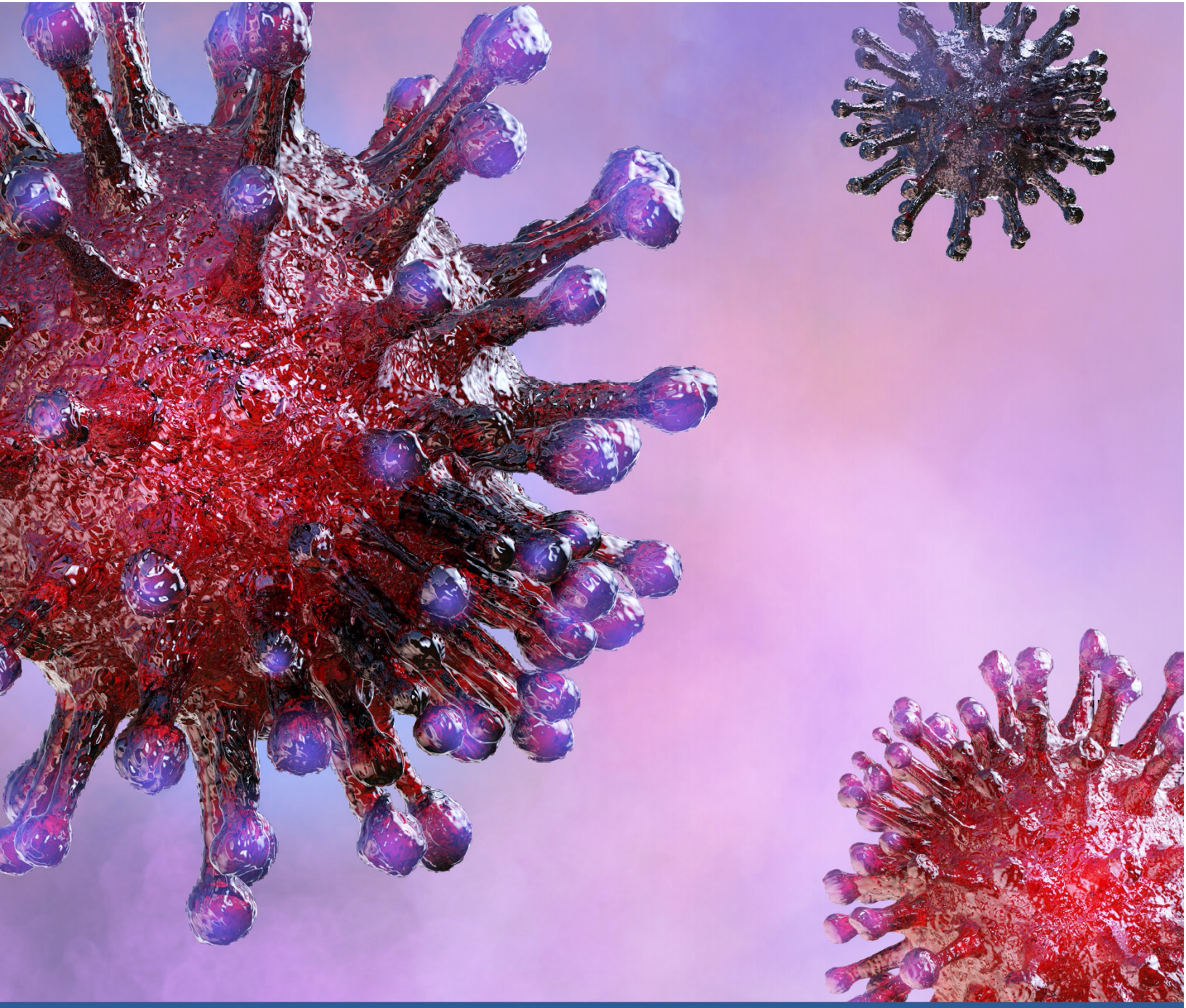

Role of Cytokine Release Syndrome (CRS) in COVID-19 Infections:

Multiplexed Cytokine Analysis Using IsoPlexis™ CodePlex™ Reveals Functional Proteomic Differences in Severe COVID-19 Patient Samples

