NOVEL DIAGNOSTIC CONSIDERATIONS IN NEONATAL SEPSIS

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Neonatal Sepsis Classification

- **Early-Onset**: acquired by transplacental, ascending or intrapartum transmission in the perinatal period shortly before or during birth (up to postnatal (PN) day 3)
- **Late-Onset**: acquired by horizontal transmission in the hospital, at home, or in the community (after PN4)

Early Onset Sepsis (EOS)
- Remains a relatively common problem
- Worldwide: 1-10/1,000 live births
- Preterm: 20-30/1,000 live births
- Accounts for 10-15% of all neonatal deaths

Risk Factors for Early Onset Sepsis (EOS)

Maternal Risk Factors for EOS
- Maternal recto-vaginal GBS colonization
- Maternal infection at time of labor and delivery
- Labor and delivery at <37 weeks gestation
- ROM >18 hours
- Intrapartum temperature >38 C
- Chorioamnionitis

Background Diagnosis
- Given their potential impact, sepsis evaluations and antimicrobial treatment while awaiting culture results (which can take at least 2 days) are a common practice in the NICU
- No consensus statement for choice or duration of antibiotic therapy while awaiting results
- In a survey of 61 US NICUs, 56% of neonatologists reported continuing antimicrobials for at least 2 days pending blood culture results

Background
Diagnosis

- In the YNCH NICU, from 7/1/2008 through 6/30/2009:
  - 460 evaluations were performed for EOS
  - 424 of these neonates were hospitalized in the NICU and exposed to antimicrobials for at least 48 hours
  - 3 had positive blood and/or CSF cultures (0.65%)

Background
Diagnosis

- Blood culture is the current gold standard
- In a post-mortem study, 18% of infants who died of a bacterial infection had negative blood cultures
- Problems with blood culture include:
  - Unable to obtain adequate blood sample
  - 60% of cultures may be falsely negative with blood volumes of 0.5 ml
  - Low bacterial load in neonates
  - Exposure to maternal antibiotics

Infection: Inflammatory response

Many investigators have looked to hematologic measures, cytokine profiles, and novel techniques for detection of micro-organisms to more accurately and expeditiously make or exclude the diagnosis

Novel Approaches to Diagnosis of EOS

- PROTEOMICS: AMNIOTIC FLUID
  - Lower complexity than maternal blood
  - Relates to compartmentalization of the disease process
  - Reflects better the state of the fetus than maternal blood
Mass Restricted scoring

Select diseased set
Select non-diseased set

Mass (Daltons)

MR SCORE: BIOMARKER IDENTIFICATION

- P1 defensin 2 (HNP-2)
- P2 defensin 1 (HNP-1)
- P3 calgranulin C (S100 A12)
- P4 calgranulin A (S100 A8)

3.3 kDa
10.8 kDa

Antimicrobial peptides involved in the innate immunity

PROTEOMICS – MR SCORE

Biomarkers:
- P1 = Defensin 2
- P2 = Defensin 1
- P3 = S100 A12 (EN-RAGE)
- P4 = Calgranulin A

INFLAMMATION

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR score 3-4</td>
<td>93</td>
<td>92</td>
<td>82</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>Glucose</td>
<td>61</td>
<td>93</td>
<td>77</td>
<td>87</td>
<td>84</td>
</tr>
<tr>
<td>LDH</td>
<td>79</td>
<td>95</td>
<td>84</td>
<td>93</td>
<td>91</td>
</tr>
<tr>
<td>Gram stain</td>
<td>35</td>
<td>92</td>
<td>63</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td>IL-6</td>
<td>45</td>
<td>95</td>
<td>75</td>
<td>83</td>
<td>82</td>
</tr>
<tr>
<td>MMP-8</td>
<td>93</td>
<td>67</td>
<td>50</td>
<td>96</td>
<td>74</td>
</tr>
</tbody>
</table>
Amniotic Fluid MR Score and EOS


