Defining Normal Cerebrospinal Fluid White Blood Cell Counts in Neonates and Young Infants: A Scholarly Pursuit

Lori A. Kestenbaum, Jessica L. Ebberson, Joseph J. Zorc, Caitlin LaRussa, Richard L. Hodinka & Samir S. Shah
Overview

- Evaluating a febrile neonate
- Meningitis
- Study background and methods
- Study results and conclusions
- Part II: Participating in Research as a Student
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Febrile Neonate

- Fever in a well appearing infant (0 to 60 days) without identifiable source of infection
- 10 to 15% will have a serious bacterial infection
- All are screened with:
  - Peripheral white blood cell count
  - Urinalysis
  - LUMBAR PUNCTURE
  - Chest X-ray/Stool smear for specific symptoms
Meningitis

- Bacterial: Group B Strep, E. Coli, Listeria
  - >6 weeks: Strep pneumoniae, Neisseria, H. flu
- Viral: Enterovirus, Herpes Simplex
- On exam: bulging fontanelle, irritability
- Studies:
  - CSF culture, cell count (white cells, glucose, protein)
  - Enterovirus or Herpes simplex PCR in certain circumstances
## Interpreting CSF Values

<table>
<thead>
<tr>
<th>Type</th>
<th>Mononuclear:</th>
<th>Neutrophils:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>Hundreds</td>
<td>100s-1000s</td>
</tr>
<tr>
<td>Viral</td>
<td></td>
<td>Hundreds</td>
</tr>
<tr>
<td>TB</td>
<td>Mononuclear</td>
<td>100s</td>
</tr>
<tr>
<td>Cryptococcal</td>
<td></td>
<td>Few-100s</td>
</tr>
</tbody>
</table>
We know what is abnormal . . .

- But what is normal in the CSF?
  - 0 white blood cells?
  - 2?
  - 19?
  - 21?
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Lumbar puncture (LP) is routinely performed in the emergency department when evaluating febrile neonates.

Clinicians require reference values to interpret cerebrospinal fluid (CSF) white blood cell (WBC) counts.
Study Background

- Determining normal values in infants is ethically problematic
  - Invasive, potentially harmful procedure
  - Participants cannot offer consent or assent
- Reference values have been based on children who are not truly normal
Study Background

- Current reference values reflect expert opinion or cite studies with significant limitations.
- Limitations include small sample sizes and inclusion of patients with conditions known to cause CSF pleocytosis:
  - Traumatic lumbar puncture
  - Seizures
  - Bacterial infections
<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>N</th>
<th>Median</th>
<th>90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarff(^1)</td>
<td>≤10 days</td>
<td>87</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Portnoy(^2)</td>
<td>&lt;6 weeks</td>
<td>64</td>
<td>3.73*</td>
<td>8.1**</td>
</tr>
<tr>
<td>Bonadio(^3)</td>
<td>&lt;4 weeks</td>
<td>35</td>
<td>8.5</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>4-8 weeks</td>
<td>40</td>
<td>4.5</td>
<td>11</td>
</tr>
<tr>
<td>Ahmed(^4)</td>
<td>≤30 days</td>
<td>108</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^{+}\) All units /mm\(^3\)

*Mean value **Calculated

\(^2\) Portnoy JM, Olson LC. Pediatrics 1985.
Objective

To determine accurate, age-specific reference values for CSF WBC counts in a population of neonates & young infants
Methods: Study Design

- Cross-sectional study
- The Children’s Hospital of Philadelphia
  - 75,000 Emergency Department visits per year
- Participants
  - All infants age ≤ 56 days who had LP in ED between January 1, 2005 and June 30, 2007
  - Infants ≤ 56 days routinely undergo LP for evaluation of fever
  - Data collected by chart review
Methods: Exclusion

- Conditions known to cause CSF pleocytosis systematically excluded
  - Traumatic LP
  - Serious bacterial infection
  - Meningitis
  - Known neurologic condition or congenital infection
  - Positive CSF enterovirus PCR
  - No CSF WBC count documented
Methods: Patient Selection

1064 Infants with lumbar puncture

- 331 Traumatic lumbar puncture
- 133 Non-CNS bacterial infections
- 34 Other CNS processes*
- 44 Without CSF WBC count

90 Transported in

- 6 Bacterial meningitis
- 46 CSF EV PCR positive

380 Infants Included

*includes VP Shunt, CNS HSV or syphilis, seizure, abnormal head imaging
Methods: Analysis

- Median values established for each age group
- Wilcoxon rank-sum test
  - Compared infants 0-28 days to 29-56 days of age
- Stratified by CSF enterovirus PCR status (negative vs not done) and season
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## Patient Population

