**Procalcitonin:**
A reliable predictive biomarker to diagnose early-onset neonatal sepsis?

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**Learning Objectives:**
1. Describe early-onset neonatal sepsis (EONS) and the role of procalcitonin (PCT)
2. Discuss consequences of inappropriate antibiotic use in neonates
3. Evaluate literature of PCT’s reliability as a biomarker for infection in neonates
4. Develop an algorithm for most dependable PCT drawn levels in the neonatal intensive care unit (NICU)
I. Neonatal sepsis

A. Incidence

i. Sepsis occurring in the first 3 days of life is a leading cause of morbidity and mortality among infants in the U.S.\(^1\)

ii. Mortality rates worldwide vary from 3-50% of live births annually\(^2,4\)

iii. Estimated national U.S. incidence in 1995 of severe sepsis in all neonates:\(^5\)

1. 3.60 per 1000 population
2. 10.3% case fatality

iv. U.S. annual estimate of invasive EONS between 2005-2008:\(^1,6,7\)

1. About 3,300 cases, including 390 deaths
   a. 1,600 cases among preterm infants with 360 deaths
   b. 1,100 cases among black infants

2. Pathogens
   a. Group B *Streptococcus* (GBS) is the leading infectious cause (~ 490 cases)
      i. Incidence declined after introduction of GBS perinatal prevention guidelines in early 2000s\(^8,9\)
      1. 1.7 cases /1,000 live births in early 1990s to 0.34–0.37 cases /1,000 live births in late 2000s
      2. Case-fatality ratio as high as 50% in 1970s decreased to 4-6% in recent years
   b. *E. coli* is the leading pathogen associated with fatal outcome (~ 90 deaths)

B. Definition

i. International pediatric sepsis consensus conference\(^10\)

1. 20 experts in sepsis and clinical research from Canada, France, Netherlands, United Kingdom, and United States modified adult definitions of systemic inflammatory response syndrome (SIRS), infection, sepsis, severe sepsis, septic shock and organ dysfunction for pediatrics

2. Pediatric definitions are similar to adult definitions but depend on age-specific heart rate, respiratory rate, and white blood cell count cutoff values\(^10,11\)

   i. For example, SIRS is defined as the presence of at least 2 of the following 4 criteria (one must be abnormal temperature or leukocyte count): (1) core temperature (2) tachycardia or bradycardia (3) increased respiratory rate (4) leukocyte count elevated or depressed

   ii. ‘Clinical sepsis’- lack of uniform definition\(^17-20\)

      1. Negative body-fluid cultures PLUS clinical signs of sepsis (as per institution)

C. Neonatal Sepsis Classifications: Early-Onset versus Late-Onset Sepsis\(^3,4,9,12,13\)

i. Most common organisms are GBS and gram negative bacteria

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Neonatal Sepsis Classifications(^3,4,9)</th>
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<tbody>
<tr>
<td><strong>Type of Sepsis</strong></td>
<td><strong>Defined Time of Onset</strong></td>
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</table>
| EONS* | Within first 7 days of life (usually < 48-72 hrs) | Maternal genital tract | Term neonates: GBS, Enterococci, *L. monocytogenes*  
Preterm neonates: GBS, *E. coli*, *H. influenzae*, gram-negative bacilli |
| Late-Onset Sepsis (LOS) | Within first month of life (varies, >48 hrs to >7 days of age) | Maternal genital tract or environment/community | GBS, *S. aureus*, Coagulase-negative staph (*S. epidermidis*), *Serratia*, *Klebsiella*, *Pseudomonas*, *Candida* species |
| Very-late-onset Sepsis | After 1 month of life | Environment: i.e. prolonged NICU stay |

* Little consensus as to what age limits apply, with EONS ranging from 48 hours to 6 days after delivery
D. Risk factors of EONS\textsuperscript{3,4,9,12-14}

i. GBS infection during pregnancy
   1. Women who previously delivered baby who developed invasive GBS sepsis or have positive cultures during current pregnancy
   2. Approximately 30\% of pregnant women have vaginal or rectal colonization
   3. Without maternal chemoprophylaxis, 1-2\% of infants develop invasive infection

ii. Preterm delivery < 37 weeks gestation or low birth weight

iii. Premature (prelabor) rupture of membranes (PROM)\textsuperscript{15}
   1. Membrane rupture before onset of uterine contractions
   2. Prolonged PROM >18-24 hours increases risk of infection

iv. Chorioamnionitis\textsuperscript{9}
   1. Microbial invasion of amniotic fluid most often associated with PROM >18-24 hours
   2. Clinical syndrome of intrauterine infection including:
      a. Maternal fever >100.4°F PLUS at least 2 of the following criteria:
         i. Uterine tenderness
         ii. Foul-smelling vaginal discharge/amniotic fluid
         iii. Maternal leukocytosis
         iv. Maternal and/or fetal tachycardia
   3. Diagnosed by amniotic fluid analysis or placenta pathologic exam

E. Diagnosis of EONS\textsuperscript{14}

i. Clinical signs are nonspecific and indistinguishable from those of noninfectious diseases making it difficult to diagnose\textsuperscript{6,13,16-20}
   1. Asymptomatic bacteremia is uncommon, but may occur
   2. Symptomatic patients presenting with nonspecific signs: hypothermia or hyperthermia, irritability, lethargy, apnea, and bradycardia

ii. No ideal marker or single test for early diagnosis\textsuperscript{6}

iii. Blood culture is considered the gold standard\textsuperscript{2,18,21}
   1. Minimum of 1-2 mL of blood obtained via umbilical artery catheter or peripheral vein
   2. Time consuming: results of culture in 24-48 hours
   3. Can yield false-positive results due to contamination or false-negative due to inappropriate amount of blood collected

iv. Lumbar puncture\textsuperscript{14}
   1. Performed in any infant with a positive blood culture, complicated clinical course or lab data suggesting bacterial sepsis, or worsening symptoms with antimicrobial therapy
   2. Cerebral spinal fluid (CSF) values
      a. Higher CSF white blood cell (WBC) counts may be due to gram-negative organisms
      b. Low CSF glucose concentration has greatest specificity for diagnosis of meningitis

v. Biomarkers\textsuperscript{14,18}
   1. WBC count and differential count\textsuperscript{20}
      a. Total WBCs have poor positive predictive value (PPV)
      b. Neutrophil indices useful in excluding infants without infection
         i. Absolute immature counts have poor sensitivity and PPV
         ii. Immature/total neutrophil ratio (I/T ratio) has poor PPV but high negative predictive value (NPV)
   2. Platelet counts
      a. Low counts are nonspecific, insensitive marker and late indicator of sepsis

3. C-reactive protein (CRP)\textsuperscript{14,17,20,22}
   a. Concentration increases within 6-8 hours of infectious cause and peaks at 24 hours
   b. Low sensitivity at birth, but improves 24-48 hours after birth
c. High NPV if concentrations persistently normal, therefore bacterial sepsis unlikely
d. Upper limit of normal cutoff level ≤ 10 mg/L in the first days of life
e. Liver synthesis of CRP induced by interleukin-6 (IL-6)
f. Lower CRP response to infection in pre-term compared to term newborns

4. Pro-inflammatory cytokines: IL-6, IL-8, tumor necrosis factor-α (TNF-α)
   a. IL-6 has high sensitivity during early states of sepsis
   b. Short half-life of IL-6 leads to rapid normalization even if infection persists
c. Same kinetics with IL-8 and TNF-α
d. IL-6 and IL-8 vary with gestational age

5. PCT
   a. Increases within 2 hours of infectious cause, peaks at 12 hours, then normalizes in 2-3 days in adult volunteers
   b. Physiologic increase in serum concentration within first 24 hours of birth

vi. Sepsis Screening Score Systems
1. Hematologic scoring system
   a. Rodwell and colleagues described a scoring system in which a score of 1 was assigned to 1 of 7 findings: (1) abnormalities of leukocyte count, (2) total neutrophil count, (3) increased immature polymorphonuclear count (PMNs), (4) increased I/T ratio, (5) immature to mature PMN ratio > 0.3, (6) platelet count < 150,000/mm³, (7) degenerative changes in PMNs
   b. Score ≥ 3: no sepsis in 90% of term infants and ~60% of preterm infants
c. Poorer sensitivity and NPV after birth than at 24 hours of age

