Detection of Previously Missed Pathogens in Immunocompromised Children with Suspected Pulmonary Infections by a Validated Next-Generation Sequencing Test (Explify™ Respiratory)

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RATIONALE

Pulmonary infections are 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[1]. We have developed and validated an NGS test for respiratory pathogens that can generate final results within 48 hours[2]. We assessed diagnostic yield by testing bronchoalveolar lavage (BAL) samples from 41 immunocompromised children receiving intensive care for suspected pulmonary infections with negative microbiology tests.

METHODS

Figure 1. *RNA and DNA from banked BAL samples was sequenced according to validated protocols to a depth of ~15 million sequencing reads per sample on illumina NextSeq sequencers. Resulting metagenomics data was analyzed with Explify, our diagnostic-grade, rapid data analysis tool, with validated reporting criteria. Explify™ is based on Taxonomer, an ultra-fast metagenomics data analysis research tool with improved speed and accuracy for microorganism detection[3]. Analytical performance was validated using >200 real and contrived BAL samples and thousands of virtual, in silico generated BAL samples.*

Test Validation

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.

Patient Population

Children (n=41, mean age 11 years) were included based on clinical and radiographic evidence of pulmonary infection (new consolidation, other infiltrates, nodules and/or pleural effusions), extensive, unrevealing, diagnostic workup,, immunosuppression, broad-spectrum antimicrobial therapy, and bronchoscopic BAL. Children were not intubated at the time of specimen collection.

RESULTS

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.

Table 1. *Reasons for immunosuppression*

Condition	n	%
Bone marrow transplantation	17	42
Solid organ transplantation	11	27
Primary immunodeficiency	7	17
Chemotherapy	6	15
High dose steroid therapy	2	5

Table 2. *Radiographic findings*

Condition	n	%
Diffuse opacities [with consolidation]	28 [7]	68 [17]
Nodules	6	15
Pleural effusions	6	15

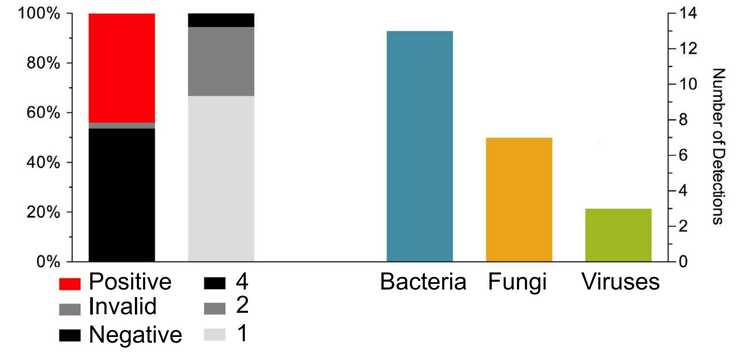

Figure 2. *Putative or possible pathogens were identified in 44% of children. In 67% of specimens, only 1 pathogen was detected. Pathogens included bacteria (n=13; K. pneumoniae, H. influenzae, Staph. aureus), fungi (n=7; Mucor spp., Fusarium spp., Pneumocystis jirovecii) and viruses (n=3; HPIV, HRV, HBoV).*

CONCLUSIONS

This first validated NGS test for detection of >200 respiratory pathogens identified putative and possible causes in 44% of test-negative, children with suspected respiratory infections. Hypothesis-free pathogen detection provided improved diagnostic yield with clinically-actionable turn-around time. This test will be available at our national reference laboratory and will enable clinicians to initiate effective treatment sooner and more often.

ACKNOWLEDGEMENTS

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