in dramatic bacterial clearance in the *Pseudomonas* case (9), but intravenous-only administration did not result in eradication of MRSA in the experimental model (8). A higher lung necrosis score was associated with nonsurvival (*see* Figure E3 in Reference 8), raising concerns about intravenous delivery of both phage and antibiotic to the lumen of cavitary pneumonia. Phage therapy is not neutral for the host: phages may transcytose host cells and stimulate Toll-like receptor 9 and other pattern recognition molecules (13). Generally, phage infusions or mucosal applications are well tolerated without an inflammatory signal, as was seen in the mouse model (8). More importantly, neutralizing antibodies are common in individuals exposed to naturally occurring phages, and may blunt the benefit of intravenous therapeutic phage therapy. Because aerosolization is less likely to induce neutralizing antiphage antibodies and less likely to be blunted by preformed antibodies, this route may be preferred for pneumonia treatment in the critically ill.

Validation of the benefit of routine phage treatment of AMR pneumonia requires much more work. Only further data will demonstrate whether phage therapy is truly a new chapter in pneumonia treatment or just another interesting footnote. ■

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

Richard G. Wunderink, M.D.
Feinberg School of Medicine
Northwestern University
Chicago, Illinois

ORCID ID: 0000-0002-8527-4195 (R.G.W.).

References

1. El Bcheraoui C, Mokdad AH, Dwyer-Lindgren L, Bertozzi-Villa A, Stubbs RW, Morozoff C, *et al*. Trends and patterns of differences in infectious disease mortality among US counties, 1980-2014. *JAMA* 2018;319: 1248–1260.
2. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, *et al*.; EPIC II Group of Investigators. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009;302:2323–2329.
3. Torres A, Zhong N, Pachl J, Timsit JF, Kollef M, Chen Z, *et al*. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. *Lancet Infect Dis* 2018;18: 285–295.
4. Wunderink RG, Giamarellos-Bourboulis EJ, Rahav G, Mathers AJ, Bassetti M, Vazquez J, *et al*. Effect and safety of meropenem-vaborbactam versus best-available therapy in patients with carbapenem-resistant Enterobacteriaceae infections: the TANGO II randomized clinical trial. *Infect Dis Ther* 2018;7:439–455.
5. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 2004;2:289–300.
6. Kollef MH, Ricard JD, Roux D, Francois B, Ischaki E, Rozgonyi Z, *et al*. A randomized trial of the amikacin fosfomycin inhalation system for the adjunctive therapy of Gram-negative ventilator-associated pneumonia: IASIS Trial. *Chest* 2017;151:1239–1246.
7. Wunderink RG, Niederman MS, Kollef MH, Shorr AF, Kunkel MJ, Baruch A, *et al*. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012;54:621–629.
8. Prazak J, Iten M, Cameron DR, Save J, Grandgirard D, Resch G, *et al*. Bacteriophages improve outcomes in experimental *Staphylococcus aureus* ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2019;200:1126–1133.
9. Maddocks S, Petrovic Fabijan A, Ho J, Lin RCY, Ben Zakour NL, Dugan C, *et al*. Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by *Pseudomonas aeruginosa* [letter]. *Am J Respir Crit Care Med* 2019;200:1179–1181.
10. Qi C, Hountras P, Pickens CO, Walter JM, Kruser JM, Singer BD, *et al*. Detection of respiratory pathogens in clinical samples using metagenomic shotgun sequencing. *J Med Microbiol* 2019;68: 996–1002.
11. Charlson ES, Bittinger K, Haas AR, Fitzgerald AS, Frank I, Yadav A, *et al*. Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Respir Crit Care Med* 2011;184:957–963.
12. Jault P, Leclerc T, Jennes S, Pirnay JP, Que YA, Resch G, *et al*. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis* 2019;19: 35–45.
13. Van Belleghem JD, Dąbrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. *Viruses* 2018;11:E10.

