for heating is associated with higher COPD prevalence among never-smokers (3).

This study has limitations, some of which highlight the unique challenges of studying rural COPD. Although NHANES was designed to be representative of U.S. demographics, it does not sample all 50 states. Rural residents faced a greater burden of COPD in the West, Midwest, and South, but data were not available for adults living in isolated rural areas of the Northeast (Figure 2). This underscores the need to build a more robust infrastructure to investigate COPD in understudied rural areas. We also note that although GOLD guidelines recommend post-bronchodilator spirometry to confirm airflow obstruction, the primary analyses used prebronchodilator spirometry to capture the most participant data; post-bronchodilator spirometry was only available for a subset of participants. Multiple large epidemiologic studies have described a strong correlation between pre- and post-bronchodilator spirometry in predicting outcomes, and lower-limit-of-normal values, which define obstruction according to ATS criteria, are determined using prebronchodilator spirometry (10). Furthermore, a sensitivity analysis that applied the GOLD criteria of $FEV_1/FVC < 0.70$ for individuals with post-bronchodilator spirometry produced results consistent with those obtained in the primary analysis. Lastly, there is a need for individual-level environmental exposure assessments in future studies to better quantify the contribution of factors such as secondhand smoke and heating with solid fuels to disease development in rural areas—a limitation of the present study.

Despite these limitations, by using an approach that allowed us to uniquely link multiple nationally representative studies, we confirmed that individuals living in rural areas are at increased risk for spirometry-defined COPD and face greater respiratory morbidity. Further studies are now needed to better understand the risk factors that are unique to rural regions and enable the development of strategies to improve respiratory health and reduce disparities. ■

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Detection of Pneumonia Pathogens from Plasma Cell-Free DNA

To the Editor:

Pneumonia is a major contributor to infectious disease mortality (1). Blood cultures in patients with pneumonia return positive in only 4–18% of cases, presumably because of distance from the site of active infection or antibiotic sterilization before sample collection (2). The degree to which microbial DNA circulates in the blood of patients with pneumonia is unknown, and this information may improve our understanding of disease pathophysiology and clinical diagnosis.

Detection of circulating microbial DNA in plasma via metagenomic next-generation sequencing (mNGS) is a recently described strategy for culture-independent assessments of infectious diseases (3–5). We hypothesized that plasma mNGS might be able to detect respiratory pathogen DNA in the bloodstream of patients with pneumonia, including those with negative blood cultures. To

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Table 1. Clinical and Demographic Characteristics of the Study Cohort

	Total	Pneumonia-Positive	Pneumonia-Negative
Total enrolled, *n*	25	18	7
Age, yr, mean	57	56	57
Sex, F	8 (32%)	6 (33%)	2 (29%)
African American	3 (12%)	3 (17%)	1 (14%)
Asian	2 (8%)	2 (11%)	1 (14%)
Caucasian	12 (48%)	12 (67%)	4 (57%)
Other race	4 (8%)	1 (6%)	1 (14%)
Hispanic ethnicity	1 (4%)	1 (6%)	0 (0%)
CAP	9 (36%)	9 (50%)	—
HAP/VAP	9 (36%)	9 (50%)	—
Subsequent VAP	2 (8%)	2 (11%)	—
Immunosuppression*	11 (44%)	6 (33%)	5 (83%)
Prior antibiotic use	13 (83%)	13 (72%)	7 (100%)
Bacteremia/viremia	4 (16%)	4 (22%)	0 (0%)
Mortality, 30 d	5 (20%)	5 (28%)	0 (0%)

Definition of abbreviations: CAP = community-acquired pneumonia; HAP = hospital-acquired pneumonia; VAP = ventilator-associated pneumonia.
Data are shown as *n* (%) unless otherwise indicated.
*Includes history of solid organ transplant, hematopoietic stem cell transplant, or treatment with a biologic immune-modulating agent, corticosteroid, or cancer chemotherapy.

address this issue, we assessed 25 critically ill adults (18 with pneumonia [four with concurrent bloodstream infections] and seven with noninfectious acute respiratory illnesses). We then asked whether mNGS of plasma cell-free DNA could detect culture-confirmed respiratory pathogens, and we compared the results against those obtained by mNGS of respiratory fluid, which has been established as an accurate diagnostic method for pneumonia (6).

