(5, 6). With increasing use of single-cell profiling, the breadth and potential clinical importance of AM heterogeneity have become even more apparent. Our group has used single-cell RNA sequencing to identify novel populations of alveolar macrophages expressing profibrotic genes in patients with pulmonary fibrosis (7). In addition, a single-cell approach was recently used to identify five transcriptionally distinct clusters of AMs within the inflamed lung (8).

High-resolution studies aimed at splitting apart relevant populations of AMs in the injured lung can be limited by high cost and complex downstream analysis. In addition, it remains to be seen whether these novel approaches can support discovery of uniquely informative biomarkers or clinical phenotypes. However, it is important to consider how these splitting approaches may complement or improve on studies that analyze an immune cell population in the broadest terms. As an example, in the work by Morrell and colleagues, there were no differences in genome-wide expression profiles between patients with good versus poor clinical outcomes after adjustment for multiple testing (1). This may be because of the noise present in genomic datasets derived from critically ill patients, but it may also be driven in part by the limited resolution of the analysis; an overly broad view can make even complex systems look uniform.

Should we lump or split immune cells when studying the injured lung? If the goal is to advance our understanding of the pathobiologic mechanisms of disease, then splitting using high-resolution approaches offers particular promise. If the aim is to identify clinically informative disease phenotypes, then both approaches may prove useful and synergistic. However, when we choose the 10,000-foot view, we should remember and be informed by the complexity that lies below. ■

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

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Reply to Walter and Reyfman

From the Authors:

We appreciate Drs. Walter and Reyfman's correspondence regarding our study (1). We agree that immune cell heterogeneity—particularly alveolar macrophage (AM) diversity—likely plays a key role in the disease pathogenesis of acute respiratory distress syndrome (ARDS). Recent studies by our group (2) and others (3, 4) have used single-cell approaches to better characterize alveolar immune subtypes in ARDS and animal models of acute lung injury. However, we caution against solely relying on "splitting" approaches such as single-cell RNA sequencing to understand the pathobiology of complex human syndromes. Highly granular approaches performed on limited numbers of subjects may not capture the diversity of clinical phenotypes that exist in critical illness, and there remain significant technical and computational limitations (5) regarding single-cell approaches.

Critical care translational studies rely on analyzing data from relatively large patient cohorts to overcome external confounders that can bias results such as variation in clinical interventions, timing in the onset of risk factors, and baseline genetic diversity. The complexity and cost of single-cell approaches currently limit the number of samples that can be practically analyzed. In addition, many important genes are not captured with commonly used single-cell RNA sequencing platforms because of the limited depth of sequencing coverage and amplification bias (6). For example, *Myd88* (myeloid differentiation primary response 88) and *Tlr9* (toll-like receptor 9) are two important macrophage effector genes that were not detected in a recent single-cell RNA sequencing experiment identifying AM subtypes in an animal model of acute lung injury (3).

Our bulk microarray approach was inclusive of 18,415 unique genes; however, we did not identify any differentially expressed genes in AMs from subjects with good versus poor clinical outcomes after adjustment for multiple hypothesis testing. We concur with

Originally Published in Press as DOI: 10.1164/rccm.201907-1309LE on July 17, 2019

Walter and Reyfman that because our bulk approach did not capture the relative contributions that specific AM subtypes made to the overall transcriptional signal, our ability to detect differentially expressed genes may have been weakened. Despite this limitation, our bulk transcriptomic approach has advanced our understanding of AM function in ARDS by identifying AM-specific genetic programs that were associated with good versus poor outcomes. Future work is needed to identify the AM subtypes that might be responsible for the bulk transcriptional signatures we identified in our clinical cohort.

We believe that "lumping" and "splitting" approaches are complementary in furthering our understanding of the pathobiology of syndromes such as ARDS and sepsis. Analytical approaches such as cellular deconvolution (7) may be able to bridge bulk transcriptomic datasets and clinical cohorts like ours with highly granular single-cell datasets to fully leverage the strengths of both lumping and splitting approaches to understand the mechanisms of complex human syndromes. ■

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

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Diagnostic Classification of Bronchopulmonary Dysplasia: A Compromise between Defining Lung Disease versus Long-Term Outcome Prediction

To the Editor:

There are important limitations to current bronchopulmonary dysplasia (BPD) definitions, and many groups are working to come up with diagnostic criteria that are better adapted to current clinical presentation and treatment modalities and can also predict long-term outcomes.

Jensen and colleagues analyzed data from the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network to explore 18 different combinations of respiratory support and identify the definition that best predicts death or poor long-term respiratory and neurological outcomes (1). The authors concluded that a definition that includes nasal cannula flow, nasal continuous positive airway pressure, and mechanical ventilation at 36 weeks corrected age offers the best prediction for these composite outcomes. Surprisingly, many of the combinations of respiratory support tested in this study showed very similar accuracy for predicting long-term outcomes.

Although the proposed definition is appealing because of its simplicity and ability to predict outcomes and was validated in a large, multicenter population, it may not accurately reflect the severity of lung disease. This analysis assumes that the use of respiratory support in preterm infants is driven mainly by parenchymal lung disease. In reality, the use of respiratory support in this population can be related to many different respiratory and nonrespiratory problems, and indications for such support are subjective and vary considerably among institutions. Therefore, many patients may be inappropriately labeled as having BPD when in fact they are receiving respiratory support for indications other than lung disease.

More surprisingly, and in contrast to previous evidence (2–4), the authors concluded that inspired oxygen administered at 36 weeks postmenstrual age did not add strength to the prediction models. Oxygen administration may vary among centers, but in most instances, oxygen is titrated to maintain a narrow range of Sa_{O_2}. In the absence of extrapulmonary shunts, inspired oxygen is the simplest and most sensitive single indicator of the severity of

Originally Published in Press as DOI: 10.1164/rccm.201906-1130LE on July 19, 2019