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Leveraging Transcriptomics to Disentangle Sepsis Heterogeneity

Critical illness is not a discrete disease but a matrix of heterogeneous and overlapping syndromes. This heterogeneity presents impediments to both research and clinical practice. Nowhere is this more evident than in sepsis (1), and identifying robust models to improve clinical and biologic stratification has become a research priority (2, 3). The field of oncology has provided insight into how this might be accomplished (4); however, sepsis presents unique and complex challenges.

Transcriptomics, assay of global differential gene expression within a cell population, provides a potential approach to sepsis stratification, using clustering algorithms to identify discrete patterns of cellular response (5). This clustering is ideally conducted in an unsupervised manner, without reference to clinical outcomes and response to therapy. The resultant groupings are then assessed for their correlation with outcome or response to treatment.

In this issue of the *Journal*, Burnham and colleagues (pp. 328–339) provide an example of a transcriptomic approach to sepsis stratification (6). Earlier work by this group identified two distinct sepsis response signatures, designated SRS1 and SRS2, among patients with sepsis secondary to community-acquired pneumonia (CAP) (7). Patients expressing the first pattern, characterized by a gene expression signature reflecting immune suppression, had increased mortality compared with those in the second group. The current study was undertaken to determine whether the stratification strategy also applies to patients with sepsis secondary to fecal peritonitis (FP) and to determine whether the transcriptomic response of patients with FP differs from that of patients with CAP.

The investigators used two complementary approaches to determine the relevance of the SRS1/2 classification in the FP cohort. After unsupervised clustering of the FP cohort and subsequent assignment to SRS1/2, they compared differential gene expression in the FP and CAP groups, according to SRS assignment, and found a modestly high correlation. They subsequently applied a previously reported seven-gene set (7), *a priori*, to assign SRS1/2 membership among the FP cohort subjects. This model showed excellent agreement with the results obtained by unsupervised clustering, but, more importantly, the subjects allocated to SRS1 had higher mortality than those allocated to SRS2, consistent with previous findings (7).

The authors found a relatively small number of genes differentially regulated between the CAP and FP cohorts. It is entirely possible this particular finding reflects the statistical noise inherent in such analyses, because the number of gene probes analyzed greatly exceeds the number of study subjects. There is little reason to believe that the transcriptomic response reflected in whole blood–derived RNA should vary with the anatomic site of infection. In the absence of a control cohort undergoing major laparotomies for indications unrelated to sepsis, it is unclear whether the differentially expressed genes reflect the anatomical source of infection, or a generic response to major abdominal surgery.

Previous stratification studies in pediatric septic shock revealed endotypes A and B, based on a gene signature reflecting adaptive immunity and glucocorticoid receptor signaling (8, 9). These genes are repressed among the endotype A subjects, and this group has higher mortality and organ failure burden than endotype B subjects. This pattern is reminiscent of the SRS1 grouping with respect to biology and outcome, but the current analysis revealed minimal overlap between the pediatric and adult gene expression data sets. Whether this reflects age-related differences in the host response or the statistical approach requires further exploration. It is recognized in oncology that widely variable gene signatures can yield very similar clinical and prognostic stratification

models, likely because the component variables in these complex datasets are highly related (10).

There are four critical considerations that must be addressed in moving the insights from unsupervised transcriptomics toward clinical or research utility (Table 1):

1. What is the most relevant cell population to study?
2. What is the study population, and what is the comparator?
3. What is the outcome that is being predicted?
4. How can the information be used to inform a clinical decision?

The lessons of oncology are instructive. Transcriptomic studies in oncology evaluate cells from the cancer itself. They compare the results to those of healthy cells and assess the capacity of these alterations to predict recurrence, the expression of tumor markers, and response to specific therapy. Thus, the information is readily used to identify a subgroup of patients at increased risk of recurrence and to guide a decision for a specific adjuvant therapy (11).

The optimal approach to transcriptomic studies in sepsis is less clear. Investigators typically evaluate gene expression in circulating blood leukocytes, although cells from the organs that are dysfunctional or fixed cells such as macrophages or endothelial cells might be more appropriate. An assumption implicit in the study of leukocytes is that the altered transcriptome is causally related to the development of organ dysfunction. It is plausible, however, that the clinical sequelae of sepsis result from the potentially modifiable alterations in gene transcription in target tissues. Circulating leukocytes are a mixture of cell populations having different roles in innate and adaptive immunity. Differential responses may reflect different patient responses but may also reflect relative differences in the percentage of blood leukocytes.

The study population is patients who meet physiologic criteria for sepsis and have evidence of infection as the cause. The comparator may be healthy control subjects, patients who do not meet sepsis criteria, patients who do not have infection, or, as in the present study, patients whose septic episode arises from different sites or causes of infection. The conclusions of the study and the utility of the resultant signature are entirely dependent on the control population selected.

