human lungs injured with live bacteria. *Am J Respir Crit Care Med* 2013;187:751–760.
9. Lee JW, Fang X, Gupta N, Serikov V, Matthay MA. Allogeneic human mesenchymal stem cells for treatment of *E. coli* endotoxin-induced acute lung injury in the *ex vivo* perfused human lung. *Proc Natl Acad Sci USA* 2009;106:16357–16362.
10. Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol* 2007;179:1855–1863.
11. Pedrazza L, Cunha AA, Luft C, Nunes NK, Schimitz F, Gassen RB, *et al*. Mesenchymal stem cells improves survival in LPS-induced acute lung injury acting through inhibition of NETs formation. *J Cell Physiol* 2017;232:3552–3564.
12. Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, *et al*. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005;33:145–152.
13. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, *et al*. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA* 2003;100:8407–8411.
14. Moodley Y, Vaghjiani V, Chan J, Baltic S, Ryan M, Tchongue J, *et al*. Anti-inflammatory effects of adult stem cells in sustained lung injury: a comparative study. *PLoS One* 2013;8:e69299.
15. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* 2008;132:598–611.
16. Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006; 441:1075–1079.
17. Nelson CM, Bissell MJ. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 2006;22:287–309.
18. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev Biol* 2010;341:126–140.
19. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS One* 2010;5:e10088.
20. Ren J, Stroncek DF, Zhao Y, Jin P, Castiello L, Civini S, *et al*. Intra-subject variability in human bone marrow stromal cell (BMSC) replicative senescence: molecular changes associated with BMSC senescence. *Stem Cell Res (Amst)* 2013;11:1060–1073.
21. Kizilay Mancini O, Shum-Tim D, Stochaj U, Correa JA, Colmegna I. Age, atherosclerosis and type 2 diabetes reduce human mesenchymal stromal cell-mediated T-cell suppression. *Stem Cell Res Ther* 2015; 6:140.
22. Islam D, Huang Y, Fanelli V, Delsedime L, Wu S, Khang J, *et al*. Identification and modulation of microenvironment is crucial for effective mesenchymal stromal cell therapy in acute lung injury. *Am J Respir Crit Care Med* 2019:199:1214–1224.
23. Iyer SS, Torres-Gonzalez E, Neujahr DC, Kwon M, Brigham KL, Jones DP, *et al*. Effect of bone marrow-derived mesenchymal stem cells on endotoxin-induced oxidation of plasma cysteine and glutathione in mice. *Stem Cells Int* 2010;2010:868076.
24. Huleihel L, Sellares J, Cardenes N, Álvarez D, Faner R, Sakamoto K, *et al*. Modified mesenchymal stem cells using miRNA transduction alter lung injury in a bleomycin model. *Am J Physiol Lung Cell Mol Physiol* 2017;313:L92–L103.



# Teaching an Old Intensivist Neutrophil Tricks: Using Alveolar Neutrophilia to Diagnose Ventilator-associated Pneumonia

An insidious belief pervades the modern ICU—that in cases of diagnostic uncertainty, broad-spectrum antibiotic therapy is "safest for the patient." This idea is likely a vestige of that bygone era before multidrug-resistant bacteria, when faith in the everlasting efficacy of antimicrobials was strong, and responsible doctors protected their patients from infection without regard for pretest probability.

Fortunately, this belief has been vigorously challenged. Antibiotic overuse is now known to be a clear contributor to the spread of drug resistance, which the World Health Organization has declared to be one of the greatest current threats to human health. It has further predicted that in the absence of improved stewardship, we will enter a postantibiotic era by 2050, with an associated 10 million deaths due to multidrug-resistant bacteria per year (1).

If this apocalyptic prediction were not deterrence enough, it is also clear that inappropriate antimicrobial therapy poses an immediate threat to the patient (2). Risks include life-threatening drug reactions such as Stevens–Johnson syndrome and anaphylaxis, as well as more common complications such as drug–drug interactions and nephrotoxicity. A more recently appreciated hazard is the profound disruption of gut microbiota produced by antibiotics, a condition termed dysbiosis. Although the full consequences of dysbiosis have yet to be elucidated, it is clearly linked to *Clostridioides difficile* colitis, a highly prevalent and often deadly infection (3). Altogether, the adverse effects associated with unnecessary antibiotics have been shown to worsen mortality in a number of studies by independent groups (4, 5).

