</td>
<td>Indeterminate</td>
<td>True Positive</td>
</tr>
<tr>
<td>&lt;1000 g</td>
<td>16</td>
<td>810</td>
<td>0</td>
</tr>
<tr>
<td>1000-1499 g</td>
<td>14</td>
<td>447</td>
<td>0</td>
</tr>
<tr>
<td>1500-2499 g</td>
<td>388</td>
<td>1134</td>
<td>1</td>
</tr>
<tr>
<td>&gt;=2500 g</td>
<td>305</td>
<td>1090</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>723</td>
<td>3481</td>
<td>24</td>
</tr>
</tbody>
</table>

**Table:**

- Tier 1: Screen Positive, Indeterminate, True Positive
- Tier 2: Screen Positive, True Positive
- Overall: Screen Positive, True Positive, PPV
Our endeavor

- Identify cost-effective changes in CAH Tier 1 screening algorithm to improve overall screening performance
- Our goal: assess risk factors and cutoffs →
  - lower the number of false positives called out
  - increase overall PPV
  - Step 1. Retrospective population-based cohort analysis to identify neonatal factors associated with 17-OHP level using multiple regression.
  - Step 2. Presumptive positive only cohort analysis to simulate positive predictive value (PPV) based on the results from Step 1.
  - Identify potential cutoffs with visualizing logistic regression predictive curve
Step 1. Identify the neonatal factors for 17-OHP level

• Using 2017 California NBS data (N≈480,000), 3 random subsets of 50,000 and a 90 percentile subset (for verification purpose)

• Multiple regression model on 17-OHP (natural log transformed) and neonatal factors, including age at collection, sex, gestational age (GA), birth weight, nursery type
  – Interaction between factors also explored

• Results
  – GA is the strongest factor
  – followed by nursery type
  – birth weight is also a significant factor, but not as strong as GA
Correlation between 17-OHP and birth weight/GA

Scatter plot and regression line: birth weight (X-axis) and log 17-OHP (Y-axis)  
\[ R^2 = 0.10 \]

Scatter plot and regression line: GA (X-axis) and log 17-OHP (Y-axis)  
\[ R^2 = 0.20 \]
NICU effect

A combination of prematurity and the presence of neonatal medical conditions potentially lead to higher 17-OHP levels in NICU settings.

<table>
<thead>
<tr>
<th></th>
<th>Presumptive Positive</th>
<th>Indeterminate</th>
<th>True Positive</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NICU</td>
<td>891</td>
<td>2954</td>
<td>7</td>
<td>0.79%</td>
</tr>
<tr>
<td>Non-NICU</td>
<td>89</td>
<td>527</td>
<td>17</td>
<td>19.10%</td>
</tr>
<tr>
<td>Total</td>
<td>990</td>
<td>3481</td>
<td>24</td>
<td>2.42%</td>
</tr>
</tbody>
</table>
17-OHP value and gestational age, by nursery type

- In both NICU and regular nursery, the earlier the GA, the higher the 17-OHP level.
- 17-OHP level is higher in NICU newborns, especially among premature babies.
- **Nursery type difference diminishes among older gestational age newborns.**
17-OHP value and birth weight, by nursery type

- 17-OHP level is higher in NICU low birth weight newborns compared to regular nursery.
- Nursery type difference diminishes among normal birth weight newborns.
- 17-OHP level does not significantly correlate with birth weight among regular nursery newborns.
Conclusion and Reflection for Step 1

• Among neonatal factors, gestational age (GA) has the strongest association with 17-OHP.
  – 17-OHP cutoff adjustment based on gestational age (GA) could lead to improved screening performance.

• Similar results are found using smaller verification subsets, including the 90 percentile sample.

• 17-OHP cutoff based on birth weight could be fine-tuned.

• Nursery type is a moderator for both GA and birth weight in relation to 17-OHP, and could be built into interpretation algorithm.
Step 2. Identify new 17-OHP cutoffs

- Using 2012-2015 and 2017 California CAH presumptive positive data (N≈4,000), confirmed true positive cases (classical) included.
  - Before 2012, gestational age (GA) data are not available
- Logistic regression predictive curve (CAH true positive = 1) sliced with nursery type and gestational age (GA) group
  - To help visualize and locate potential cutoff points
  - Two cutoffs, Tier 1 Positive and Tier 1 Indeterminate
- Using 2016 and 2018 NBS data to validate the performance of the new cutoffs.
  - Further fine-tuning if needed.
Predicting CAH true positive with 17-OHP for non-NICU presumptive positives

It's actually a PPV!