Subjects were enrolled within the first 72 hours of ICU admission for acute respiratory failure as part of an ongoing prospective cohort study (approved by the Institutional Review Board of the University of California, San Francisco, #10-02701) (Figure 1). Adjudication by two physicians, based on retrospective medical record review (blinded to mNGS results) and the CDC surveillance case definitions of pneumonia (7), was used to identify 18 subjects with culture-confirmed bacterial pneumonia, including two who later developed clinically diagnosed ventilator-associated pneumonia (VAP), and 7 subjects with respiratory illnesses due to noninfectious etiologies (Tables 1 and 2). The first 18 pneumonia-positive and first 7 pneumonia-negative subjects enrolled in the study with available matched plasma (from an indwelling line, central or arterial) and respiratory samples available for study underwent mNGS. Before enrollment, 83% of the subjects (13/18 pneumonia-positive, 7/7 pneumonia-negative) had received empiric broad-spectrum antibiotics.

Plasma specimens were collected in citrate tubes on the day of study enrollment (Day 0) and underwent DNA extraction (ZR-Duet kit; Zymo Research) followed by sequencing library preparation (NEBNext; New England BioLabs) according to previously described methods (6). After paired-end Illumina sequencing, we used ID-Seq v3.2 to detect microbes from raw sequence data (8). Matched respiratory samples underwent paired DNA and RNA sequencing, and microbes that were detected concordantly in both nucleic acid types were aggregated to the genus level and ranked by abundance (reads per million reads mapped [rpM]) as previously described (6). The sequencing data are available via BioProject accession number PRJNA525157 (http://www.ncbi.nlm.nih.gov/bioproject/525157).

For plasma samples, we performed pathogen assessments and background contaminant correction using a previously described Bayesian scoring metric (9): score $= [(|\text{genus nt } Z| \times \text{species nt } Z \times \text{species nt rpM}) + (|\text{genus nr } Z| \times \text{species nr } Z \times \text{species nr rPM})]$, where nr = nonredundant and nt = nucleotide. We optimized the metric for plasma by incorporating the following rules: *1*) percent identity of nt alignment $\geq$95%; *2*) nt reads $>$10; *3*) $\log(1/e) \geq 75$; *4*) sequencing read length $>$135 nt; *5*) score + 0.05 $>$ 0; and *6*) exclusion of background environmental microbiota representing common mNGS library preparation contaminants (such as skin flora), as described in Reference 10. *Z*-scores were calculated using a cohort-optimized background model, and microbes were aggregated to the genus level and ranked by rpM, with the top five most abundant microbes in each patient carried forward for analysis (10).

We first examined positive control subjects with culture-confirmed bacteremia ($n = 3$) or cytomegalovirus viremia ($n = 1$) and found that plasma mNGS identified the etiologic bloodstream pathogen in every one of these cases (Table 1). We next assessed the ability of plasma mNGS to detect pathogens identified by lower-respiratory culture (mini-BAL or tracheal aspirate) or serum antigen testing from the patients with pneumonia. Plasma mNGS identified one or more clinically confirmed pneumonia pathogens in 67% (12/18) of cases, and in 44% (8/18) of cases the pathogen was the most abundant microbe detected. These results compared with 100% and 61% of cases, respectively, for mNGS of respiratory samples (6). Notably, in addition to bacterial pathogens, plasma mNGS also identified the invasive fungal pathogen *Histoplasma capsulatum* in a patient with disseminated disease (Tables 1 and 2).

Two patients in the study received a clinical diagnosis of VAP after enrollment. In these cases, plasma cell-free mNGS detected the culture-confirmed bacterial pathogens at the time of study enrollment, 4 days before the VAP diagnosis (Table 1). The first of these cases was a patient hospitalized for intracranial hemorrhage who developed VAP secondary to *Enterobacter cloacae* and *Staphylococcus aureus*, both of which were detectable by plasma mNGS performed at the time of enrollment.

Table 2. Comparison of Cell-Free Plasma mNGS versus Respiratory mNGS for Detection of Clinically Confirmed Pneumonia Pathogens, with Microbes Ranked by Abundance (Reads per Million)