It is therefore imperative for both the community as a whole and the individual patient that we develop highly sensitive diagnostic tools to rule out bacterial infection and enable safe and prompt cessation of antibiotics. Such diagnostics are particularly needed for pneumonia, which is responsible for a substantial proportion of antibiotic misuse and is a well-established driver of resistance (6–8).

In this issue of the *Journal*, Walter and colleagues (pp. 1225–1237) take an important step toward solving this problem in the ICU (9). To do so, they make use of the defining host immune response in bacterial pneumonia, namely, neutrophilic alveolitis. Indeed, most of the clinical manifestations of pneumonia stem from this process, including *1*) respiratory symptoms such as cough and purulent sputum; *2*) systemic signs such as fever, which results from inflammatory signals derived in part from polymorphonuclear cells; and *3*) radiographic infiltrates, which in pneumonia represent pus in the lung. Although relatively nonspecific, the BAL



Originally Published in Press as DOI: 10.1164/rccm.201811-2163ED on December 13, 2018

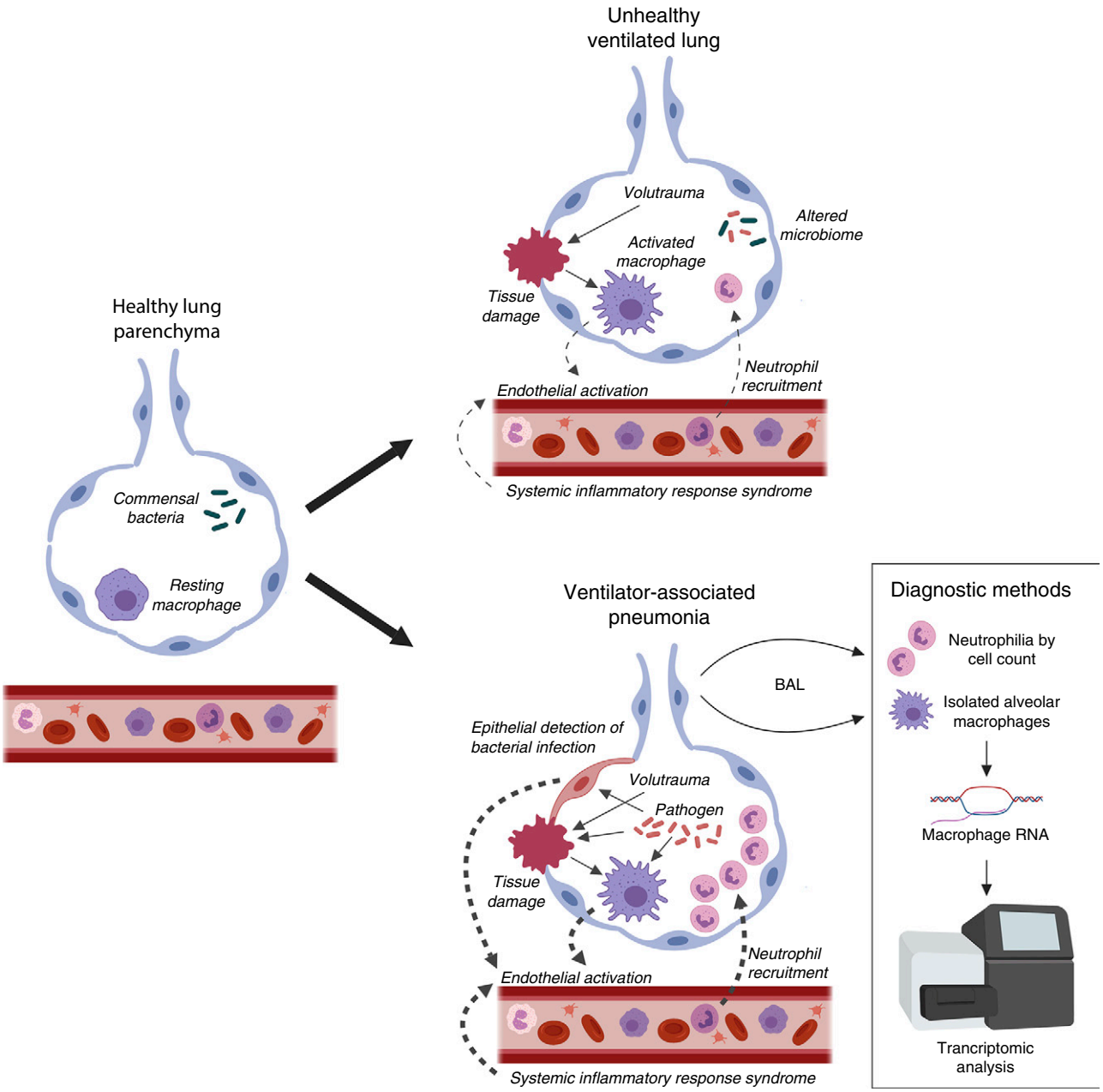


**Figure 1.** Pneumonic immune responses. Under healthy conditions (left panel), low levels of commensal bacteria and resting, noninflammatory macrophages predominate. The mechanically ventilated lung (top right panel) has numerous immune stimuli, for example, relating to volutrauma-induced tissue damage and systemic inflammation. These factors lead to endothelial activation and immune infiltrate, but not overt pneumonia. During ventilator-associated pneumonia (depicted in the bottom right panel), pathogenic bacteria further activate macrophages and induce both damage and stimulation of epithelial cells. Together, these drive a robust pyogenic inflammatory response, which may be detected by either one of the diagnostic methods described here.

neutrophilia test is believed to be highly sensitive for pneumonia, as previously shown in critically ill (10) and nonneutropenic immunocompromised patients (11).

In keeping with this canonical pathophysiologic principle, Walter and colleagues demonstrate here that nonbronchoscopic BAL (NBBAL) neutrophil counts of $<$50% provided a negative predictive value of 91.5% in patients with suspected ventilator-associated pneumonia, and coupled with a negative gram stain, the negative predictive value approached 100%. Diagnosis of pneumonia was confirmed by quantitative culture, a method that is highly stringent but not widely available.

This is a remarkable finding in terms of not only test performance but also practicality. NBBAL does not require pulmonologists; it may be performed by trained respiratory therapists, which reduces the cost and potential delay involved with formal bronchoscopy, with no decrease in diagnostic yield (12).

