Inflammation and Endothelial Injury Profiling in COVID-19 Pediatric Multi-system Inflammatory Syndrome

Douglas Fraser (douglas.fraser@lhsc.on.ca)
Western University https://orcid.org/0000-0002-5635-3791
Eric Patterson
Western University
Mark Daley
Western University
Gedaminas Cepinskas
Western University

Case Report

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Abstract

Background

COVID-19 is associated with a novel multi-system inflammatory syndrome that shares some characteristics with Kawasaki's Disease. The syndrome manifestation is delayed relative to COVID-19 onset, with a spectrum of clinical severity. Clinical signs may include persistent fever, gastrointestinal symptoms, cardiac inflammation and/or shock.

Case Presentation:

We measured 59 inflammatory and endothelial injury plasma analytes in an adolescent girl that presented with malaise, fever, cough, strawberry tongue and jaundice. Her COVID-19 status was positive with detection of 2 SARS-CoV-2 viral genes using polymerase chain reaction. She was treated with intravenous immunoglobulin prior to blood draw, but our plasma measurements suggested a unique analyte expression pattern associated with inflammation, endothelial injury and microvascular glycocalyx degradation.

Conclusions

COVID-19 is associated with a multi-system inflammatory syndrome and a unique inflammatory and endothelial injury signature. Analyte markers of inflammation and endothelium injury might serve as putative biomarkers and/or be investigated further as potential therapeutic targets.

Backgound

COVID-19 has been associated with a novel multi-syndrome inflammatory syndrome (1, 2). Presentation is typically delayed after SARS-CoV-2 exposure, with clinical symptoms that share features of Kawasaki's Disease (KD) and are attributable in part to an acute vasculopathy. A 'cytokine storm' has been suggested to underlie the syndrome, with tissue injury secondary to the host innate response (3). The inflammatory and endothelial injury mediators have not yet been described, but knowledge of these analytes is critically important for earlier syndrome recognition and for potential interventions.

Case Presentation

A 15-year-old female presented to hospital to a tertiary care emergency department with a history of malaise, dry cough, strawberry tongue, rash and jaundice. COVID19 was confirmed by detection of two SARS-CoV-2 viral genes using polymerase chain reaction. Her complete blood count, electrolytes, coagulation profile and blood gas were normal. C-reactive protein and ferritin were mildly elevated at 25.7 mg/L and 302 µg/L, respectively. She had a mild hepatitis with alanine aminotransferase 142 U/L,

aspartate aminotransferase 87 U/L, alkaline phosphate 405 U/L, total bilirubin 92.6 μmol/L. She was admitted to hospital with a presumptive diagnosis of atypical KD and treated with intravenous immunoglobulin (IVIg) and Aspirin. Her inpatient electrocardiogram and echocardiogram were normal.

Blood was drawn for inflammation/endothelial injury profiling after the patient's COVID-19 status was confirmed, but IVIg had already been administered approximately 48 hours earlier. Thus, analyte measurements must be evaluated in the context of this immune modulator (see below). Nonetheless, we measured 59 inflammation- and endothelium-related analytes using multiplexed biomarker immunoassay kits or enzyme-linked immunosorbent assay (ELISA). As only one COVID-19 pediatric patient was admitted to our hospital, we compared the measured analyte values from this COVID-19 case patient to analyte reference ranges that we obtained from a cohort of 20 pediatric healthy control subjects [median 15 years of age (IQR 8)].

The analyte data from the COVID-19 patient and the 20 healthy control subjects were first visualized with a nonlinear dimensionality reduction on the full data matrix using the t-distributed stochastic nearest neighbour (t-SNE) embedding algorithm (Fig. 1) (4). t-SNE assumes that the 'optimal' representation of the data lies on a manifold with complex geometry, but low dimension, embedded in the full dimensional space of the raw data. Based on analyte measurements, the COVID-19 case patient is a clear outlier with respect to her inflammation and endothelial injury profile.

We then generated confidence intervals (CIs) for the expected value of each analyte using the plasma measurements from the 20 healthy pediatric controls. The plasma values for each analyte were not normally distributed, so we computed 99.9% (95%, Bonferonni corrected for comparison across 59 plasma analytes) CIs via the bias corrected and accelerated bootstrap. Plasma analyte values in the COVID-19 case patient that were outside the CIs for healthy control subjects were therefore considered significant ($p < 0.05$, corrected; Table 1). We found significant elevations in 21 inflammation and endothelial analyte markers, while 1 endothelial glycocalyx degradation marker (heparan sulfate) was significantly depressed (Table 1).

