pathogenesis of COPD, and this study has established p73 as a new player in the complex dysregulated biology underlying the development of COPD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Ronald G. Crystal, M.D.
Department of Genetic Medicine
Weill Cornell Medical College
New York, New York

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Respiratory Metagenomics: Ready for Prime Time?

Pneumonia is one of the most common infectious reasons for hospital admission, but current standard-of-care pneumonia diagnostics leave much to be desired. In a population-based survey of community-acquired pneumonia in hospitalized adults in the United States, only 38% had a pathogen identified despite exhaustive clinical testing with culture, multiplex PCR, and urinary antigens (1). This diagnostic gap leads to the overuse of broad-spectrum antibiotic agents, contributing to the ever-increasing global burden of antimicrobial resistance, which is outpacing novel antimicrobial agent development and cited by the World Health Organization as one of the top 10 global threats facing humanity (2). Furthermore, as the number of immunocompromised patients steadily increases (3), so does the risk of infection with unusual pathogens often missed by standard microbiologic testing, resulting in delayed or missed diagnoses in our most vulnerable patients (4). There is an urgent need for new respiratory diagnostics that are less biased and more sensitive and provide rapid results.

A sequencing-based approach can overcome many of the limitations of existing pneumonia diagnostics. Metagenomic sequencing permits unbiased assessment of all nucleic acid in biological samples, enabling the detection of potential pathogens, the wider microbiome, and the human host response in a single assay (5). Presently, Clinical Laboratory Improvement Amendments (CLIA) certified metagenomic tests are clinically available for plasma and cerebrospinal fluid (6, 7), but technical and bioinformatic complexity delays turnaround time, and high sensitivity makes it challenging to distinguish signal from noise (8). Respiratory metagenomics (RMg) presents an even greater challenge because the respiratory tract is a nonsterile environment with a well-described microbiome, further complicating analysis and clinical interpretation.

In this issue of the *Journal*, Charalampous and colleagues (pp. 164–174) conducted a prospective clinical pilot study (9) implementing a previously established RMg workflow (10) in the ICU of an academic hospital (Figure 1). Although others have explored metagenomics for the diagnosis of lower respiratory tract infections (11–15), this study is notably the first to truly implement RMg in a clinical care setting and assess its impact. Generation and analysis of high-complexity metagenomic data involves multiple steps, including nucleic acid extraction, library preparation, sequencing, removal of human reads, taxonomic alignment, and modeling to distinguish pathogens from the background microbiome (5). Here, the authors report a median turnaround time from sample acquisition to result of just 6.7 hours, which is impressive and much faster than traditional culture-based diagnostics. This pilot study had the capacity to perform RMg on three samples per day, so follow-up studies regarding scale-up and cost effectiveness will be important.

Beyond the rapid turnaround time, this study demonstrated that RMg had a compelling impact on clinical management. When clinical testing results were negative, RMg detected a clinically

Originally Published in Press as DOI: 10.1164/rccm.202311-2039ED on November 29, 2023

