

Title: Gram-Positive Diplococci in a Cerebrospinal Fluid Gram Stain

Running Title: Gram-Positive Diplococci in the CSF

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A five-month-old previously healthy female presented with fever and new-onset seizures. Four days before presenting to medical attention, she developed a temperature up to 38.6°C, cough, nasal congestion, and vomiting. She had been evaluated at an urgent care center at that time and was diagnosed with bilateral otitis media. She was prescribed amoxicillin. She continued to have fever and presented with respiratory failure and a prolonged seizure. Her physical exam was notable for meningismus. A computed tomography scan of the head noted bilateral middle ear and mastoid opacification. Analysis of the patient's cerebrospinal fluid (CSF) revealed a leukocyte count of 478 cells/ μ L (69% polymorphonuclear cells), a glucose concentration of 4 mg/dL, and a protein concentration of 213 mg/dL. A cytopsin-prepared CSF Gram stain revealed Gram-positive cocci arranged in pairs, some of which were within leukocytes (Figure 1). A clear area ("halo") was apparent around the intra-leukocytic organisms, suggesting an encapsulated bacterial species. The patient's blood and CSF cultures grew *Streptococcus pneumoniae* serotype 35B, a capsular serotype not included in the 13-valent pneumococcal conjugate vaccine. Notably, the isolates exhibited a penicillin minimum inhibitory concentration value of 2 μ g/mL, which is considered resistant when interpreted using the parenteral penicillin meningitis breakpoints for *S. pneumoniae*.(1)

Marked declines have occurred in invasive pneumococcal serotypes included in or related to the 13-valent pneumococcal conjugate vaccine (PCV-13) in both children and adults (through herd protection) in the U.S. Previously uncommon serotypes are now responsible for an increasing proportion of invasive disease, and serotype 35B was the fourth most common invasive isolate (8%) among U.S. children less than five years of age in 2012 – 2013.(2) Overall, antibiotic-resistant invasive pneumococcal cases declined significantly among young children by three

years after PCV13 licensure.(2) In a study of invasive and non-invasive pneumococcal isolates from across the U.S. in 2012 - 2013, serotype 35B had become the most common penicillin non-susceptible serotype (24.8%) and the fourth most common multi-drug resistant serotype (6.4%)(3).

Cells of *S. pneumoniae* are elongated (“lancet-shaped”) cocci that are surrounded by a polysaccharide capsule and predominantly organized in pairs (diplococci). Other Gram-positive organisms that cause meningitis in neonates and infants include *Streptococcus agalactiae* (Group B Streptococcus), a coccus ordered in pairs and chains, and *Listeria monocytogenes*, a short rod.

Figure 1. Cytospin-prepared Gram stain of the patient’s CSF (original magnification $\times 1,000$).

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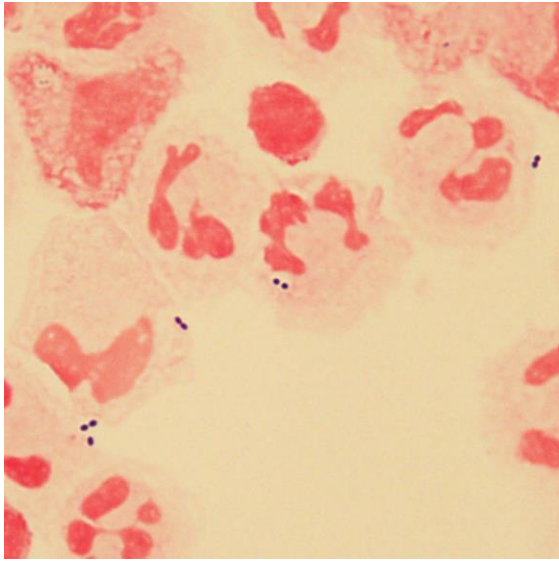
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Conflict of Interest:

Evan J. Anderson reports personal fees for consulting from AbbVie and clinical trial funding and editorial assistance from MedImmune, outside the submitted work. Jennifer P. Collins and Lars F. Westblade declare no competing interests.

Figure 1



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