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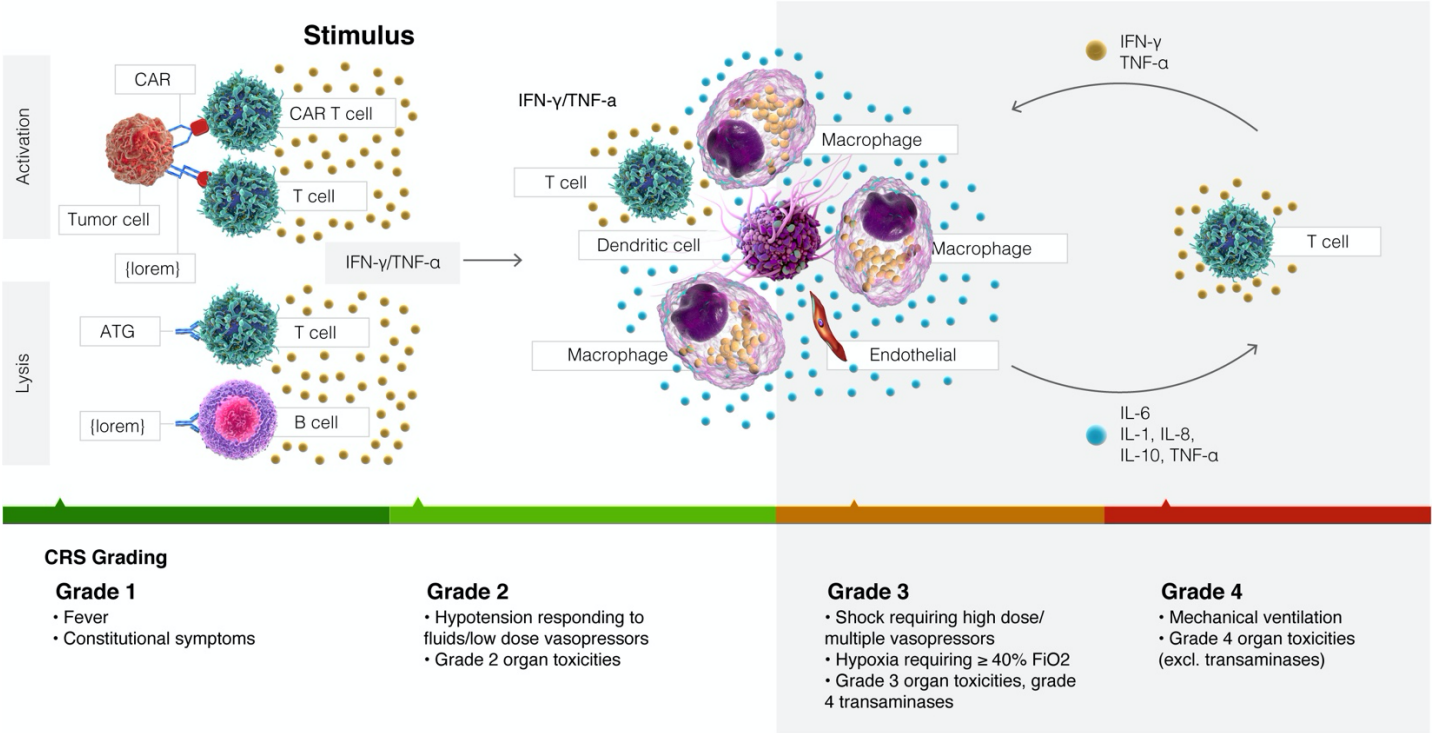
Impact of Cytokine Release Syndrome (CRS) in Severe COVID-19 Infections.

Cytokine-associated toxicity, also known as cytokine release syndrome (CRS), is a non–antigen-specific toxicity that occurs as a result of high-level immune activation and can be triggered by a variety of factors such as certain drugs and infections. As the name implies, a number of cytokines are released and elevated during CRS. The main cytokines implicated during pathogenesis of CRS include interleukin- 6 (IL-6), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) and interleukin-10 (IL-10). Cytokine release syndrome (CRS) represents a potentially life-threating toxicity that recently has been observed in those patients presenting with severe COVID-19 infections. [1] The proposed mechanism of how CRS is induced comes from the activation of mainly T cells or by the lysis of immune cells which induces a release of IFN-γ and TNF-α as observed in severe viral infections. Release of these cytokines triggers a chain reaction due to activation of innate immune cells like macrophages and endothelial cells which release additional cytokines, hence the term “cytokine storm or cytokine release syndrome”.