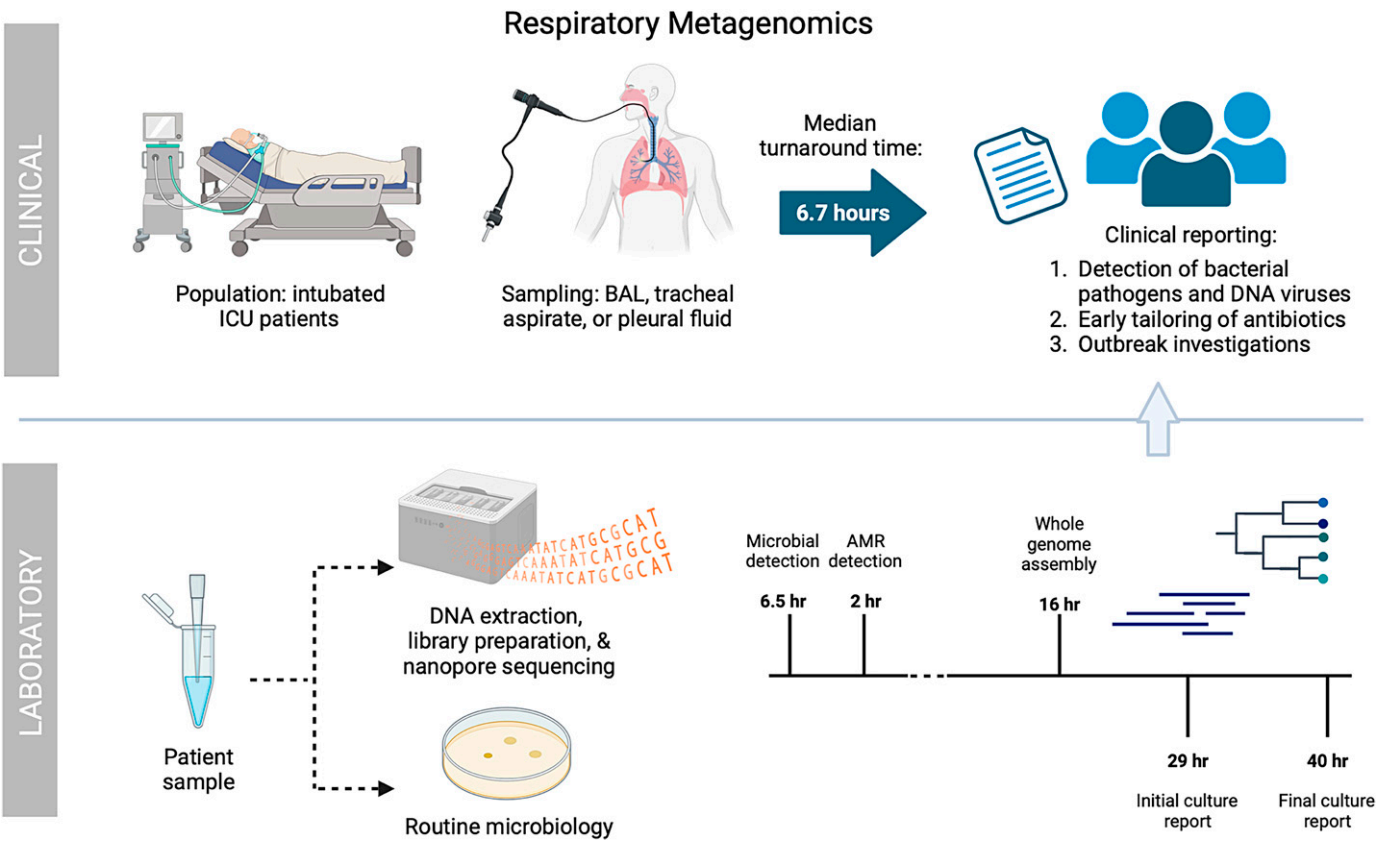

Figure 1. Clinical implementation of respiratory metagenomics. Nanopore respiratory metagenomics were performed on BAL fluid, tracheal aspirates, and pleural fluid samples from intubated patients in the ICU with suspected respiratory infection. Routine clinical testing with culture was simultaneously performed. Sequencing reports were generated after 30 minutes and 2 hours for microbes and resistance mutations, respectively. Median turnaround time from sample acquisition to the reporting of results to clinicians was 6.7 hours. In comparison, preliminary culture results were reported after an average of 29 hours and were finalized in 40 hours. The impacts on clinical care, antibiotic agent prescribing, and infection control involvement were evaluated. AMR = antimicrobial resistance; BAL = bronchoalveolar lavage; ICU = intensive care unit.

relevant pathogen 19% of the time, often with organisms not initially suspected by clinicians, such as *Legionella* or *Cytomegalovirus*, and resulted in a management change. Conversely, RMg had a high level of agreement (93%) when standard culture results were positive. The high sensitivity gave clinicians confidence to discontinue antibiotic therapy when RMg returned negative results and, in some cases, even start early immunosuppression for suspected autoimmune inflammatory pulmonary conditions. RMg had the added benefit of early detection of resistance genes for many organisms, allowing clinicians to appropriately tailor antimicrobial agents more expediently. In total, the authors estimate that RMg contributed to prescribing decisions in a noteworthy 80% of cases.