17-OHP=150
Predicting CAH true positive with 17-OHP for non-NICU presumptive positives, GA ≥ 37 weeks

Predicted Probabilities for CAH=1
With 95% Confidence Limits

Tier 1 positive
17-OHP=90

Tier 1 Indeterminate
17-OHP=60
How to spot a cutoff point using retrospective data?

• A decent PPV (Y axis value) based on the predictive curve, at least an observable PPV (> 0)

• Try not to leave too many true positives to be called out in second tier
  – Second tier testing may have false negative

• Be conservative with indeterminate cutoff
Predicting CAH true positive with 17-OHP among all NICU presumptive positives
What does the previous slide tell us?

- Overall, CAH predictive model in NICU is not “perfect”
  - PPV is not observable until 17-OHP=150
  - Difficult to spot a good point for positive cutoff
    - Too many false positives
    - Too many true positives specimens would be called out by Tier 2 testing
- Sliced by gestational age (GA) may help
  - Recall the analysis from Step 1, GA effect is stronger in NICU
Predicting CAH true positive with 17-OHP among NICU presumptive positives, GA ≤30 weeks
Predicting CAH true positive with 17-OHP among NICU presumptive positives, GA 31-36 weeks

Observable

17-OHP = 75
17-OHP = 130

Predicted Probabilities for CAH True Positive, NICU and GA 31-36 Weeks
Potential CAH Tier 1 interpretation algorithm based on nursery type and gestational age

<table>
<thead>
<tr>
<th></th>
<th>Gestational age</th>
<th>Indeterminate (nmol/L)</th>
<th>Positive (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NICU</td>
<td>&lt;30 weeks</td>
<td>120</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>31-36 weeks</td>
<td>70</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>≥37 weeks</td>
<td>65</td>
<td>90</td>
</tr>
<tr>
<td>Non-NICU</td>
<td>&lt;37 weeks</td>
<td>80</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>≥37 weeks</td>
<td>55</td>
<td>90</td>
</tr>
</tbody>
</table>

Current birth weight-based cutoffs

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>Indeterminate</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000 g</td>
<td>80</td>
<td>300</td>
</tr>
<tr>
<td>1000-1499 g</td>
<td>80</td>
<td>200</td>
</tr>
<tr>
<td>1500-2499 g</td>
<td>55</td>
<td>80</td>
</tr>
<tr>
<td>&gt;=2500 g</td>
<td>50</td>
<td>70</td>
</tr>
</tbody>
</table>
The RESULTS: 1\textsuperscript{st} Tier after suggested cutoff

- Using 2016 and 2018 NBS data (N ≈ 1 million screened) as testing datasets, the new cutoffs would yield:
  - 75% reduction in 1\textsuperscript{st} tier presumptive positives (PP).
  - 60% reduction in indeterminate cases for 2\textsuperscript{nd} tier testing.
  - 95% of true positive cases would be called out in the first tier.
  - No missing cases (0 false negative)
  - PPV could increase 400% after 2\textsuperscript{nd} tier screen positives are counted.

<table>
<thead>
<tr>
<th>Year</th>
<th>Indeterminate</th>
<th>1\textsuperscript{st} Tier PP</th>
<th>Indeterminate</th>
<th>1\textsuperscript{st} Tier PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>3315</td>
<td>660</td>
<td>1260</td>
<td>168</td>
</tr>
<tr>
<td>2018</td>
<td>3481</td>
<td>723</td>
<td>1455</td>
<td>171</td>
</tr>
</tbody>
</table>
Conclusions and discussion

• Our existing framework of using birth weight-specific cutoffs is reasonable but needs improvement.
  – 17-OHP is a reliable biomarker for CAH screening except for premature newborns in NICU. (If practical, a re-draw would be desirable.)

• Modifying cutoffs by taking nursery type and gestational age into consideration can significantly reduce false positives and increase PPV.
  – Similar process can be used to find new cutoffs for different birth weight groups.