2. Töllner Scoring System
   a. Based on retrospective analysis of 83 neonates and prospective studies of 39 neonates with sepsis, 183 neonates as control group, 42 with amniotic infection, 28 with post-asphyxia syndrome and 28 premature babies with cerebral hemorrhage
   b. Symptoms before, at the beginning, and at the peak of septicemia were studied and a score system was created on the basis of clinical and hematological symptoms

<table>
<thead>
<tr>
<th>Score &lt; 5</th>
<th>Score 5-10</th>
<th>Score ≥ 10</th>
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<tbody>
<tr>
<td>no sepsis</td>
<td>possible sepsis</td>
<td>clinical sepsis</td>
</tr>
</tbody>
</table>

F. Antibiotic treatment of EONS
   i. Standard empirical therapy: ampicillin PLUS aminoglycoside (gentamicin)
   ii. 3rd generation cephalosporin (cefotaxime) is alternative to aminoglycoside
      1. Rapid development of resistance has been reported
      2. May use in addition to aminoglycoside with meningitis caused by gram-negative organisms until susceptibility is known
   iii. Treatment Duration
      1. If culture negative, (usually 48-72 hours to obtain results), discontinue antibiotics
      2. If bacteremic with no identifiable focus of infection, treat for 10 days
      3. If uncomplicated meningitis due to GBS occurs, treat for a minimum of 14 days
   iv. GBS Prevention: Maternal Intrapartum Antibiotic Prophylaxis
      1. Indicated in the following situations:
a. Positive antenatal cultures for GBS scheduled for vaginal delivery
b. Unknown maternal colonization status with gestation < 37 weeks, prolonged PROM > 18 hours, temperature > 100.4°F
c. GBS bacteriuria during current pregnancy
d. Previous infant with invasive neonatal sepsis

2. Prophylaxis with penicillin (drug of choice) or ampicillin given > 4 hours before delivery
a. Alternatives if penicillin allergic: cefazolin (if nonserious allergy), clindamycin, or vancomycin

II. Inappropriate antibiotic use

A. Iatrogenic effects
   i. Prolonged antibiotic therapy > 5 days in infants with suspected EONS and negative blood culture associated with an increased risk of death, necrotizing enterocolitis (NEC), and LOS
   ii. Altered gut colonization and increased risk Candida colonization
   iii. Separation of mother and child
   iv. Pain from IV punctures

B. Promoting selective pressure for the emergence of multi-drug resistant organisms
   i. Hyde et al. conducted a population-based surveillance study in San Francisco and Atlanta from 1998-2000, that revealed a significant increase in resistance of *E. coli* to ampicillin among preterm infants from 29% in 1998 to 84% in 2000. Possible contributing factors:
      1. Maternal exposure to antibiotics may select for resistant organisms
      2. Antibiotic resistance in community-acquired *E. coli* infections in neonatal population
   ii. A prospective study by Shah and colleagues conducted in India in 2011 of NICU infants showed:
      1. 75% of isolates were ampicillin resistant
      2. 90% of gram negative isolates had increased resistance against amikacin and gentamicin
      3. 14% of gram negative isolates were extended spectrum beta-lactamase (ESBL) producers
      4. 29% were carbapenemamase producers
   iii. In 2005, under-resourced countries of Asia and Africa reported:
      1. 70% of neonatal sepsis isolates were resistant to ampicillin and gentamicin
      2. 51% of *Klebsiella* species were ESBL producers
      3. 38% of *S. aureus* strains were methicillin resistant staph aureus (MRSA)

C. Increased health care costs
   i. A multicenter retrospective cohort study found that preterm infants receiving antibiotics for 7 days or more had a significantly longer average length of hospitalization and more ventilator days compared with infants who received 3 days or less.
   ii. Costs incurred from antibiotic treatment, laboratory costs, hospital staffing, and longer hospitalization stay

D. Challenges in antimicrobial stewardship
   i. Differentiating true infection versus inflammatory non-infectious causes
      1. Low blood volume obtained from neonates (often <1 mL) decreases sensitivity of blood culture
      2. Lack of reliable, gold standard biomarkers to rule-out sepsis

III. Procalcitonin (PCT)

A. Definition
   i. 116 amino acid peptide divided into 3 sections: amino terminus of PCT, immature calcitonin, and katacalcin (CCP-1)
   ii. Increases significantly during systemic bacterial and fungal infections but not viral infections

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*Figure 1. PCT Structure*
B. Pathophysiology\textsuperscript{23}
   i. Encoded by CALC-1 gene located on chromosome 11
   ii. Expressed in tissue-specific manner (produced predominantly by monocytes and hepatocytes)
      1. PCT is synthesized to smaller peptides and mature calcitonin which is stored in secretory granules and secreted in the blood to regulate calcium concentrations
      2. Absence of infection: CALC-1 gene transcription in non-neuroendocrine tissue is suppressed except for thyroid gland C cells producing the precursor of calcitonin in healthy and non-infected individuals
      3. Presence of infection: non-neuroendocrine tissue (parenchymal tissue and differentiated cell types) express the CALC-1 gene to produce increased levels of PCT; function of this increase is currently unknown

C. Kinetics in neonates
   i. In uninfected, healthy neonates there is physiologic increase that occurs over the first few days of life due to direct stress on baby or adaptation to extrauterine environment\textsuperscript{16-20}
      1. PCT peaks at 18-30 hours of life then normalizes at 42-48 hours
      2. Short half-life ~24-30 hours in peripheral blood\textsuperscript{18,23}
   ii. PCT increase not dependent on gestational age\textsuperscript{16,17,20}
   iii. In healthy subjects, PCT concentration rises rapidly, within 3-4 hours, in response to bacterial endotoxin and reaches max concentration at ~18-24 hours; remains elevated for at least 24-48 hours\textsuperscript{32}
   iv. Non-infective events may cause increase in circulating PCT concentrations
   v. For sepsis, most studies in adults and children use an optimum cut-off value of 0.5 to 2 ng/mL\textsuperscript{16}
   vi. Neonatal age-specific reference values should be considered\textsuperscript{16-20}

D. PCT Assays\textsuperscript{33}
   i. Quantitative and qualitative (semi-quantitative) assays available for measuring PCT
      1. Qualitative tests: rapid test strips for point-of-care testing (results available in < 30 minutes)
      2. Quantitative tests: use luminescence immunoassay (results available in a few hours)
         NOTE: Studies being evaluated used the LumiTest PCT (BRAHMS, Hennigsdorf, Germany) as their assay
   ii. Unknown if quantitative testing yields similar results to semi-quantitative testing
   iii. 3 FDA approved PCT quantitative assays commercially available
      1. Assay labeled indication: for use in with other laboratory findings and clinical assessments to aid in the risk assessment of critically ill patients for severe sepsis and septic shock
      2. Blood or plasma samples used with a minimum volume of 20-50 microliters\textsuperscript{17-20}
      3. Lowest detection limit: 0.1 – 0.3 ng/ml
   iv. Cost\textsuperscript{20,44}
      1. Moderate cost
         a. Depends on institution and whether or not it is a send out lab versus in-hospital lab
            i. In-hospital lab ~$25-30 (Methodist Hospital- San Antonio)

E. Role in neonatal sepsis
   i. Early detection of absence of infection\textsuperscript{18}
      1. A single center, prospective study conducted in France in NICU infants with clinical suspicion of late-onset sepsis (after 72 hours of life) found that a PCT cut-off value of 0.6 ng/ml provided a sensitivity, specificity, PPV and NPV of 100%, 65%, 67%, and 100% respectively. Therefore a rapid measurement of PCT could help rule out nosocomial infection in newborn infants.\textsuperscript{34}
   ii. Decrease inappropriate antibiotic use\textsuperscript{18}
      1. Decreases unnecessary risk to patients
      2. Decrease multi-drug resistant (MDR) organisms
      3. Decrease healthcare expenditures by shortening hospital stay and reducing antibiotic duration
4. Studies using PCT-guided decision-making on antibiotic duration
   a. Another single-center, prospective, randomized intervention study (n =121) conducted in a Switzerland NICU provided moderate evidence that PCT guidance reduces the use of antibiotic therapy for EONS. Antibiotic duration was overall reduced by 22.4 hours (22.0%) and a 27% reduction in neonates on antibiotics for > 72 hours.35,36
   b. A larger cohort study is currently being conducted to test the reliability of a PCT-based strategy in neonates (age 0-3 days of life) with EONS. Primary endpoints are the duration of antibiotic therapy and the proportion of infants with a recurrence of infection along with a secondary outcome measure of hospital length-of-stay.2