Cytokines and chemokines have long been understood to have an important role in immunity, however, when the immune response becomes dysregulated and exuberated by these factors, they have been shown to potentially cause lung damage and diminish patient survival. When β-coronaviruses, such as SARS-CoV-2 (COVID-19), infect monocytes, macrophages, and dendritic cells they can become activated and secrete IL-6 along with other inflammatory cytokines (Fig.1) CRS is common in patients with severe COVID-19, and elevated levels of serum IL-6 correlates with respiratory failure and adverse clinical outcomes. [2]. In COVID-19 infected individuals, IL-6, IL-10 and TNF-α surge during infection and decline during recovery. Patients with severe disease have significantly higher levels of IL-6, IL-10 and TNF-α and decreased IFN-γ expression in CD4+ T cells, indicating that cytokine release syndrome may dampen adaptive immunity against the virus and may contribute to the leading cause of death in these patients [3]. Understanding immune dysregulation in patients with COVID-19 will provide not only a greater understanding of the viruses’ pathogenesis but also identifies potential targets for immune-therapeutics and provide insights into effective vaccine designs. Targeted immunomodulation that reduces CRS may effectively reduce severe pulmonary inflammation and hopefully reduce patient mortality.

Figure 1. Cytokine Release Syndrome in COVID-19 Patients.



IsoPlexis CodePlex Secretome Solution: Fully Automated Functional Proteomics Solution for Predicting Cytokine Release Syndrome with Serum Cytokine Monitoring During COVID-19 Infections

The ability to detect and measure levels of multiple serum protein cytokines is critical in predicting cytokine release syndrome ("cytokine storm") and associated toxicity. The CodePlex Secretome Solution performs automated multiplexed, bulk proteomic detection of 30+ cytokine markers, to provide insight into early predictive markers of functional and inflammatory cytokines. The system required very small sample volumes (5-11µl), with a limit of detection ranging from 5-5000 pg/ml. Assay sensitivity: intra-chip CV=21% and intra-assay CV=22%. Note: accurate determination of cytokine levels above 5000pg/mL are achievable using 1:2 or 1:4 dilution of samples.

The CodePlex Secretome Cytokine Storm Panel detects and quantifies the following markers: IL-10, TNF-α, IL-6, IL-2, IL-7, MCP-1, MIP-1α, IP-10, IL-4, IL-8, IL-9, IFN-γ, GM-CSF, MIP-1β, IL-17A, IL-5, TGF-β1, IL-13, which can be used in a highly multiplexed protein assay as predictive markers to stratify patients and identify those most at risk of sever response and poor outcome.

Case Study

The scope of the cellular immune response to SARS-CoV-2 infection is far from clear, and with a tidal wave of studies now being published, there is significant confusion and conflicting data on the underlying pathogenic drivers. We describe here a prospective study, in which we characterized cytokine profiles and anti-SARS-CoV-2 IgG titers within serum samples from COVID-19 patients for comparison with both healthy donors and serum from pre-COVID samples.

Method: Serum sample from healthy and COVID-19 patients were thawed and diluted 1:4 in 2% BSA in PBS. Samples of 5-11μl of each patient serum was loaded onto the CodePlex Human Cytokine Storm chip for analysis. Background control samples consisted of 2% BSA in PBS.

Cytokine profiles were determined and reported using batch processing on the fully automated IsoPlexis IsoLight™ data analysis package, to generate a comparison of the secretome profiles determined in a cohort of COVID-19 patients, with those of healthy and pre-COVID-19 patient cohorts.

Anti-SARS-CoV-2 antibodies were measured on a semi-quantitative platform using beads-conjugated with SARS-CoV-2 nucleocapsid and RBD proteins. Bound patient IgG was measured by the binding of fluorescently labelled anti-human IgG conjugate on a flow cytometry platform. Note: all healthy and pre-COVID samples were also examined on this platform- all had non-detectable levels of anti-SARS-CoV-2 antibodies (data not shown).

