premorbid blood pressures; however, it is unclear if this rate is generalizable to settings with less interconnected electronic health records (e.g., the United States). Out of the 302 included patients, none had a right-heart catheterization; premorbid central venous pressure was instead estimated from echocardiography (25%) or cardiac disease history (75%). Using MAP deficit may be more pragmatic, therefore, given its relatively similar performance characteristics and reliance on less premorbid data.

Randomized controlled trials to assess the value of individualized blood pressure targets are warranted. Such personalization is not antithetical to protocolization. Rather, protocolized tailoring of vasopressor titration based on individualized targets—akin to protocolized tailoring of ventilator settings based on predicted body weight (10)—may allow us to realize the best of both worlds: standardization with a personal touch. ■

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Spelunking in Sputum: Single-Cell RNA Sequencing Sheds New Insights into Cystic Fibrosis

Cystic fibrosis (CF) is autosomal recessive disease caused by mutations in the CFTR (CF transmembrane conductance regulator) gene, which leads to chronic pulmonary disease and gastrointestinal abnormalities through the loss of CFTR-mediated chloride and bicarbonate transport (1, 2). Clinically, the lung disease is characterized by chronic neutrophilic inflammation with bacterial airway infection, especially by *Pseudomonas aeruginosa*, which can lead to progression of CF lung disease, the primary cause of morbidity and mortality in CF (3). Although the dominant inflammatory cells in CF sputum are neutrophils, other cells including macrophages, eosinophils, T cells, and B cells have been reported in sputum and BAL fluid (4). However, much of this analysis has been morphological or based on flow cytometry with prespecified antibody panels, which by definition introduce some bias to the analysis. There have been prior bulk RNA sequencing (RNAseq) studies that found clear evidence of excessive inflammation, dominated by neutrophils, as well as type 1 and type 17 inflammation (5, 6). In this issue of the *Journal*, Schupp and colleagues (pp. 1419–1429) conducted an unbiased analysis by performing single-cell RNAseq analyses in sputum between nine CF subjects and five healthy control subjects (7).

The authors found a cluster of recruited lung mononuclear phagocytes in CF sputum and identified three different archetypes of monocytes: activated monocytes, monocyte-derived macrophages,

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and heat shock–activated monocytes. The authors used pseudotime analyses, which is a bioinformatic tool to infer a cell trajectory that is highly relevant to recruited myeloid cells in the lung. In accordance with prior data (8), some monocytes had a proinflammatory trajectory with increasing expression of inflammatory genes (*IL1B*, *CXCL2*, *CCL3*, *CCL4*, *CCL20*, *VEGFA*, and *EREG*), calprotectin (*S100A8*, *S100A9*), antiapoptotic genes such as *MCL1* and *BCL2L1*, the inflammasome subunit *NLRP3*, inducible cyclooxygenase 2 (*PTGS2*), and expression of the transcription factors *NFKB1*, *NFKB2*, *ETS*, and *IRF1*. Expression of the proinflammatory cytokines *TNF* and *IL1A* were observed in the most activated subset. There was also a significant decrease in monocyte maturation gene expression (*APOC1* and *APOE*) and impaired phagocytic function (*MARCO*). This transcriptomic feature might account for phagocytic dysfunction, contributing to the perpetuation of infection in CF lungs. Most macrophages in CF sputum originated from the circulating monocytes as opposed to tissue-resident alveolar macrophages observed in healthy control subjects.

Also, the authors observed a complex population of polymorphonuclear neutrophils (PMN) based on the inflammatory genes (*S100A8*, *S100A9*, *S100A11*, *CSF3R*, *BL2A1*, and MIP [*CCL3* and *CCL4*]) and genes involved in PMN maturation (*FCGR3B*, *ALPL*, *CXCR2*, *CEBPB*, and *NFIL3*), which was quite distinct from healthy control subjects. Consistent with a prior report (9), the CF airway PMNs consisted of an overall proinflammatory phenotype, but single-cell RNAseq identifies cells in different stages of PMN differentiation. In addition, the authors found evidence of an antiapoptotic program in PMNs that may be due to high levels of G-CSF (granulocyte colony–stimulating factor) in the CF lung (10).

The authors also found B cells in CF sputum. This feature is quite interesting, as B cells are believed to largely reside in the submucosal space (11). Considering that *P. aeruginosa*–specific antibodies in patients with CF decrease rapidly after transplantation (12), tissue-resident B cells in the CF airway might play a role to exert humoral immune responses in CF. Given that class II MHC (major histocompatibility complex) is a gene modifier in CF (13), it would be of keen interest to know the antigen specificity of these cells. Also, are antibodies protective, or do they contribute to CF lung disease? Newer single-cell techniques that allow simultaneous BCR (B-cell receptor) and mRNA sequencing of the same cell will be a valuable tool to understand the role of B cells in CF. Notably, not fresh but cryopreserved sputum samples were able to be used for transcriptome analysis, which allows investigation on archived specimens. In addition, unlike lung tissues or bronchial blushing (14, 15), sputum sampling is noninvasive, on hand at the clinic, and low cost.

However, as the authors point out, there are still some limitations in this study. One issue is the minimal sputum and cell populations in health, so what are the appropriate controls for CF? Also, with the use of modulators, sputum production has declined, and thus it remains to be determined if this technique may aid our understanding of residual disease after modulator therapy.

These results provide one of the first unbiased transcriptomic data sets in CF sputum. Moreover, the data could be successfully generated from archived sputum samples as opposed to more invasive samples such as BAL or bronchial brushings. Whether sputum single-cell analysis will be useful in other diseases remains to bet determined. However, this report could be a blueprint for the future applications of this technology to advance our basic understanding of chronic lung diseases. These data may be useful in understanding responses to therapy as well as potentially informing clinical trial design. This technology will likely accelerate our understanding and, ultimately, management of chronic lung disease. With a more granular understanding of the cellular basis of lung disease, we can use this knowledge to reduce the burden of these diseases, which are major diseases affecting human health. ■

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