Table 6. Performance of the four proteomic biomarkers in identifying infants and neonates with confirmed neonatal sepsis

<table>
<thead>
<tr>
<th>Sero</th>
<th>Score</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Bacteria</td>
<td>-</td>
<td>98 (25-69)</td>
<td>94 (85-94)</td>
<td>90 (68-95)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>B. Staph.</td>
<td>-</td>
<td>95 (94-95)</td>
<td>90 (86-93)</td>
<td>86 (79-90)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C. Coag.</td>
<td>-</td>
<td>80 (64-98)</td>
<td>85 (79-90)</td>
<td>75 (41-90)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>D. Other</td>
<td>-</td>
<td>75 (66-83)</td>
<td>75 (66-83)</td>
<td>65 (49-81)</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Amniotic Fluid MR Score and EOS

Neonatal Diagnosis: Molecular Assays

- Polymerase chain reaction:
  - A molecular tool for DNA amplification
  - In neonates, PCR has been utilized to screen for genetic disorders and to diagnose viral infections
  - The use of PCR to diagnose bacterial infection in the neonate has also been studied with a turn-around time reported of 4-6 hours

- Polymerase chain reaction:
  - Jordan et al.* were able to validate this technique in term neonates.
  - Using PCR on neonatal whole blood inoculated with various species of bacteria, the investigators could accurately differentiate between diverse bacterial groups
  - The same group compared PCR as a diagnostic technique with blood culture in near term (>34 weeks’ gestation) neonates
  - Blood cultures were collected under sterile techniques
  - Left-over blood from the CBC was used for PCR

Culture Results

<table>
<thead>
<tr>
<th>PCR Results</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>1186</td>
<td>1196</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>1216</td>
<td>1233</td>
</tr>
</tbody>
</table>

Overall agreement of 96.8%

Negative predictive value of PCR was 99.2%, specificity 97.5%

Of the 10 PCR negative, culture positive cases:
-4 were GBS
-4 were CONS
-2 were S. viridans
-1 was Micrococcus spp.

Cases of Late-Onset Sepsis per 1,000 NICU Admissions, 1979-2012
Scientific Basis for Quantitative PMN CD64 as an Improved Diagnostic Test of Sepsis

- High-affinity Fcγ receptor I on surface of nucleated blood cells
- PMN CD64 expression is negligible in the healthy state (<2,000 molecules per cell) - low false positive rate
- PMN CD64 becomes elevated in the presence of infection/sepsis
  - Final cytokine pathway effect at cellular level – barometer of sickness
  - Better performance than cell counts, left shift, and CRP
- High specificity – PMN CD64 expression is not elevated in:
  - Malignancy of myeloid cells (CML, MPD, MDS)
  - Any drug therapy (other than cytokines)
  - Clinical conditions with localized tissue damage (myocardial ischemia, uncomplicated surgery, and exercise injury)
  - Pregnancy
  - Auto-immune disorders (Rheumatoid Arthritis, Systemic Lupus Erythematosus)

PMN CD64 as a Sepsis Marker in Neonates


Similar reports by:

PMN CD64 Index correlates best with CRP and presence of sepsis

Inflammatory Markers in Neonates

Neonatal Sepsis at Yale, 1928 to 2012

Clinical features used to identify patients for LOS work-ups:

1. Respiratory compromise (tachypnea (respiratory rate of > 60/min); increased spnea (cessation of respiration ≥ 20 seconds occurring at a rate of >2 per hour); severe spnea (any single episode requiring positive pressure ventilation); increased ventilatory support (with no other obvious cause, e.g. pneumothorax); and desaturation (pulse oximetry readings ≤ 85%));
2. Cardiovascular compromise (bradycardia (heart rate <100 beats per minute); pallor; decreased perfusion (capillary refill ≥ 3 seconds or cold extremities); hypotension;
3. Metabolic changes (hypothympia (rectal temperature < 36°C), hyperthermia (rectal temperature > 38°C); feeding intolerance (increased gastric residuals of >30% of food volume in at least 2 feedings within 24 hours); glucose instability (blood glucose <45 or >125 mg/dL); metabolic acidosis (pH < 7.25));
4. Neurologic changes (lethargy; hypotonia; decreased activity).
**Study Protocol**

