smoking history would lend more depth of understanding about these patients and their disease and could be utilized in more granular phenotyping toward COVID-19 risk. In contrast to some previously published work, in this study, markers of type 2 inflammation were not protective against COVID-19. It is possible that a more objective definition of atopy, including levels of specific or total IgE with the relevant associated diagnoses, would clarify this finding. A full understanding of these relationships would require translational work, such as experiments using nasal epithelial or sputum samples among individuals before and after infection, stratified by disease and treatment, but such a study is unlikely to be completed. However, there are cohorts with COVID-19 that have been evaluated longitudinally and will likely provide more detailed clinical and immunologic data that should lend clarity to the role of asthma and atopy in SARS-CoV-2 infection (7). ■

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From Mass to Flow: Emerging Sepsis Diagnostics Based on Flow Cytometry Analysis of Neutrophils

Sepsis mortality decreases dramatically with timely initiation of standardized clinical management protocols (1), including source control, adequate antibiotic therapy (2), and aggressive hemodynamic resuscitation (3). Despite best practice recommendations by international critical care societies for the implementation of sepsis screening tools in health systems (4), the ability to diagnose sepsis early and accurately remains a major challenge for emergency and critical care clinicians (5). Current diagnostic criteria primarily reflecting sepsis-related organ dysfunctions rather than pathobiological mechanisms lack specificity, often leading to under- (6) or overdiagnosis (7). New translational research approaches to study the pathogenesis of sepsis in humans are needed to identify immunologic, metabolic, and microbial dysfunctions specific to sepsis and to provide the biological elements of accurate diagnostic tools.

In this issue of the *Journal*, Meghraoui-Kheddar and colleagues (pp. 46–59) used a high-dimensional mass cytometry approach for an in-depth and single-cell immune profile comparing patients with sepsis with those with sterile inflammation (8). Using a state-of-the-art unsupervised analysis to capture the cellular diversity of the neutrophil compartment, the authors identified and prospectively validated a neutrophil immunological signature that accurately differentiates patients with sepsis from control subjects. The results provide the foundation for the development of a sepsis-specific diagnostic test that will enhance the precision of managing critically ill patients.

High-dimensional mass cytometry and other single-cell technologies have revolutionized our ability to study complex immunologic states at the bedside and identify clinically relevant biological markers of human diseases (9, 10). In the context of sepsis, the study by Meghraoui-Kheddar and colleagues is the first to harness the cellular complexity of the neutrophil compartment to identify novel and diagnostically relevant biology. Using a 42-parameter mass cytometry immunoassay, the authors quantified the phenotype and abundance of circulating immune cell subsets in whole blood samples from 17 patients with sepsis and 12 control subjects with noninfectious inflammation (i.e., patients recovering from cardiac surgery) on Day 1 and Day 7 after hospital admission in addition to analysis of 11 samples

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from healthy donors. Application of the t-distributed stochastic neighbor embedding (t-SNE) dimension reduction algorithm to agnostically characterize the neutrophil compartment enabled identification of two novel neutrophil subsets enriched in samples from patients with sepsis at Day 1: the cluster of differentiation (CD)10^{-}CD64^{+}CD16^{+} programmed death-ligand 1 (PD-L1)$^{+}$ and CD10^{-}CD64^{+}CD16^{low}CD123^{+} immature neutrophils. The phenotype and abundance of the sepsis-specific neutrophils was confirmed using two complementary cell-type identification approaches, including the Uniform Manifold Approximation and Projection (UMAP)/Flow Self Organizing Maps (FlowSOM) algorithms and a conventional gating strategy. Importantly, the authors validated their findings in an independent patient cohort, using a seven-parameter fluorescence flow cytometry assay that identified patients with sepsis with high accuracy (area under the receiveroperating characteristic curve = 0.89).

The combination of rigorous methodologies for the processing of whole blood samples, the development and validation of a high parameter mass cytometry assay informed by sound pathophysiological hypotheses, and the use of validated unsupervised cell-identification algorithms are all essential factors of the study that contributed to the identification of strong immune correlates of sepsis. Another major strength of this study is the validation of the findings in an independent patient cohort, using a reduced fluorescence flow cytometry antibody panel. The authors should be lauded for "going the extra mile" by pruning down their 42-plex mass cytometry panel to a seven-marker flow cytometry immunoassay. The reduced assay targeted the CD123^{+} and PD-L1^{+} neutrophil populations to differentiate patients with sepsis from control subjects successfully. Although mass cytometry is a powerful discovery platform, reducing the dimensions of a mass cytometry assay to a small set of predictive immune cell attributes, identifiable using cost-effective clinical platforms, remains an essential step toward clinical translation and adoption.