Another Warning Sign: High Nicotine Content in Electronic Cigarettes Disrupts Mucociliary Clearance, the Essential Defense Mechanism of the Lung

Electronic cigarette (e-cigarette) usage has become popular at an alarming rate and continues to rise, especially among younger populations in the United States. In 2018 alone, there was an ~40% increase in usage (from 12% to 21%) compared with 2017 among high school students, according to a recent Centers for Disease Control and Prevention report (1). Many incorrectly believe that smoking e-cigarettes (vaping) is not harmful to health. The general misconception by the public that "e-cigarettes are safe," however, has been challenged or overturned by many recent studies revealing the association between e-cigarettes and adverse cardiovascular, pulmonary, and systemic health effects.

As a nicotine delivery system, e-cigarette liquids typically contain *1*) a vehicle (propylene glycol/vegetable glycerin [PG/VG]), *2*) a chemical that gives an appealing flavor, and *3*) various

Supported by the NIH/NHLBI/FDA (P50HL120100).

Originally Published in Press as DOI: 10.1164/rccm.201905-1080ED on June 14, 2019

concentrations of nicotine. Previous studies have focused on the toxic effects of these ingredients either as a whole or as separate components of e-cigarettes. Using airway epithelial cell lines, Sassano and colleagues showed that PG/VG itself adversely affected cell viability, and that certain e-liquids, including the most common ones on the market, are more toxic than PG/VG alone (2). White blood cells in the airways, such as neutrophils and macrophages, are crucial for the innate defense of the airways. However, when they are exposed to constant stimulation, such as from cigarette smoke, they can contribute to the initiation and progression of chronic lung diseases such as chronic obstructive pulmonary disease. It has been shown that e-cigarettes can also trigger neutrophils and macrophages to release their enzymes such as elastase and MMP9 (matrix metalloproteinase 9) (3, 4), which are known to cause tissue damage in the long run. Furthermore, e-liquids, even without nicotine, can trigger an inflammatory and oxidative response and cytotoxicity on the human monocytes (5). Moreover, *in vivo* human unbiased proteomics studies using human induced sputum and epithelial cells derived from human airways from smokers and e-cigarette users revealed that vaping causes marked adverse changes in the airways, including altered epithelial and sputum proteomes and mucus/mucin composition (3, 6) in both a similar and a unique way relative to cigarette smokers.

Nicotine is a highly addictive substance, and the high level of nicotine delivered by the current generation of e-cigarette devices can cause addiction in never-smokers (7) and could subsequently provide a foundation to start traditional cigarette smoking (8). The nicotine content of e-cigarettes typically varies between 3 and 36 mg/ml. Most recent generations of e-cigarettes contain much more nicotine (up to 60 mg/ml), typically in a salt form, to speed up and increase the delivery of nicotine to the brain at rates comparable to those found with cigarette smoking. The adverse effect of nicotine on the airways and its consequences, however, is an understudied area. As reported in this issue of the *Journal*, Chung and colleagues (pp. 1134–1145) addressed this critical issue by performing a comprehensive mechanistic study using *in vivo* (sheep) and *in vitro* models (human primary bronchial epithelial cell cultures) to observe lung hemostasis and pathobiology in response to nicotine (9). The authors previously showed that chronic e-cigarette exposure caused chronic obstructive lung disease in mice in a nicotine-dependent manner (10). In this work, they used primary airway epithelial cells derived from nonsmoking healthy individuals for *in vitro* exposure experiments. They then assessed the effects of e-cigarette vapor on airway cells via sophisticated mucociliary transport (MCT), mRNA and protein expression, calcium imaging, mucus concentration, and complex viscosity assays. For *in vivo* animal (sheep) studies, tracheal mucus velocity (TMV), a surrogate marker for mucociliary clearance, was measured after nebulized e-cigarette exposure.

The relative viscosity analysis of cell secretions showed that compared with an air-only control and e-cigarette vapor with no nicotine, e-cigarette vapor with nicotine increased mucus viscosity and decreased the airway surface liquid height in a dose-dependent fashion. Also, human primary bronchial epithelial cell mucus concentrations, measured by percent solids, increased approximately 1.5- to 2-fold. Given the fact that increased mucus concentration and viscosity are inversely related to mucociliary clearance rates, they measured the MCT in cell cultures. As predicted, MCT was significantly reduced, by approximately sixfold on average, in the cultures exposed to nicotine. To understand the mechanism of the adverse effect of nicotine on the cells, the authors focused on a Ca^{2+}-selective ion channel, TRPA1 (transient receptor potential ankyrin 1), as a possible nicotine receptor. In fact, using known inhibitors of TRPA1, the authors averted the adverse effects of the nicotine on airway surface liquid, mucus concentration, and viscosity, which strongly suggests that the effects of nicotine were transmitted through TRPA1 and not the nicotinic acetylcholine receptor.

