

## **A multidisciplinary approach to identify biomarkers of Post-Acute Sequelae of SARS-CoV-2 infection.**

### **Background**

SARS-CoV-2 is the etiological agent of COVID-19, a disease declared pandemic in March 2020, which has caused about half billion confirmed cases, with a case/fatality rate of 1.4%. An increasing number of recovered people report a long-term condition, that has been named Post Acute Sequelae of COVID-19 (PASC) or Long COVID (LC). A significant proportion of individuals develops PASC several weeks after recovery, independently from the severity of the disease. Symptoms associated with LC are very heterogeneous and include exercise intolerance and muscle weakness, which are reported in two-thirds of COVID-19 survivors. Interestingly, post-infectious fatigue has been widely observed across a variety of other non-persistent viral infections, such as Epstein-Barr virus (EBV), SARS, Ebola and Zika virus, where an abnormal innate immune response and subsequent chronic inflammation are proposed to play a key role. Of note, herpesvirus reactivation has been frequently reported during acute SARS-CoV-2 infection, as a consequence of immune alterations. Thus, long COVID-19 symptoms may result from persistent immune activation, inflammation and/or reactivation of latent viruses.

### **Aim of the PhD project**

To identify an immunological signature associated with the presence of PASC in patients undergoing periodic clinical assessment after discharged from COVID-19.

Patients attending the post-COVID outpatient clinic at IRCCS Fondazione San Matteo will be recruited and followed-up intervals over several months. A control group of patients recovered from COVID without signs of PASC shall be simultaneously enrolled. We are planning to perform an in depth immunophenotyping analysis of peripheral blood monocytes, NK and T cells in a prospective cohort of COVID-19 recovered patients with or without persisting muscle weakness and/or fatigue. NK cell cytotoxicity and monocyte ability to produce proinflammatory cytokines will be analyzed. When clinically needed, muscle biopsies will be performed and tissue samples will be analyzed for immune infiltrate. Moreover, single-cell transcriptomic analysis will be used to identify inflammatory elements associated with neuromuscular disorders. Circulating levels of proinflammatory cytokines will also be evaluated in the patient serum.

The ultimate goals of this project are (i) to identify host and viral factors associated with neuromuscular impairment, (ii) to characterize neuromuscular determinants of long-term muscle weakness and decreased exercise tolerance in LC patients and (iii) to apply machine learning and statistical analyses to identify potential clusters of characteristics and classify patients as either with or without PASC.

Figure A summarizes the study design, methods and analysis.

**Techniques:** Peripheral blood isolation, flow cytometry, transcriptome analysis, ELISA, single cell transcriptome profiling.

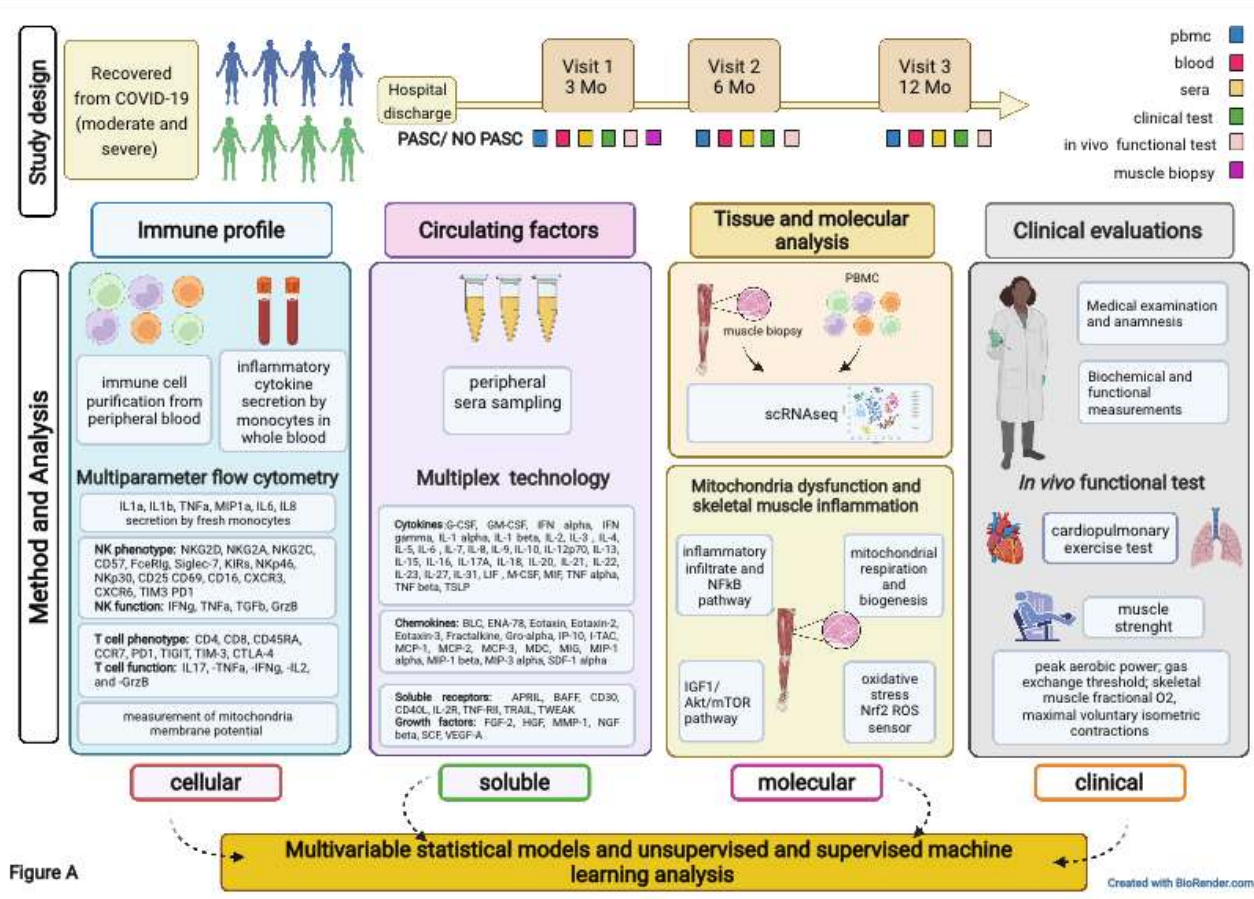


Figure A