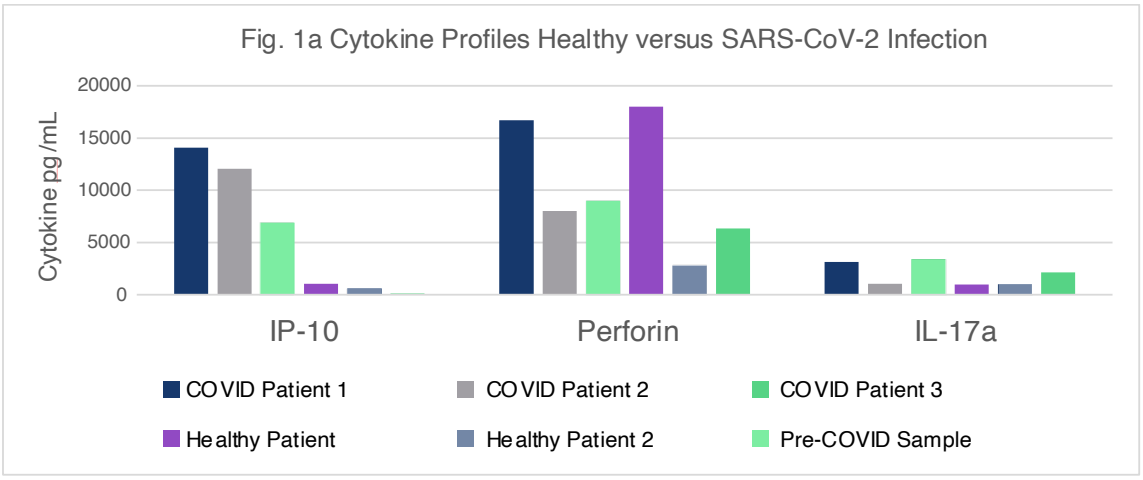
Results: The CodePlex Human Cytokine Storm multiplexed assay clearly identified elevated levels of IP-10, MCP-1, TGF-β and IL-17a in the COVID-19 patient serum compared with healthy controls and pre-COVID samples (Fig 1a and 1b).

IL-6 levels were highly elevated in one COVID-19 patient sample from an individual with severe symptoms. This individual displayed no detectable titers of anti-SARS-Cov2 IgG antibodies. IP-10 expression has been shown to correlate with disease severity, alongside HGF and MCP-3 and in this small cohort, IP-10 levels were indeed significantly higher in the COVID-19 patients compared with healthy controls. However, there was however direct correlation between IP-10 levels and anti-SARS-CoV-2 IgG levels, although there did appear to be correlation between IL-2 levels and anti-SARS-CoV-2 antibody titers.

MCP-1 (monocyte chemoattractive protein-1, CCL2) was elevated in all COVID-19 patients samples compared with healthy controls and pre-COVID-19 samples. This protein has a key role in regulating the migration of monocytes and macrophages during an inflammatory response.

No significant differences in levels of perforin, TNF-α, TNF-β, sCD137 were found between COVID-19 patient and control cohorts.

Anti-SARS-CoV-2 antibody titers were determined on a semi-quantitative platform using beads coated with SARS-CoV2 nucleocapsid and RBD proteins. Bound IgG was measured using fluorescently labelled anti-human IgG conjugate. The relative MFI signals are represented here and compared with cytokine profiles of COVID-19 patients with typical mild symptoms (fever, cough) versus severe requiring hospitalization.