IV. Literature Review

A. Clinical question: How reliable is PCT as a biomarker in diagnosing early onset neonatal sepsis?

B. Literature criteria:
   i. Newborn infants in NICUs
   ii. Included studies with infants < 7 days old and defined EONS as 7 days of life or less
   iii. Excluded studies with late-onset sepsis (defined as > 48-72 hours) because there was no method of abstracting results of patients who were < 7 days old
   iv. Endpoints: PCT concentration sensitivity, specificity, PPV, and/or NPV

C. Four Studies for Literature Review of PCT Reliability
   iii. Altunhan et al, 2011. Procalcitonin measurement at 24 hours of age may be helpful in the prompt diagnosis of early-onset neonatal sepsis.
### Table 3: Reliability of Procalcitonin Concentration for the Diagnosis of Sepsis in Critically Ill Neonates

<table>
<thead>
<tr>
<th>Objective</th>
<th>Evaluate the reliability of PCT serum concentrations for early- and late-onset sepsis diagnosis in NICU setting</th>
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<tbody>
<tr>
<td><strong>Methods</strong></td>
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<tr>
<td><strong>Study Design</strong></td>
<td>• Prospective study in Rome, Italy from February 1996 to February 1997</td>
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<td>• Case-control study during same period evaluating effects of noninfectious complications from extended NICU hospitalization on PCT concentrations for diagnosing late-onset infection</td>
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<td>• PCT analyzed using LumiTest® PCT kit (limit of detection = 0.08 ng/mL) completed in 2 hrs using 40 μL of serum</td>
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<td><strong>Endpoints</strong></td>
<td>• Define normal PCT ranges for healthy neonates during first 48 hrs after birth</td>
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<td></td>
<td>• Compare PCT levels for noninfectious complications in NICU patients to established reference range</td>
</tr>
<tr>
<td></td>
<td>• Determine sensitivity/specificity of PCT concentrations for EONS diagnosis for first 48 hrs after birth</td>
</tr>
<tr>
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<td>• Determine sensitivity/specificity of PCT concentrations for the diagnosis of sepsis 3-30 days after birth</td>
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<thead>
<tr>
<th><strong>Patient Selection</strong></th>
<th><strong>Period 1 (0-48 hrs of age)</strong></th>
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<tbody>
<tr>
<td><strong>First Group:</strong> healthy neonates</td>
<td><strong>Period 2 (3-30 days of age)</strong></td>
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<tr>
<td><strong>Group 0:</strong> Uncomplicated pregnancy/labor and normal postnatal course x 3 days</td>
<td><strong>Included:</strong></td>
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<tr>
<td><strong>Second Group:</strong> all symptomatic preterm and term neonates evaluated for sepsis</td>
<td>• Presented with systemic infectious conditions during their NICU stay</td>
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<tr>
<td><strong>Group 1A:</strong> Early-onset documented infection◊</td>
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<tr>
<td>o 1 or more + blood cultures</td>
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<tr>
<td>o Clinical signs of infections◊</td>
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<tr>
<td>o CRP findings of pneumonia</td>
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<tr>
<td><strong>Group 1B:</strong> ‘Clinical septicemia’</td>
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<tr>
<td>o Negative body-fluid cultures</td>
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<tr>
<td>o Positive sepsis screen◊</td>
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<tr>
<td>o Clinical signs of infection◊</td>
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<tr>
<td>o CRX findings of pneumonia</td>
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<tr>
<td><strong>Group 2:</strong> No infection</td>
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<td>o Presented with various types of distress but apparently well within 48-72 hrs</td>
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<tr>
<td><strong>Group 3:</strong> Uncertain – not included in groups 1 or 2</td>
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<tr>
<td>o Negative body-fluid cultures</td>
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<tr>
<td>o Less than 3 clinical signs of infection◊</td>
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<tr>
<td>o 1 or none abnormal values for sepsis screening◊</td>
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* All PCT levels drawn between 0-48 hrs of age were recorded and timed for both study populations @ 0.6,12,18,24,30,36,42,48 hrs

1 Infection diagnosis defined as ≥ 2 of the following abnormal sepsis screening criteria: (1) WBC count (2) absolute neutrophil count (3) immature/total neutrophil ratio (4) CRP level > 1.0 mg/dL

◊ Clinical signs of infection defined as ≥ 3 of the following categories: apnea/tachypnea/cyanosis/respiratory distress; bradycardia/tachycardia; hypotonia/seizures; poor perfusion/hypotension; irritability/lethargy/poor feeding; or hepatosplenomegaly/jaundice/abdominal distension

<table>
<thead>
<tr>
<th><strong>Baseline Characteristics</strong></th>
<th><strong>Period 1:</strong> No significant differences among groups in regards to gestational age (mean ~34-35 weeks); however, patients in group 2 had significantly lower birth weights (2 kg vs 2.5 kg) [P &lt; 0.01]. Healthy neonates were significantly larger (average birth weight of 3.2 kg) and more mature (average gestational age of 39 weeks) [P &lt; 0.001]</th>
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<tbody>
<tr>
<td><strong>Period 2:</strong> No significant differences among groups regarding gestational age (~32-33 weeks) and birth weight (~1.6 kg)</td>
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<tr>
<th><strong>Statistical Analysis</strong></th>
<th><strong>Period 1 (0-48 hrs of age)</strong></th>
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<tbody>
<tr>
<td>• All statistical tests based on significance level p ≤ 0.05</td>
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<tr>
<td>• Multiple linear regression analyses performed to determine association between the PCT response during the first 48 hrs and variables i.e. age, birth weight, gestational diabetes, prolonged rupture of membranes &gt;24 hrs, etc.</td>
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<thead>
<tr>
<th><strong>Results</strong></th>
<th><strong>First Group:</strong> 83 healthy neonates</th>
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<tbody>
<tr>
<td><strong>Period 1:</strong></td>
<td>124 levels drawn (Soon after birth: &lt; 0.08 - 0.7 ng/mL; Peak levels @ 21-24 hrs: 0.6 - 21 ng/mL; At 48 hrs: &lt; 0.08 - 2 ng/mL)</td>
</tr>
<tr>
<td><strong>Second Group:</strong> 126 newborns admitted to NICU - 6 excluded for serious congenital malformations</td>
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**Group 1A:** 14 neonates with culture proven sepsis
- 5 neonates with CRX + pneumonia

**Group 1B:** 14 neonates with negative cultures but positive sepsis screens and clinical signs
- All 14 born to mothers receiving antibiotics
- 4 neonates with CRX + pneumonia

**PCT levels:** 54 levels drawn
- 50 levels above upper limits of reference ranges
- No infants with EONS delivered by mothers with GDM
- Sensitivity = 92.6%; Specificity = 97.5%; PPV = 94.3%; NPV = 96.8%

**PCT vs CRP on initial evaluations:**
- 24/28 infants abnormal PCT levels (Sensitivity 85.7%)
- 4 levels falsely-negative but demonstrated abnormal values within subsequent 18-24 hrs
- 13/28 infants abnormal CRP levels (Sensitivity 46.6%, P = 0.004)
- 15 infants’ levels subsequently proved abnormal value within 24-48 hrs

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TABLE 3. Reliability of Procalcitonin Concentration for the Diagnosis of Sepsis in Critically Ill Neonates (continued)

Results cont.