Furthermore, the laboratory analytics required—cell counts and Gram stain—are universally available, inexpensive, and rapid. For these reasons, the current findings have the potential to make a broad and immediate impact on clinical practice in the ICU.

The diagnostic insufficiency of BAL neutrophilia as a standalone test is worth discussing. One explanation may relate to a phenomenon that is observed in advanced sepsis and is known as neutrophil paralysis, wherein polymorphonuclear cell extravasation and chemotaxis are impaired (13). Thus, the 8.5% of patients in the study who had bacterial infection without neutrophilia may have had insufficient recruitment of neutrophils into the alveolar space due to sepsis-induced immunosuppression. Fortunately, these few cases could be identified via Gram stain.

Walter and colleagues also present a provocative series of genomic studies in which they assessed the ability of alveolar macrophage transcriptomes to predict the diagnosis of ventilator-associated pneumonia. Because alveolar macrophages serve as the central innate immune sensor of alveolar bacteria and principal coordinator of neutrophil recruitment, one might predict their transcriptional status to be a highly accurate reporter of lung infection. Surprisingly, however, the operating characteristics of an RNA sequencing–based test did not significantly outperform the NBBAL neutrophilia test alone. A number of reasons may explain this finding.

First, alveolar macrophages do not operate in isolation in the generation of pneumonic immune responses (Figure 1). Numerous other components of the innate immune system contribute to pathogen detection in the lung, including epithelial cells, innate lymphocytes, platelets, and plasma constituents (e.g., complement) (14). Indeed, immune signaling in epithelial cells alone was shown to be sufficient to recruit neutrophils and control infection in a murine model of *Pseudomonas* pneumonia (15). Therefore, alveolar macrophage–independent neutrophilic responses may help to explain the findings reported here.

Second, alveolar macrophages process immune signals from not only microbes (Figure 1) but also systemic inflammatory sources and damaged tissue (induced by pathogens, ventilator-induced volutrauma, etc.). It may be that the integration of these various stimuli produces genomic responses that are simply more similar than they are different. Studies in pneumonia and sepsis support this hypothesis and further indicate that the ability to distinguish features such as the site of infection, microbial etiology, and even the presence of infection appears to diminish as the illness progresses (16).

Thus, in patients with advanced critical illness, such as those studied here, host transcriptomics may be more challenging to interpret. Perhaps sampling patients earlier, combining transcriptomics with other biomarkers (including the alveolar neutrophilia described here), or synthesizing host genomics with microbiomic sequencing would lead to further diagnostic refinement. Further omics-based investigations of such questions are certainly warranted.

