COVID-19

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Immunologic prediction of long COVID

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In addition to the acute phase of SARS-CoV-2 infection, a significant percentage of patients experience a prolonged illness with varying symptomatology. Longitudinal SARS-CoV-2 patient-centric immunologic, inflammatory and metabolic data collection has allowed the generation of a composite signature to predict recovery.

The SARS-CoV-2 pandemic has ravaged the planet, with over 6.5 million people dead worldwide from COVID-19 (https://coronavirus.jhu.edu/data/mortality) and children suffering from post-SARS-CoV-2 multisystem inflammatory syndrome in children (MIS-C)[1]. Immunization rates have lowered incidences of both severe COVID-19 and MIS-C, but beyond surviving viral infection, many individuals experience prolonged consequences of SARS-CoV-2 infections. Long COVID, or post-acute sequelae of SARS-CoV-2 infection (PASC), affects up to 1 in 3 infected individuals, producing a variety of symptoms[2]. In this issue of *Nature Immunology*, Ruffieux et al. explore the biology and immunology of individual patient responses to SARS-CoV-2 infection in order to generate a predictive model of systemic recovery, or lack thereof, for those with PASC[3].

Symptoms of PASC comprise cough, fatigue, brain fog, myalgias and arthralgias, chest pain and many others, including disruption of smell and taste[2]. Many of these somatic complaints are relatively nonspecific and have been noted with other viral infections, including Epstein–Barr virus (EBV)[4]. The anosmia (loss of smell) and aguesia (loss of taste) may be fairly COVID-19 specific, with SARS-CoV-2 infecting the support cells for the olfactory nerves[5]. However, much of what is experienced during long-term post-SARS-CoV-2 infection overlaps with chronic fatigue syndrome/myalgic encephalitis, and it has been hypothesized to result from EBV reactivation[6]. Similarly, multiple sclerosis has been linked with EBV infection[7]. Nonetheless, the biology and immunology of PASC remain unclear, with commonly suggested hypotheses ranging from effects of pathogen remnants to autoimmunity to microbiome dysbiosis to direct tissue damage[4]. Multiple reports have explored risk definition for PASC development[8], but the study by Ruffieux et al. explores patient-centric clinical, inflammatory, immunologic and metabolic parameters longitudinally to predict outcomes[3].

In this study, 215 individuals infected with SARS-CoV-2 (across a spectrum of disease severity) were assessed, and compared to 45 healthy controls, over a 12-month period after infection[3]. The authors found intrapatient covariation of innate immune cell numbers, as well as levels of kynurenine and lipid metabolites, that predicted resolution, mortality and PASC[3]. From these data, they generated (and posted online) a composite signature to predict patient recovery based on molecular and cellular parameters measured soon after disease onset. Data from 70% percent of the 215 individuals were used as the training set, and the remaining 30% as the test set. Parameters measured

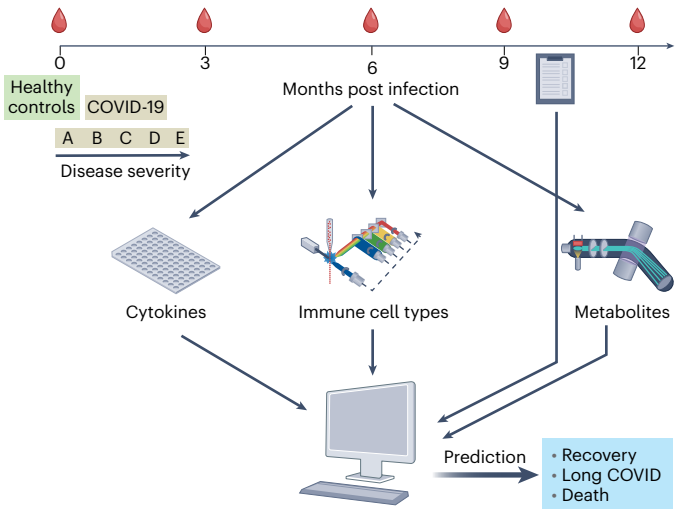

Fig. 1 | Development of a tool to predict recovery from SARS-CoV-2 infection. Immunological, inflammatory and metabolic samples were collected longitudinally in a patient-centric manner from peripheral blood of 215 individuals infected with SARS-CoV-2 with various degrees of disease severity and compared to 45 healthy controls. Analyses of cytokines, immune cell subsets (by CyTOF), and kynurenine and lipid metabolites (by NMR and mass spectrometry) and symptoms collected from questionnaires were used to generate a composite signature predictive of systemic recovery.