- Most recent CBC used for flow cytometry
- Blood sample collected in a lavender top tube or a microtainer (EDTA)
- Assay requires 50 uL whole blood

**Hematologic criteria used as categorical indicators for sepsis:**

- (1) Absolute neutrophil count (ANC) <7500 or >14500/mm^3
- (2) Absolute band count (ABC) >1500/mm^3 by manual differential
- (3) Immature to Total neutrophil ratio (I/T ratio) >0.16 - dependent on band count
- (4) Platelet count <150,000/ mm^3.
- Positive sepsis score ≥ 2 criteria = suspected sepsis

**RESULTS**

- 293 separate episodes of sepsis work-ups
- Confirmed (n=10) and suspected sepsis episodes (n=118, total n=128) compared with no sepsis (n=165) episodes
- As expected, sepsis characterized by:
  - higher WBC
  - higher ANC
  - higher ABC
  - higher I/T ratios
  - lower platelet counts.
- Sepsis episodes had higher neutrophil CD64 indices.

**Characteristics of the sepsis episodes**

<table>
<thead>
<tr>
<th>Variables</th>
<th>No Sepsis (n=165)</th>
<th>Sepsis present (n=128)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at sepsis work up (days) *</td>
<td>12.3 ± 1.5</td>
<td>22.1 ± 3.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>42.7 ± 0.8</td>
<td>41.7 ± 0.9</td>
<td>0.371</td>
</tr>
<tr>
<td>White Blood Cell Count (WBC) *</td>
<td>14.0 ± 0.5</td>
<td>10.2 ± 1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Segmented neutrophils (%) *</td>
<td>41.9 ± 1.3</td>
<td>40.9 ± 1.5</td>
<td>0.596</td>
</tr>
<tr>
<td>Bands (%)</td>
<td>4.5 ± 0.3</td>
<td>14.0 ± 0.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (k/mm^3) *</td>
<td>285.3 ± 8.5</td>
<td>176.0 ± 8.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Absolute Neutrophil Count (ANC) *</td>
<td>6324 ± 326</td>
<td>8929 ± 704</td>
<td>&lt;0.0004</td>
</tr>
<tr>
<td>Absolute Band Count (ABC) *</td>
<td>698 ± 60</td>
<td>2716 ± 242</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Immature/Total neutrophil ratio *</td>
<td>0.05 ± 0.00</td>
<td>0.14 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD64 index *</td>
<td>2.83 ± 0.20</td>
<td>3.41 ± 0.85</td>
<td>&lt;0.0002</td>
</tr>
</tbody>
</table>

*Mean ± SEM

**LOS: ROC curves**

- For all sepsis (n=128) episodes, CD64 had an AUC of 0.74

**CD64 for all sepsis episodes [confirmed and suspected]**

- Using the ROC-defined cutoff value of 2.30 for the CD64 index:
- CD64 in combination with the standard ANC criteria had the following:
  - Highest negative predictive value (93%) for ruling out sepsis.
  - 95% sensitivity for diagnosing sepsis.
Confirmed Sepsis in neonates: ROC curves

For culture-positive sepsis episodes, CD64 had the highest AUC (0.852) of all hematologic variables, with a sensitivity of 80%, and a specificity of 79%, using the ROC-defined cutoff value of 4.02.