Table 1
Statistically significant analyte concentration changes for the COVID-19 case patient and 20 healthy control subjects (EC, endothelial cell). The data is in rank order of magnitude of change.

Plasma Analyte	Units	20 Healthy Controls (CI: 5%, 95%)	Case Patient
1. MMP7	pg/ml	3356, 4424	51788
2. IP-10	pg/ml	86, 242	1098
3. Resistin	pg/ml	7.3, 11.1	41.8
4. IL-3	pg/ml	0.1, 2.4	7.3
5. Hyaluronic acid (EC)	ng/ml	17.6, 40.4	119.2
6. Thrombospondin-1	pg/ml	620, 1275	3286
7. Elastase 2	pg/ml	2.1, 4.4	11.3
8. PDGF-AB/BB	pg/ml	769, 2537	6390
9. MIG	pg/ml	1205, 2684	6531
10. MCP-1	pg/ml	190.3, 269.5	529.4
11. MMP1	pg/ml	384, 709	1286
12. Lactoferrin	pg/ml	338.3, 521.3	845.8
13. IL-1RA	pg/ml	8.1, 73.3	112.0
14. IL-18	pg/ml	30.3, 64.6	98.3
15. IFNα2	pg/ml	13.2, 120.7	179.6
16. P-selectin (EC)	ng/ml	16.3, 22.4	30.4
17. MIP-1β	pg/ml	22.8, 72.0	89.16
18. Eotaxin	pg/ml	49.4, 81.6	97.4
19. MMP8	pg/ml	288.4, 643.6	762.7
20. PDGF-AA	pg/ml	84.4, 652.9	766.8
21. MMP10	pg/ml	385.2, 751.4	876.1
22. Heparan sulfate (EC)	ng/ml	22.7, 294.5	20.6

After 3 days of observation, and partial resolution of her symptoms, our COVID-19 case patient was discharged home on Aspirin (3 mg/kg/day) with a 2-week follow up echocardiogram.

Discussion And Conclusions

We present an COVID-19 positive adolescent female with a unique analyte expression pattern associated with inflammation, endothelial injury and microvascular glycocalyx degradation. Given the timing of the blood draw, some plasma analytes may have been altered by IVIg administration or down-regulated to the levels of healthy control subjects. Nonetheless, we briefly summarize the purported actions of the top 5 analytes that were elevated 3-fold or greater in the COVID-19 case patients relative to the upper confidence limit for healthy controls.

Matrix metalloproteinase 7 (MMP7) was the most elevated analyte in the COVID-19 case patient relative to healthy control subjects. Also called matrilysin, MMP7 is expressed in endothelial cells, monocytes and macrophages and it is capable of degrading multiple extracellular membrane components (proteoglycans, laminin, fibronectin, casein and basement membrane collagen type IV). MMP7 is significantly upregulated in KD and it is implicated in acute vasculopathy (5). Specifically, MMP7 degrades endothelial junctions, which can promote vascular leak/edema and/or leukocyte migration into tissues (6).

Interferon-γ-inducible protein 10 (IP-10), an inflammatory cytokine secreted primarily by monocytes and endothelial cells in response to interferon-γ (IFNγ), was also significantly elevated in the COVID-19 case patient. IP-10 has multiple roles including lymphocyte chemoattraction and adhesion to endothelial cells. IP-10 is a promising target for the treatment of infectious diseases as it aids cellular targeting to threatened tissues where it modulates innate and adaptive immune responses. High serum IP-10 is found in KD, and it has been suggested as a KD biomarker (7).

Resistin is highly expressed in macrophages, bone marrow and the non-fat fraction of adipose tissue, and it stimulates several pro-inflammatory pathways and cytokines. Microvascular tone, as well as endothelial cell barrier function and nitric oxide production, are all altered by resistin. Similar to our COVID-19 case patient, elevated resistin is found in plasma from KD patients (8, 9).

Interleukin 3 (IL-3), released by activated T-cells, was elevated in our COVID-19 case patient. IL-3 promotes the production of inflammatory monocytes and neutrophils, thereby contributing to the cytokine storm that is implicated in sepsis from multiple etiologies. The microvascular endothelial cell response to inflammation and immunity is also regulated by IL-3 (10) and vasculopathy is suggested to be a primary feature of the novel multi-system inflammatory syndrome.

