The Axis of Contamination

Dextrose and Amino Acids

False Positives due to TPN for VLBW Infants

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The title I would like to use…

FAST FOOD FOR PREEMIES
* Hypothesis:
  * Extremely high concentrations of amino acids in DBS are **not** due to a biochemical defect.

  * False Positive Results are **in part** due to mixing (contamination) of IV feeds (total parenteral nutrition solutions) that contain very high concentrations of amino acids.

  * A marker for contamination by TPN would help identify infants whose DBS sample is invalid / unacceptable and reduce presumptive positives.
    * Would not reduce repeat sampling in the short term.
    * Reduce repeat sampling in the long term by identifying laboratories that do not collect samples properly.
Brief History

* High FP rate for premature infants historically.
  * even with better analytical instruments and methods.

* False Positives characterized by very high Leu/Ile, Phe, Met, Ala but not Tyr.
  * Interpretation guidelines in some labs act on milder elevations of Tyr and/or Met but not on much higher concentrations of Met when Leu/Phe/Ala are also high.

* Clinical trial of 100 premature infants with multiple collections points on TPN did not have these very abnormal profiles.

* Examination of all very high AA profiles revealed markers in the acylcarnitine profiles that were not acylcarnitines.
  * Process of elimination indicated dextrose
  * Mass spectra indicated a carbohydrate
  * Mayo suggested dextrose in an abstract at an SIMD meeting.
What is TPN?

- Source of Energy
  - DEXTROSE (D-glucose)
    - 5, 10 and 12.5% (% = g per 100mL, dL)

- Source of Protein
  - Free L-Amino Acids
    - 2 – 4 g per kg per day
  - Proprietary mix of AA

- Isotonic Saline
  - Salts, minerals etc...
Unusual m/z values in the AC-BE Profiles (Pre 85)

* Nearly all false positive results with high amino acid concentrations had the following markers in the acylcarnitine profile:

\[ m/z: \quad 325 \quad 399 \quad 473 \]

* Dextrose suspected based on
  * Product ion profile had fragmentation profiles similar to carbohydrates.
  * Eliminated other components of TPN

* Conclusive evidence for dextrose not easily obtained because:
  * Analysis of pure dextrose did not show markers
    * (subsequently found that preparation in same manner of a DBS did show markers)
  * m/z values did not add up to dextrose (MW = 260)
Pre – 85 (AC profile)
Dextrose  – *(prepared in same manner as DBS – butyl esterification)*
Solution: Isotope Labeled D-glucose (m/z 180)

Isotope Labeled D-glucose (m/z 180)

Dextrose (MS scan)

$^{13}$C$_6$H$_{12}$O$_6$ 186 Da

$^{13}$C$_6$-Dextrose (MS scan)

Shift of fragments by 12 Daltons

Can only occur if “2” dextrose molecules

Found a dextrose monomer at m/z 237
<table>
<thead>
<tr>
<th>m/z</th>
<th>Dx</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>Formula</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>237</td>
<td>Dex</td>
<td>Butyl</td>
<td>-</td>
<td>-</td>
<td>$[C_{10}H_{21}O_6]^+$</td>
<td>butyl ether</td>
</tr>
<tr>
<td>325</td>
<td>Dex</td>
<td>Dex-H$_2$O</td>
<td>-</td>
<td>-</td>
<td>$[C_{12}H_{21}O_{10}]^+$</td>
<td>Dimer – water</td>
</tr>
<tr>
<td>399</td>
<td>Dex</td>
<td>Dex</td>
<td>Butyl</td>
<td>-</td>
<td>$[C_{16}H_{31}O_{11}]^+$</td>
<td>Dimer + butyl ether</td>
</tr>
<tr>
<td>417</td>
<td>Dex</td>
<td>Dex</td>
<td>Butyl</td>
<td>-</td>
<td>$[C_{16}H_{33}O_{12}]^+$</td>
<td>237 + 180</td>
</tr>
<tr>
<td>473</td>
<td>Dex</td>
<td>Dex</td>
<td>Butyl</td>
<td>Butyl</td>
<td>$[C_{20}H_{41}O_{12}]^+$</td>
<td>2 x 237 − H$^+$</td>
</tr>
</tbody>
</table>

**Butyl ethers with dextrose!**

**Monomers, dimers and trimers – Oh My!**
DEXTROSE IDENTITY CONFIRMED

BUT WAS THE CRIME SCENE CONTAMINATED?
Classic High FP from a Preemie DBS

- **Internal Standards**

- Acylcarnitine Profile (Pre 85)
  - C3
  - C4
  - C5
  - C14
  - C16
  - C18:1
  - C18

- Amino Acid Profile (NL 102)
  - Ala
  - Leu+Ile
  - Met
  - Phe
  - Tyr

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**+Prec (85.00): Exp 2, 0.479 to 1.148 min from Sample 1 (Sciex-5) of ar2010023660.wiff (Ion Spray)**

Max. 2.3e4 cps.

