FLUIDIGM®

MASS CYTOMETRY PUBLICATIONS

TRENDING NOW

July 2020

Welcome to the July issue of Trending Now, a quarterly anthology of recent impactful publications by researchers using CyTOF technology.

This month's issue focuses on the latest in COVID-19 disease research.

WHAT'S NEW IN MASS CYTOMETRY

The ease of designing high-parameter panels and standardized assays, an advantage that is unique to mass cytometry, supports the rapid pace of current infectious disease research targeting SARS-CoV-2.

Researchers have stepped up to COVID-19, and mass cytometry is there to support them

COVID-19 has made a global impact on human health, and it continues to spread (Figure 1). The science community is scrambling to learn not only about the new coronavirus and its biology, but more so the infection it causes and its immunopathology. Mass cytometry has become integral to this objective, supporting a steadfast response with the significant benefits of broad-scale immune discovery and profiling, low-volume sample use and quick start-to-finish experiments. Researchers worldwide continue to publish their data in a timely manner using mass cytometry, even with strict limitations on laboratory openings.

During the initial discovery phase, the unknowns about severe COVID-19 cases seemed insurmountable. Research focused on the intense symptoms and what caused a rapid decline in many patients. As a complement, some studies targeted mild cases and how the disease is more easily overcome in much of the population. What followed was a new understanding of disease pathology that led to inquiries into relevant biomarkers and characteristics in those more likely to be affected, allowing recognition of critical progression and facilitating early treatment. Here, we outline publications using mass cytometry to characterize and comprehend COVID-19 disease.

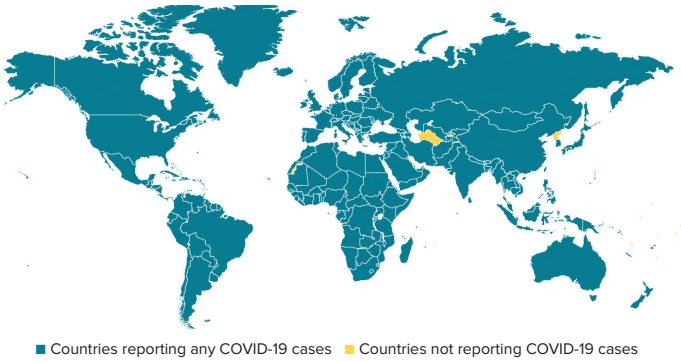

Figure 1. Confirmed COVID-19 cases by country, as reported by the World Health Organization. Source: cdc.gov/coronavirus/2019-ncov/global-covid-19/world-map.html

Solving the immunopathology puzzle in severe cases

Once the initial COVID-19 spread occurred in China, a group from You'an Hospital in Hubei worked quickly to understand COVID-19 pathology in critical patients in order to help direct treatments. To date, this prolific lab has published more than four studies using mass cytometry to investigate COVID-19. These studies illuminate patient response to treatment and demonstrate the unique changes in immune response across patients with varying disease progression.

The group first used Imaging Mass Cytometry™ (IMC™) to analyze the immune cell clusters in lung tissue after biopsy of two patients with COVID-19: one who had died from acute respiratory distress syndrome (ARDS) and the other of severe pneumonia (Zhang et al.). "Imaging Mass Cytometry with CyTOF® technology is our method of choice for these studies because the limited samples are very valuable. CyTOF allows more than 30 markers in one panel, and we don't have to worry about color compensation or background autofluorescence issues," explains **Dr. Dexi Chen, PhD, MD**, of the Beijing Institute of Hepatology and You'an Hospital.

Moving forward with these new findings, the team examined gene expression levels and T cell proportions associated with the modified immune response observed during disease progression (Ouyang et al.). Results revealed decreased T cell numbers and down-regulated gene expression involved in T cell activation and differentiation in severe COVID-19 patients, indicating suppression in the T cell immune response. The team also applied mass cytometry to analyze PBMC from patients with different disease progressions, observing obvious differences in composition of immune cells in severe patients (Figure 2. Wang et al.).