Although it is surprising that rapid, inexpensive, and widely available tests such as BAL cell count and Gram stain perform as well as cutting-edge personalized techniques like RNA sequencing, the finding is all the more valuable given the practical advantages of the former. Prospective trials are needed to demonstrate that the algorithm presented here can indeed reduce antibiotic use without compromising patient safety, but these results should certainly encourage more studies on the implementation of NBBAL sampling in the ICU. Finally, we submit that the present work, which describes a cost-effective rule-out test for infection inspired by sound pathophysiology, represents an important model for future studies aimed at reducing the unnecessary use of antibiotics—an often underappreciated but nonetheless rampant problem with potentially dire consequences for both society and the patient. ■

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

Samir Gautam, M.D., Ph.D.
Lokesh Sharma, Ph.D.
Charles S. Dela Cruz, M.D., Ph.D.
*Section of Pulmonary, Critical Care, and Sleep Medicine*
*Yale University School of Medicine*
*New Haven, Connecticut*

## References

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance; 2016 [accessed 2018 Apr 16]. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.
2. Gautam S, Sharma L, Dela Cruz CS. Personalizing the management of pneumonia. *Clin Chest Med* 2018;39:871–900.
3. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, *et al*. Burden of *Clostridium difficile* infection in the United States. *N Engl J Med* 2015;372:825–834.
4. Garnacho-Montero J, Gutiérrez-Pizarraya A, Escoresca-Ortega A, Corcia-Palomo Y, Fernández-Delgado E, Herrera-Melero I, *et al*. De-escalation of empirical therapy is associated with lower mortality in patients with severe sepsis and septic shock. *Intensive Care Med* 2014;40:32–40.
5. Schuetz P, Wirz Y, Sager R, Christ-Crain M, Stolz D, Tamm M, *et al*. Effect of procalcitonin-guided antibiotic treatment on mortality in acute respiratory infections: a patient level meta-analysis. *Lancet Infect Dis* 2018;18:95–107.
6. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States; 2013 [accessed 2018 Apr 15]. Available from: http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
7. Cookson WOCM, Cox MJ, Moffatt MF. New opportunities for managing acute and chronic lung infections. *Nat Rev Microbiol* 2018;16:111–120.
8. Guest JF, Morris A. Community-acquired pneumonia: the annual cost to the National Health Service in the UK. *Eur Respir J* 1997;10:1530–1534.
9. Walter JM, Ren Z, Yacoub T, Reyfman PA, Shah RD, Abdala-Valencia H, *et al*. Multidimensional assessment of the host response in mechanically ventilated patients with suspected pneumonia. *Am J Respir Crit Care Med* 2019;199:1225–1237.
10. Choi SH, Hong SB, Hong HL, Kim SH, Huh JW, Sung H, *et al*. Usefulness of cellular analysis of bronchoalveolar lavage fluid for predicting the etiology of pneumonia in critically ill patients. *PLoS One* 2014;9:e97346.
11. Stolz D, Stulz A, Müller B, Gratwohl A, Tamm M. BAL neutrophils, serum procalcitonin, and C-reactive protein to predict bacterial infection in the immunocompromised host. *Chest* 2007;132:504–514.
12. Lavigne MC. Nonbronchoscopic methods [nonbronchoscopic bronchoalveolar lavage (BAL), mini-BAL, blinded bronchial sampling, blinded protected specimen brush] to investigate for pulmonary infections, inflammation, and cellular and molecular markers: a narrative review. *Clin Pulm Med* 2017;24:13–25.
13. Sônego F, Castanheira FV, Ferreira RG, Kanashiro A, Leite CA, Nascimento DC, *et al*. Paradoxical roles of the neutrophil in sepsis: protective and deleterious. *Front Immunol* 2016;7:155.

14. Quinton LJ, Walkey AJ, Mizgerd JP. Integrative physiology of pneumonia. *Physiol Rev* 2018;98:1417–1464.
15. Mijares LA, Wangdi T, Sokol C, Homer R, Medzhitov R, Kazmierczak BI. Airway epithelial MyD88 restores control of *Pseudomonas aeruginosa* murine infection via an IL-1-dependent pathway. *J Immunol* 2011;186:7080–7088.
16. Burnham KL, Davenport EE, Radhakrishnan J, Humburg P, Gordon AC, Hutton P, *et al*. Shared and distinct aspects of the sepsis transcriptomic response to fecal peritonitis and pneumonia. *Am J Respir Crit Care Med* 2017;196:328–339.



