microbiology identifies organisms that are viable using the appropriate media and culture conditions, and the load can be easily quantified.
2. The greater the viable load, the more likely it is that secondary defense systems, including neutrophilic recruitment, will be activated. This turns the sputum green and is associated with airway inflammation and destructive neutrophil products that may also impede host defenses (9).
3. The presence of inflammation and the response to it can be monitored even by simple observation of sputum purulence, and a change to mucoid sputum with a reduction in microbial load or clearance reflects a successful intervention.
4. If that is the aim, those are the patients!
5. The persistent production of purulent sputum should be investigated and, when possible, treated to decrease airway neutrophilia and the accompanying damaging neutrophil products, as this also improves the patient's well-being. This can be achieved with long-term inhaled therapy. The wheel is rediscovered: it is still a wheel, but certainly a more robust one.
6. Such a strategy points the way not only to a more personalized approach to patient management but also to a more focused clinical trial design.
7. Sometimes simple methodologies and clinical understanding outrank modern technological approaches. The authors conclude that "molecular techniques may be better" at identifying all airway bacteria (alive or dead). However, it remains to be seen whether such information will lead to better focused management strategies, as the load of the dominant species will likely remain the critical clinical factor in driving inflammation, as was confirmed in a previous study that used such a methodology in COPD (10). ■

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Robert A. Stockley, M.D., D.Sc.
University Hospitals Birmingham NHS Foundation Trust
Queen Elizabeth Hospital Birmingham
Birmingham, United Kingdom

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Peeking under the Hood of Acute Respiratory Distress Syndrome Phenotypes: Deeper Insights into Biological Heterogeneity

Given the biological heterogeneity inherent to acute respiratory distress syndrome (ARDS), it is unsurprising, or perhaps even inevitable, that a pharmacotherapy "magic bullet" targeting a specific mechanistic pathway has failed to emerge. A silver lining in the plethora of failed clinical trials is the foresight of the original trial investigators to judiciously collect and store biospecimens, which, coupled with the richness of the trial data, has permitted informative secondary analyses. Investigators have used these data to perform unsupervised subgrouping analyses and have consistently identified two biologically and clinically distinct phenotypes in ARDS, referred to as the "hypoinflammatory" and "hyperinflammatory" phenotypes (1–4). The hyperinflammatory phenotype is associated with elevated plasma levels of proinflammatory biomarkers and an increased incidence of shock and organ dysfunction. More recently, researchers identified two phenotypes using only plasma biomarkers in an observational cohort of patients with ARDS (5). These phenotypes, termed "uninflamed" and "reactive," share similarities with the hypoinflammatory and hyperinflammatory

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phenotypes, respectively. In both classification schemes, the two phenotypes have divergent clinical outcomes, and the phenotypes derived from trial data were also associated with distinctly different responses to randomized treatments (6).

Although the nomenclature attached to these ARDS phenotypes focuses on inflammation, in reality, our understanding of the biological pathways associated with the phenotypes remains very superficial. A deeper understanding of the true biological differences between the phenotypes will be required before we can move toward personalized therapies directed at one or the other specific subtype. In a study reported in this issue of the *Journal*, Bos and colleagues (pp. 42–50) used whole-blood transcriptomic data to study key differences in biological pathways associated with the reactive and uninflamed phenotypes in an observational cohort of 210 patients with sepsis-related ARDS (7). First, the authors used a previously developed five-biomarker-based classifier model (5) to assign either the reactive or the uninflamed phenotype. Next, in plasma samples drawn simultaneously, they used microarray technology to compare mRNA expression of 11,443 genes between the two phenotypes. A significant difference in expression was observed in 29% of these genes. In the reactive phenotype, genes associated with leukocyte activation were more highly expressed, with *MMP8*, *OLFM4*, *RETN*, and *GPR84* showing the greatest fold difference in expression compared with the uninflamed phenotype. By pathway analysis, the gene enrichment pattern of the reactive phenotype was most associated with oxidative phosphorylation, mitochondrial dysfunction, and cholesterol metabolism pathways.

In contrast, the uninflamed phenotype was associated with higher expression of genes that were previously found to be negatively associated with ARDS (e.g., *MME* and *HCAR3*) (8). Pathway analysis revealed expression patterns associated with cell proliferation/survival (mitogen-activation protein kinase and RAF-1 pathways). Many of the discriminatory genes associated with pathways in the uninflamed phenotype are ubiquitously expressed during cellular stress from a multitude of insults (9). Interestingly, in contrast to the reactive phenotype, where the altered pathways were associated mostly with the innate immune system, in the uninflamed phenotype several of the altered pathways were associated with the adaptive immune system.

What are the key "big-picture" implications of this study? In the reactive phenotype, several of the top differentially expressed genes were neutrophil related and known to be immune modulatory (10, 11). Coupled with elevated levels of proinflammatory biomarkers, these data suggest that the innate immune response is a key contributor to the pathogenesis of the reactive phenotype (6). From a biological standpoint, these findings further undermine our current approach to defining ARDS, which mandates "lumping" these disparate patients together (12). Another notable finding of this study is the analysis of pathways that are relatively overexpressed in the uninflamed phenotype. To date, the uninflamed phenotype has been primarily defined by its relative lack of inflammatory markers, rather than by any distinct biology of its own. Along these lines, it is interesting to note that the gene-expression pattern of the uninflamed phenotype overlapped more closely with sepsis than with the reactive phenotype of ARDS in a principal component analysis, bringing into question the accuracy with which the current definition of ARDS identifies the pathophysiologic entity it seeks.