Further study with CyTOF and cytokine assays on additional patients confirmed these findings, showing a decrease in T cells, B cells and NK cells along with a progressive decrease of interleukin-2 (IL-2) in plasma (Shi et al.). Association of immune cell suppression with IL-2 could serve as a warning of disease deterioration in patients with COVID-19 pneumonia. Such a combination of approaches to uncover the diverse mechanisms activated in the COVID-19 response strengthens our understanding of the disease and its progression.

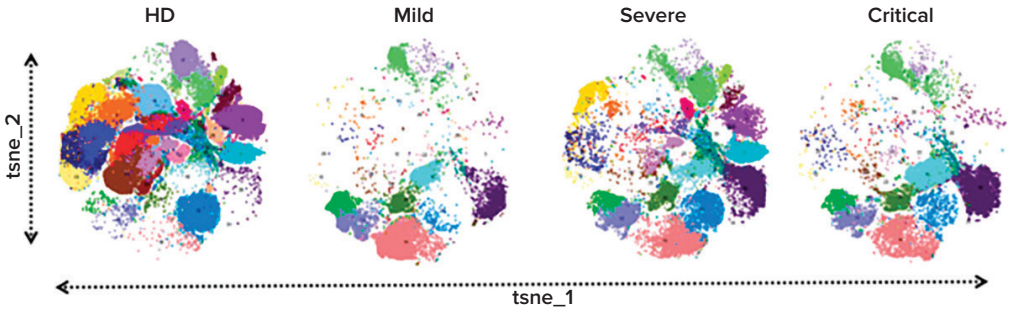

Figure 2. CyTOF based analysis identified immune cell signatures in peripheral blood of mild to critical COVID-19 patients, compared to healthy donors (HD). A representative viSNE plot of immune cell populations from healthy donors and COVID-19 patients. Wang et al.

Defining mild vs. severe disease

One of the most confounding challenges with COVID-19 is the unknown immune response of each individual, ranging from asymptomatic to fatal. Mass cytometry has helped identify immune cell subsets, particularly T cell subtypes that are either activated or suppressed in patients with mild cases compared to those with severe to critical cases.

One approach to deciphering the disease reaction has been to pinpoint the immune response in individuals who experience mild or no symptoms. A group from the Gladstone Institute at the University of California, San Francisco, used a 38-parameter CyTOF panel to phenotype a small set of patients who recovered from mild COVID-19 (Neidleman et al.). Their results (Figure 3) demonstrated specific T cell activation that revealed common features of effective immunity against SARS-CoV-2.

A collaboration of researchers from University of Bonn and Berlin University of Medicine applied both genomics and proteomics approaches to study the mechanisms of protective immunity in mild cases and the pathogenesis of a distressed response in severe courses of COVID-19 (Schulte-Schrepping et al.). Comparing two independent patient cohorts with cases ranging from mild to severe, they determined changes in immune cell composition and activation, providing detailed insights into systemic immune response to SARS-CoV-2 infection.

The COVID-19 pandemic has also offered the ironic opportunity to perform both cross-sectional and longitudinal studies on current patients for clinical trials. An active clinical trial, COntAGIouS, was initiated by KU Leuven and UZ Leuven to provide an in-depth characterization of the dynamic host immune response to SARS-CoV-2. The team will apply a transdisciplinary approach to identify host factors, compared between mild and severe COVID-19 patients and between COVID-19 positive and negative patients (NCT04327570).