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<thead>
<tr>
<th>Second Group:</th>
<th>Period 1 (0-48 hrs of age)</th>
</tr>
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</table>
| • **Group 2**: 75 neonates presented with 1 or more conditions, no clinical/lab evidence of infection  
  o Received antibiotics ≤ 3 days  
  o 4 neonates had abnormal CRP levels | PCT levels: 137 levels drawn  
  • 16% fell outside the established reference range; 17 levels above the 97.5 percentile  
  • If age and GDM only 2 variables analyzed, then PCT values were significantly higher for neonates born to diabetic mothers [P < 0.05] |
| • **Group 3**: 17 neonates in whom systemic infection not confirmed nor excluded  
  o Antibiotics administered ≥ 6 days | PCT levels: 20 levels drawn  
  • 11 levels above reference range  
  • Sensitivity/specificity not calculated |

<table>
<thead>
<tr>
<th>Period 2: (3-30 days of age)</th>
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</table>
| • 23 cases and 92 controls  
  • 20/23 case infants had positive blood cultures, clinical signs of septicemia, and positive sepsis screens  
  • 3 infants had negative blood cultures but diagnosed with NEC | PCT vs CRP on initial evaluations:  
  • Abnormal PCT levels [P <0.001] (sensitivity/specificity=100%)  
    o 23/23 Cases (mean conc. = 42 ng/mL, range 2.0-249 ng/mL)  
    o 92/92 Controls (mean conc. = 0.2 ng/mL, range 0.08-1.0 ng/mL)  
  • Abnormal CRP levels  
    o 7/23 Cases missed abnormal value (sensitivity = 69.5%)  
    o Subsequently abnormal 24-48 hrs after onset of sepsis | PCT levels returned to normal (< 1.0 ng/mL) in 21 survivors 3-7 days after initiation of appropriate antibiotic therapy  
  Patients with infections caused by coag-negative staph (n=11) had lower PCT concentrations (~4.4 ng/mL) as compared with other systemic infections (~63 ng/mL) [P < 0.001] |

Limitations
Small sample size; single-center study

Author’s Conclusion
PCT appears to be a highly specific and sensitive marker for early-onset neonatal sepsis as well as an effective laboratory marker for accurately identifying NICU patients early in the course of late-onset sepsis.

Critique
**Strengths**: prospective study; blind classification of infants; detailed infant grouping; separate analyses for each group; separation of time periods in an attempt to distinguish from early- and late-onset neonatal sepsis  
**Weaknesses**: small sample size; subsequent PCT levels not enforced; timing of antibiotic initiation not discussed

Clinical Significance
Results strengthen reliability of using PCT as a biomarker to monitor for infants in the NICU and can help ruling in/out neonatal sepsis.

NICU = neonatal intensive care unit; PCT = procalcitonin; CRP = C-reactive protein; EONS = early-onset neonatal sepsis; CXR = chest X-ray; GDM = gestational diabetes;  
PPV = positive predictive value; NPV = negative predictive value; NEC = necrotizing enterocolitis
### TABLE 4. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection.


<table>
<thead>
<tr>
<th>Objective</th>
<th>To investigate variation of diagnostic biomarker values due to differences in population baseline severity and risk status as well as specific ante- and perinatal variables, independent of the presence of neonatal infection.</th>
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</thead>
</table>
| Methods   | **Study Design**<br>• Prospective study conducted over 6-month period (July-December 2001) from 2 NICUs in Rome, Italy<br>• Illness severity scores: SNAP and SNAP-PE*; Ante- and perinatal data recorded; cultures and CRX<sup>‡</sup><br>• Routine blood sampling of CRP, IL-6, and PCT at birth (cord blood), 24 and 48 hrs of life<br>• Designation of infection status made retrospectively, yet done in a blinded fashion to the biomarker values<br>• Biomarker assays: IL-6 (Endogen™) detection limit <1 ng/L; PCT (LumiTest PCT™) detection limit 0.1 mcg/L<br>• ROM >18 hrs, maternal GBS colonization, uterine tenderness, foul smelling/cloudy amniotic fluid; preterm labor; intrapartum antimicrobial administration<br>• Weaknesses: biomarker assays: IL-6 associated to ante-natal and antibiotic treatment, placental/fetal abnormalities, fetal presentation, mode of delivery, duration of active labor, use of anesthesia, birth asphyxia (defined as need of ET ventilation or CPR)<br>• Risk factors for infection: maternal fever, PROM >18 hrs, maternal GBS colonization, uterine tenderness, foul smelling/cloudy amniotic fluid; preterm labor; intrapartum antimicrobial administration<br>• Clinical signs of infection defined ≥ 3 of the following categories: temp instability (hyper/hypothermia); respiratory (grunting, intercostal retractions, apnea, tachypnea, cyanosis); CV (bradycardia/tachycardia, poor perfusion, hypotension); neurologic (hypotonia, lethargy, seizures); GI (feeding intolerance, abdominal distention)<br>**Endpoints**<br>• Sensitivity/specificity of CRP, IL-6, and PCT; association between biomarkers, disease severity/ ante-/intrapartum variables<br>• **Patient Selection**<br>- **Group 1**: early onset infection; **Group 2**: no infection; **Group 3**: uncertain<br>• **Baseline Characteristics**<br>No significant difference in gestational age (~33 weeks), birth weight (2.2 grams), and gender between infected/uninfected<br>• **Statistical Analysis**<br>All comparisons of CRP, IL-6, PCT values & all regression analyses done after logarithmic transformation (normal distribution)<br>Log-linear regression used for association between disease severity, ante- and intrapartum variables, & biomarker concentration<br>Geometric mean with 95% CI of infected versus uninfected neonates compared by t-test; Statistically significance (P ≤ 0.01)<br>**Results**<br>219 critically ill neonates: Excluded patients (15 preadmission deaths, 3 lethal abnormalities, 16 cord blood samples not containing all 3 biomarkers)<br>Final enrollment = 185 neonates (insufficient blood sampling in 31 neonates) ➔ 154 neonates potentially available for analysis<br>**Group 1**: 19 infants (9 term and 10 preterm), received ABX tx > 5days<br>• 11 positive cultures w/ clinical signs<sup>†</sup><br>• 8 w/ persistent ‘clinical septicemia’: all mothers received ABX before delivery (n=4), GBS culture (n=2), or PROM (n=2)<br>**Group 2**: 115 asymptomatic infants: negative body cultures, apparently well within 24-48 hrs, received ABX tx ≤3 days<br>**Group 3**: 20 infants (excluded from study analysis)<br>134 neonates available for analysis: infected (n= 15) vs noninfected (n=115)<br>**Biomarkers (95% CI):**<br>- CRP (mg/L) [P<0.0001]<br>- IL-6 (ng/L) [P<0.0001]<br>- PCT (mcg/L) [P<0.0001]<br>**Status:**<br>- Infected<br>- Uninfected<br>**CRP, mg/L**<br>- At birth ≥ 4<br>  73 (43-90)<br>  74 (51-88)<br>19 (71-203)<br>  3.3 (3.1-3.5)<br>  622.7 (203.4-621.9)<br>19.9 (16.1-26.6)<br>  3.79 (1.7-8.43)<br>  0.22 (0.18-0.27)<br>**IL-6, ng/L**<br>- At 24 hrs ≥ 200<br>  73 (43-90)<br>  74 (51-88)<br>12 (7.7-158.8)<br>  4.7 (4.1-5.4)<br>  45.0 (12.7-158.8)<br>  11.6 (8.8-15.0)<br>  252.5 (164.3-396.4)<br>  5.60 (4.28-7.30)<br>**PCT, mcg/L**<br>- At birth ≥ 1<br>  82 (52-95)<br>  79 (57-92)<br>1 (89-97)<br>  3.3 (2.3-4.3)<br>  54 (28-79)<br>  53 (32-73)<br>  70 (62-78)<br>**Sensitivity %, (95% CI):**<br>- Culture positive (n=11)<br>- All patients (n=19)<br>- Specificity %, (95% CI):<br>- CRP, mg/L<br>- At birth ≥ 4<br>  73 (43-90)<br>  74 (51-88)<br>83 (75-89)<br>**IL-6, ng/L**<br>- At birth ≥ 200<br>  73 (43-90)<br>  74 (51-88)<br>89 (82-93)<br>**PCT, mcg/L**<br>- At birth ≥ 1<br>  82 (52-95)<br>  79 (57-92)<br>95 (89-98)<br>**Limitations**<br>Small sample size; 2 center study<br>**Author’s Conclusion**<br>PCT specificity/sensitivities for infection was greater than that for CRP/IL-6 at all 3 neonatal ages; the magnitude of PCT response to infection was much greater than those caused by ante-/perinatal events<br>**Critique**<br>Strengths: blind classification of septic vs nonseptic; validated objective scores used; endpoint comparison charts provided<br>Weaknesses: unequal # pts in septic & nonseptic groups; authors do not discuss limitations; ABX initiation not mentioned<br>**Clinical Significance**<br>Results add to PCT reliability as a more sensitive/specific biomarker over CRP/IL-6 during the first 24 hrs of life