longitudinally included C-reactive protein (CRP), serum cytokines (IFN-γ, IL-6, IL-10, IL-1β and TNF), peripheral blood immune cell subsets (studied using cytometry by time of flight (CyTOF)) and plasma-derived metabolites (analyzed by NMR and mass spectroscopy) (Fig. 1). Differential abundance analysis between individuals infected with COVID-19 allowed for linear mixed modeling to study associations with CRP levels, and functional principal component analysis was employed to characterize inter- and intrapatient variability so as to estimate individual disease trajectories[3]. A variety of statistical approaches were applied to ultimately develop longitudinal modeling to enable the creation of a predictive modeling tool. The tool and the data used to generate it are shared online (http://shiny.mrc-bsu.cam.ac.uk/apps/covid-19-systemic-recovery-prediction-app).

Using CRP trajectories over 7 weeks after symptom onset, the authors identified three 'recovery groups'. Over the 7 weeks, group 1 patients had no or mild inflammation, group 2 had early-resolving inflammation and group 3 had persisting inflammation[3]. Although all severity classes of SARS-CoV-2 infection (asymptomatic to intubated) were represented in recovery group 3, the group was dominated by those who were most severely ill, including all of those who died. Recovery groups 2 and, particularly, 3 had the highest percentages of males and of individuals over 55 years of age. The data generated from

these studies, amassed at the population level and comparing individuals infected with SARS-CoV-2 across all severity groups to healthy controls, revealed that levels of kynurenine pathway intermediates (such as quinolinic acid) were increased, whereas tryptophan, the upstream amino acid degraded by this pathway, was depleted. This is intriguing, as kynurenine regulates the balance between regulatory T (T_{reg}) cells and type 17 helper $CD4^+$ T (T_H17) cells, and, in this study, group 3 patients had reduced T_{reg} cell counts[3]. Some B cell and T cell subsets were also decreased, whereas neutrophils, plasmablasts and activated $CD8^+$ T cell subsets were increased, in COVID-19 patients across all severity groups[3].

Group 3 patients with unresolving serum CRP levels showed markedly low natural killer (NK) cell numbers, possibly as a result of ongoing inflammation[3]. Similarly, hypertriglyceridemia was associated with hyperinflammation in this cohort. Both low NK cell numbers and hypertriglyceridemia are associated with, and criteria for, hemophagocytic lymphohistiocytosis, a frequently fatal cytokine storm syndrome (CSS)[9]. Many individuals with severe COVID-19 develop a CSS[10], but in the current study[3], it is unclear whether the NK cell lymphopenia contributes to COVID-19 CSS development or is a consequence of the SARS-CoV-2 infection, in a chicken-or-egg, cause-or-effect scenario. Nonetheless, both SARS-CoV-2 and EBV can trigger CSS[10] and, interestingly, both viral infections can be associated with long-term suffering, often irrespective of original disease severity.

In the current report[3], clinical associated symptoms were collected from questionnaires provided to individuals infected with SARS-CoV-2 between 2 and 11 months after symptom onset. Recovery group 3 patients experienced the greatest array of long-term symptoms, particularly neurological (fatigue, muscle weakness, pain and difficulty eating, swallowing and drinking). Nevertheless, even subsets of recovery group 1 patients experienced a variety of subjective complaints, despite the restoration of hemostasis in their cellular, inflammatory and molecular parameters. However, the prediction model developed from this work supports early measurements of metabolites being strong predictors of long COVID/PASC. In particular, high kynurenic acid and low tryptophan were early markers of poor prognosis[3]. Proven or suspected secondary infections (70% of which were in patients in recovery group 3) were also associated with recovery scores. Many of these infections were bacterial pneumonias, which were likely hospital acquired secondary to assisted ventilation. Removing individuals with secondary infections from the principal component analyses, however, did not notably alter the severity or recovery scores from the original analyses, indicating the robust nature of the framework[3].

Through longitudinal patient-centric data collection, Ruffieux et al. have explored the biological and immunological underpinnings of SARS-CoV-2 infection in an attempt to create a predictive tool for recovery and PASC development. They have largely succeeded, with robust performance of their model based and evaluated on the training and test cohorts, respectively. The model is not yet ready for clinical prime time, and many of the analytes involved are not easily measured. However, the predictive tool has been made available for others to study in their own cohorts to confirm or refute its utility. Whether the predictive tool will be useful for predicting outcomes for other infections (such as EBV) is also worthy of exploration. Perhaps a pared-down version requiring a subset of measurements for practicality at the expense of precision will eventually find clinical utility. For now, the model brings us one step closer to understanding the factors linking SARS-CoV-2 infection with recovery and prolonged disease course.

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Competing interests

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