Background. Blood cultures (BC) fail to detect a pathogen in most patients with neutropenic fever (NF). We examined the performance of the Karius next generation sequencing plasma test (plasma NGS) compared with that of BC in chemotherapy-induced NF.

Methods. Patients 5 years or older with absolute neutrophil count <500 cells/mm$^3$ anticipated for >7 days were enrolled at the time of BC collection (T0) due to fever. Plasma samples were collected at T0 and twice weekly until neutrophil recovery or discharge. Samples were shipped to the Karius CLIA/CAP laboratory (Redwood City, CA) where cell-free plasma was prepared, DNA extracted, and NGS performed. After removing human reads, remaining sequences were aligned to a curated pathogen database. Organisms present at a significance-level above a predefined threshold were reported. BC and plasma NGS results were compared, excluding organisms (eg, viruses) not recoverable by BC. Positive agreement was defined as plasma NGS identification of at least one isolate seen on BC. Plasma NGS+/BC- results underwent adjudication by 3 infectious diseases specialists. Diagnosis was Definite if microbiology confirmed NGS result within 7 days of enrollment; Probable if clinical, radiologic, and laboratory data were compatible with plasma NGS test result; and otherwise Indeterminate.

Results. Three hundred sixty-two patients were evaluated. At the time of enrollment, 6 patients were on antibiotics and 3 were on antifungals. There were 4 positive BC (each monomicrobial) at T0 and all were concordant with plasma NGS. The plasma NGS test identified additional organisms in 2 of the BC+ patients with surgical abdomen. Compared with BC, plasma NGS showed positive agreement in 100% (106/106) of plasma samples. Concordance between plasma and single or mixed organisms in patients with enterococcal or severe mucositis. In one sample, Aspergillus fumigatus was detected in a patient with new lung nodule.

Conclusion. The etiologic diagnosis of NF is frequently unknown, leading to broad antibiotics and sometimes delay of targeted treatment. Plasma NGS may provide useful data for managing NF given its ability to detect a breadth of pathogens even when samples are collected with antibiotics.

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2085. Metagenomic Analysis reveals Importance of Anaerobes in Development and Clinical Outcome of Necrotizing Soft-tissue infections

Hannah Zhao-Fleming, BS; Jeremy Wilkinson, PhD and Kendra Rumbaugh, PhD

Department of Surgery and Burn Center of Research Excellence, Texas Tech University Health Sciences Center, Lubbock, Texas, Research and Testing Laboratory, Lubbock, Texas

Background. Skin and soft-tissue infections can manifest in a variety of ways, ranging from a self-resolving abscess to a rapidly spreading necrotizing soft-tissue infection (NSTI). Based on culture data, the microbiology of both infections are similar, both involving Gram-positive cocci such as Staphylococcus species. This begs the question – why would different patients start with seemingly similar infections and end with drastically different clinical courses? One factor is the patient’s immune response, but it does not fully account for many NSTIs that occur in otherwise healthy individuals. We hypothesize that anaerobes, which are difficult to detect via culture and thus are under-detected, worsen the infection, favoring the NSTI pathology. Our objective in this study was to better understand the impact of anaerobes in NSTIs.

Methods. We enrolled adult patients that had been diagnosed either with NSTIs or abscesses. We collected samples of their infections via routine skin debridements or incision and drains, respectively. We then extracted DNA from each sample and sequenced the variable regions 1–2 of the 16S rRNA. The sequences were compared against an in-house database and for species identification.

Results. From December 2011 to the present, we have collected 26 NSTI samples and 19 abscess samples. We found that a higher proportion of obligate anaerobes in the wound is correlated with increased BMI and mortality in NSTIs. The NGS microbiomes of patients who died from their infections also seem to cluster together. Moreover, our preliminary data suggest that NGSs may also have a higher proportion of obligate anaerobes and higher microbial diversity compared with abscess infections. Lastly, we noted significant discordance between data obtained by our institution’s clinical microbiology laboratory and our 16S data.

Conclusion. The presence of obligate anaerobes, traditionally difficult to detect via culture, plays a significant role in the development and worsened clinical outcome of NSTI. Their presence may be one of the determining factors analyzing the NSTI pathology over the easily-treated abscess. If true, this should lead to more aggressive and comprehensive anaerobe coverage in NSTIs, especially in culture-negative infections.