NICU = neonatal intensive care unit; SNAP = Score for Neonatal Acute Physiology; SNAP-PE = SNAP Perinatal Extension; CXR = chest X-rays; CRP = C-reactive protein; IL-6 = interleukin 6; PCT = procalcitonin; ABX = antibiotics; GBS = group B streptococcus; PROM = prolonged rupture of membranes

* SNAP and SNAP-PE scoring system can be found on Appendix B, Table 4
† Ante- and perinatal data: maternal age, race, parity, prenatal care, multiple pregnancy, preexistent or pregnancy-related diseases (hypertension, diabetes, preeclampsia), medications during pregnancy, drug abuse, prenatal steroid exposure, maternal GBS colonization; duration/characteristics of rupture of amniotic membranes, clinical evidence of choioamnionitis and antibiotic treatment, placental/fetal abnormalities, fetal presentation, mode of delivery, duration of active labor, use of anesthesia, birth asphyxia (defined as need of ET ventilation or CPR)
‡ Risk factors for infection: maternal fever, PROM >18 hrs, maternal GBS colonization, uterine tenderness, foul smelling/cloudy amniotic fluid; preterm labor; intrapartum antimicrobial administration
§ Clinical signs of infection defined ≥ 3 of the following categories: temp instability (hyper/hypothermia); respiratory (grunting, intercostal retractions, apnea, tachypnea, cyanosis); CV (bradycardia/tachycardia, poor perfusion, hypotension); neurologic (hypotonia, lethargy, seizures); GI (feeding intolerance, abdominal distention)
TABLE 5. Evaluation of procalcitonin for diagnosis of neonatal sepsis of vertical transmission

Objective: To determine PCT levels in uninfected/infected neonates & assess value of PCT for neonatal sepsis of vertical transmission diagnosis

Methods

Study Design
- Prospective, multicenter study: 13 hospitals in Spain between January 2000- January 2001
- PCT analyzed using LumiTest® (limit of detection= 0.08 ng/mL) completed in 2 hrs using 20 µL of serum

Endpoints
- Diagnostic efficacy of PCT at birth, 12-24 hrs, and 36-48 hrs of life evaluated by calculating sensitivity, specificity, and +/- LR

Patient Selection
- **Inclusion**: neonates < 48 hrs old; blood samples available for timed PCT measurements according to the 3 postnatal periods: shortly after birth, within 12-24 hrs of life and 36-48 hrs of life
- **Exclusion**: infants born to mothers with GDM; parental consent refusal

<table>
<thead>
<tr>
<th>Group 1 (1st population)</th>
<th>Group 2 (2nd population)</th>
</tr>
</thead>
</table>
| Asymptomatic infants admitted during first 24 hrs of life for prematurity, low birth weight, or ≥ 2 risk factors for infection | Symptomatic infants admitted during first 48 hrs of life
  - **Group 2A**: (confirmed vertical sepsis) ≥ 3 clinical signs of infection with ≥ 1 bacteriologic evidence of infection
  - **Group 2B**: (vertical clinical sepsis) ≥ 3 clinical signs of infection, ≥ 1 lab finding consistent with infection (WBC >30 cells/ml or < 5 cells/ml, CRP >5 mg/L, - culture, and ≥ 2 vertical transmission risk factors +/- intrapartum antibiotics
  - **Group 2C**: (non-infectious disease) Uninfected, - cultures

Baseline Characteristics
- Group 2C had significantly lower birth weights and gestational ages (1.5 grams vs ~2.5 grams; 30 weeks vs ~35 weeks) [P<0.001]

Statistical Analysis
- Nonparametric tests (Kruskal-Wallis test and Mann-Whitney U test): (data not normally distributed)
- PCT values expressed as median and interquartile (25th-75th) ranges
- Multiple linear regression assessed association of PCT values and different perinatal variables; extreme variables were removed and PCT values not normally distributed were log transformed
- ROC curves used to calculated optimal PCT cutoffs for sensitivity, specificity, +/- LR calculated with 95% CI. P < 0.05

Results
- Total = 827 blood samples from 317 neonates

<table>
<thead>
<tr>
<th>1st population (Group 1): 169 asymptomatic newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT significantly higher at 12-24 hrs (1.54 ng/mL) than at birth (0.35 ng/mL) or at 36-48 hrs (0.73 ng/mL). [P &lt; 0.0001]</td>
</tr>
<tr>
<td>PCT levels independently associated with resuscitation at birth &amp; 36–48 h of life, and with chorioamnionitis at birth</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2nd population (Group 2): 148 symptomatic newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 2A</strong>: 31 confirmed vertical sepsis; <strong>Group 2B</strong>: 38 vertical clinical sepsis; <strong>Group 2C</strong>: 79 non-infectious diseases</td>
</tr>
</tbody>
</table>

PCT levels
- Higher than those of patients in group 1 at each postnatal period [P < 0.0001]
- Group 2A had consistently higher serum PCT values than those of group 2B at each postnatal period
- No significant differences between PCT levels of neonates with respiratory diseases and clinical sepsis
- Group 2C excluded from PCT levels due to antibiotics given in majority of this group’s infants

<table>
<thead>
<tr>
<th>Threshold cutoff [ng/mL] (cutoff to achieve 90% sensitivity)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth: ≥ 0.55 (± 0.15)</td>
<td>75.4% (91.2%)</td>
<td>72.3% (10.8%)</td>
</tr>
<tr>
<td>12-24 hrs: ≥ 4.7 (± 1.2)</td>
<td>73.8% (90.2%)</td>
<td>80.8% (43.0%)</td>
</tr>
<tr>
<td>36-48 hrs: ≥ 1.7 (± 0.75)</td>
<td>77.6% (91.8%)</td>
<td>79.2% (51.4%)</td>
</tr>
</tbody>
</table>

Limitations
- Small sample size
- Heterogeneity (i.e. sepsis definition) and different nature of control groups led to difference in sensitivity/specificty
- No comparison to CRP or other infection markers (not standardized or performed at study hospitals)
- Control group of asymptomatic infants without evidence of infection may have led to an overestimate of reliability

Author’s Conclusion
- PCT levels showed a moderate diagnostic value in detecting neonatal sepsis of vertical transmission with better results after 12 hrs of birth; specific cutoff values at each evaluation point are needed to improve diagnostic accuracy of PCT in first 48 hrs

Critique
- **Strengths**: PCT assays performed by 2 centralized labs; multicenter trial; comparison charts of endpoints provided
- **Weaknesses**: No comparison to other biomarkers; timing of antibiotic initiation not mentioned

Clinical Significance
- Moderate diagnostic value of PCT seen; no effect of perinatal variables on PCT at 12-24 hrs of life

PCT = procalcitonin, GDM = gestational diabetes; GBS = group B streptococci; LOS = late-onset sepsis; ROC = receiver-operating characteristics; LR = likelihood ratio
TABLE 6. Procalcitonin measurement at 24 hours of age may be helpful in the prompt diagnosis of early-onset neonatal sepsis

Objective: To determine the diagnostic value of PCT at birth and at 24 hrs of age in the prompt diagnosis of EONS

Methods

Study Design
- A prospective study in Konya, Turkey from June 2008 to January 2011
- Diagnosis of EONS: (1) Clinical signs consistent with infection based on Töllner score* (2) CRP values ≥ 1 mg/dl (3) Positive culture (blood, urine, CSF) or CXR indicating pneumonia
- Serum PCT (measured with LumiTest PCT™) and CRP (normal < 5 mg/l) measured at birth (1st) & at 24 hrs (2nd)
- First sample of levels drawn before initiation of antibiotic therapy or at moment of inclusion of study