**+NL (102.10): Exp 3, 0.513 to 1.182 min from Sample 1 (Sciex-5) of ar2010023660.wiff (Ion Spray)**

Max. 1.8e6 cps.
DBS spiked with TPN for Comparison

1:40 TPN/Blood (TPN 12.5% Dex, 2.5 g/kg/day amino acids)

Internal Standards

Internal Standards

Acylcarnitine Profile (Pre 85)

Amino Acid Profile (NL 102)

1:40 dilution of TPN solution in Blood before making DBS
Dextrose Marker Quantification – Amino Acid Relationship

Can I use data in my interpretation?

Is it linear?

What is the detection limit?

Is there a direct relationship between Dextrose and Amino Acids?
Dextrose Blood Concentration vs Sum of Dextrose Marker Pseudo-concentrations

Dextrose Marker Quantification

Note:
Diluted TPN solutions

- 10%
- 12.50%
- 5%
- Control (0)
- Linear (10%)
- Linear (12.50%)
- Linear (5%)
Methionine blood Concentrations (a representative marker of amino acids)

Methionine Quantification

Note:
Diluted TPN solutions

- 3 g/kg/day
- 2.5 g/kg/day
- 4 g/kg/day
- Control (0)

 Linear (3 g/kg/day)
 Linear (2.5 g/kg/day)
 Linear (4 g/kg/day)

Met (umol/L)

endogenous

Met added umol/L
A bit more data....

Note that a 1:80 contamination increases Leu+Ile above most cutoffs and Met and Phe are borderline. Tyr remains normal.

1:80 is a detectable dex marker concentration.

Dextrose / Amino Acids correlate with calculated concentrations in TPN.
* Molar Ratios

* As the TPN contamination increases, the molar ratios of amino acids approach a signature TPN profile rather than a normal endogenous profile

* Reducing FP for PKU via an elevated Phe/Tyr ratio does not work.
  * As published – a secondary ratio (Phe/Leu) is necessary to rule out PKU in preemies. These results confirm why.

<table>
<thead>
<tr>
<th>3.0 g/kg</th>
<th>Phe/Tyr</th>
<th>Phe/Leu</th>
<th>Leu/Phe</th>
<th>Met/Phe</th>
<th>Leu/Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous</td>
<td>1.12</td>
<td>0.35</td>
<td>2.86</td>
<td>0.39</td>
<td>0.41</td>
</tr>
<tr>
<td>1:5 saline/endo</td>
<td>1.08</td>
<td>0.35</td>
<td>2.88</td>
<td>0.38</td>
<td>0.41</td>
</tr>
<tr>
<td>1:80</td>
<td>2.48</td>
<td>0.22</td>
<td>4.50</td>
<td>0.53</td>
<td>1.15</td>
</tr>
<tr>
<td>1:5</td>
<td>7.26</td>
<td>0.20</td>
<td>5.00</td>
<td>0.68</td>
<td>2.05</td>
</tr>
</tbody>
</table>
Approach for use in the NBS lab...

- Verify markers on your instrument...
  - Ionization efficiency of instruments vary thus the three markers may also vary in relative intensity
    - It is why the sum of the markers was used in the study

- An exact determination of the increase concentration of amino acids cannot be made based on the dextrose marker sum.
  - It can be approximated based on information of what was given to infant.

- Further study in ongoing in a 1000 premature infant clinical trial where the marker is measured at 5 different time points.
Conclusions

* Detection of dextrose markers together with elevated amino acids indicate that a DBS was contaminated by TPN solution.
  * Profile does not reflect infants metabolism

* Higher concentrations of TPN contamination more closely reflect the TPN solution.

* It is likely that dextrose markers may be more frequent in certain collection facilities/nurseries.
  * An opportunity to revisit collection procedures

* False positive rates reduced. Short term follow-up / repeat sample increased. Long term follow up /repeat sample reduced with improved collection.
Tandem mass spectrometric identification of dextrose markers in dried-blood spots from infants receiving total parenteral nutrition

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b Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mail Stop F-19, Atlanta, GA 30341, USA

Detection of TPN contamination of dried blood spots used in newborn and metabolic screening and its impact on quantitative measurement of amino acids

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b Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mail Stop F-19, Atlanta, GA 30341, USA
Evidence Suggest Markers are Present in UNDERIVATIZED METHOD USING HYDRAZONE

Hydrazine derivatizes succinylacetone and...

New Markers at m/z 177,195,339,357

This crime (not doing butylesters) is under investigation!

PS – if you do butyl esterification and hydrazine you get even more markers! Just what we need in our profiles!
AMERICA’s MOST Wanted .... (Scientists at the CDC)

Partners in Crime Research – The Line-Up spot check