**Streptococcus pneumoniae**-Related Hemolytic-Uremic Syndrome Identified by the Karius Test

The Karius Test can detect culture-negative infections and has the potential to identify clusters of disease.

### PATIENTS

Four pediatric patients (ages ranging 11 - 42 months) from two separate hospitals (Washington, DC and San Diego, CA) were part of this case series.

All patients had severe pneumonia with two patients having negative blood and respiratory cultures. These patients also had clinical symptoms consistent with hemolytic-uremic syndrome with three patients requiring hemodialysis via continuous renal replacement therapy.

### RESULTS

The Karius Test detected *Streptococcus pneumoniae* in all 4 patients at extremely high levels ranging from ~1 to 150 million MPMs (Molecules of cell-free DNA fragments of the pathogen Per Microliter of plasma).

Further analysis of the sequencing data revealed that *S. pneumoniae* serotype 3 was present in 3 out of the 4 patients. Serotype 3 continues to cause significant invasive disease despite its inclusion in the Pneumococcal Conjugate Vaccine.

This cluster of patients HUS and *S. pneumoniae* would otherwise have gone undetected if they were not all identified via the high levels of *S. pneumoniae* detected by the Karius Test.
Streptococcus pneumoniae Related Hemolytic Uremic Syndrome (pHUS) and the Identification of Matched Cross-Country Strains by Next-Generation Sequencing (NGS)

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Introduction

Hemolytic uremic syndrome (HUS) describes a presentation of acute kidney injury, microangiopathic hemolytic anemia and thrombocytopenia, which often occurs following infection, though it can be genetic or autoimmune in origin. Five to 15% of HUS cases are related to S. pneumoniae infection, most often meningitis or pneumonia. Despite the introduction of PCV13 and an overall decrease in incidence of invasive pneumococcal disease in children, the incidence of pneumococcal related HUS (pHUS) cases is rising. Efforts have been made to determine if certain factors increase the risk of development of pHUS in patients with suspected pneumococcal disease. These efforts are often hampered by culture collection occurring after empiric antibiotic administration, which may inhibit culture growth and limit identification. Alternative methods of microbiologic identification, such as next-generation sequencing (NGS), may be useful in determining specific etiologies of syndromes such as HUS that can have infectious triggers. We present the cases of 4 children, from two distant institutions, with concern for HUS in the setting of pneumonia. These efforts are often hampered by culture collection occurring after empiric antibiotic administration.

Methods

Patient Characteristics

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>PCV 13 UTD</th>
<th>Presenting Illness</th>
<th>Positive S. pneumoniae culture?</th>
<th>Other major interventions</th>
<th>Viral Co-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rady 1</td>
<td>11 mo</td>
<td>Yes</td>
<td>Multifocal PNA with effusion</td>
<td>Yes (B,R)</td>
<td>CRRT, Epi/Norepi, chest tubes, ventilator, steroids</td>
<td>hMPV</td>
</tr>
<tr>
<td>CNMC 1</td>
<td>18 mo</td>
<td>Yes</td>
<td>Bilateral multifocal PNA with effusion</td>
<td>No</td>
<td>CRRT, Epi/Norepi, chest tubes, ventilator, eculizumab</td>
<td>RSV B</td>
</tr>
<tr>
<td>Rady 2</td>
<td>26 mo</td>
<td>Yes</td>
<td>Lobar PNA</td>
<td>No</td>
<td>CRRT, Epi, chest tubes, ventilator</td>
<td>Influenza A</td>
</tr>
<tr>
<td>Rady 3</td>
<td>42 mo</td>
<td>No</td>
<td>Lobar PNA with effusion; bacteremia</td>
<td>Yes (B,R)</td>
<td>Epi/Norepi, chest tubes, ventilator, IVlg, steroids</td>
<td>Influenza A</td>
</tr>
</tbody>
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R-Respiratory, B-blood, U-urine, P-pleural fluid

Background

• pHUS is seen in cases of complicated pneumonia (PNA).1,2
• Incidence of pneumococcal related HUS (pHUS) cases is rising3 for unclear reasons, despite use of PCV13 and an overall decrease in invasive pneumococcal disease in children3
• PCV13 includes serotypes 1,3,4,5, 6A, 6B, 7F, 9V, 14, 18C, 19 A, 19F, 23F
• Yield of blood cultures in patients with pneumonia is poor (<5%)4 and often impaired by culture collection occurring after empiric antibiotic administration.

Results

All four samples were found to be positive for S. pneumoniae at extremely high levels (Figure 1). Three (3) out of 4 samples were identified as serotype 3 by NGS. The fourth sample was similar to the others but ultimately identified as serotype 12A. S. pneumoniae culture isolates from R1 and R3 were both independently confirmed as serotype 3.

Table 1: Karius NGS serotype determination was performed via two methods: 1. Analysis of reads aligning to the cps locus and 2. Inference of the serotype based on S. pneumoniae strain determination from all sequencing reads.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Identification</th>
<th>MPM (molecules/μL)</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>S. pneumoniae</td>
<td>9,122,698</td>
<td>3</td>
</tr>
<tr>
<td>C1</td>
<td>S. pneumoniae</td>
<td>1,957,238</td>
<td>3</td>
</tr>
<tr>
<td>R2</td>
<td>S. pneumoniae</td>
<td>151,941,207</td>
<td>12A</td>
</tr>
<tr>
<td>R3</td>
<td>S. pneumoniae</td>
<td>1,435,748</td>
<td>3</td>
</tr>
</tbody>
</table>

• NGS is useful for pathogen detection and quantitation of culture-negative infections
• Karius NGS has potential to identify clusters of disease that would likely otherwise have gone undetected.
• The extremely elevated levels of pathogen DNA, 1000-100,000 fold higher than in non-HUS cases of S. pneumoniae disease, may inform the pathophysiology of pHUS.
• NGS may be useful in determining specific etiologies of syndromes such as HUS that can have infectious triggers
• Serotype 3 strains of S. pneumoniae continue to be a common cause of pneumococcal invasive disease and pHUS, despite inclusion in PCV13.

References

3. www.CDC.gov/pneumococcal/surveillance