#90: Outcomes of Gram-negative Bacteremia Managed According to a Risk-based Algorithm

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Background. Management of gram-negative bacteremia (GNB) in children with cancer (CWC) is complicated by increasing rates of antimicrobial resistance. Guidelines for the management of CWC with suspected serious bacterial infections at St. Jude Children's Research Hospital (SJ) recommend cefepime (CEF) monotherapy for empirical therapy for most patients. Children with evidence of sepsis, recent treatment with CEF or known colonization with a CEF-resistant (CEF-R) organism are initially treated with meropenem and/or amikacin. We compared outcomes of CWC with infections caused by CEF susceptible (CEF-S) and CEF-R bacteria treated according to this algorithm.

Methods. Demographic information on patients, and clinical and microbiological characteristics of 100 episodes of GNB treated from May 2018 to April 2019 at SJ were retrospectively reviewed.

Results. Patients' median age was 8 years; 48% were female. Overall, 46% of patients had leukemia or lymphoma, 40% solid tumors, and 14% were HSCT recipients. Patients were neutropenic [absolute neutrophil count (ANC) <1000] during 70% of episodes and severely neutropenic (ANC <100) in 59%. The most common bacteria identified included *E. coli* (42%), *Ps. aeruginosa* (17%), and *Klebsiella* spp. (10%); 26% of episodes were polymicrobial and 24% of patients had focal infections complicating their bacteremia. Overall, 52% of episodes were caused by CEF-R organisms, including 57% of infections caused by *E. coli*. Demographic characteristics of CWC with infections caused by CEF-R organisms were similar to those with CEF-S organisms, except that these patients were more likely to have leukemia (adjusted OR 3.6, 95% CI 1.3–9.8) and to have had previous colonization or infection caused by a CEF-R organism (adjusted OR 14.4, 95% CI 2.7–76.0). CWC with infectious caused by CEF-R organisms were more likely to receive intensive care at admission and in the first 3 days of hospitalization than those with CEF-S isolates, but these differences were not statistically significant. The median time to administration of effective antimicrobial therapy was 0.53 hours for episodes caused by CEF-S bacteria and 12.05 hours for episodes caused by CEF-R organisms ($P < 0.001$), and CEF-R bacteremia was associated with increased time to microbiological resolution ($P = 0.002$) and a greater likelihood of early modification of treatment because of poor clinical response (5% vs. 14%, $P = 0.043$). CWC with CEF-R bacteremia had a longer median duration of fever (1 day vs. 2 days, $P = 0.015$), time to resolution of other signs and symptoms of infection (2 days vs. 4 days, $P = 0.005$), and median duration of hospitalization (6 vs. 11.5 days, $P < 0.001$) relative to CEF-S infections. All-cause mortality at hospital discharge and at 30 days was 8% in both CEF-S and CEF-R infections and was associated with the presence of relapsed or refractory malignancy and other significant medical comorbidities.

Conclusion. Over half of the episodes of GNB in this study were caused by CEF-R organisms. Infection with CEF-R organisms was associated with a delay in the administration of effective therapy and greater morbidity, but not greater mortality. Further studies are warranted to clarify the relationship between antimicrobial resistance, disease severity, treatment, and infection outcomes.

#97: Rapid, Noninvasive Detection of *Kingella kingae* Pediatric Vertebral Infections Using a Microbial Cell-free DNA Sequencing Test for Pathogen Identification

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Background. *Kingella kingae* is a recognized cause of bone and joint infections (BJI) in infants. The diagnosis of *Kingella kingae* BJI can be challenging due to its fastidious growth with conventional culturing methods even when infected tissue is obtained. *Kingella kingae* spinal infections are likely an underdiagnosed entity given the limitations of culture-based methods and the reluctance to biopsy spinal locations of infection (in favor of empiric treatment). This approach often necessitates MRSA coverage. A sensitive, rapid, noninvasive diagnostic approach to pediatric vertebral infections would enable targeted therapy. Detection of circulating microbial cell-free DNA (mcfDNA) in the plasma originating from areas of sequestered infection through next-generation sequencing (NGS) has shown utility in pediatric pneumonia (Farnaes *et al.* DMID 2019) and a wide variety of infections in the immunocompromised host (Rossoff *et al.* OFID 2019) and potentially offers promise in resolving the etiology of pediatric vertebral infections.

Methods. The Karius test is a CLIA-certified/CAP-accredited NGS plasma test that detects circulating mcfDNA in the blood. After mcfDNA is extracted and NGS performed, human sequences are removed and remaining sequences are aligned to a curated pathogen database of >1400 organisms. Organisms present above a statistical threshold are reported and quantified. The time to result reporting is on average 24 hours from sample receipt. Karius Test results over the prior 2 years were reviewed for detections of *Kingella kingae* in the context of spinal infections. Clinical chart review was performed by the treating pediatric infectious diseases physicians at each participating institution after IRB notification and approval.

