Newborn Screening of Premature Infants: Clinical Trial Data Suggests Gestational Age and Chronological Age are Key to Interpretation

D. CHACE, R. CLARK, A. SPITZER, A. KELLEHER, PEDIATRIX MEDICAL GROUP, SUNRISE, FL
Objectives

To describe the influence that gestational age and chronological age have on amino acid and acylcarnitine profiles in an at risk population of premature infants.
Patient Samples: 3,579
Premature Infants Studied: 995
Collection Times (Days <1,7,24,42): 4
Metabolic Disorders Confirmed: 0
The Analyses

Tandem Mass Spectrometry

Amino Acid Profile (NL 102, butyl esters)
Acylcarnitine Profile (Pre 85, butyl esters)

179,500 data points (3,579 x 50 metabolites)
Gestational Ages of Premature Infants

- 23 – 31 weeks

3 Groups
- Group 1: 23 to 26 weeks (n = 293)
- Group 2: 27 to 28 weeks (n = 277)
- Group 3: 29 to 31 weeks (n = 425)

Median Birth Weight
- 1057 grams (644–1514 g)

Study performed at 23 sites in 17 states
- 1 Central lab performed all tests
- PE Genetics
- Interpretation of results
- Pediatrix Analytical

Samples collected using NBS consensus protocols
- State data shared from all abnormal results.
- Received 15/17 state alert values
Nutrition

**Parenteral (IV) Nutrition**
- 2 Hours (median, initiated)
  - Dextrose, Amino Acids
- 24 Hours
  - Lipids
- 33% of patients
  - Carnitine
    - Increased use with Decreased gestational age
- Major Source of Nutrition < 7 days

**Enteral Nutrition**
- > 24 Hours (median, initiated)
  - Human Breast Milk, Formulas
- Day 7
  - % Enteral > Parenteral
  - Highest % AA and Lipids
- Day 42
  - All Enteral
  - Max nutrition
Study Results
Amino-Acids and Ratios

Median (10-90th) Z score vs Day (1-42)

Quick Peak
Amino Acid Example: Leu + Ile

From NBS Data

Primarily TPN

Primarily Enteral
Acylcarnitines (OA) Example: Isovaleryl Carnitine

From NBS Data

Primarily TPN

Primarily Enteral

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Quantiles90, Quantiles10, Median

Terms:
- 0.2
- 0.4
- 0.6
- 0.8
- 1
- 1.2

Weeks:
- 23 to 26
- 27 to 28
- 29 to 31

Data Primarily TPN

Data Primarily Enteral
Acylcarnitines (FA) Example: Linoleyl Carnitine

Quantiles
- 90th Percentile
- 10th Percentile
- Median

Term
- 23 to 26 weeks
- 27 to 28 weeks
- 29 to 31 weeks

From NBS Data

Primarily TPN
Primarily Enteral
Generalized Findings (AA and AC)

MS/MS Study (AC and AA)
- 214 alerts (21.5% based on standard NBS interpretation protocols)
  - 29% from Group 1 • 17% from Group 2 (*all days*)
  - 12% Day 1 • 2% Day 28 (*all groups*)
  - Highest rate of abnormal results were on day 7 from Group 1

State NBS Screening (all tests)
- 461 alerts% (21.5% based on standard NBS interpretation protocols)
  - Premature Infants had most abnormal results: 64% versus 36% term
  - T4/TSH and CAH: highest percentage of abnormal results.
  - MS/MS: 8.9% and 2.6% for AC and AA, abnormal results respectively

Study and State NBS results were similar in terms of abnormal % and metabolite elevations (harmonization)?
Key Amino Acid Findings

- **Phe**
  - Higher in the 23-26 week group 1 than other groups.
  - Above median at day 1 (62 µM). Median day 7 (55 µM).
  - **Well below median days 28-42 (38 µM).**
  - Phe/Tyr ratio above median day 7 and below median days 28-42.
  - Tyrosine below median day 7, above median day 1

- **Citrulline**
  - Increased with time for all groups (At days 28-42, 40% greater than day 1 and 7). Lowest on day 7.
  - Interesting note *(Citrulline not contained in protein or amino acid solution in TPN)*
    - Made in small intestine, potential marker of gut health (low in unhealthy or abnormal GI tract)
Key Acylcarnitine Findings

- Organic acid acylcarnitines higher in group 1 (C5, C5:1, C4DC, C5OH, C5DC) compared to group 2 and 3.

- Most ACs higher on day 0 than decrease through day 7. More variable pattern days 28-42.

- **Linoleic Acid**
  - Significant increase by day 7. Return to normal levels days 28-42
  - Due to Intralipid in TPN. Direct correlation between Intralipid C18:2 content and C18:2 acylcarnitine

- Free Carnitine
  - Supplemented patients with L-carnitine showed highest FC on day 7 (40 µM versus 18µM)
  - Higher FC on day 1 compared to normal. Lower FC on day 7 for non supplemented patients)
Summary

- 21% of infants had an abnormal alert value.
- 0% could be explained by TPN contamination with amino acids.
- Cutoff values inappropriate for premature infants (median values 1 SD above and below medians)
  - Normative values must correct for time and gestational age.
  - Must also correct for illness and nutritional support.
- The most premature infants metabolically different than most mature premature infants.
- Organic acid acylcarnitines, branched chain amino acids, long chain acylcarnitines highest on day 7.
  - All feed into beta oxidation pathway.
  - Elevating more toxic metabolites.