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2086. Evaluation of a Multiplex PCR Assay with Molecular Beacon Probes to Rapidly Detect Bacterial Pathogens Directly in Bronchial Alveolar Lavage (BAL) Samples from Patients with Hospital-Acquired Pneumonia (HAP)

Kalyan D. Chaudhry, PhD; Michael Satlin, MD, MS; Liang Chen, PhD; Bhaskar Chand, MD; Claudia Manca, PhD; Lars Westblade, PhD; Stephen J. Gross, PhD; Thomas J. Lurie, MD; James E. Tompkins, PhD; Barry Naiman, MD, MS

Public Health Research Institute, Rutgers New Jersey Medical School, Newark, New Jersey, New York-Presbyterian Hospital/Weill Cornell Medical Center, New York, New York, Weill Cornell Medicine, New York, New York, Pathology and Laboratory Medicine, Weill Cornell Medical, New York, New York, Internal Medicine/Infectious Diseases, Weill Cornell Medicine, New York, New York

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Background. Bacterial pneumonia is a common complication in hospitalized patients and it is associated with high morbidity and mortality. Standard culture-based methods may take 2–3 days to identify the etiologies of HAP, leading to delays in appropriate therapy. A rapid molecular assay that could diagnose the etiology of bacterial pneumonia directly from BAL samples within a few hours could facilitate faster and more directed administration of antimicrobial therapy.

Methods. BAL samples were collected from hospitalized patients with suspected pneumonia, including ventilated patients, from December 2016 through April 2017. Genomic DNA was isolated from BAL samples using NucliSENS easyMAG. A panel of target-specific molecular beacon probes in a real time PCR assay (MB-PCR) was used to identify the following pathogens: universal bacterial Identification (16S RNA), E. coli (uidA), K. pneumoniae (gpyA), S. aureus (papA), P. aeruginosa (rplA), A. baumannii (Ab-ITS) and the following resistance determinants: ESBLs (CTX-M, TEM and SHV), carbapenemases (NDM, VIM, IMP, OXA-48 and KPC) and mecA. The results of MB-PCR were then compared with quantitative culture results performed by the clinical microbiological lab.

Results. We evaluated 53 BAL samples to identify the bacterial pathogen and key resistance determinants. Thirty-one samples yielded growth of $<1 \times 10^6$ CFU/mL of bacteria by quantitative culture. The bacterial identification using MB-PCR for 16S RNA correctly identified the presence of bacteria in all 31 samples (100% sensitivity). The MB-PCR identified P. aeruginosa (n = 5), S. aureus (n = 5), E. coli (n = 1), A. baumannii (n = 1), and K. pneumoniae (n = 1) in BAL samples that yielded $<1 \times 10^3$ CFU/mL of the same pathogen by culture (100% sensitivity). The MB-PCR also identified bla$_{\text{CTX-M}}$, harboring E. coli that grew ampicillin-resistant E. coli by culture. The specificity of the 16S RNA probe was 79%, while the MB-PCR was false positive, whereas the specificity for the MB-PCR was 100% for P. aeruginosa, S. aureus, E. coli, and A. baumannii, and 98% for K. pneumoniae.

Conclusion. Multiplex MB-PCR assay is a rapid, sensitive and specific tool for detection of common bacterial causes of nosocomial pneumonia and important resistance determinants directly from BAL samples.

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2087. A Novel Real Time PCR Assay for the Detection of Babesia microti in a Highly Endemic Area Using Luminex Aries System

Alamelu Chandrasekaran, PhD; Fan Zhang, MD; Ansamma Joseph, PhD; Maureen Bythow, BS; Reeti Khare, PhD, DA(BMM) and Stefan Juretschko, PhD, DB(ABMM)

Pathology and Laboratory Medicine, Northwell Health Laboratories, New Hyde Park, New York, Hoffman-La Roche’s New School of Medicine, Hemstead, New York

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Background. Babesiosis is a tick-borne infection of erythrocytes caused by parasites of the genus Babesia. In United States most of the reported cases occur in the Northeast and upper Midwestern states, New York being an endemic area. Majority of the cases in the United States is due to infection with Babesia microti. Most patients with babesiosis are asymptomatic or have a mild illness, but some may develop fatal illness. Babesiosis is diagnosed by a microscopic demonstration of intraerythrocytic Babesia parasites in blood films which requires specially trained personnel. We have developed a simple and rapid PCR assay for the detection of B. microti 185 RNA gene (BMPCR) which can be used as an alternative screening assay.

Methods. BMPCR assay was developed based on Luminex Multicycle technology using Luminex Aries instrument and in-house developed primers. Babesia positive clinical samples confirmed by NY State Department of Health and clinical samples negative for Babesia were used to determine accuracy. Specificity was evaluated using malaria positive samples, several bacterial isolates, selected B. microti strains and other Babesia species. Limit of Detection was determined using known copies of B. microti DNA. Reproducibility of the assay was assessed by testing samples on different days and run by different analysts and PCR cycles.

Results. Accuracy of the BMPCR assay was 100% for the 30 Babesia positive and 30 negative samples. All B. microti ATCC strains were positive by BMPCR. 95% LOD of the assay as determined by probit analysis was 1177 copies/50µl of blood. Sensitivity of the assay was higher than that of microscopic detection and specificity was 100%.