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RAPID RESEARCH IN COVID-19 PROGRAMME

Host Biomarkers of Acute Respiratory Distress Syndrome in COVID-19 infection

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AIMS

Most patients with COVID-19 infection do not require hospitalization, and most that require hospitalization will recover. It is currently not possible to accurately predict which patients will deteriorate and develop acute respiratory distress syndrome (ARDS), the most feared complication of COVID-19. The immune response to the virus, rather than the virus itself is responsible for most of the serious consequences of the disease. This study therefore set out to investigate, in detail, the immune system in patients with COVID-19 compared to controls, in order to achieve two objectives. First, to identify biomarkers that could be used to identify patients at risk, and secondly to identify pathways involved in severe disease that could be used to develop new treatments.

KEY FINDINGS

- High resolution mass spectrometry, a technique which accurately identifies and quantifies proteins within a cell, can be used to identify laboratory tests that can aid clinical diagnosis of COVID-19 and assessment of disease severity.
- COVID-19 causes profound changes in the immune system which can be measured through changes in the immune proteins in blood cells.
- A specific family of proteins that are controlled by the bodies antiviral defence system called “interferon” were shown to be increased in COVID19 and could identify which patients had COVID-19 and which did not.
- This study also found proteins that could discriminate between patients with severe and mild COVID-19 infections. Crucially, we were also able to identify patients with apparently “mild” COVID-19 infection who subsequently deteriorated.
- This study has identified both potential biomarkers of prognosis, and potential targets for future development of treatments.

WHAT DID THE STUDY INVOLVE?

We conducted a large research programme called PREDICT-COVID that involved studying patients admitted to Ninewells Hospital in Dundee with confirmed or suspected COVID-19 from May 2020 onwards. All eligible patients were approached to ask their permission to take blood and other samples as well as collecting detailed clinical data. All patients in the study have specialised immune cells called neutrophils and peripheral blood mononuclear cells (PBMCs) isolated from their blood, as well as serum/plasma and other samples such as sputum and throat swabs. These samples were taken on the first day of hospitalization and then repeat samples taken during their hospital course and at day 29 once patients had recovered in a subset.

A pipeline for sample processing for high-resolution quantitative mass spectrometry, a technique that allows all of the proteins in a sample to be identified, was established to profile the proteins of neutrophils and PBMCs isolated from individuals recruited to the study. This mass spectrometry approach has allowed reproducibly identification and quantification on average of 4900 proteins from each donor neutrophil sample and 6000 proteins from each donor PBMC sample. These data provide an in-depth overview of the protein landscape in immune cells of individuals with COVID-19 and control populations.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

We enrolled 144 individuals with COVID-19 infection, 91 patients with respiratory disease who tested negative for COVID-19 (disease controls) and 39 healthy controls without evidence of infection. High levels of proteins involved in the bodies natural antiviral response (interferon induced proteins) was a signature of COVID-19 in immune cells. The interferon signature could tell the difference between patients with COVID-19 and those with suspected COVID-19 who ultimately tested negative. The levels of expression of these interferon regulated proteins did not correlate with disease severity. Longitudinal analysis revealed that the levels of these interferon regulated proteins were highest at the early stages of infection and dropped in abundance over time returning to baseline as the infection cleared over 14-29 days. These data suggest that measuring interferon proteins could be useful to detect the disease in its early stages, since these markers are raised in patients regardless of disease severity, but that interferon related proteins would not be useful to identify which patients were at highest risk of complications.

There were, however, clear markers of disease severity when we examined all the proteins within PBMC and neutrophils. The array of proteins found within a cell is termed the proteome. The PBMC proteome signature indicated changes in the proportion of different immune cells in the blood. For example, T cells were lost while myeloid cells involved in inflammation during infection were increased in individuals with severe COVID compared with mild COVID. The PBMC signature also indicated changes in B cell populations in the blood of patients with COVID notably evidence for an increased number of a particular type of antibody producing B cell termed IgA plasmablasts. The B cell proteome signature distinguished the PBMC from COVID19 positive patients versus controls but this was not a predictor of disease severity. One marker of severe COVID-19 disease from the PBMC analysis was the loss of two proteins which help cells to kill, PRG2 and Galectin 10.

The neutrophil signature also identified markers of COVID disease severity. Out of >4990 proteins quantified there were approximately 411 proteins that were found in different ammounts in neutrophils isolated from severly ill COVID-19 patients (those requiring high levels of oxygen) compared to controls or hospitalized COVID-19 patients that did not require supplemental oxygen. For example, neutrophils from patients with severe COVID-19 disease expressed low levels of a subset of proteins termed granule effector molecules which are important in the killing functions of neutrophils. They expressed high levels of certain proteins found on the surface of the cell which allow it to sense and respond to its sorroundings including TLR2, IL-18 receptors, IL1R2, and VISTA (V-domain Ig-containing Suppressor of T-cell Activation) which controls the switching on of the neutrophil defence response. Importantly, higher levels of neutrophil TLR2 and VISTA were also seen in the neutrophil proteomes from patients who had low COVID disease severity on hospital but then whose condition subsequently deteriorated.
These results mean that it is possible to identify patients with COVID-19 who appear clinically "mild" but will deteriorate, and suggests potential pathways for treatment that are not currently being targeted by drugs in development.

WHAT IMPACT COULD THE FINDINGS HAVE?

The study has generated a world leading resource of protein data and clinical samples which can be exploited by researchers to learn about the immune response to COVID-19 with relevance both to the current and future pandemics.
The study shows that by accurately measuring the amount of thousands of proteins within a cell we can identify biomarkers to aid clinical diagnosis. The expression of specific proteins including VISTA and TLR2 on neutrophils, are thus candidate biomarkers to predict COVID disease progression in hospitalised COVID-19 patients. Moreover, in the context of VISTA there is already intense interest in this protein as a druggable target for other diseases. Further work should explore the feasibility of targeting VISTA for the treatment of severe COVID. The interferon induced protein signature identified in COVID-19 patients was striking because some of these proteins were expressed at a very high level in PBMC and neutrophils making them good candidates as biomarkers for the development of lateral flow tests that could rapidly assess the viral infections in patients with respiratory disease. This could be valuable for future pandemic preparedness.

HOW WILL THE OUTCOMES BE DISSEMINATED?

A manuscript reporting these findings is being prepared for publication in a peer reviewed journal. Data will be presented at international conferences and released to the general public in the form of press releases. The researchers are active in both traditional and social media and will use these platforms to ensure wide dissemination and to maximise impact of the findings.

CONCLUSION

This study has identified novel biomarkers of COVID-19 disease and disease severity while generating a valuable data and biosample resource for ongoing research into the disease.

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ADDITIONAL INFORMATION

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