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Detection of Pathogen DNA using a Novel Plasma-Based Next-Generation Sequencing Assay in Patients with Respiratory Infection

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Stanford MEDICINE

ABSTRACT

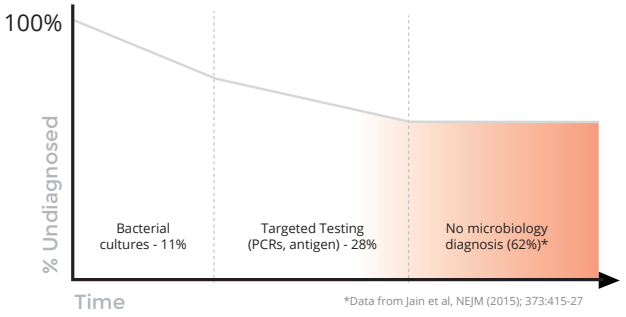
Background: Determining the etiology of lower respiratory tract infection is challenging. Blood cultures are positive in only 5-14% of patients with pneumonia and it can be difficult to distinguish colonization from infection among organisms identified in sputum or endotracheal cultures. The low sensitivity and specificity current diagnostic tests results in empirical treatment, which can lead to antibiotic overuse, particularly in the treatment of hospital-acquired pneumonia. There is a need for better diagnostics to aid in the management of respiratory infections.

Methods: We have developed a plasma next-generation sequencing (NGS) assay capable of detecting a wide breadth of over 5,000 bacteria, viruses and eukaryotic pathogens. Patients with positive respiratory microbiology tests were identified from a cohort of subjects with blood culture and plasma NGS results . NGS was performed on plasma samples that were obtained on the same day as the blood culture samples. DNA was extracted from plasma and NGS performed. After filtering human sequences, remaining sequences were aligned to a pathogen reference sequence database. Relative abundance of each individual microorganism was calculated and pathogens estimated to be present with high statistical significance were identified.

Results: We identified 25 patients with positive respiratory samples up to 5 days before, or 1 day after, blood cultures and plasma for NGS were collected. Of these 25 patients, eleven had positive NGS plasma assays that identified the same species as was found in the respiratory test. These included confirmation of *Enterobacter aerogenes* (3), *Escherichia coli* (2), *Enterococcus* (2), *Moraxella catarrhalis*, *Serratia marcescens*, *Staphylococcus aureus*, and adenovirus. In contrast, only three patients had positive blood cultures that matched the respiratory tests. The 14 remaining patients with NGS assays that did not match respiratory tests had sputum or BAL cultures with a mix of gram-negative, gram-positive bacteria, and yeast.

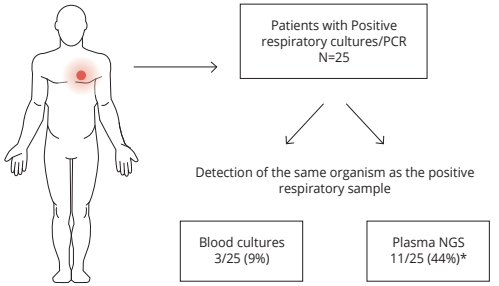
Conclusion: A novel plasma-based NGS assay was able to detect pathogen DNA from patients with microbiologically confirmed respiratory infection despite negative blood cultures. This open-ended assay was able to detect both bacterial and viral causes of respiratory infection and may be useful in the diagnosis of the etiology of acute respiratory tract infection directly from blood.

