Detection Of Previously Missed Pathogens In Immunocompromised Children With Pneumonia by A Fully-Validated Next-Generation Sequencing Test

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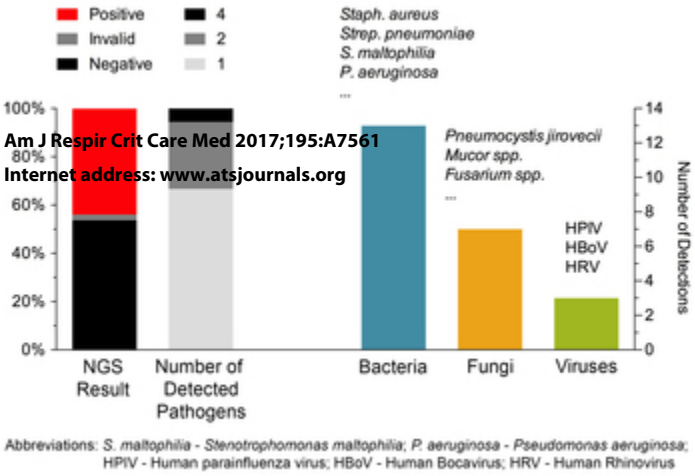
RATIONALE. Pneumonia is the most common tissue-invasive infection in immunocompromised patients and can be caused by a wide range of common and opportunistic pathogens. Early etiologic diagnosis and initiation of effective treatment is critical to reduce morbidity and mortality but current tests remain negative in up to 60% of patients. Next-generation sequencing (NGS) can be used to detect any known pathogen with a single test. This catch-all approach eliminates the need for large test panels, is faster than culture for slow-growing organisms, and can shorten diagnostic odysseys. However, complex workflows and long turn-around-times have hindered adoption in routine practice. We have developed and extensively validated an NGS test for respiratory pathogens that can generate final results within 48 hours. We assessed diagnostic yield by testing bronchoalveolar lavage (BAL) samples from 41 immunocompromised children receiving intensive care for pneumonia with negative microbiology tests.

METHODS. RNA and DNA from BAL samples was sequenced (~15 million sequencing reads/sample) and analyzed with TaxonomerDx, our diagnostic-grade, rapid data analysis tool. Sensitivity and specificity for >200 respiratory pathogens were validated using >200 real and contrived BAL samples and thousands of virtual, *in silico* generated BAL samples. Banked BAL samples from children with leukemia, solid organ or hematopoietic stem cell transplant, severe combined immunodeficiency and/or prolonged high dose steroid therapy were included based on radiographic and clinical evidence of pneumonia and extensive, broad, unrevealing diagnostic workup.

RESULTS. Test validation demonstrated high agreement with conventional microbiology tests (90.2% for bacterial, 94.1% for virus, 66.7% for fungal detection) and >98.8 accuracy and specificity with virtual BAL samples. Previously missed putative pathogens were identified in 18 of 41 immunocompromised children (44%) with life-threatening pneumonia, including 7 of 11 children (64%) with fatal infections. The NGS test detected a single pathogen in 12 (63%), 2 pathogens in 5 (26%), and 4 pathogens in 1 (5%) patient. Bacterial pathogens were detected in 13, fungal pathogens in 7, and viral pathogens in 3 patients (Figure).

CONCLUSIONS. We have developed the first fully-validated NGS test for detection of >200 respiratory pathogens with rapid turn-around time. This universal pathogen detection test identified putative pathogens in 44% of previously test-negative, immunocompromised children, including in 64% of children with fatal outcome. Universal, NGS-based pathogen detection can provide rapid etiologic diagnosis with improved yield over conventional tests and may help initiate effective treatment sooner and more often. This test will be available at our national reference laboratory.



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