Compared with standard culture, sequencing-based approaches pair nicely with infection control and public health efforts. With usual practice, in a suspected outbreak, cultured organisms from the microbiology laboratory are sent for whole-genome sequencing to assess relatedness with other isolates. However, with metagenomics, if sequencing depth is adequate and genome coverage is high enough, the sequencing data can be used for proactive and timely infection control interventions. The authors demonstrate this impact with a case of *Legionella pneumophila* that originated from a bedside water faucet and a confirmed patient-to-patient *Klebsiella variicola* transmission.

As with any study, there are some limitations. Because the RMg assay studied was exclusively DNA-based, the workflow is unable to detect RNA viruses, which are the most common cause of lower respiratory tract infection in adults and children (1, 16). This could potentially hamper antimicrobial stewardship efforts because a positive viral test result in the setting of negative bacterial testing results can provide the confidence to stop antimicrobial therapy. Ideally, the RMg workflow could be modified to include DNA and RNA sequencing to better capture the most common causes of respiratory infections and enable the detection of emerging viral pathogens that may not be detectable by standard PCR assays. This could also enable profiling of host gene expression, which could inform whether the detected microbes are matched to an immune response consistent with infection. Another consideration for future studies is the inclusion of a noninfectious control group to more rigorously define specificity, optimize the differentiation of pathogens from commensal organisms, and understand the impact on antibiotic agent use when incidental microbiota are detected.

All said, the authors have performed an impressive clinical pilot study of RMg and have successfully moved the needle toward future implementation of this technology in routine clinical practice, in which current methods often fall short. Although some challenges and limitations persist, this study opens the door for future research

in assay optimization, launching of a randomized controlled trial, and cost-effectiveness analyses to propel RMg to prime time. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Emily Lydon, M.D.
Department of Medicine
University of California, San Francisco
San Francisco, California

Charles R. Langelier, M.D., Ph.D.
Department of Medicine
University of California, San Francisco
San Francisco, California
and
Chan Zuckerberg Biohub
San Francisco, California

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Portable Air Purifiers to Mitigate the Harms of Wildfire Smoke for People with Asthma

Wildfires are increasing in frequency and intensity across the world. Changing temperatures, drought, and vegetation stemming from anthropogenic climate and land-use change have interacted to boost conditions for their development and spread (1). Wildfires produce harmful emissions and are a significant source of environmental air pollution, including fine particulate matter (i.e., particles <2.5 μm in diameter [$PM_{2.5}$]), ozone, and carbon monoxide; as much as one fourth of ambient $PM_{2.5}$ in the United States can be attributed to wildfire smoke, a trend that is projected to increase (2).

There is a clear relationship between short-term rise in $PM_{2.5}$ and adverse respiratory (and many other) health outcomes (3). This is especially relevant for individuals with asthma, in whom the link between short-term $PM_{2.5}$ exposure with worse of symptoms and higher risk of exacerbation is well established, particularly for children (4, 5). For these individuals, air filtration has been proposed as an option to reduce personal exposure. High-efficiency particulate air (HEPA) filters remove 99.97% of particles with a size of 0.3 μm, and, perhaps contrary to popular belief, capture a greater percentage of particles both larger and smaller than this worst-case size. HEPA purifiers can reduce indoor $PM_{2.5}$ concentrations by approximately 50–80%, even in countries with relatively high ambient pollution levels, suggesting that they are effective in a wide range of real-world conditions (6). Government programs that encourage the purchase of portable air filters to mitigate poor air quality caused by wildfires are being tried in some jurisdictions, but many barriers exist for their widespread adoption. Chief among them is uncertainty regarding

Originally Published in Press as DOI: 10.1164/rccm.202311-2012ED on December 4, 2023