The etiology of most pneumonia cases is unknown



*Data from Jain et al, NEJM (2015); 373:415-27

Study Design



*Plasma NGS was positive in all patients where blood culture matched respiratory sample

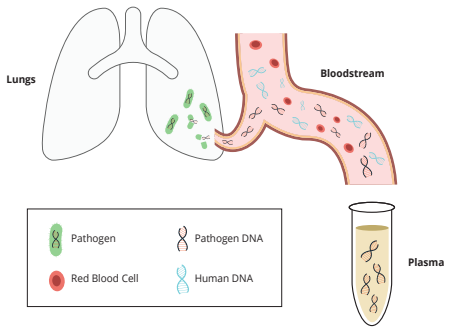
Plasma NGS did not detect the organism in respiratory culture/PCR

Blood Culture	Karius Test	Positive Respiratory Test	Day relative to Sample
Negative	Negative	BAL – 1+ K. *pneumoniae*, rare number *Bacillus* sp.	0
Negative	Negative	Sputum - 2+ E. *cloacae* complex, 2+ S. *maltophilia*	0
Negative	Negative	Sputum – 20-50 colonies yeast, 3 colonies *A. fumigatus* complex	0
Negative	Negative	BAL - 2+ S. *aureus*	0
Negative	Negative	Endotracheal (ET) - 1 colony Yeast ET – 1+ *E. cloacae*	0 -5
Negative	Negative	ET culture - 1+ S. *aureus*	-1
Negative	Negative	BAL – 50-100 colonies C. *glabrata*	+1
Negative	Negative	ET – 3+ *E.cloacae*, 3+ *Acinetobacter sp*, 3+ *S.aureus*	+1
Negative	Negative	Sputum – 1+ *S.aureus* Sputum – 2+ *S.aureus*	-3 0
Negative	*F. nucleatum* *B. thetaiotamicron* *B. fragilis* *S. constellatus*	Respiratory culture – 1+ *S. aureus* Abdominal fluid culture – *S.anginosus*, *E. faecalis*	+1
Negative	*M. catarrhalis*	NP swab - +Parainfluenza 4, adenovirus	+1
Klebsiella pneumoniae	*Klebsiella pneumoniae*	Induced sputum - 10-50 colonies of yeast	-5
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	Induced sputum - 4 colonies of *Aspergillus fumigatus*	+1
Escherichia coli	*Escherichia coli*	Sputum - 4+ S. *maltophilia*	+1

Plasma NGS detected the same organism as respiratory culture/PCR

Blood Culture	Karius Test	Positive Respiratory Test	Day relative to Sample
Negative	*Enterobacter sp*	Sputum culture - + ***Enterobacter aerogenes***	-3
Negative	*Enterobacter aerogenes*	ET culture- + ***Enterobacter aerogenes***	-2
Negative	*Human adenovirus B*	NP respiratory PCR panel - ***+Adenovirus***	-1
Negative	*Enterobacter aerogenes*	ET culture - ***+Enterobacter aerogenes*** and *A. baumannii*	0
Negative	*Escherichia coli*	ET culture - 4+ *S. aureus*, 4+ ***Escherichia coli***	0
Negative	*Moraxella catarrhalis*	Sputum culture - 4+ ***Moraxella catarrhalis***, 1+ *S. aureus*	-5
Negative	*Escherichia coli*	Sputum culture - ***3+ Lactose fermenting rods***, 3+ diphtheroid	0
Coag negative staphylococcus	*Serratia marcescens*	ET Culture - ***2+ Serratia marcescens***, 1+ *S.aureus*	0
Staphylococcus aureus	*Staphylococcus aureus*	Sputum - ***1+ Staphylococcus aureus***	0
Klebsiella pneumoniae	*Klebsiella pneumoniae*, *Enterococcus faecium*	Sputum - ***2+ Klebsiella pneumoniae, 4+ Enterococcus species***	-4
Enterococcus faecium	*Enterococcus faecium*	Sputum - 3+ Candida sp, ***3+ Enterococcus sp.***	-2

Pathogen DNA in Cell-Free Plasma



SUMMARY

- This novel plasma NGS assay can detect pathogen DNA from patients with respiratory infection despite negative blood culture
- Both viral and bacterial causes of respiratory infection were detected
- Plasma NGS can aid in identifying the causal pathogen in patients with respiratory infections