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<tr>
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<th>29-56 Days</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>142</td>
<td>238</td>
</tr>
<tr>
<td>Male Sex</td>
<td>82 (57%)</td>
<td>125 (52%)</td>
</tr>
<tr>
<td>White</td>
<td>47 (34%)</td>
<td>64 (29%)</td>
</tr>
<tr>
<td>Fever on presentation</td>
<td>92 (65%)</td>
<td>213 (90%)</td>
</tr>
<tr>
<td>Preterm</td>
<td>22 (15%)</td>
<td>35 (15%)</td>
</tr>
<tr>
<td>EV Season</td>
<td>66 (46%)</td>
<td>82 (34%)</td>
</tr>
<tr>
<td>Hospital Stay Length Median (IQR)</td>
<td>2 days (2-3)</td>
<td>2 days (1-2.5)</td>
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# Cerebrospinal Fluid WBC Counts

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<tr>
<td>Mean (SD)</td>
<td>9.2 (32.1)</td>
<td>3.1 (5.0)</td>
</tr>
<tr>
<td>Upper Limit 95% CI</td>
<td>14.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Median*</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>90%</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
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<td>19</td>
<td>9</td>
</tr>
<tr>
<td>IQR</td>
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† All units are /mm$^3$

* p<0.001
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CSF WBC Counts Infants 0-28 Days of Age

- **EV Negative**
  - N=37
  - Median: 4/mm³
  - 95th Percentile: 78

- **EV Not Tested**
  - N=70
  - Median: 3/mm³
  - 95th Percentile: 19

- **EV Not Tested Not EV Season**
  - N=70
  - Median: 3/mm³
  - 95th Percentile: 19

Box plots show the distribution of CSF WBC counts for each category.
CSF WBC Counts Infants 29-56 Days of Age

- **EV Negative**
  - Median: 2.5/mm³
  - 95th Percentile: 34
  - N=38

- **EV Not Tested**
  - Median: 2/mm³
  - 95th Percentile: 7
  - N=146

- **EV Season**
  - Median: 2/mm³
  - 95th Percentile: 7
  - N=54

Median: 2.5/mm³ 95th Percentile: 34

Median: 2/mm³ 95th Percentile: 7

Median: 2/mm³ 95th Percentile: 7
Enterovirus Testing

- Higher WBC counts in EV negative group
  - May be physician driven
  - May be a virus not tested for by PCR
  - May represent spectrum of normal

- Implications
  - Reference values can potentially shift with advances in PCR
Limitations

- Preterm Infants included
  - CSF WBC dependent on postnatal age, not postconceptional age
  - Analyzed with term infants

- Antibiotics prior to LP included
  - Additional criteria of gram stain, protein and glucose used
  - Unlikely that infants with bacterial meningitis are included
Conclusions

- Infants 0-28 Days: 95th percentile: $19/\text{mm}^3$
- Infants 29-56 Days: 95th percentile: $9/\text{mm}^3$
- Largest sample population to date
- Strict exclusion criteria used
- Age dependent reference values are essential to interpret results of lumbar puncture
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Research As a Medical Student

- Define your goals
- Carrying out a project
- What to do with your finished work
- Funding
Defining your goals

- Are you curious what research is about?
- Are you ‘checking off’ your scholarly pursuit?
- Do you want a full year experience?
- Do you want a full project without taking a year out?
Meeting your goals

- Summer projects
- Scholarly pursuit
- Year out
  - Doris Duke Fellowships
  - NIH fellowships
  - Pathology fellowship
  - And so on . . .
- Scholarly pursuit+
Choose your project

- Pick a topic that interests you
  - Clinical vs. basic science
  - Can be related to your future field but not essential

- Pick a mentor
  - Lecturers?
  - Clerkship attendings?
  - Mentors from previous years?
A Timeline

- Driven by your goals
- August of your clerkship year: Start thinking
- Email people, set up meetings
- Build your fourth year around your goals
  - Do your project done before interview season?
  - Do you want a letter from your advisor?
  - Do you want to do all of your research in the fall during interviews?
Your project!

- You have a mentor, a topic, and a time to start!
- You can work on ANY part of a project
  - IRB submission
  - Background research
  - Data collection
  - Data Analysis
  - Abstracts/Manuscripts/Posters
Things to Expect

- Everything takes longer than expected
- Good research takes time
  - IRB revisions
  - Data collection is tedious
  - Data cleaning prompts review of data
  - Writing requires multiple drafts, revisions
Preparing your work

- Abstracts
  - Short 10 sentence summaries of your work
  - Submitted to conferences or journals

- Posters
  - Visual presentations
  - Present at conferences/Research Day

- Manuscripts
  - Submit to journals
Funding

- Small projects can be done without grants
- Funding always helps
  - Off set small project expenses
  - Living for a summer
  - Support you for a year
- Requires ambition on your part
  - Read your email & check websites
  - Apply!
Thank you

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