To validate their *in vitro* observations, the authors used an *in vivo* (sheep) exposure model. They found that TMV diminished by approximately half after exposure to e-cigarette liquid with 15–20 mg/ml nicotine as compared with e-cigarette liquid with 10 mg nicotine or e-cigarette vapor with no nicotine. This effect was prevented when a TRPA1 inhibitor was added to the mixture, which is consistent with the cell culture results and confirms that the nicotine effect occurs via the TRIPA1 receptor. However, this important *in vivo* observation (i.e., impaired mucociliary clearance via nicotine and its consequences) needs to be tested and replicated in the future in *in vivo* human studies involving e-cigarette users with no history of smoking cigarettes. Although this study focused on certain e-cigarette liquids with nicotine up to 36 mg/ml, some new e-cigarette devices and some e-liquids contain a much higher nicotine content and can potentially deliver more nicotine to the airways (10). Given the nicotine concentration–dependent nature of the impaired TMV shown here, it can therefore be speculated that these new devices could be more harmful to the airways.

Mucus abnormalities, such as increased viscoelasticity, elevated mucus/mucin concentrations, and impaired MCT, are commonly seen in cigarette smokers with and without chronic bronchitis, and are closely related to chronic inflammatory muco-obstructive lung diseases such as chronic obstructive pulmonary disease (11). Altogether, this important report published by Chung and colleagues provides novel and unique data showing the harmful effect of e-cigarettes on mucociliary clearance, a crucial part of the lungs' first line of defense. Along with previously reported adverse effects of e-cigarettes, such as increased oxidative stress, neutrophil and macrophage activation, impaired and altered innate defense, and inflammatory response and cytotoxicity, the current findings provide further convincing evidence that e-cigarette smoking is harmful to the airways. ■

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

Mehmet Kesimer, Ph.D.
Marsico Lung Institute
and
Department of Pathology and Laboratory Medicine
University of North Carolina
Chapel Hill, North Carolina

ORCID ID: 0000-0003-3867-1873 (M.K.).

References

1. Centers for Disease Control and Prevention. Tobacco use by youth is rising. Vital signs; February 2019 [accessed 2019 May 28]. Available from: https://www.cdc.gov/vitalsigns/youth-tobacco-use/index.html.
2. Sassano MF, Davis ES, Keating JE, Zorn BT, Kochar TK, Wolfgang MC, *et al*. Evaluation of e-liquid toxicity using an open-source high-throughput screening assay. *PLoS Biol* 2018;16: e2003904.

3. Reidel B, Radicioni G, Clapp PW, Ford AA, Abdelwahab S, Rebuli ME, *et al*. E-cigarette use causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. *Am J Respir Crit Care Med* 2018;197: 492–501.
4. Scott A, Lugg ST, Aldridge K, Lewis KE, Bowden A, Mahida RY, *et al*. Pro-inflammatory effects of e-cigarette vapour condensate on human alveolar macrophages. *Thorax* 2018;73:1161–1169.
5. Muthumalage T, Prinz M, Ansah KO, Gerloff J, Sundar IK, Rahman I. Inflammatory and oxidative responses induced by exposure to commonly used e-cigarette flavoring chemicals and flavored e-liquids without nicotine. *Front Physiol* 2018;8:1130.
6. Ghosh A, Coakley RC, Mascenik T, Rowell TR, Davis ES, Rogers K, *et al*. Chronic e-cigarette exposure alters the human bronchial epithelial proteome. *Am J Respir Crit Care Med* 2018;198: 67–76.
7. Menakuru S, Inzamam Ali M. Beliefs and reality of e-cigarette smoking. *BMJ Case Rep* 2018;pii: bcr-2018-225683.
8. Berry KM, Fetterman JL, Benjamin EJ, Bhatnagar A, Barrington-Trimis JL, Leventhal AM, *et al*. Association of electronic cigarette use with subsequent initiation of tobacco cigarettes in US youths. *JAMA Netw Open* 2019;2:e187794.
9. Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, *et al*. Electronic cigarette vapor with nicotine causes airway mucociliary dysfunction preferentially via TRPA1 receptors. *Am J Respir Crit Care Med* 2019;200:1134–1145.
10. Garcia-Arcos I, Geraghty P, Baumlin N, Campos M, Dabo AJ, Jundi B, *et al*. Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. *Thorax* 2016;71: 1119–1129.
11. Omaiye EE, McWhirter KJ, Luo W, Pankow JF, Talbot P. High-nicotine electronic cigarette products: toxicity of JUUL fluids and aerosols correlates strongly with nicotine and some flavor chemical concentrations. *Chem Res Toxicol* 2019;32:1058–1069.
12. Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, *et al*. Airway mucin concentration as a marker of chronic bronchitis. *N Engl J Med* 2017;377:911–922.