Endpoints
- Calculating sensitivity, specificity, PPV, and NPV for PCT concentrations

Patient Selection

<table>
<thead>
<tr>
<th>Group 1: patients with suspected sepsis</th>
<th>Group 2: control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion:</td>
<td></td>
</tr>
<tr>
<td>• + clinical sepsis signs (Töllner score ≥ 10)</td>
<td>• No antibiotic treatment</td>
</tr>
<tr>
<td>• OR factors associated with increase infection risk°</td>
<td>• No clinical signs of sepsis for 1st week of life</td>
</tr>
<tr>
<td>• Parental informed consent</td>
<td>• Töllner score &lt; 5 -OR- Töllner score 5-10 with no factors associated with increased risk of infection° and negative CRP and blood culture</td>
</tr>
<tr>
<td>• If negative culture, neonate must have clinical signs of sepsis and/or CXR with pneumonia, positive sepsis screen, factors associated with increase infection risk°</td>
<td></td>
</tr>
</tbody>
</table>

Exclusion:
- mother or newborn given antibiotics; congenital malformations; congenital infections associated with TORCH complex; refusal of parental consent

Baseline Characteristics
- No statistical significant difference between groups regarding gestational age (~34 weeks) or birth weight (~2.1-2.3 kg)

Statistical Analysis
- Mann-Whitney U-test (data not normally distributed)
- ROC cutoff PCT levels used to measure sensitivity, specificity, PPV, NPV
- 95% confidence intervals (CI) for all parameters; statistical significance assumed for p < 0.05

Results

<table>
<thead>
<tr>
<th>Group 1: 190 neonates (preterm and term)</th>
<th>Group 2: N = 89 neonates (preterm and term)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 excluded for antibiotics being given</td>
<td>No positive cultures</td>
</tr>
<tr>
<td>N = 171 neonates with suspected clinical sepsis</td>
<td>No deaths during first week of life</td>
</tr>
<tr>
<td>Blood cultures positive in 67 patients</td>
<td>Neonates discharged home before 7 days of life were followed to ensure no development of LOS</td>
</tr>
<tr>
<td>20 patients died</td>
<td></td>
</tr>
</tbody>
</table>

PCT levels [ng/ml]: mean (range)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>First level at birth</td>
<td>0.51 (0.09-28.6)</td>
</tr>
<tr>
<td>Cutoff ≥ 0.59 ng/ml</td>
<td>Sens: 48.7% Spec: 68.6% PPV: 48.71 NPV: 68.57</td>
</tr>
<tr>
<td>Second level at 24 hrs</td>
<td>16.17 (0.17-100)</td>
</tr>
<tr>
<td>Cutoff ≥ 5.83 ng/ml</td>
<td>Sens: 83.8% Spec: 88.6% PPV: 83.33 NPV: 88.57</td>
</tr>
</tbody>
</table>

CRP levels [mg/l]: mean (range)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>First level at birth</td>
<td>1.9 (0.0-68.2)</td>
</tr>
<tr>
<td>Cutoff &gt; 5 mg/l</td>
<td>Sens: 44.5% Spec: 59.4% PPV: 45.62 NPV: 64.25</td>
</tr>
<tr>
<td>Second level at 24 hrs</td>
<td>24.3 (0.0-104.6)</td>
</tr>
<tr>
<td>Cutoff &gt; 12 mg/l</td>
<td>Sens: 76.4% Spec: 78.9% PPV: 79.75 NPV: 81.62</td>
</tr>
</tbody>
</table>

No statistically significant difference in PCT or CRP levels between patient and control group at birth [p >0.05]; at 24 hrs of PCT and CRP levels were significantly higher in the patient group that in the control [p <0.001]

Limitations
- Small sample size; single center study

Author’s Conclusion
- Serum PCT measurements may be useful for diagnosis of EONS. Serial measurements of PCT and CRP at birth and at 24 hrs of age may be more helpful

Critique
- Strengths: prospective study; use of validated sepsis score; comparison charts of endpoints provided
- Weaknesses: no limitations noted by authors; no definition of ‘positive sepsis screen’; no mention of differences in PCT levels among control group infants w/ hypoglycemia, hypocalcemia, fetal distress, or those born to GDM moms

Clinical Significance
- Results strengthen reliability of using PCT as a more sensitive biomarker over CRP during the first 24 hrs of life and can help rule out neonatal sepsis.

PCT = procalcitonin; EONS = early-onset neonatal sepsis; CSF = cerebrospinal fluid; CXR = chest X-ray; PROM = prolonged rupture of membranes; LOS = late-onset sepsis; ROC = receiver operating characteristics; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; GDM = gestational diabetes

* Töllner Scoring System (See Table 2, page 4 of handout)
I. Discussion

A. Meta-analysis of PCT in EONS\textsuperscript{26}
   i. Vouloumanou et al performed a systematic review and meta-analysis of a total of 16 studies
   ii. Six of these studies, involving a total of 780 neonates, were included in the analysis of PCT for the diagnosis of EONS
      1. Sensitivity was 76\% (95\% CI 68–82\%); Specificity was 76\% (95\% CI 60–87\%)
      2. Positive & negative likelihood ratios: 3.2 (95\% CI 1.8–5.7) & 0.32 (95\% CI 0.23–0.43), respectively

B. Reasons for contradicting results of PCT reliability for EONS diagnosis\textsuperscript{16,26}
   i. Variations in study design
   ii. Confounders such as intrapartum antibiotics, postnatal antibiotics, neonatal hypoxemia, respiratory distress syndrome (RDS), and intracranial hemorrhage
   iii. Lack of uniform definition of clinical septicemia
   iv. Different cut-off points used for PCT concentrations

II. Conclusions

A. PCT as biomarker for EONS
   i. No biomarker has demonstrated complete reliability in detecting all septic infants
   ii. Used as single biomarker, PCT not 100\% reliable in diagnosing septic neonates but may be useful as part of a sepsis evaluation
   iii. Factors affecting sensitivity/specificity:
      1. Lack of universally acceptable definition of neonatal sepsis, particularly ‘clinical septicemia’
         a. Variability in criteria for sepsis definition could affect the outcomes of measure
      2. PCT may perform differently due to different pathogens i.e. gram-positive, gram-negative, or fungal
   iv. PCT is a fairly moderate biomarker to rule out sepsis in certain neonatal populations
      1. Preterm or term neonates excluding those who have congenital malformations
      2. Not neonates with mothers who had GDM, intrapartum antibiotics, chorioamnionitis

B. Further study of PCT
   i. Larger studies comparing PCT with other biomarkers for EONS and determining optimal cut-off level
   ii. PCT assay differences and PCT-guided antibiotic duration

---

Figure 2. PCT Algorithm for EONS

- Early Onset Neonatal Sepsis\textsuperscript{10,17–20}
  - Onset < 48 hours of life (pre-term and term neonates)\textsuperscript{17–20}
  - Meet at least 2 of the 4 SIRS criteria PLUS suspected infection
  - Risk factors for infection: maternal fever ≥ 38°C, PROM >18 hours, maternal GBS colonization

- Congenital malformations, mother had GDM, intrapartum antibiotics, chorioamnionitis\textsuperscript{16–20}

- Draw PCT level after birth
  - 0-12 hours after birth ≥ 0.8 ng/ml\textsuperscript{17,18}

- No antibiotics. Monitor patient.
  - Draw subsequent PCT level 12-24 hrs of life\textsuperscript{17,18}

- Start antibiotics, draw blood cultures

- Do NOT start antibiotics. Monitor patient.
REFERENCES


REFERENCES (continued)

42. Allen J. Applying Study Results to Patient Care: Glossary of Study Design and Statistical Terms. Pharmacist’s Letter/Prescriber’s Letter. 2004;20: Number 200512.
APPENDIX A