# Developmental Milestones in Pediatric Research: A Case for Including Efficacy as Part of Interventional Trials in Infants with Cystic Fibrosis

In a study reported in this issue of the *Journal*, Stahl and colleagues (pp. 1238–1248) provide new evidence for the safety, tolerability, and potential efficacy of inhaled 6% hypertonic saline in infants with cystic fibrosis (CF) (1). PRESIS (Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis), a randomized, double-blinded trial, included just over 40 subjects enrolled at an average age of 3 months and followed for 1 year after being assigned to inhalation of twice daily nebulized isotonic versus hypertonic saline. No subject experienced a study-related serious adverse event or withdrew from the trial for intolerance. Studies done in children so young are often limited to safety and tolerability, but the PRESIS investigators were able to move beyond this and include measures of potential benefit to pulmonary health.

The study found that 6% hypertonic saline nebulized twice a day for 52 weeks provided a statistically significant improvement in lung function as measured by the primary efficacy outcome, lung clearance index (LCI). When compared with infants assigned to isotonic saline, those given 6% saline experienced a reduction (i.e., improvement) in LCI that was sustained over the 12-month observational period. The improvement in LCI, although not large, is similar to the difference reported between healthy children and those with CF at this age (2, 3). Larger improvements in LCI were seen in a small number of preschool-aged children after they started ivacaftor, but most of the children had higher (i.e., worse) baseline LCI values, and the few patients with normal baseline LCI tended to show little change with therapy (4).

The feasibility and successful use of LCI in the PRESIS study is perhaps as informative as the results. This is the first publication to report the successful use of LCI in a multicenter, randomized controlled clinical trial in infants. A single-center pilot substudy within the Infant Study of Inhaled Saline trial previously demonstrated that subjects receiving inhaled 7% saline had better LCI values than those receiving isotonic saline, but the participants had a median age of >2 years at enrollment (5). Stahl and colleagues now extend the argument that LCI can identify the health impacts of pulmonary interventions in trials that include very young children across multiple centers. Although the clinically meaningful change in LCI at this age is uncertain, they should be commended for this work requiring repeated study-related procedures for both LCI and chest magnetic resonance imaging (MRI).

There are a number of interesting observations beyond the key findings reported in this study. The PRESIS trial was set up to take advantage of early diagnosis of CF after newborn screening, with the goal of delaying or reducing the development of CF lung disease. This demonstrates the need for sensitive measures such as LCI to detect relatively small differences in lung function when focused on very young patients who may have been diagnosed before the onset of any pulmonary symptoms. Such measures were not available in initial studies of the effect of newborn screening on CF lung disease (6). Improvement in LCI was not associated with improvement in lung morphology as measured by MRI or the risk of predefined acute pulmonary exacerbations. Caution against overinterpreting data from a study of this size is necessary, but the findings suggest that either lung functional and structural abnormalities at this age do not align or lung imaging outcome measures may have less value in a population this young. Clearly, alternative interventions or imaging modalities may produce different results, but prior research also found a poor correlation between results from LCI and computed tomography imaging in subjects under 1 year old (7, 8). Less than one-third of children with CF appear to have bronchiectasis on computed tomography at 1 year of age (9).

The modified Fuchs criteria were used to define acute pulmonary exacerbation in this trial. This definition is a common, valuable tool in CF clinical research (10), but it may be less useful in such young children. One recognizes that infants, even when acutely ill, often lack many of the symptoms required by the Fuchs criteria (e.g., change in sputum, hemoptysis, dyspnea, sinus pain, chest X-ray changes, and change in lung function), which may explain why less than half of the subjects in the trial experienced a protocol-defined exacerbation over the 12-month period. Although hypertonic saline may not significantly impact the risk of acute pulmonary exacerbations in young children (11), this also underscores the ongoing need to consider alternative quantitative efficacy measures for the youngest populations. The CF community has recently been encouraged, if not surprised, by potentially important benefits reported in studies testing CFTR (cystic fibrosis transmembrane conductance regulator) modulator drugs in infants and toddlers with CF (12). Thus, accumulating evidence suggests that using chronic preventive therapies before the onset of observable symptoms may provide clinically meaningful benefits. More work is needed to understand long-term safety and whether



Originally Published in Press as DOI: 10.1164/rccm.201811-2103ED on November 13, 2018