From a mechanistic standpoint, the results from the study by Meghraoui-Kheddar and colleagues integrate well with an extensive body of evidence pinpointing innate immune cell dysfunction as a hallmark of sepsis pathobiology (11), including decreased monocytes HLA-DR expression (12), increased antigen presenting cells PD-L1 expression (13), and increased circulating CD10^{-}CD64^{+} neutrophil frequency (14). In their study, the authors demonstrate that neutrophils from patients with sepsis have a decreased phagocytotic capacity compared with those from control subjects, thereby anchoring the novel neutrophil signature of sepsis in biologically plausible mechanisms. Their findings dovetail with other studies suggesting that immature neutrophils release antiinflammatory cytokines (IL-10 and TGF-β [transforming growth factor-β]) and contribute to sepsis associated with the immune suppression (15). In addition, the study highlights CD123, the α-chain of the IL-3 receptor, as a potential mediator of the pathophysiology of sepsis. These findings are consistent with retrospective and prospective studies showing significant association between circulating levels of its ligand, IL-3, and hospital mortality during sepsis (16). The results have important implications for the development of novel therapies targeting CD123 for the treatment of patients with sepsis that could echo similar advances for the treatment of hematological malignancies (17).

The study has certain limitations. The number of patients included in the discovery and the validation patient cohorts is small (<20 patients per group in two university hospitals), which is inherent to translational research (18) and limits the generalizability of the results to the broader population. In addition, the number of time points (only two) limits the temporal resolution of the analysis. The immune response in patients with sepsis is highly dynamic and additional time points may have shed light on further pathophysiological mechanisms discriminating sepsis from sterile inflammation. In addition, the mass cytometry panel focused on cell phenotypes, which did not allow for functional analysis of the newly described cellular subsets. The assessment of proximal signaling pathways and effector immune cell responses is readily feasible with mass or flow cytometry and may be useful in the future to improve the predictive performance of the diagnostic test, while revealing novel mechanisms implicated in the pathogenesis of sepsis (19, 20).

Despite decades of research, delay in sepsis diagnosis and inaccurate prediction of sepsis outcomes remain key clinical challenges that contribute to its high morbidity and mortality (15). Meghraoui-Kheddar and colleagues provide promising clues for the development of a diagnostic test and for future mechanistic studies aiming to elucidate the role of the novel CD123^{+} and PD-L1^{+} neutrophil subsets in the pathogenesis of sepsis. The approach and study results resonate with other recent efforts leveraging various omic technologies for the diagnosis of sepsis and the prediction of sepsis outcomes (21). However, a single omic modality can only capture certain aspects of the complex pathobiology of sepsis in which multiple biological systems are dysregulated (microbial, immunological, and metabolic systems). Future studies integrating biological data across multiple omic modalities will be critical to improve the diagnostic accuracy and identify actionable biological dysfunctions that can be targeted therapeutically to improve the clinical outcomes of patients with sepsis. ■

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Cut from the Same Cloth: Similarities between Hypersensitivity Pneumonitis and Idiopathic Pulmonary Fibrosis

Hypersensitivity pneumonitis (HP) is a clinically and molecularly heterogeneous immune-mediated interstitial lung disease (ILD). Although newer classification of HP into fibrotic HP (fHP) and nonfibrotic HP has simplified diagnostic objectivity (1), the molecular pathways driving fHP have yet to be fully defined. This is important, as fHP is associated with worse outcomes compared with nonfibrotic HP (2). Furthermore, although fHP and idiopathic pulmonary fibrosis (IPF) are believed to be pathologically distinct, recent reports suggest they may have clinical and pathophysiologic similarities (3–5). Further insight of shared mechanisms between these ILDs may have practice implications for fHP as well as other inflammatory or fibrotic ILDs.

In this issue of the *Journal*, De Sadeleer and colleagues (pp. 60–74) sought to characterize the molecular determinants of fHP and whether they are shared with IPF (6). To achieve this goal, lung transcriptomic data were compared between control, IPF, and multiple samples within the same fHP lung to account for heterogeneity in regions of disease severity. Novel micro–computed tomography technology was used to stratify fHP samples by disease severity into mild, moderate, and severe groups as a proxy for morphological disease progression. Gene expression profiles were validated using publicly available data and BAL and computed tomography data from a separate fHP cohort. Distinct patterns of differential gene expression were defined, including those with an overall increase in fHP compared with controls but decreasing with local severity (degressive increase), an increase in fHP and increase with local severity (progressive increase), or an overall decrease in expression in fHP but further decrease with local severity (progressive decrease). These distinctions identified pathways implicated in fHP (disease specific) as well as implicated in the progression of fibrosis (disease severity specific).

Six molecular traits were associated with fHP. There was a degressive increase in extracellular matrix (ECM) genes and collagen functions, which have been previously implicated in HP as well as fibrotic lung diseases (7, 8). A similar pattern was seen in T cell signatures, including increased antigen presentation and T

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