ID	Respiratory Culture, Day 0	Blood Culture or PCR, Day 0	Plasma mNGS Rank, Day 0	Respiratory mNGS Rank, Day 0
Pneumonia-Positive, Blood Culture/PCR-Positive Cases				
252	*Streptococcus*	*Streptococcus*	1	2
	Enterobacter		ND	3
	Staphylococcus		ND	>10
289	*Staphylococcus*	*Staphylococcus*	1	2
298	*Enterobacter**	Cytomegalovirus†	ND*, 2†	1*, >10†
334	*Streptococcus*	*Streptococcus*	1	1
Pneumonia-Positive Cases				
213‡	*Klebsiella*	—	4	4
	Escherichia	—	ND	7
239	*Staphylococcus*	—	2	4
	Pseudomonas	—	ND	1
257	*Enterobacter*	—	ND	1
268	*Pseudomonas*	—	ND	1
	Stenotrophomonas	—	ND	2
278	*Moraxella*	—	1	1
277	*Enterococcus*	—	4	1
288	*Staphylococcus*	—	2	2
297	*Pseudomonas*	—	2	2
	Enterobacter	—	ND	3
	Morganella	—	ND	4
	Klebsiella	—	ND	7
314	*Staphylococcus*	—	1	2
	Streptococcus	—	2	4
335	*Histoplasma*§	—	1	1
350	*Serratia*	—	ND	1
382	*Serratia*	—	ND	1
386	*Staphylococcus*	—	ND	2
VAP Cases				
ID	VAP Respiratory Culture, Day 4	Plasma mNGS Rank, Day 0	Respiratory mNGS Rank, Day 0	Respiratory mNGS Rank, Day 2
203	*Enterobacter*	1	ND	1
	Staphylococcus	2	ND	2
213‡	*Serratia*	1	7	n/a

Definition of abbreviations: mNGS = metagenomic next-generation sequencing; n/a = respiratory mNGS data not available; ND = not detected; VAP = ventilator-associated pneumonia.
Pathogen detection was performed using respiratory mNGS as previously described (4).
*Culture-identified pneumonia pathogen.
†Detected by clinical PCR of blood.
‡Patient developed *Serratia* VAP 4 days after enrollment.
§Detected by serum antigen testing.

The second patient developed VAP secondary to *Serratia marcescens* while undergoing ceftriaxone treatment for a primary pneumonia due to *Escherichia coli* and *Klebsiella pneumoniae*. mNGS of plasma cell-free DNA obtained 4 days before the VAP diagnosis identified *Serratia* as the most abundant microbe. In addition, plasma mNGS detected the *SRT-1* β-lactamase gene, which is known to confer inducible resistance to ceftriaxone (8), providing a potential molecular explanation for emergence of the VAP pathogen.

As negative controls, we sequenced seven critically ill pneumonia-negative subjects with respiratory failure. In two subjects (29%), no microbes were identified (Table 2). Microbes identified in the remaining subjects included human herpes virus 6, which may be incidentally present in healthy individuals and reactivate in the setting of sepsis (9), as well as several potential pathogens and commensals. These results suggest that circulating microbial DNA is detectable in the bloodstream of critically ill patients, even in the setting of negative blood cultures. Microbes detected in such cases could represent true but missed pneumonia pathogens or circulating microbial DNA in the bloodstream derived from gut translocation, the respiratory tract, skin, or other sources.