2nd CD64 Study: Demographics


2nd CD64 Study: ROC curves for culture-proven EOS and LOS


SUMMARY: 1st CD64 Study

- CD64 index had the highest AUC compared to commonly utilized hematologic parameters (including band count and I/T ratio) to diagnose confirmed neonatal sepsis
- The combination of the CD64 index and the ANC criteria, without any other hematologic indices, demonstrated 95% sensitivity for all sepsis episodes and a 93% incidence for predicting which episodes did not fit septic criteria

SUMMARY: 2nd CD64 Study

- For culture-proven sepsis, using a cut-point CD64 Index value of 2.38 for EOS, the test had a sensitivity of 100%, a specificity of 68%, and an NPV of 100%
- For culture-proven sepsis, a cut-point CD64 Index value of 3.62 for LOS, the test had a sensitivity of 75%, a specificity of 77%, and an NPV of 96%
CONCLUSIONS: EOS

- **Proteomics:**
  - Promising technique with rapid turnaround
  - High specificity and negative predictive value
  - Low sensitivity
  - Cost is an issue

- **Molecular Assays:**
  - Meta-analysis of 23 studies utilizing molecular assays for detecting neonatal sepsis
  - Mean sensitivity and specificity were 90% and 96%, respectively
  - Real-time PCR and broad-range conventional PCR had higher sensitivity and specificity than other assays
  - Molecular assays do not have sufficient sensitivity to replace microbial cultures in the diagnosis of neonatal sepsis but may perform well as adjunctive tests


CONCLUSIONS: EOS and LOS

- **PMN CD64:**
  - Promising technique with rapid turnaround
  - High sensitivity and negative predictive value
  - Low specificity
  - Given the high NPV value of CD64, it has strong potential to influence the initiation, early termination and duration of antibiotic therapy
  - Larger sample size needed for combination studies with Hematological Indices and PCT
  - Enhanced CD64 i.e. monocyte/neutrophil CD64 may be another useful approach
Results

There were 293 separate episodes of sepsis work-ups in 163 infants.

Infants with sepsis episodes (confirmed or suspected, n=40) were compared to those (n=123) with no sepsis:

- **Sepsis was associated with**:
  - higher gestational age
  - similar birth weights and Apgar scores at 1 and 5 minutes.

- There was no difference in the duration of hospitalization in the 2 groups.


<table>
<thead>
<tr>
<th>Variables</th>
<th>No Sepsis (n=123)</th>
<th>Sepsis present (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)*</td>
<td>32.55 ± 0.47</td>
<td>34.72 ± 0.95</td>
<td>0.031</td>
</tr>
<tr>
<td>Birth weight (gms)*</td>
<td>1969 ± 94</td>
<td>2325 ± 200</td>
<td>0.058</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>68 (55)</td>
<td>24/39 (62)</td>
<td>0.492</td>
</tr>
<tr>
<td>Vaginal delivery (%)</td>
<td>98 (80)</td>
<td>33 (80)</td>
<td>0.826</td>
</tr>
<tr>
<td>1-min. Apgar score**</td>
<td>7 (1 - 9)</td>
<td>8 (1 - 9)</td>
<td>0.876</td>
</tr>
<tr>
<td>5-min. Apgar score**</td>
<td>8 (1 - 9)</td>
<td>9 (3 – 9)</td>
<td>0.419</td>
</tr>
<tr>
<td>Ventilation days*</td>
<td>14.0 ± 2.3</td>
<td>12.0 ± 4.8</td>
<td>0.674</td>
</tr>
<tr>
<td>NCPAP days*</td>
<td>3.6 ± 0.3</td>
<td>2.3 ± 1.0</td>
<td>0.290</td>
</tr>
<tr>
<td>Days on Oxygen*</td>
<td>10.1 ± 2.8</td>
<td>10.8 ± 6.3</td>
<td>0.865</td>
</tr>
<tr>
<td>Length of Stay(days)*</td>
<td>50.7 ± 3.6</td>
<td>26.8 ± 7.1</td>
<td>0.156</td>
</tr>
</tbody>
</table>

*Mean±SEM; ** Median (range); NCPAP: nasal continuous positive airways pressure