Phenotypes: Deeper Insights into Biological Heterogeneity

Peeking under the Hood of Acute Respiratory Distress Syndrome Phenotypes

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
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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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 (li) 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...