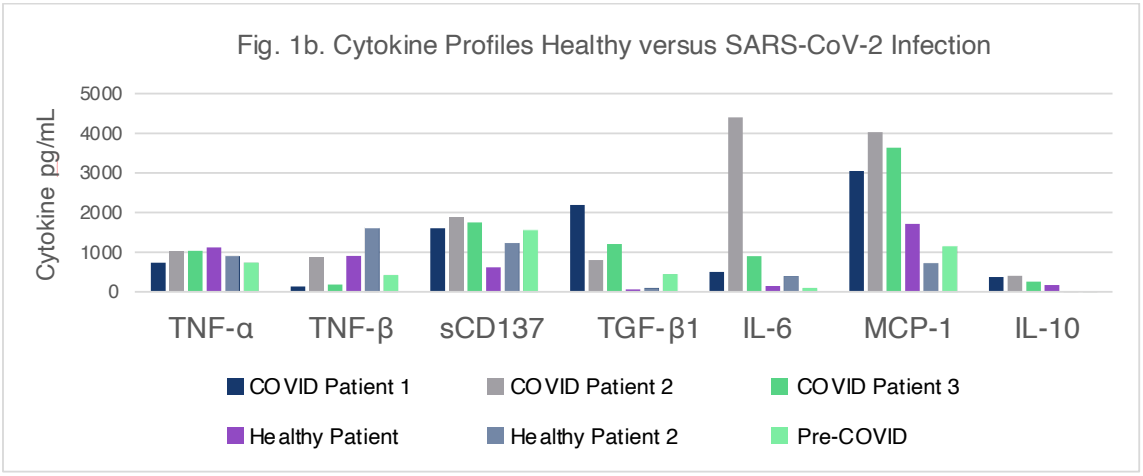
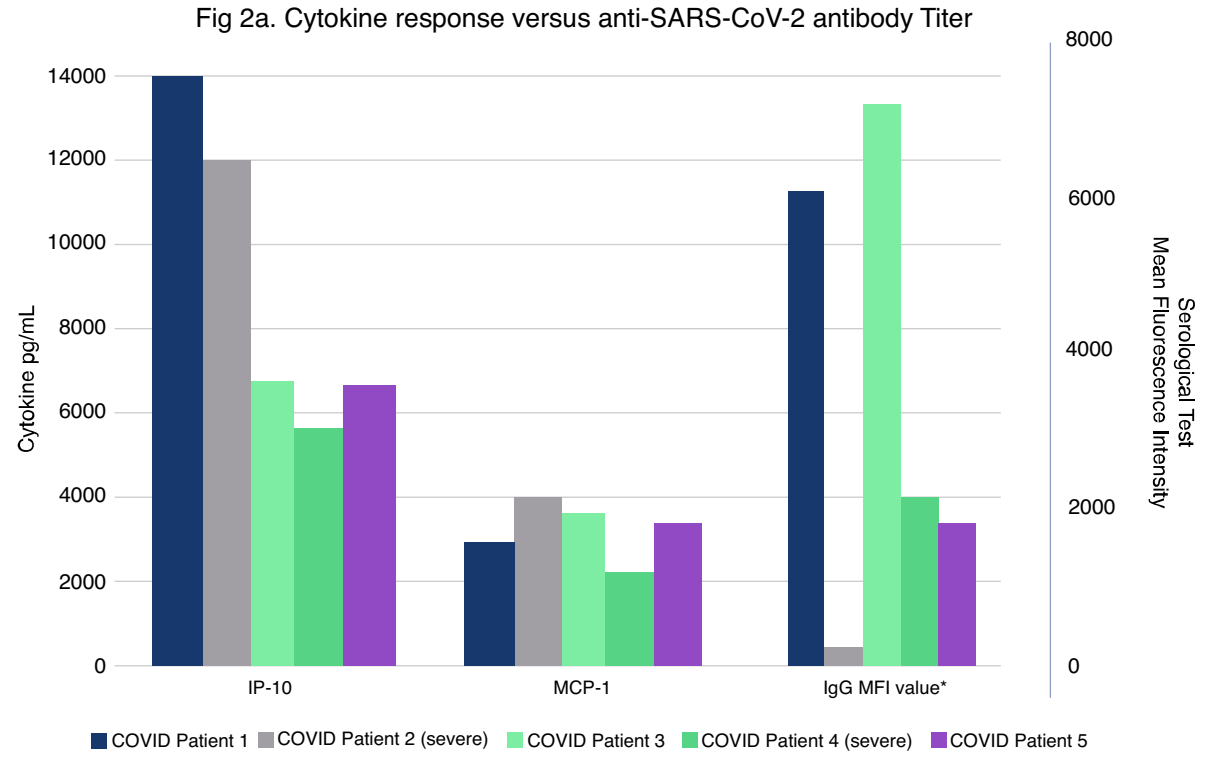


Fig. 1a and 1b. Comparison of cytokine in representative samples of patient serum from healthy controls, pre-COVID-19 controls and from individuals diagnosed with COVID-19.