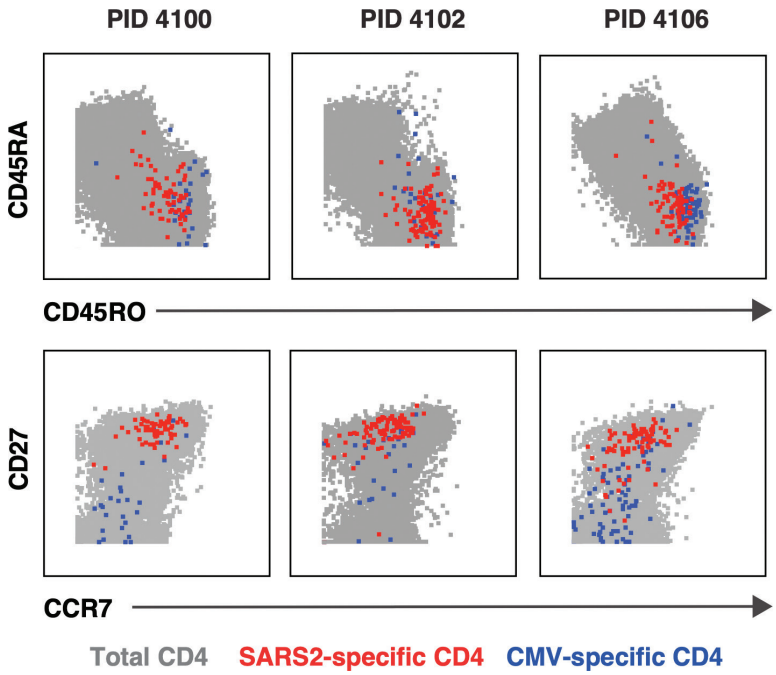

Figure 3. SARS-CoV-2 specific but not cytomegalovirus (CMV)-specific CD4+ T cells are predominantly T central memory cells (Tcm). The phenotypes of total (gray), SARS-CoV-2 specific (red), and CMV-specific (blue) CD4+ T cells are shown as dot plots. Top: Both SARS-CoV-2 specific and CMV-specific CD4+ T cells are predominantly CD45RA-CD45RO+, features of canonical memory cells. Bottom: Most SARS-CoV-2 specific CD4+ T cells are CD27+CCR7+, characteristic of Tcm, while most CMV-specific CD4+ T cells are CD27-CCR7-, characteristic of T effector memory cells (Tem). Neidleman et al.

Hallmark signatures could help point the way to better treatment

Research on the immunopathology of COVID-19 and its pathogenesis has helped the medical community take a more preventative approach to hospitalized patients, potentially identifying who is more at risk. For example, University of Louisville researchers found a possible clinical marker to monitor COVID-19 disease status and progression (Morrissey et al.). Their longitudinal study involved multiple analyses, including a 44-marker CyTOF panel with PBMC from severe and moderate COVID-19 patients compared to healthy donors. Results revealed that low-density neutrophils significantly contribute to COVID-19 associated inflammation.

The [Maxpar Direct] panel was used as is with the simple addition of two markers, PD-1 and Tim-3, allowing the team to work quickly to generate quality data, analyze the results and submit a preprint publication within one month.

A study from the University of Paris (Hadjadj et al.) discovered a hallmark of severe COVID-19, using the Maxpar® Direct™ Immune Profiling Assay™ to characterize distinct patterns of disease progression in patients tested 8 to 12 days following initial symptoms. The panel was used as is with the simple addition of two markers, PD-1 and Tim-3, allowing the team to work quickly to generate quality data, analyze the results and submit a preprint publication within one month. The unique phenotype, featuring impaired interferon type I response, was identified using combined results from mass cytometry, whole blood transcriptomics and cytokine quantification. The phenotype was also associated with a persistent blood virus load and an exacerbated inflammatory response.

Exploratory investigations on larger cohorts are forthcoming as well, focusing on specific comorbidities associated with patient outcome or in search of biomarkers for effective host-directed therapeutic interventions. A group at the National Institute of Environmental Health Sciences with an interest in related comorbidities initiated a longitudinal study that will explore the interaction between smoking, immune system characteristics and COVID-19 (NCT04403386). With early evidence suggesting that smokers have higher risk for morbidity and mortality associated with COVID-19 infection, the group is correlating smoking-associated altered epigenetics, transcription and changes in immune cell profiles.

The Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC) (NCT04378777) is studying the pathogenic mechanisms underlying severe pneumonia often seen with COVID-19. The study is using mass cytometry and the Maxpar Direct Immune Profiling Assay, as outlined in a recent publication (Geanon et al.), “because they provide a robust and standardized solution for comprehensive immune monitoring that is technically easy to execute and harmonize across multiple study sites,” says Adeeb Rahman, PhD, a core lab director for the study and Associate Professor and Director of Technology Development at the Icahn School of Medicine at Mount Sinai Human Immune Monitoring Center. As IMPACC study patients recover, investigators will continue to evaluate their immune responses to identify factors that may relate to long-term protection against re-infection.