TABLE 1. Age Stages Defined According to NICHD Pediatric Terminology

<table>
<thead>
<tr>
<th>Stage</th>
<th>Defined ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm neonatal</td>
<td>Newborn born before full gestational period (&lt;37 weeks)</td>
</tr>
<tr>
<td>Term neonatal</td>
<td>Birth – 27 days</td>
</tr>
<tr>
<td>Infancy</td>
<td>28 days – 12 months</td>
</tr>
<tr>
<td>Toddler</td>
<td>13 months – 2 years</td>
</tr>
<tr>
<td>Early childhood</td>
<td>2-5 years</td>
</tr>
<tr>
<td>Middle childhood</td>
<td>6-11 years</td>
</tr>
<tr>
<td>Early adolescence</td>
<td>12-18 years</td>
</tr>
<tr>
<td>Late adolescence</td>
<td>19-21 years</td>
</tr>
</tbody>
</table>

Adapted from NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

TABLE 2. Definitions of systemic inflammatory response syndrome (SIRS), infection, and sepsis

<table>
<thead>
<tr>
<th>SIRS: Presence of at least 2 of the following criteria (one must be abnormal temperature or leukocyte count):</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Core temperature of &gt;38.5°C (&gt;101.3°F) or &lt;36°C (&lt;96.8°F). (measured by rectal, bladder, oral or central catheter probe)</td>
</tr>
<tr>
<td>• Heart rate (HR)°</td>
</tr>
<tr>
<td>o Tachycardia: mean HR &gt;2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or unexplained persistent elevation over a 0.5- to 4-hr time period</td>
</tr>
<tr>
<td>o Bradycardia: (children &lt;1 yr old) mean HR &lt;10th percentile for age in the absence of external vagal stimulus, β-blocker drugs, or congenital heart disease; or unexplained persistent depression over a 0.5-hr time period</td>
</tr>
<tr>
<td>• Respiratory rate° &gt;2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or receipt of general anesthesia</td>
</tr>
<tr>
<td>• Leukocyte count° elevated or depressed for age or &gt;10% immature neutrophils</td>
</tr>
</tbody>
</table>

| Infection: Suspected or proven (by positive culture, tissue stain, or PCR test) infection caused by any pathogen OR clinical syndrome associated with a high probability of infection |
| Evidence of infection: positive findings on clinical exam, imaging, or laboratory tests (e.g., WBCs in a normally sterile body fluid, CXR consistent with pneumonia, petechial or purpuric rash, or purpura fulminans) |

| Sepsis |
| SIRS in the presence of or as a result of suspected or proven infection |

| Severe Sepsis |
| Sepsis plus one of the following: |
| • Cardiovascular organ dysfunction- hypotension (despite administration of IV fluid bolus) or need for vasoactive drugs (i.e. dopamine, dobutamine, epinephrine, or norepinephrine) OR 2 of the following: unexplained metabolic acidosis, increased arterial lactate 2 x upper limit of normal, prolonged capillary refill >5 seconds. |
| OR |
| • ARDS - PaO2/FIO2 < 300 in absence of cyanotic heart disease or preexisting lung disease, PaCO2 > 65 torr or 20 mmHg over baseline PaCO2, need of > 50% FIO2 to maintain saturation > 92%, or need for mechanical ventilation |
| OR |
| • 2 or more other organ dysfunctions: |
|   o Neurologic – GCS < 11, or acute mental status change with a decrease in GCS > 3 points from baseline |
|   o Hematologic – Platelet count < 80,000/mm³, 50% decline in platelet count, or INR >2 |
|   o Renal – SCr > 2 x upper limit of normal or 2-fold increase in baseline creatinine |
|   o Hepatic – Total bilirubin > 4 mg/dL (not applicable for newborn), or ALT 2 times upper limit of normal |

| Septic Shock |
| Sepsis and cardiovascular organ dysfunction as defined above. |

SD = standard deviation; CXR = chest X-ray; WBC = white blood cell; ARDS = acute respiratory distress syndrome; GCS = Glasgow Coma Score
APPENDIX B

TABLE 3. Age-specific vital signs and laboratory variables

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Heart Rate (beats/min)</th>
<th>Respiratory Rate (breaths/min)</th>
<th>Leukocyte Count (WBC x 10^9/mm)</th>
<th>Systolic Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tachycardia</td>
<td>Bradycardia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 days to 1 week</td>
<td>&gt; 180</td>
<td>&lt; 100</td>
<td>&gt; 50</td>
<td>&gt; 34</td>
</tr>
<tr>
<td>1 week to 1 month</td>
<td>&gt; 180</td>
<td>&lt; 100</td>
<td>&gt; 40</td>
<td>&gt; 19.5 or &lt; 5</td>
</tr>
<tr>
<td>1 month to 1 year</td>
<td>&gt; 180</td>
<td>&lt; 90</td>
<td>&gt; 34</td>
<td>&gt; 17.5 or &lt; 5</td>
</tr>
<tr>
<td>2-5 years</td>
<td>&gt; 140</td>
<td>--</td>
<td>&gt; 22</td>
<td>&gt; 15.5 or &lt; 6</td>
</tr>
<tr>
<td>6-12 years</td>
<td>&gt; 130</td>
<td>--</td>
<td>&gt; 18</td>
<td>&gt; 13.5 or &lt; 4.5</td>
</tr>
<tr>
<td>13 to &lt;18 years</td>
<td>&gt; 110</td>
<td>--</td>
<td>&gt; 14</td>
<td>&gt; 11 or &lt; 4.5</td>
</tr>
</tbody>
</table>


Note: Lower values for heart rate, leukocyte count, and systolic blood pressure are for the 5th; upper values for heart rate, respiration rate, or leukocyte count for the 95th percentile

TABLE 4. SNAP and SNAP-PE Scoring System

<table>
<thead>
<tr>
<th>Score for Neonatal Acute Physiology (SNAP)</th>
<th>OUTCOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Applicable to any infant admitted to a NICU (reduced sensitivity in premature infants)</td>
<td>• SNAP predicted death better than birth weight alone (predictive value 0.87 v 0.77), and SNAP-PE was even better (predictive value 0.93)</td>
</tr>
<tr>
<td>• SNAP scores based on 28 items collected over the first 24 hours of life (worst physiologic derangements in each organ system in timeframe)</td>
<td>• Higher SNAP scores should be associated with a higher risk of adverse outcome</td>
</tr>
<tr>
<td>• Variables weighted according to degree of derangement from physiologic normal:</td>
<td></td>
</tr>
<tr>
<td>▪ 0 = no derangement</td>
<td></td>
</tr>
<tr>
<td>▪ 1 = clearly abnormal requiring careful monitoring</td>
<td></td>
</tr>
<tr>
<td>▪ 3 = severe derangement requiring immediate monitoring</td>
<td></td>
</tr>
<tr>
<td>▪ 5 = an acute life-threatening value</td>
<td></td>
</tr>
<tr>
<td>• <strong>Variables</strong>: blood pressure, heart rate, respiratory rate, temperature, PO2, PO2/FIO2 ratio, PCO2, oxygenation index, packed cell volume, WBC, immature/total ratio, absolute neutrophil count, platelet count, BUN, SCR, urine output, indirect bilirubin, direct bilirubin, sodium, potassium, calcium (ionized and total), glucose, serum bicarbonate, serum pH, seizure, apnea, stool guaiac</td>
<td></td>
</tr>
<tr>
<td><strong>Score for Neonatal Acute Physiology—Perinatal Extension (SNAP-PE score)</strong></td>
<td></td>
</tr>
<tr>
<td>• SNAP variables PLUS birth weight, small for gestational age (weight, 5th percentile for gestation), and low Apgar score (&lt;7) at 5 min</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5. APGAR Score

<table>
<thead>
<tr>
<th>5 Components</th>
<th>Signs</th>
<th>Symptoms and associated scores</th>
<th>Calculating Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Score = 0</td>
<td>Score = 1</td>
</tr>
<tr>
<td>Appearance</td>
<td>Color</td>
<td>Blue, pale</td>
<td>Body pink; extremities blue</td>
</tr>
<tr>
<td>Pulse</td>
<td>Heart rate</td>
<td>Absent</td>
<td>&lt;100 beats/min</td>
</tr>
<tr>
<td>Grimec</td>
<td>Reflex irritability</td>
<td>No response</td>
<td>Grimace</td>
</tr>
<tr>
<td>Activity</td>
<td>Muscle tone</td>
<td>Limp, flaccid</td>
<td>Some flexion of extremities</td>
</tr>
<tr>
<td>Respiration</td>
<td>Respiratory effort</td>
<td>Absent</td>
<td>Groping; slow, irregular</td>
</tr>
</tbody>
</table>