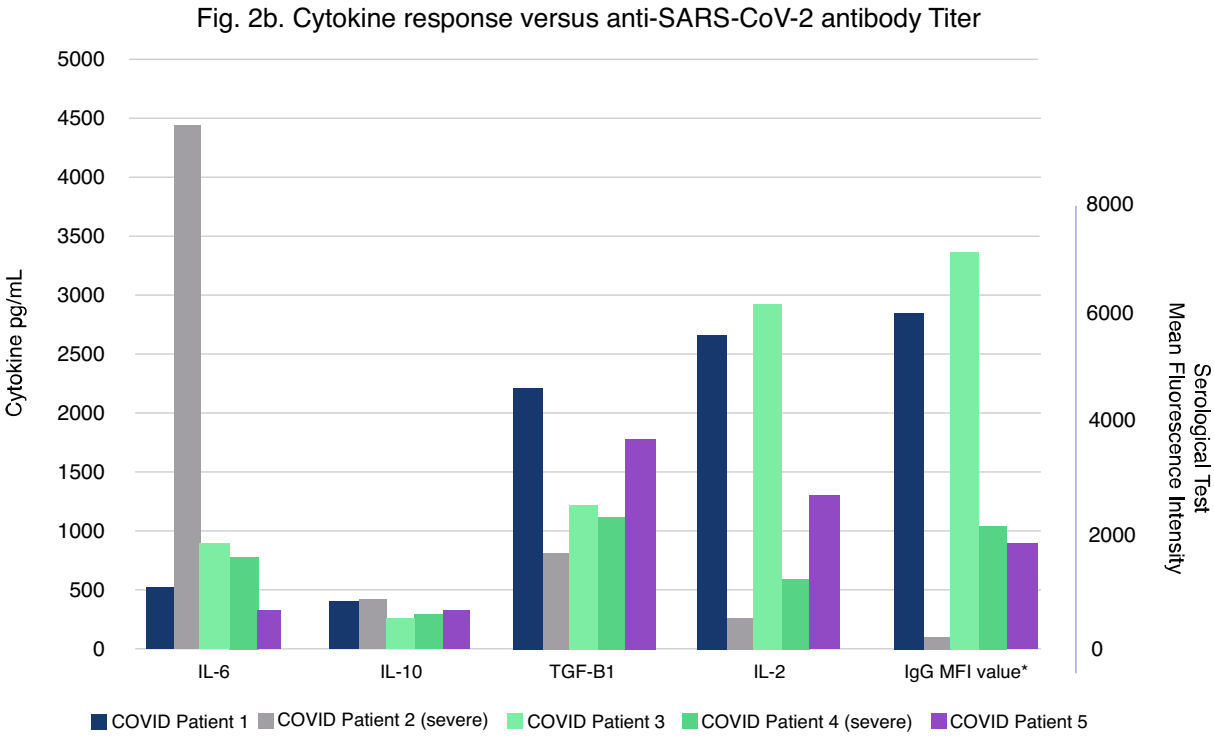


Fig. 2 a. and b. comparison of COVID-19 patient sample cytokine profiles and comparative anti-SARS-CoV-2 nucleocapsid IgG levels.

Conclusions:

These findings demonstrate the value of the CodePlex system for cytokine profiling of patients with COVID-19. The Isoplexis platform provides unprecedented multiplexed analysis of immune cell signaling, from low volumes of clinical samples. Coupling this analysis with serological testing enables both the cell mediated and humoral immune responses to be interrogated.

This small study suggests that there is an initial presentation of the following regulatory cytokines as measured using the CodePlex Human Cytokine Storm system: IP-10, MCP-1, TGF-β, and IL-17a in COVID-19 patients as compared to either normal healthy donors or pre-COVID samples and that the acute respiratory inflammation that ensues may be associated with manifestation and progression of disease.

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