The search for potential treatments

The ferocity that the scientific community has applied to learning about SARS-CoV-2 and COVID-19 has further stimulated work on potential treatment options. Early in the fight against COVID-19, a group from Shanghai University investigated mesenchymal stem cells (MSCs) as a preventive therapy to reverse the severe cytokine storm witnessed in critical patients (Leng et al.). MSCs are thought to have immunomodulatory characteristics and were tested by transplantation into patients with pneumonia caused by COVID-19. After assessing inflammatory levels, immune function and any adverse effects, the study provided evidence that MSC transplantation could significantly improve patient outcomes and serve as a safe and effective treatment for patients with COVID-19 pneumonia.

Expanding on the potential use of MSCs, an ongoing clinical trial (NCT04416139) examines how the plasticity of MSCs can regulate inflammation and immunity. The trial will outline clinical changes after intravenous administration of allogeneic MSCs in patients with bilateral COVID-19 pneumonia complicated by severe ARDS from cytokine storm.

With severe disease typically characterized by overactive inflammatory responses in the lung, efforts to control this hyperinflammation and resulting ARDS depend on learning how immune cell interactions and cytokines drive a response. Karolinska Institutet researchers performed a systems-level analysis to simultaneously capture all immune cell populations and their protein mediators (Rodriguez et al.) for a look into cell-to-cell communication (Figure 4). Longitudinal monitoring of each patient's immune response enabled an understanding of immune function in different stages during the infection. By mapping an immune trajectory from severe COVID-19 to recovery, the group provides data on which potential treatments could help modulate lung hyperinflammation.

With a growing number of studies and clinical trials underway, patient prognoses are likely to improve through a more comprehensive understanding of the disease and its progression. Mass cytometry continues to offer the foundation for new and creative approaches to learn more about COVID-19 and what we can do to prevent and treat it.

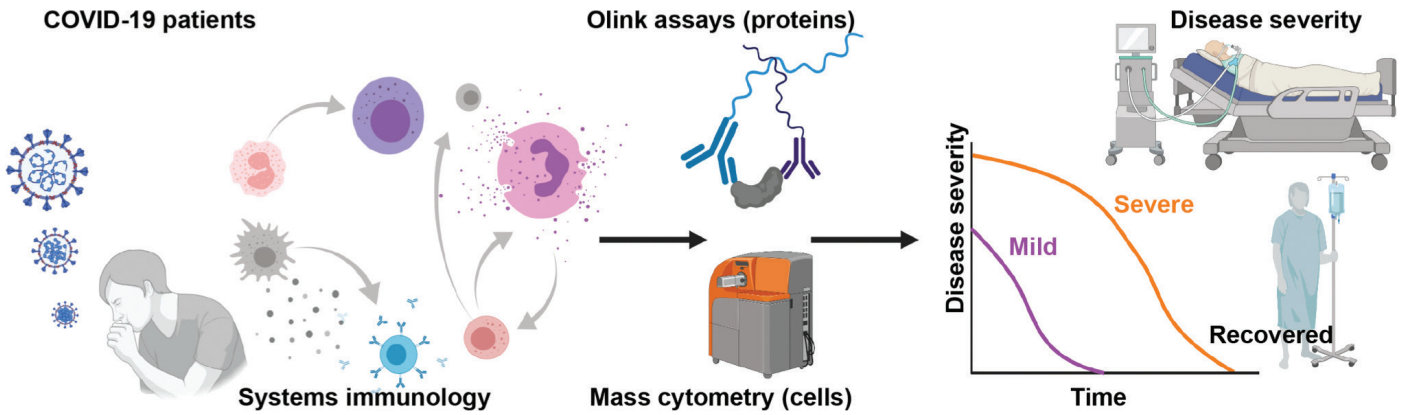

Figure 4. Experimental design for longitudinal profiling of the immune system in moderate to severe COVID-19 patients. Rodriguez et al.

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CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000 | South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada | Fax: 650 871 7152

SALES
North America | +1 650 266 6170 | info-us@fluidigm.com
Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com
Latin America | +1 650 266 6170 | info-latinamerica@fluidigm.com
Japan | +81 3 3662 2150 | info-japan@fluidigm.com
China (excluding Hong Kong) | +86 21 3255 8368 | info-china@fluidigm.com
All other Asian countries | +1 650 266 6170 | info-asia@fluidigm.com

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