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A Pilot Study of the Noninvasive Assessment of the Lung Microbiota as a Potential Tool for the Early Diagnosis of Ventilator-Associated Pneumonia

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BACKGROUND: Ventilator-associated pneumonia (VAP) remains a common complication in critically ill surgical patients, and its diagnosis remains problematic. Exhaled breath contains aerosolized droplets that reflect the lung microbiota. We hypothesized that exhaled breath condensate fluid (EBCF) in hygroscopic condenser humidifier/heat and moisture exchanger (HCH/HME) filters would contain bacterial DNA that qualitatively and quantitatively correlate with pathogens isolated from quantitative BAL samples obtained for clinical suspicion of pneumonia.

METHODS: Forty-eight adult patients who were mechanically ventilated and undergoing quantitative BAL (n = 51) for suspected pneumonia in the surgical ICU were enrolled. Per protocol, patients fulfilling VAP clinical criteria undergo quantitative BAL bacterial culture. Immediately prior to BAL, time-matched HCH/HME filters were collected for study of EBCF by real-time polymerase chain reaction. Additionally, convenience samples of serially collected filters in patients with BAL-diagnosed VAP were analyzed.

RESULTS: Forty-nine of 51 time-matched EBCF/BAL fluid samples were fully concordant (concordance > 95% by κ statistic) relative to identified pathogens and strongly correlated with clinical cultures. Regression analysis of quantitative bacterial DNA in paired samples revealed a statistically significant positive correlation ($r = 0.85$). In a convenience sample, qualitative and quantitative polymerase chain reaction analysis of serial HCH/HME samples for bacterial DNA demonstrated an increase in load that preceded the suspicion of pneumonia.

CONCLUSIONS: Bacterial DNA within EBCF demonstrates a high correlation with BAL fluid and clinical cultures. Bacterial DNA within EBCF increases prior to the suspicion of pneumonia. Further study of this novel approach may allow development of a noninvasive tool for the early diagnosis of VAP.

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ABBREVIATIONS: BALF = BAL fluid; CFU = colony-forming unit; DPPC = dipalmitoylphosphatidylcholine; EBCF = exhaled breath condensate fluid; HCH/HME = hygroscopic condenser humidifier/heat and moisture exchanger; PCR = polymerase chain reaction; RT-PCR = real-time polymerase chain reaction; SICU = surgical ICU; SP-B = surfactant-associated protein B; VAP = ventilator-associated pneumonia; VUMC = Vanderbilt University Medical Center

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Ventilator-associated pneumonia (VAP) is among the most common health-care-associated infections in severely ill and injured patients, accounting for substantial morbidity, increased length of ICU and hospital stay, and excess cost and mortality.[1-3] Critically ill and injured patients are particularly prone to VAP, due to several factors that both increase the risk and confound the diagnosis of pneumonia, making accurate and timely diagnosis of pneumonia problematic.[1,4-9] Atelectasis, pulmonary contusions, acute lung injury, aspiration pneumonitis, and the systemic inflammatory response syndrome are all common following major operative interventions or severe trauma and can mimic pulmonary infections. The standard clinical criteria for the diagnosis of VAP overestimates the rate twofold when compared with quantitative culture using BAL or protected brush specimens.[10-13] Quantitative scoring systems of clinical and radiographic findings have failed to increase the diagnostic accuracy when compared to quantitative cultures.[14-18] Quantitative culture techniques increase the specificity of the diagnosis and may improve overall outcomes.[11] However, these techniques mandate the development of clinical symptoms to introduce clinical suspicion, requiring approximately three additional days for culture and sensitivity results, necessitating empirical antibiotic coverage while results are pending. Thus, existing diagnostic strategies delay therapy until the infection is well established and require significant empirical therapy, contributing to unnecessary antibiotic exposure.[19]

While the diagnosis of pneumonia is difficult to establish in a rapid or specific fashion, significant evidence supports reduced morbidity and mortality when appropriate antibiotic therapy is initiated early in patients with VAP.[20-23] Inadequate empirical antibiotic coverage for VAP is associated with a twofold increase in mortality, and retrospective studies suggest that delays as short as 30 min from the onset of fever in infected patients may increase mortality.[20-24] However, unnecessary antibiotic exposure is associated with increased risk for subsequent infectious complications, colonization, and infection with resistant

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pathogens, and increased hospital costs.[21,25-31] Thus, early identification of patients with pneumonia is necessary to improve outcomes in this population.

The goal of our research is to develop tools that can improve the timeliness, specificity, and sensitivity of VAP diagnosis in critically ill patients. This would enable clinicians to diagnose pneumonia earlier in its course, select more-specific antimicrobial therapies, and more accurately monitor response to treatments. Exhaled breath contains aerosolized droplets of widely varying size (majority between 5 μm and 100 μm) that carry bacteria, as first described by Flugge in 1897.[32,33] These droplets reflect the pathogens in the lower respiratory tree, transmitting them to the environment.[32-35] Thus, we hypothesized that bacteria within these aerosolized breath droplets would collect within the hygroscopic condenser humidifier/heat and moisture exchanger (HCH/HME) filters between the endotracheal tubes and ventilator circuit and provide a quantitative assessment of pulmonary bacterial growth. The purpose of this current study is to examine if polymerase chain reaction (PCR) analysis of exhaled breath condensate fluid (EBCF) would correlate quantitatively and qualitatively with fluid samples from time-matched, semiquantitative BAL fluid (BALF) obtained for the clinical suspicion of pneumonia, thus providing qualitative and quantitative results in hours rather than the 3 days required by current techniques.

Materials and Methods

We previously reported that HCH/HME filters (Fig 1) serve as a reservoir for pathogens carried in exhaled breath condensate but do not allow bacterial proliferation, since they are bacteriocidal. As a result, bacterial cells remain intact and can be reliably quantified by real-time PCR (RT-PCR) (Fig 2).[36] Additionally, we have demonstrated that bacterial recovery is independent of species and that these isolated bacteria can be uniformly lysed and quantified without bias or loss of specific species. Finally, we have demonstrated that samples can be normalized by surfactant content via surfactant-associated protein B (SP-B) and dipalmitoylphosphatidylcholine (DPPC) measurements.

Patient Recruitment and Sample Collection

The study was approved by the Vanderbilt University Medical Center (VUMC) institutional review board (No. 101185) and funded by the Vanderbilt Institute for Clinical and Translational Research (CTSA 1 UL1 RR024975). The current standard of care for critically ill patients who were mechanically ventilated in the Vanderbilt surgical ICU (SICU) with suspected pneumonia is to undergo protocol directed, semiquantitative BAL for standard microbiologic culture and empirical antibiotic therapy until BAL results are available. Antibiotics are discontinued for BAL culture results $< 10^4$ colony-forming unit (CFU) of bacteria and deescalated to cover only pathogens $> 10^4$ CFU.

Between April and August of 2011, adult patients who were ventilated in the SICU, with a clinical suspicion of pneumonia, were enrolled. Two to 4 h prior to BAL, the existing HCH/HME filter unit was exchanged for a new "study" unit. Immediately prior to BAL, the study filter was removed and placed in a biohazard bag. Following the BAL, 2 mL excess BALF was placed in a sterile specimen vial for PCR analysis and residual BALF was sent to the clinical microbiology laboratory, per standard of care. The BALF sample and the time-matched EBCF collected from the study filter were processed separately in identical fashion, with positive and negative control samples. PCR findings from matched BALF