BRIEF REPORT

‘The long tail of Covid-19’ - The detection of a prolonged inflammatory response after a SARS-CoV-2 infection in asymptomatic and mildly affected patients [version 1; peer review: 1 approved]

Ivan Doykov1*, Jenny Hällqvist1,2*, Kimberly C. Gilmour3, Louis Grandjean3, Kevin Mills1, Wendy E. Heywood1

1Translational Mass Spectrometry Research Group, University College London Institute of Child Health, London, UK
2Clinical and Movement Neurosciences, University College London Queen Square Institute of Neurology, London, UK
3Great Ormond Street Children’s Hospital NHS Foundation Trust, Great Ormond Street, London, WC1N 3JH, UK

* Equal contributors

First published: 19 Nov 2020, 9:1349
https://doi.org/10.12688/f1000research.27287.1
Latest published: 19 Nov 2020, 9:1349
https://doi.org/10.12688/f1000research.27287.1

Abstract

‘Long Covid’, or medical complications associated with post SARS-CoV-2 infection, is a significant post-viral complication that is being more and more commonly reported in patients. Therefore, there is an increasing need to understand the disease mechanisms, identify drug targets and inflammatory processes associated with a SARS-CoV-2 infection. To address this need, we created a targeted mass spectrometry based multiplexed panel of 96 immune response associated proteins. We applied the multiplex assay to a cohort of serum samples from asymptomatic and moderately affected patients. All patients had tested positive for a SARS-CoV-2 infection by PCR and were determined to be subsequently positive for antibodies. Even 40-60 days post-viral infection, we observed a significant remaining inflammatory response in all patients. Proteins that were still affected were associated with the anti-inflammatory response and mitochondrial stress. This indicates that biochemical and inflammatory pathways within the body can remain perturbed long after SARS-CoV-2 infections have subsided even in asymptomatic and moderately affected patients.

Keywords
Sars-CoV-2, mass spectrometry, inflammation, biomarker, proteomics

Open Peer Review

Reviewer Status ✔
 Invited Reviewers
1
version 1
19 Nov 2020

1. William J. griffiths* ID, Swansea University
Medical School, Swansea, UK
Swansea University, Swansea, UK

Any reports and responses or comments on the article can be found at the end of the article.
Introduction
As more and more people are recovering from SARS-CoV-2 infection, one of the growing concerns is the increasing reports of the post viral fatigue symptoms or 'long Covid'. This phenomenon is defined as not recovering for several weeks or months following the start of symptoms and whereby patients present with chronic and recurrent fatigue for weeks and even many months after a SARS-CoV-2 infection\(^1\),\(^2\). Understanding the effects and complications of 'long Covid', and then managing it, is the next challenge for public health services. Currently the UK is increasing its testing capacity for virus detection and antibody detection, but there still remains a gap in the understanding and diagnosis of long Covid.

Work has been performed to characterise the inflammatory response to SARS-CoV-2 infection in relation to disease severity. There has been controversy as to whether severity is associated with a hyperinflammatory cytokine storm or failure of host protective immunity that results in unrestrained viral dissemination and organ injury. What has made addressing this question challenging has been the lack of diagnostic tools to evaluate immune function in Covid-19 infections. There are sets of simple but expensive immunoassay panels that are available to look at known key inflammatory proteins such as cytokine panels; however, these only give information on known pathways and limit discovery of novel or less defined inflammatory responses. Targeted proteomics using mass spectrometry to quantitate multiple diagnostic proteins can help address this. Novel assays for virus detection have been already developed using targeted mass spectrometry\(^3\),\(^4\) but no assays are available to look at the symptoms for the diagnosis or understanding of ‘long Covid’.

From a previous study (unpublished reports) we developed a custom targeted mass spectrometry based assay panel that looks at up to 96 pro- and anti-inflammatory associated proteins (Figure 1a; see Table 1 on protocols.io\(^5\)). Our hypothesis was long Covid symptoms could be related to a lingering ‘tail’ and an abnormal inflammatory response to an infection, by a type of virus the body has not seen before. We applied this assay to a cohort of samples taken from healthcare workers who had tested positive for SARS-CoV-2 infection by PCR and were either asymptomatic or had only a mild infection. Samples were taken at least 40–45 days post infection and demonstrated a positive antibody test. We compared these with serum from healthcare workers with a negative antibody test, no reported infection and no positive PCR test.

Methods

Ethical statement
Samples were identified from the Health Research Authority approved project Co-Stars (Great Ormond Street Hospital NHS Trust COSTARS, IRAS 282713, ClinicalTrials.gov Identifier: NCT04380896, registered May 8\(^{th}\) 2020) and all participants provided informed written consent.

Samples
A pilot group of 10 positive and 10 negative samples covering a broad age range was selected as proof of principle for this assay. The negative group was 60% female with an age-range of 21–57, median 38 years. The positive group was 69% female, with an age range 31–66 and median age of 44 years. Of the positive patients, seven were asymptomatic and six had loss of taste/smell or had abnormal taste/smell. None were admitted to hospital or reported other symptoms.

Multiplex assay
The detailed method for the multiplex assay is published and available at protocols.io\(^5\). Briefly, serum sample proteins were precipitated and trypsin digested to peptides. Peptides were desalted, separated by reverse phase chromatography and analysed on a Waters Aquity UPLC system coupled to a Xevo TQ-S mass spectrometer.