Results. Six cases of *Kingella kingae* pediatric vertebral infections were identified across five institutions; clinical data were available for five cases across four institutions (see Table). Four cases were male; the average age was 15.3 months. Four of five cases had an antecedent URI. The clinical presentations were characterized by decreased mobility and relatively bland inflammatory response (lack of fever, bland inflammatory markers). The lumbar region was the most commonly affected vertebral location (80%). Blood cultures were negative in all cases; empiric anti-MRSA therapy was initiated in all cases. The time to result of *Kingella kingae* mcfDNA detection in the plasma was one day from sample receipt in all cases. McfDNA from co-pathogens were detected in 66.7% of cases (*Haemophilus influenzae* was the most common). The detection of *Kingella kingae* by the Karius test influenced a decision to narrow coverage in 80% of cases and a decision to forego biopsy in 60% of cases.

Conclusion. Plasma NGS for circulating mcfDNA offers a rapid, noninvasive means of detecting *Kingella kingae* pediatric vertebral infection. This culture-independent approach may enable specific diagnosis despite antibiotic pretreatment and obviate the need for an invasive procedure. Accurate identification of *Kingella kingae* has important implications on antibiotic stewardship enabling targeted therapy without the reliance on empiric MRSA coverage. Given the capacity to detect over 1400 organisms from a single sample NGS for mcfDNA offers a means to detect a broad variety of pathogens known to have predilection to cause pediatric spine infection.

#99: *Streptococcus pyogenes* Utilizes the Peptide-Based Rgg 2/3 Quorum Sensing System During Oropharyngeal Colonization

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Background. *Streptococcus pyogenes* is a human-restricted pathogen most often found in the human nasopharynx. Multiple bacterial factors have been found to contribute to persistent colonization of this niche, and many of these factors are important in mucosal immunity and vaccine development. In this work, we infected mice intranasally with transcriptional regulator mutants of the Rgg2/3 quorum sensing (QS) system—a peptide-based signaling system conserved in all sequenced isolates of *S. pyogenes*.

Methods. Three-week-old CD1 mice were intranasally infected with ~10^7 CFU of *S. pyogenes* strain MGAS315. Calcium alginate throat swabs were used to monitor nasopharyngeal colonization by the bacteria over time. Luciferase reporters used alongside an IVIS camera were able to show quorum sensing activity levels after inoculation into the mouse nose. Bacterial RNA was isolated from the throat of the mice and quantitative RT–PCR was performed on the samples to corroborate the luciferase reporter data. The nasal-associated lymphoid tissue (NALT) was excised and its supernatants were subjected to 32-plex murine cytokine and chemokine analysis (Millipore).

Results. Deletion of the QS system's transcriptional activator (*Δrgg2*) dramatically diminished the percentage of colonized mice. Deletion of the transcriptional repressor (*Δrgg3*) increased the percentage of colonized mice compared with wild type. Stimulation of the QS system using synthetic pheromones prior to inoculation did not significantly increase the percentage of animals colonized, indicating that activity of the QS system is responsive to conditions of the host nasopharynx. Mice inoculated with QS-dependent luciferase reporters were subjected to *in vivo* imaging and showed activation within 1 hour. Bacterial RNA extracted directly from oropharyngeal swabs and evaluated by quantitative RT–PCR subsequently confirmed QS upregulation within 1 hour of inoculation. In the nasal-associated lymphoid tissue (NALT), a muted inflammatory response to the Δ*rgg2* bacteria suggests that their rapid elimination fails to elicit the previously characterized response to intranasal inoculation of GAS.

Conclusions. Deletion of the Rgg2 transcriptional activator of the Rgg 2/3 quorum sensing system eliminates colonization of the murine nasopharynx and changes the transcriptional profile of the bacteria in this niche. An existing small-molecule inhibitor of the Rgg2/3 system was unable to inhibit QS activation *in vivo*, likely due to the suboptimal achievable doses; however, results of our study indicate inhibition of QS may diminish the oropharyngeal colonization of *S. pyogenes* and argue for further development.

#63: Antibodies to Peptides Representing *Plasmodium falciparum* Circumsporozoite Protein Reflect Acquisition of Naturally Acquired Immunity in Malian Adults and Children

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Background. An effective vaccine against *Plasmodium falciparum,* the most common and deadly cause of malaria, is a global priority. Circumsporozoite protein (CSP) is a major *P. falciparum* vaccine target. Previously recognized CSP epitopes include the immunodominant NANP repeat region, the conserved junction between Region 1 (R1) and the NANP repeats, and the polymorphic Th2R and Th3R in the