Ramirez 17
**APPENDIX C**

### TABLE 6. Biostatistics Definitions\textsuperscript{42,43}

<table>
<thead>
<tr>
<th>Definition</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity:</strong> Ability of a test to reliably detect presence of a disease. The proportion of patients with the disease who have a positive test.</td>
<td>( \frac{100 \times \text{true positives}}{\text{true positives} + \text{false negatives}} )</td>
</tr>
<tr>
<td><strong>Specificity:</strong> Ability of a diagnostic test to reliably rule out a disease. The proportion of patients without the target disease who have a negative test.</td>
<td>( \frac{100 \times \text{true negatives}}{\text{true negatives} + \text{false positives}} )</td>
</tr>
<tr>
<td><strong>Positive Predictive Value (PPV):</strong> Proportion of people who actually have the disease when a diagnostic test is positive.</td>
<td>( \frac{100 \times \text{true positives}}{\text{true positives} + \text{false negatives}} )</td>
</tr>
<tr>
<td><strong>Negative Predictive Value (NPV):</strong> Proportion of people who actually do not have the disease when a diagnostic test is negative.</td>
<td>( \frac{100 \times \text{true negatives}}{\text{true negatives} + \text{false positives}} )</td>
</tr>
</tbody>
</table>

**Receiver operating characteristic (ROC):** Created by plotting fraction of true positives out of the total actual positives (sensitivity) vs. the fraction of false positives out of the total actual negatives (specificity), at various threshold settings. The area measures discrimination or ability of the test to correctly classify those with and without the disease. Classifying the accuracy of a diagnostic test:

- .90-1 = excellent
- .80-.90 = good
- .70-.80 = fair
- .60-.70 = poor
- .50-.60 = very poor

![ROC curve](image)

### TABLE 7. Abbreviations

<table>
<thead>
<tr>
<th><strong>ABX:</strong> Antibiotics</th>
<th><strong>HR:</strong> Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APGAR:</strong> Appearance, Pulse, Grimace, Activity, and Respiration (APGAR scoring)</td>
<td><strong>IL:</strong> Interleukin</td>
</tr>
<tr>
<td><strong>ARDS:</strong> Acute respiratory distress syndrome</td>
<td><strong>IT Ratio:</strong> Immature/total neutrophil ratio</td>
</tr>
<tr>
<td><strong>BUN:</strong> Blood urea nitrogen</td>
<td><strong>INR:</strong> International normalized ratio</td>
</tr>
<tr>
<td><strong>CALC-1:</strong> Calcitonin gene</td>
<td><strong>LOS:</strong> Late-onset sepsis</td>
</tr>
<tr>
<td><strong>Cl:</strong> Confidence intervals</td>
<td><strong>LR:</strong> Likelihood ratios</td>
</tr>
<tr>
<td><strong>CPP-1:</strong> Katakalcin</td>
<td><strong>MDR:</strong> Multi-drug resistant</td>
</tr>
<tr>
<td><strong>CR:</strong> C-reactive protein</td>
<td><strong>MRSA:</strong> Methicillin resistant staph aureus</td>
</tr>
<tr>
<td><strong>CSF:</strong> Cerebral spinal fluid</td>
<td><strong>NEC:</strong> Necrotizing enterocolitis</td>
</tr>
<tr>
<td><strong>CXR:</strong> Chest X-ray</td>
<td><strong>NICU:</strong> Neonatal intensive care unit</td>
</tr>
<tr>
<td><strong>EONS:</strong> Early-onset neonatal sepsis</td>
<td><strong>NICHD:</strong> National Institute of Child Health and Human Development</td>
</tr>
<tr>
<td><strong>ESBL:</strong> Extended spectrum beta-lactamase</td>
<td><strong>NPV:</strong> Negative predictive value</td>
</tr>
<tr>
<td><strong>FDA:</strong> United States Food and Drug Administration</td>
<td><strong>PaO2:</strong> Partial arterial pressure of O2</td>
</tr>
<tr>
<td><strong>GBS:</strong> Group B Streptococcus</td>
<td><strong>PCR:</strong> Polymerase chain reaction</td>
</tr>
<tr>
<td><strong>GDM:</strong> Gestational diabetes</td>
<td><strong>PCT:</strong> Procalcitonin</td>
</tr>
<tr>
<td><strong>PPV:</strong> Positive predictive value</td>
<td><strong>PMN:</strong> Polymorphonuclear</td>
</tr>
<tr>
<td><strong>PROM:</strong> Premature (prelabor) rupture of membranes</td>
<td><strong>Sens.:</strong> Sensitivity</td>
</tr>
<tr>
<td><strong>RDS:</strong> Respiratory distress syndrome</td>
<td><strong>SIRS:</strong> Systemic inflammatory response syndrome</td>
</tr>
<tr>
<td><strong>ROC:</strong> Receiver-operating characteristics</td>
<td><strong>SNAP:</strong> Score for Neonatal Acute Physiology</td>
</tr>
<tr>
<td><strong>Scr:</strong> Serum creatinine</td>
<td><strong>SNAP-PE:</strong> Score for Neonatal Acute-Physiology Perinatal Extension</td>
</tr>
<tr>
<td><strong>SD:</strong> Standard deviation</td>
<td><strong>Spec.:</strong> Specificity</td>
</tr>
<tr>
<td>**S: ** Tumor necrosis factor-α</td>
<td><strong>WBC:</strong> White blood cell(s)</td>
</tr>
<tr>
<td><strong>Year:</strong> Year</td>
<td><strong>Yr:</strong> Year</td>
</tr>
</tbody>
</table>
## APPENDIX D

### TABLE 8. PCT Studies in EONS

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Design</th>
<th>Subgroups compared</th>
<th>PCT level in healthy infants</th>
<th>PCT level in infected infants</th>
<th>PCT cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiesa et al. (1998)</td>
<td>Prospective, single center</td>
<td>Culture-proven or clinical septicemia (n=28) vs healthy infants (n=83)</td>
<td>&lt;0.08-0.7 ng/mL</td>
<td>0.6-21 ng/mL</td>
<td>50/54 infants’ levels (N=28 of Group 1)* above upper limit of reference range</td>
<td>@ birth: &gt;0.7 @ 21-24*: &gt;21</td>
<td>92.6</td>
<td>97.5</td>
<td>94.3</td>
</tr>
<tr>
<td>Chiesa et al. (2003)</td>
<td>Prospective, single center</td>
<td>Culture-proven or clinical vs. no sepsis: 19/134 vs. 115/134</td>
<td>0.22 (0.18-0.27 mcg/L)</td>
<td>5.6 (4.28-7.3 mcg/L)</td>
<td>3.79 (1.7-8.43 mcg/L)</td>
<td>255.2 (164-396.4 mcg/L)</td>
<td>@ birth &gt;1</td>
<td>79</td>
<td>95</td>
</tr>
<tr>
<td>Lopez-Sastre et al. (2007)</td>
<td>Prospective, multicenter</td>
<td>Culture-proven or clinical vertical sepsis vs. no sepsis: 57/205 vs. 148/205</td>
<td>0.35 ng/mL</td>
<td>1.54 ng/mL</td>
<td>Proven: 13.59 (0.50–101.3 ng/mL)</td>
<td>Proven: 30.65 (6.38–95.9 ng/mL)</td>
<td>@ birth &gt;0.55</td>
<td>75.4</td>
<td>72.3</td>
</tr>
<tr>
<td>Altunhan et al. (2011)</td>
<td>Prospective, single center</td>
<td>Suspected sepsis (n=171) vs no sepsis (n=89)</td>
<td>0.48 (0.07-3.48 ng/mL)</td>
<td>1.72 (0.21-18.23 ng/mL)</td>
<td>0.51 (0.09-28.6 ng/mL)</td>
<td>16.17 (0.17-100 ng/mL)</td>
<td>@ birth &gt;0.59</td>
<td>48.7</td>
<td>68.6</td>
</tr>
</tbody>
</table>

* Group 1 = Culture-proven or clinical septicemia