Analysis
Raw data was acquired using MassLynx v 4.1 in multiple reaction monitoring mode. Raw files were processed using Skyline v 19. Protein-Peptide sequences were obtained from www.uniprot.org and settings optimised using custom synthesised peptides (Genscript USA). Normalised peak intensity data

Figure 1. Multivariate analysis of post infection serum inflammation profile. a) Representative overlaid chromatogram of the multiplex inflammation panel. Protein identifiers indicated by name and followed by first three amino acids of the peptide. b) Principle component analysis score plot of 10 SARS-Cov-2 infected patients >40 days post infection and 10 negative controls.
were exported to Microsoft Excel and data analysed using SIMCA v 15 (Umetrics, Sweden) for multivariate analysis and Graphpad prism v 6 was used for statistical analysis.

Results

Multivariate analysis of all inflammatory proteins measured in the control and SARS-CoV-2 positive patients are shown in Figure 1b and 1c. The score plot shows a clear separation of the positive and negative samples indicating the serum immune profile from people infected with SARS-CoV-2 is still significantly affected even 40 days post-infection. Figure 2 shows the univariate analysis of six proteins from our panel that were significantly altered. The majority of these proteins are either anti-inflammatory or associated with the stress response. Two proteins originate from the mitochondria, peroxiredoxin 3 (PRDX3) and carbamoyl phosphate synthase (CPS1). PRDX3 is a known antioxidant. Its increase in serum of patients infected with SARS-CoV-2 is likely indicative of continued mitochondrial stress response. CPS1 is a major mitochondrial urea cycle enzyme in hepatocytes. Serum CPS1 originates from the bile duct and is usually rapidly cleared by peripheral blood mononuclear cells. It is possible that basal levels of CPS1 in serum are reduced in patients infected by SARS-CoV-2 due to increased circulation and activity of peripheral blood mononuclear cells.

N-Myc downstream regulated gene 1 (NDRG1) is a cytosolic protein with many biological functions. Its role in the immune response is undefined but deficiency of NDRG1 affects the differentiation process of macrophages and maturation of mast cells. Collagen triple helix repeat containing 1 (CTHRC1) is anti-inflammatory and promotes wound healing by recruiting M2 macrophages and regulating the TGF-β and Notch pathways. This increase of CTHRC1 indicates tissue damage has occurred even in moderately affected patients.

Cystatin C is a protease inhibitor and extracellular levels are used as a biomarker for disease prognosis in cancer, cardiovascular disease, and inflammatory lung disorders. In mice serum cystatin C is controlled by the anti-inflammatory cytokine IL10 of which increasing levels suppress cystatin C expression. A longitudinal study looking at immune mediators show IL10 levels are significantly elevated in only severe cases of SARS-CoV-2 infection at four weeks post infection and are not affected at four weeks in mild cases. This would corroborate with what we observe for cystatin C as the mild patients have increased cystatin C that is not being suppressed by higher IL10 levels. We also observe a slight reduction in serum progranulin. Progranulin plays a fundamental role in the immune response which is better defined within its role in neurodegenerative disorders but the relevance of serum progranulin is not fully understood. It appears to have a pro-inflammatory role in adipocytes in diabetes and an anti-inflammatory protective role in the vascular endothelium against inflammatory reactions.

Conclusions

Remarkably, even in patients who have suffered from an asymptomatic or mild SARS-CoV-2 infection, after 40 days post-infection they still exhibit a significantly raised group of biomarkers involved in inflammation and the stress response.
This initial data using a custom designed inflammatory marker panel applied to mildly affected patients identifies potential drug targets, provides insight into the post infection inflammatory response. This approach using targeted proteomic technology has potential for application on further well-defined sample cohorts to understand what is abnormal about post infection inflammatory response in ‘long Covid’ patients.

Data availability

Underlying data

ProteomeXchange: Underlying mass spectrometry data on ProteomeXchange. Accession number PXD022159.

Underlying mass spectrometry data is also available on PanoramaWeb at https://panoramaweb.org/x1eZmn-url.

Acknowledgments

We wish to thank Annabelle Lea Mai Immunology department Great Ormond Street Hospital for help with samples and the Peto Foundation for their continuing support.

This work is partly funded by the NIHR GOSH BRC. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

References

   PubMed Abstract | Publisher Full Text

   PubMed Abstract | Publisher Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   Publisher Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text

   PubMed Abstract | Publisher Full Text

   PubMed Abstract | Publisher Full Text | Free Full Text
Open Peer Review

Current Peer Review Status: ✔

Version 1

Reviewer Report 07 December 2020

https://doi.org/10.5256/f1000research.30151.r75166

© 2020 griffiths W. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✔ William J. griffiths

1 Institute of Life Science, Swansea University Medical School, Swansea, UK
2 Swansea University, Swansea, UK

This is an interesting study looking at a panel of inflammation-related protein signatures in a small cohort of patients 40+ days post infection. The authors see some changes in inflammatory proteins even this far after infection.

The methods and underlying data are available at open access repositories.

I just have a few minor suggestions:

1. Analysis section - It would be good to read a few lines on how the data was normalised (i.e. normalisation of protein concentration).
2. Results - I think the authors mean Figure 1a and 1b?

In summary, a nice brief study with some interesting results.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes
Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mass spectrometry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

The benefits of publishing with F1000Research:

• Your article is published within days, with no editorial bias
• You can publish traditional articles, null/negative results, case reports, data notes and more
• The peer review process is transparent and collaborative
• Your article is indexed in PubMed after passing peer review
• Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com