This study has several important strengths, including the fact that it is the first to explore differences in gene expression and associated pathway analyses in ARDS phenotypes. The study was performed in a carefully curated and prospectively accrued population of patients with ARDS. In the context of a clinical syndrome already plagued by heterogeneity, studying gene expression in unselected and/or poorly defined populations that are extracted from registries can often lead to increased noise-to-signal ratios, resulting in data that are difficult to interpret (13). Interestingly, some of the key neutrophil-related upregulated genes identified in the reactive phenotype have also been identified in prior ARDS studies (8, 13). Kangelaris and colleagues identified upregulation of several genes in sepsis-related ARDS that were also identified by the investigators of the current study, including OLFM4 and LCN2, among others (8). This corroboration of previous findings is notable and adds to the validity of the study.

The study also has some limitations. First, the use of microarray technology rather than RNA sequencing may lead to some inherent bias, as only the genes on the array can be detected. Second, as with all transcriptomic analyses, definitive causal mechanistic pathways and downstream functional protein expression (other than that of MMP-8, which the authors did measure in plasma) remain unknown. Third, the nature of the studied population (sepsis specific) limits the generalizability of the findings, and it may be that the authors' findings are sepsis related rather than ARDS specific. Future analyses should include true replication in a general independent ARDS cohort.

Many important questions regarding ARDS phenotypes remain unanswered. It is unknown whether the presented reactive phenotype is the same as or similar to the hyperinflammatory phenotype described elsewhere in the literature (6). In contrast to studies of sepsis (14), investigators have not yet used gene-expression data in unsupervised analyses to seek *de novo* discrete subtypes of ARDS, and it is unknown whether the same two phenotypes, or more, would emerge with such an approach. Although the authors have made a start in understanding the underlying biology of the uninflamed/hypoinflammatory phenotype, much remains unknown about this phenotype that constitutes 38–70% of the ARDS population. Biological differences between phenotypes in the lung compartment also remain unknown. Observational studies in humans cannot identify causal pathways, so experimental work will be necessary to truly test mechanistic hypotheses. Finally, to identify the phenotypes rapidly, at the bedside, real-time tests to quantify key biomarkers are urgently needed.

As the field of seeking phenotypes in ARDS matures, a wider array of high-dimensional data, either in isolation or in concert, will be used to explore systems biology in clinical syndromes. Bos and colleagues have elegantly demonstrated that phenotypes identified using plasma biomarkers have distinct gene-expression profiles and biological attributes. This study represents a substantial step forward on the challenging journey toward delivering precision medicine in ARDS. ■

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Pratik Sinha, M.D., Ph.D.
Department of Medicine
and
Department of Anesthesia
University of California, San Francisco
San Francisco, California

Carolyn S. Calfee, M.D., M.A.S.
Department of Medicine
Department of Anesthesia
and
Cardiovascular Research Institute
University of California, San Francisco
San Francisco, California

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PP2A: A Novel Target to Prevent Cathepsin S–mediated Damage in Smoking-induced Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a recognized global health crisis, with smoking being the most important and well-studied risk factor for disease development and progression (1). The World Health Organization estimates that 80 million individuals live with moderate to severe COPD, and this disease will become the third leading cause of death worldwide by 2030 (2). COPD is characterized by chronic inflammation and obstructed airflow, usually originating from long-term exposure to particulates, with the most egregious offender being cigarette smoke. Various signaling pathways are implicated in the induction of lung inflammation associated with COPD pathogenesis. Dysregulation of phosphatases such as PP2A (protein phosphatase 2A), protein tyrosine phosphatase 1B, and pTEN (phosphatase and tensin homolog) are known to occur (3, 4). Imbalances in the activities of proteases, including serine, aspartic, metal-activated, and cysteine proteases, are also linked to the severity and progression of COPD (5).

CTSS (cathepsin S) is an endopeptidase member of the C1 family of cysteine proteases. Unlike most cathepsin proteases, which exhibit maximal activity at acidic pH, it has a relatively unusual ability to exhibit activity across a wide range of pH values. Accordingly, CTSS plays diverse physiological roles, including participation in immune responses, lysosomal protein catabolism, and extracellular matrix remodeling (6). It is particularly important in inflammation and immunity, participating in antigen presentation by cleaving invariant chain (Ii) to CLIP, which permits associated major histocompatibility complex II protein to load and present antigen. CTSS activity is implicated in many pulmonary diseases, including asthma and allergic inflammation (7), as well as alveolar remodeling and pulmonary emphysema in COPD (8, 9).

In this issue of the *Journal*, Doherty and colleagues (pp. 51–62) report two novel and interrelated findings obtained using a mouse model of chronic exposure to cigarette smoke (10). First, they establish that CTSS gene and protein expression is induced by

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