Table 1. Comparative Transcriptomics in Cancer and Sepsis

	Cancer	Sepsis
Tissue	Tumor	Circulating leukocytes or leukocyte subpopulations Alternatively, cells that are the target of injury: endothelial cells, epithelial cells of lung or kidney
Comparator	Healthy tissue from same organ	Cells from healthy volunteers Alternatively, cells from critically ill patients without clinical manifestations of sepsis, or with these manifestations in the absence of infection
Outcome	Survival; recurrence	Survival Alternatively, progression of organ dysfunction, resolution of manifestations of infection
Clinical decision	Therapy targeting transcriptional alterations	In absence of effective mediator-targeted therapy, evaluate impact on response to fluids, vasopressors, duration of antimicrobial therapy

Most studies of transcriptomics in sepsis use survival as an outcome; in consequence, they emphasize the prognostic implications of the findings. Mortality is an imminent and unquestionably relevant consequence of sepsis. Moreover, although there are multiple interventions that effectively alter the course of specific cancers by targeting specific gene products, no such treatments are available for sepsis. But mortality is also impacted by factors not directly related to the episode of sepsis, including preexisting comorbidities and patient preferences for end-of-life care. It is further influenced by events occurring over the course of the intensive care unit stay, for example, the approach used to provide mechanical ventilator support. Finally, its timing is usually the consequence of a clinical decision to terminate support, and so it reflects a clinical decision as much as a clinical characteristic of the patient.

Transcriptomic and other high-dimensional data hold the promise of informing stratification strategies for sepsis and other forms of critical illness (12, 13). Publically available datasets provide powerful opportunities for multicohort analyses and the important validation studies (14, 15). The critical care community is likely to be cautiously skeptical of these approaches, because of unfamiliarity with the complex computational methods they use. It is therefore incumbent on the bioinformatics field to present these complex data in a clinician-friendly manner; conversely, it is incumbent on clinicians to become more familiar with these complex approaches. This convergence of disciplines can enable the promise of precision critical care medicine. ■

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More Than Meets the Eye: Cigarette Smoke Induces Genomic Changes in the Small Airway Epithelium Independent of Histologic Changes

Cigarette smoke–induced lung diseases, including lung cancer and chronic obstructive pulmonary disease (COPD), are leading causes of morbidity and mortality. The airway "field of injury" hypothesis suggests that exposure to a disease or environmental insult, such as cigarette smoke, leads to molecular alterations throughout the whole respiratory system, and that these alterations occur even in the absence of histologic changes. This concept, well developed in the cancer literature, suggests exposure-associated molecular alterations can be measured in histologically normal airway epithelium by gene expression profiling (1). These genomic signatures can then be used both to gain insights into disease mechanisms and to generate biomarkers for disease onset, progression, prognosis, and treatment.

In COPD, the earliest pathological changes appear to occur in the small airways (2–4). Cigarette smoke induces squamous cell metaplasia and mucous cell hyperplasia in the small airway epithelium (SAE) (5, 6). Further, there is evidence of decreased SAE repair (7), suggesting a detrimental effect of cigarette smoke on basal cells (BCs), the airway stem or progenitor cells (8). Although cigarette smoke–induced, SAE-specific molecular alterations have been identified (9–11), whether these molecular alterations precede these early pathologic changes is less well studied. The progression of this early injury to the heterogeneous pathologic changes in COPD, including emphysema and bronchitis, is also poorly understood, especially in former smokers.

In this issue of the *Journal*, Yang and colleagues (pp. 340–352) advance our understanding of the cigarette smoke–induced airway field of injury (12). They focus on molecular alterations induced in the SAE compared with the larger bronchi, leveraging the group's small airway brushing collection technique. By comparing global gene expression profiles of the large and small airway epithelium from healthy control patients, they developed proximal and distal airway transcriptome signatures (P- or D-signatures). Using immunohistochemistry, the authors established that the genomic differences between regions was not simply a result of distinct compositions of known cell types by demonstrating that certain proximal gene expression markers are expressed by ciliated cells, a cell type also abundant in the distal airways in which these genes have lower expression. They next compared the SAE gene expression of smokers with and without COPD with that of nonsmokers. Smokers exhibited a down-regulation of ~50% of D-signature genes compared with nonsmokers, whereas P-signature genes were up-regulated. These smoking-induced SAE molecular alterations were termed "distal-to-proximal repatterning." The study further shows that the degree of proximalization was associated with lung function (FEV_1/FVC ratio) and age in healthy smokers, suggesting these genomic lesions have functionally measurable consequences.

As pathway analysis revealed EGFR as a major upstream regulator of the P-signature genes, the authors demonstrated evidence for its relevance *in vitro* by culturing primary human BCs at an air–liquid interface. Treatment of proximal airway BC cultures with an EGFR inhibitor decreased the expression of P-signature genes and increased D-signature genes. SAE BC cultures exhibited opposite changes when treated with EGF. EGF was further found to be up-regulated in the SAE of smokers, a finding reproduced by exposing cultures to cigarette smoke extract.

The changes induced *in vitro* by cigarette smoke extract support the concept that SAE proximalization represents early