A False Negative CPTII Case: Using the \((C16+C18:1)/C2\) Ratio to Improve both FN & FP Metrics

Mary Seeterlin, PhD
Michigan Department of Community Health
CPTII - Three Clinical Phenotypes

Lethal Neonatal

- Most severe form
- Symptoms begin within days of birth:
  - Liver failure
  - Respiratory failure
  - Weakened and/or inflamed heart
  - Irregular heartbeat leading to heart attack, kidney disease, and brain abnormalities
- These infants typically die within the first year of life.
Severe Infantile Hepatocardiomyoskeletal

- Symptoms begin between 6 months and 2 years of age
  - Liver failure
  - Weakened and/or inflamed heart
  - Seizures
  - Low blood sugar
  - Abdominal pain
  - Headache
  - Muscle weakness in the arms and legs
  - Irregular heartbeat which can result in sudden death during infancy. Severe episodes are often triggered by fasting, infection, or fever.
Myopathic

- Most common (infancy to adulthood)
- Least severe form
- Symptoms can begin at any time from childhood to one’s 60s.
  - Periodic attacks involving their muscles
  - Muscle pain and weakness.
  - Rhabdomyolysis
  - Brown or red-colored urine


“The MS/MS acylcarnitine profile revealed marked elevations of C16 and C18:1.”
Histogram of C16 (Age of Collection <180 hr., No NICU/TPN, N>500K)

Mean = 2.9

99.9%ile = 8.37
C2 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN=No)

C16 ≥ 8.37
### 2006 – 2011 Disease Logic

<table>
<thead>
<tr>
<th>Disorder Name</th>
<th>Disorder(s)</th>
<th>Primary Analyte(s)</th>
<th>Cutoff 0-179 hrs. old</th>
<th>Action</th>
</tr>
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<tbody>
<tr>
<td>Carnitine: acylcarnitine translocase def./Carnitine palmitoyltransferase II deficiency</td>
<td>CACT/CPT II</td>
<td>C16</td>
<td>&gt;=8.37</td>
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<tr>
<td></td>
<td></td>
<td>C18</td>
<td>&gt;=2.74</td>
<td>S+ Referral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:1</td>
<td>&gt;=3.1</td>
<td></td>
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<td>If 2 of the 3 analytes above the cutoff</td>
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If 2 of the 3 analytes above the cutoff, B+ Repeat Screen.
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr, NICU= No, TPN=No)

- C16 ≥ 8.37
- C18:1 ≥ 3.10

Legend:
- Normal
- Referred - Normal
- B+FU Normal
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN=No)

- C16 ≥ 8.37
- C18:1 ≥ 3.10

Legend:
- Normal
- CPT II
- Referred - Normal
- B+ FU Normal
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN=No)

**2006 - 2011**

<table>
<thead>
<tr>
<th>True Positives</th>
<th>False Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>a = 1</td>
<td>c = 23</td>
</tr>
</tbody>
</table>

Positive Predictive Value: 4.17%
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr, NICU= No, TPN=No)

- C16 ≥ 8.37
- C18:1 ≥ 3.10

Legend:
- Normal
- CPT II
- Referred - Normal
- B+ F U Normal
CPTII False Negative Case

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Age (hrs.)</th>
<th>C16 (μM) (≥8.37)</th>
<th>C18 (μM) (≥2.47)</th>
<th>C18:1 (μM) (≥3.10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>6.0</td>
<td>1.61</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(≥3.4)</td>
<td>(≥1.71)</td>
<td>(≥2.91)</td>
</tr>
<tr>
<td>2</td>
<td>298</td>
<td>1.49</td>
<td>1.00</td>
<td>0.82</td>
</tr>
</tbody>
</table>
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN=No)

C16 ≥ 8.37

C18:1 ≥ 3.10

- Normal
- CPT II
- Referred - Normal
- B+FU Normal
- False Negative CPTII
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN= No)

- C16 ≥ 6.00?
- C18:1 ≥ 1.82?
2006 - 2011

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<td>( a = 2 )</td>
<td>( c = 1443? )</td>
</tr>
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</table>

Positive Predictive Value \( .14\% \)
Screening for carnitine palmitoyltransferase II deficiency by tandem mass spectrometry

K. Gempel\*, S. Kiechl\^2, S. Hofmann\^1, H. Lochmüller\^4, U. Kiechl-Kohlendorfer\^3, J. Willeit\^2, W. Sperl\^5, A. Rettinger\^1, I. Bieger\^1, D. Pongratz\^4, K. D. Gerbitz\^1 and M. F. Bauer\^1