Hyaluronic acid is a major constituent of the microvascular glycocalyx, an extracellular matrix that coats the luminal surface of the endothelium (11). Hyaluronic acid degradation products are significantly elevated in plasma from the COVID-19 case patient, suggesting that the microvascular endothelial cell luminal surface has been pathologically altered. Disruption of the endothelial glycocalyx is associated with vascular lesions in KD (12), as well as decreased endothelial nitric oxide production and increased platelet/endothelium adhesion (11), Endothelial cell injury was supported in the COVID-19 case patient by the parallel elevation of soluble P-selectin, an endothelial glycoprotein that mediates adhesive intercellular interactions (13).

Our measurements showed minimal alterations in 37 inflammation and endothelial analyte markers: epidermal growth factor (EGF), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFNγ, interleukin 1a (IL-1a), IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17a, IL17e/IL25, IL-17f, IL-22, macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein 1α (MIP-1α), tumor necrosis factor α (TNFα), TNFβ, vascular endothelial growth factor A (VEGFA), regulated upon activation, normal T Cell expressed and presumably secreted (RANTES), MMP2, MMP3, MMP9, MMP12, MMP13, neutrophil gelatinase-associated lipocalin (NGAL), Granzyme B, heat shock protein 70 (HSP-70), chondroitin sulfate and syndecan-1. As some of these measurements may have been depressed by IVIg administration (14), no significant conclusions can be made with regards to their pre-treatment level. It is also plausible that some inflammatory analytes were transiently increased with inflammation onset, with TNF and IL-6 as typical examples (15). TNF-α is a pro-inflammatory cytokine released primarily by monocytes and macrophages that enhances the adaptive immune response. IL-6 is produced by monocytes and macrophages, and induces T-cell activation, B cell proliferation and stimulates the acute phase reaction, all of which lead to augmentation of the immune response.

In summary, pediatric COVID-19 patients can present with a novel multi-system inflammatory syndrome with some features similar to KD. The analyte measurements presented in this study, albeit post IVIg treatment, support a systemic inflammatory process that resulted in significant endothelial injury. These data should aid future hypothesis-generating research, as some of the identified analytes might be putative disease biomarkers and/or potential therapeutic targets.

Abbreviations

KD, Kawasaki's disease; IVIg, intravenous immunoglobulin, t-SNE, t-distributed stochastic nearest neighbour; CI, confidence interval; MMP, matrix metalloproteinase; IP, interferon-γ-inducible protein; IL, interleukin; PDGF, platelet-derived growth factor; MIG, monokine induced by interferon-gamma; MCP, monocyte chemoattractant protein; IL-1RA, interleukin-1 receptor antagonist; IFN, interferon; MIP, macrophage inflammatory protein; EGF, epidermal growth factor, G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; TNF, tumor necrosis factor; VEGFA, vascular endothelial growth factor A; RANTES, regulated upon activation, normal T Cell expressed and presumably secreted; NGAL, neutrophil gelatinase-associated lipocalin; HSP, heat shock protein

Declarations

- **Ethics approval and consent to participate:** This research has been approved by the Human Ethics Board, Western University.
- **Consent for publication:** Consent was obtained from the patient and their legal guardian.

- **Availability of data and materials:** The measurements collected and analyzed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The author declare that they have no competing interests.
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- **Authors' contributions: Author Contributions:** DDF conceived the study, obtained ethic approval and consent, collected the sample, measured the analytes and wrote the manuscript; EKP measure analytes and revised the manuscript; MD analyzed the analyte measurements and revised the manuscript; and GC measured the analytes and revised the manuscript.
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Figures

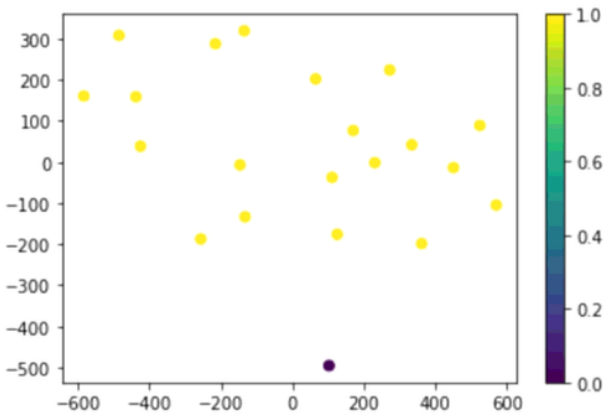

Figure 1

The COVID-19 case patient and 20 healthy control subjects plotted in two dimensions following dimensionality reduction by stochastic neighbor embedding. The Purple dot represents the COVID-19 patient, while the yellow dots represent the healthy controls. The dimensionality reduction shows that

based on 59 plasma analyte concentrations, the COVID-19 patients is distinct and easily separable. The axes are dimension-less.