Performance of a Novel Plasma-Based Next-Generation Sequencing Assay in Patients with Bacteremia

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ABSTRACT

Background: Blood cultures are routinely obtained during the diagnostic evaluation for a number of infectious conditions such as sepsis, M. tuberculosis, and pneumococcal pneumonia. Despite its ubiquitous use, blood cultures have poor sensitivity due to a number of factors including previous antibiotic therapy or the presence of poorly-growing fastidious organisms. There is a need for more comprehensive diagnostic tests that can overcome these limitations to aid in guiding therapy.

Methods: We developed a plasma next-generation sequencing (NGS) assay capable of detecting over 5,000 bacteria, viruses, and eukaryotic pathogens. To evaluate this assay in bacteremia, patients with multi-set positive and negative blood cultures were identified. We used our assay to analyze residual plasma samples that were obtained on the same day as the blood cultures. DNA was extracted from plasma and NGS performed. All filtering parameters, remaining reads were aligned to a pathogen sequence database. Relative abundance of each individual organism was calculated and pathogens estimated to be present with high statistical significance were identified.

Results: In comparing this novel NGS assay to blood culture and all other microbiologic data obtained from the patient, Positive Agreement was 82.4% (123/149) and Negative Agreement was 79.1% (132/170) and Negative Agreement was 79.1% (132/170) with an Overall Agreement of 80.7%. When comparing directly to blood culture, there was agreement in culture-positive specimens in 100 of 125 specimens (80%). For blood culture-negative specimens, NGS did not detect a pathogen in 100 of 145 specimens (70.0%). Of the 59 false positive detected by the NGS assay compared with blood culture, 10 were found to be true positives when compared with microbiologic data from the patient. These included detection of pathogens by plasma NGS that were found in endotracheal cultures, nasopharyngeal PCR, peritoneal cultures, or recently positive blood cultures.

Conclusion: We present data that show high concordance between a novel NGS assay and blood culture in patients with and without bacteremia. In addition, the assay was able to identify pathogens in plasma corresponding to pathogens identified from culture of body sites with recent or recent antibiotic therapy or the presence of poorly-growing fastidious organisms. There is a need for more comprehensive diagnostic tests that can overcome these limitations to aid in guiding therapy.

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