The Role of Surgical Lung Biopsy in Antifibrotic Therapy for Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic, fibrosing lung disease with high mortality (1). The diagnosis rests upon a compatible clinical picture of insidious dyspnea, dry cough, and the exclusion of exposure to mineral or organic dusts, connective tissue disease, fibrogenic drugs, or radiation. High-resolution computed tomography (CT) is an essential part of a clinical diagnosis, with typical features of bibasilar reticular interstitial markings and a lack of atypical features such as nodularity, upper-lobe distribution, and considerable amounts of ground-glass infiltrates or emphysematous changes. The presence of honeycombing in the bases strongly adds to the confidence of a radiographic diagnosis of usual interstitial pneumonia (UIP). When necessary, a surgical lung biopsy (SLB) can be performed to identify histologic features of UIP. A confident diagnosis of IPF is believed to be critical, as it helps direct considerations for antifibrotic medications, rehabilitation, referral to lung transplantation, enrollment in clinical trials, and, in cases of advanced disease, palliative care.

Since 2014, two antifibrotic medications, nintedanib and pirfenidone, have received U.S. Food and Drug Administration approval following the publication of two parallel phase III randomized controlled trials (INPULSIS 1 and 2, and ASCEND [Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis]) that demonstrated a slowing of the rate of decline in lung function in IPF (2, 3). However, there were no significant beneficial effects in terms of mortality, respiratory symptoms, or quality of life for either medication. The 2015 American Thoracic Society (ATS) IPF clinical practice guideline statement supported a "conditional recommendation" for the use of these medications in patients with IPF (4). In a recent study, Dempsey and colleagues used data from a large U.S. insurance database to compare patients with IPF who had been treated with antifibrotics with propensity-matched control subjects who had not received such therapy, and the authors found that the use of antifibrotic medications was associated with a decreased risk of all-cause mortality over the first 2 years of treatment, as well as decreased acute hospitalization (5). No differences were noted between patients taking pirfenidone and those receiving nintedanib. However, given the high costs of these antifibrotic agents, insurers are increasingly requiring rigorous diagnostic confidence, based on either typical CT findings or lung biopsy, to approve coverage of these agents.

In a study presented in this issue of the *Journal*, Walsh and colleagues (pp. 1146–1153) examined the level of diagnostic likelihood at which physicians prescribe antifibrotic therapy without requesting an SLB in patients suspected of having IPF (6). The study consisted of an international cohort of respiratory physicians who evaluated 60 cases of interstitial lung disease and reported differential diagnoses along with diagnostic likelihood, whether an SLB would be requested, and recommendations for initial management. IPF was included in the differential diagnosis in 41% of all physician–patient evaluations. SLB was requested in 8%, 29%, and 48% of definite, provisional high-confidence, and provisional low-confidence diagnoses of IPF. Antifibrotic therapy was prescribed without requesting an SLB in over 60% of provisional high-confidence IPF diagnoses (6). The team concluded that most respiratory physicians prescribe antifibrotic therapy without requesting an SLB if a "working diagnosis" of IPF can be rendered with high confidence. Using

Originally Published in Press as DOI: 10.1164/rccm.201907-1298ED on July 24, 2019