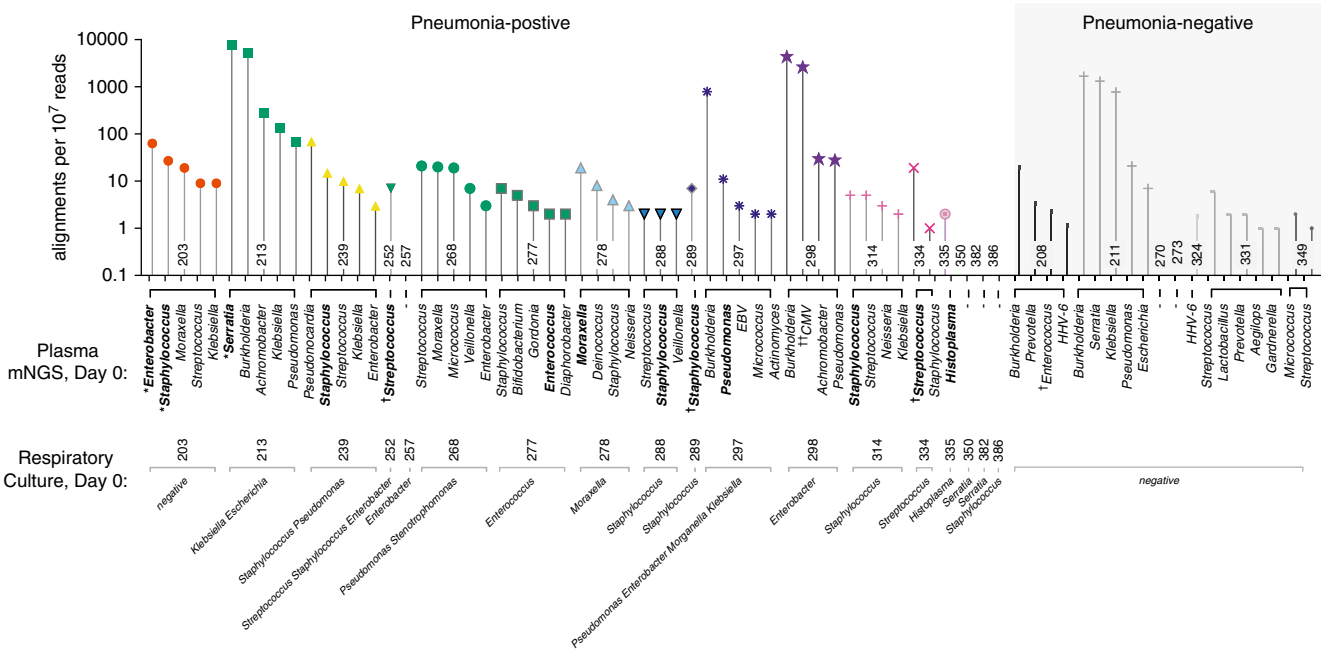

Figure 1. Microbes detected by metagenomic next-generation sequencing (mNGS) of plasma cell-free DNA performed on the day of study enrollment (Day 0). The *y*-axis corresponds to taxon abundance measured by sequencing alignments per 10^7 reads, stratified by subjects listed in order of the study ID number. Respiratory culture–identified microbes are indicated below and also highlighted in bold font. *Detected by respiratory culture performed on study Day 4 for clinically suspected ventilator-associated pneumonia. Each color/symbol combination represents a unique patient plasma sample. †Detected by blood culture. ††Detected by clinical PCR of blood. CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV-6 = human herpes virus 6.

In summary, we report that plasma mNGS can identify circulating DNA from respiratory pathogens in critically ill patients with culture-confirmed bacterial pneumonia. Our results suggest that release of pathogen DNA into the bloodstream may be more common than is appreciated in patients with pneumonia and negative blood cultures, and that plasma mNGS, although less informative than respiratory mNGS, may have value for detecting pneumonia pathogens when respiratory specimens are unavailable. Future studies in a larger cohort will be needed to assess the generalizability of these findings, evaluate the diagnostic performance of plasma mNGS, and determine its utility for pathogen detection in culture-negative pneumonia cases. ■

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Collecting Occupational Exposure Data Would Strengthen Idiopathic Pulmonary Fibrosis Registries

To the Editor:

We read with interest the article by Culver and colleagues (1), who describe the use of idiopathic pulmonary fibrosis (IPF) patient registries to capture clinically relevant data on the clinical course and impact of IPF. The authors summarize the attributes of IPF registries, including descriptions of current diagnostic and management practices and collection of biological specimens (1), but they do not discuss the role patient registries can play in the collection of occupational and environmental exposure data.

A diagnosis of IPF includes a presumption that known potential explanations have been excluded (2). The most recent diagnostic criteria for IPF recommend collection of a detailed environmental and occupational exposure history from patients who are clinically suspected of having IPF (2). Between 30,000 and 40,000 incident cases of IPF are diagnosed each year in the United States (3), and an estimated 26% of these cases are attributable to occupational exposures, including metal, silica, wood, and agricultural dusts (4).

The National Institute for Occupational Safety and Health analyzed 1999–2013 mortality data from 21 states that included supplemental employment history information and an underlying or contributing cause of death coded as International Classification of Diseases, 10th Revision J84.1 (other interstitial pulmonary diseases with fibrosis). After adjustment for age, sex, and race, IPF decedents who were employed in the engineered wood product manufacturing industry had a proportionate mortality ratio (PMR) of 2.62 (95% confidence interval, 1.58–4.09). Other industries with >100 IPF deaths and an elevated and statistically significant PMR included foundries (PMR, 1.78), offices of dentists (1.44), offices of physicians (1.36), and aerospace product and parts manufacturing (1.32) (unpublished data). These findings indicate associations between workplace exposures and IPF, but death certificate data are a lagging indicator and have poor sensitivity for identifying occupational lung diseases (5). Using IPF patient registries to capture occupation and environmental exposure data could provide more sensitive, specific, and timely data. The systematic collection of key occupational and environmental data (e.g., usual industry, occupation, and history of dust exposure) across multiple IPF registries could allow for pooled analyses to identify occupational and environmental exposures that might be contributing to the global interstitial lung disease burden.

We applaud Culver and colleagues for encouraging IPF registries to develop creative methodologies to explore specific hypotheses rather than continue to duplicate existing efforts. It is notable that not all cases of IPF are truly idiopathic, and some cases are likely attributable to occupational or environmental exposures (6). We encourage IPF registries to also collect occupational and environmental exposure data in a standardized and systematic fashion. These data could be pooled and analyzed to improve our understanding of the contribution of occupational and environmental exposures to the burden of interstitial lung disease and to inform recommendations for exposure control and medical monitoring. ■

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