**Figure 2** Distribution of ($\text{C}_{16:0} + \text{C}_{18:1}$)/$\text{C}_2$ ratios among patients with CPT II deficiency, patients on total parenteral nutrition including lipids (Intralipid), fasting healthy volunteers and patients with muscular symptoms similar to CPT II deficiency (Outpatients). The ratio was found to be significantly higher in CPT II patients, compared to non-CPT II patients with similar symptoms. The reference range is indicated by the dashed lines.
C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr, NICU= No, TPN=No)
C2 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN= No)
C2 vs (C16+C18:1) (NeoGram, N > 300,000, Age < 180 hr, NICU= No, TPN= No)

\[
\frac{(C16+C18:1)}{C2} \geq 0.40
\]

- Normal
- CPT II
- Referred - Normal
- BHFU Normal
- False Negative CPT II
2006 – 2011

C16 ≥ 6, (C16+C18:1)/C2 ≥ 0.40

<table>
<thead>
<tr>
<th>True Positives</th>
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</tr>
</thead>
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<tr>
<td>(a = 2)</td>
<td>(c = 1)</td>
</tr>
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Positive Predictive Value 66.7%
Missed Newborn Screening Case of Carnitine Palmitoyltransferase-II Deficiency.

Edmondson AC\textsuperscript{1,2}, Salant J\textsuperscript{2}, Lerardi-Curto LA\textsuperscript{1,2}, Ficioglu C\textsuperscript{3,4}.

**Abstract**

Carnitine palmitoyltransferase-II (CPT-II) deficiency can be detected through newborn screening with tandem mass spectrometry. We report a 4-year-old patient with rhabdomyolysis due to CPT-II deficiency, which was initially missed by newborn screening. The patient presented with a 2-day history of fevers, upper respiratory infection, diffuse myalgia, and tea-colored urine. Her medical history was notable for frequent diffuse myalgia when ill. She was demonstrated to have homozygous mutation c.338C>T, p. S113L in CPT2, which is typically found in the adult-onset, myopathic form of the disease. An unknown number of CPT-II deficient patients with normal newborn screening have not yet presented to medical care with the adult-onset, myopathic form of disease. We conclude that (1) not all cases of CPT-II deficiency are currently detected through newborn screening, even when blood is appropriately collected on day 2 of life and (2) CPT-II deficiency should be kept on the differential for patients presenting with rhabdomyolysis, even if the newborn screening results were normal.
Missed Newborn Screening Case of Carnitine Palmitoyltransferase-II Deficiency.

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Author information

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**C16 rule:** if case value is <90th percentile of reference range (4.17) → set case score to zero

<table>
<thead>
<tr>
<th>Differentiators</th>
<th>C0(C16+C18)</th>
<th>C3/C16</th>
<th>C12</th>
<th>C8</th>
<th>C0</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case value</td>
<td>3.24</td>
<td>0.11</td>
<td>0.13</td>
<td>0.07</td>
<td>14.52</td>
<td>13.12</td>
<td>0.38</td>
</tr>
<tr>
<td>1st ile RR</td>
<td>2.91</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99th ile RR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Overlap RR-DR</td>
<td>4%</td>
<td>9%</td>
<td>27%</td>
<td>81%</td>
<td>95%</td>
<td>98%</td>
<td>2%</td>
</tr>
<tr>
<td>Tool score</td>
<td>NI</td>
<td></td>
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Edmondson AC1,2, Salant J2, Ierardi-Curto LA1,2, Ficicioglu C3,4.
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C18:1 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU= No, TPN=No)

C16 ≥ 6.00?

C18:1 ≥ 1.82?
C3 vs C16 (NeoGram, N > 300,000, Age < 180 hr., NICU=No, TPN=No)

- Normal
- CPT II
- Referred - Normal
- B+FU Normal
- False Negative CPT II
- Fidioğlu FN

C16/C3 ≥ 8.0
C2 vs \((C16+C18:1)\) (NeoGram, \(N > 300,000\), Age < 180 hr., NICU= No, TPN= No)
CPTII False Negative Case

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<th>C16 (μM)</th>
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<tr>
<td>1</td>
<td>24</td>
<td>8.7</td>
<td>6.06</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>160</td>
<td>12.2</td>
<td>1.10</td>
<td>0.50</td>
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C16 vs Age of Collection (No NICU, No TPN)
Conclusions

- C16 and C18:1 are not elevated for the myopathic form of CPTII
- Cases of the myopathic form of CPTII are being missed by Newborn Screening
- Using ratios:
  \[
  \frac{\text{C16+C18:1}}{\text{C2}} \quad \frac{\text{C16}}{\text{C3}}
  \]
  - Will Reduce False Negatives
  - Will Reduce False Positives
Conclusions

- C16 Normalizes on FU specimens for the myopathic form of CPTII
- All CPTII presumptive positives should be Referred for FU testing
- No B+ Repeat DBS
Acknowledgments

Co-authors
• Eleanor Stanley, NBS Metabolic Unit Manager
• Harry Hawkins, NBS Section Manager

Children's Hospital of Michigan Metabolic Clinic
• Dr. Robert Conway, CHMMMC Clinic Director

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• 517-373-9779
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