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RAPID RESEARCH IN COVID-19 PROGRAMME

Viral and Immunological Correlates of Clinical Severity and Response to Anti-Viral Therapy for COVID-19 *("How viruses and the immune system relate to the severity of COVID-19 and how the body responds to antiviral treatments*")

AIMS

- To explore the longer-term consequences of surviving COVID-19 illness for patients and the healthcare service.
- To examine the long-term impact of acute illness severity to fully understand the entire course of the illness.
- To explore immune changes related to COVID-19 infection and any therapies received over 3 months from diagnosis.

KEY FINDINGS

- Severe COVID-19 was found to be strongly linked to cardiovascular complications. Notably, hypertension (high blood pressure) was more common in patients who required oxygen during the disease course.
- A strong association was observed between obesity, high blood sugar, and severe COVID-19. Patients requiring oxygen had significantly higher baseline glucose levels compared to those who did not need oxygen.
- Patients with severe COVID-19 who received supplemental oxygen had an abnormal number of white blood cells in their bloodstream compared to those with mild disease. For instance, they had lower levels of circulating lymphocytes (i.e., B cells) but higher levels of monocytes. (Lymphocytes and monocytes are both types of white blood cells that help the body fight infection, but with different functions: B cells produce antibodies to fight viruses and bacteria whilst monocytes remove pathogens and cell debris, to defend against infection.
- The immune cells in the blood (measured by a specific marker called STAT5) were less active in patients who needed oxygen. STAT5 is a protein in cells that plays an important role in how the immune system responds to infection. When the body detects a threat like a

virus, STAT5 gets "activated" and helps turn on certain immune functions to fight off the infection. Reduced activation of STAT5 in severe cases offers new insights into the complex immune mechanisms against COVID-19. Understanding STAT5's involvement in COVID-19 severity may help to develop new therapeutic strategies.

WHAT DID THE STUDY INVOLVE?

How a patient responds to being infected with the COVID-19 depends on the interaction of their immune system with the virus and the development of effective immunity to COVID-19. A protective immune response in early or late disease that results in immunological memory could help to reduce the progression of the disease in severely affected patients and reduce hospital stay. However, it is essential that we understand the physiological context the immune system is functioning in, to determine how this influences the long-term immune response in people with the range of COVID-severities; from minimal/no symptoms, to developing life threatening and often fatal COVID pneumonia.

Risk factors associated with disease severity were quickly established at the start of the first wave of the pandemic. Tools were developed to aid clinicians in their decision making based on likely prognosis, and it was proposed that these along with differing immune responses in individuals were responsible for the range of outcomes seen with COVID-19. However, very little was understood about an individual's immune responses to the virus and how this related to these risk factors and eventual disease outcomes. It was thought that those with severe disease mounted an exaggerated immune response resulting in a 'cytokine storm' and it was suggested that this cytokine storm, rather than the direct effects of the virus itself, resulted in most damage to lung tissue.

The study called COLLECT (COVID-19: Exploring Long Term Outcomes in Patients with Disease) was aimed at increasing our understanding of the immune response to COVID-19 as the disease progressed over time and how this relates to eventual outcome. An improved understanding of how an individual's immune response affects disease severity and eventual outcome could aid in management decisions for the individual and lead to improved therapeutic strategies.

In the COLLECT study, patients presenting with COVID-19 symptoms during A&E and in-patient visits at the Queen Elizabeth University Hospital, Glasgow, were invited to take part in this study. 141 patients were screened, 81 were ineligible, and 60 patients were recruited between January to July 2021. After giving informed consent, all patients had blood and nasal swab samples collected, clinical data recorded, and the patient completed a validated health and well-being questionnaire.

Depending on disease severity/progression, individuals were placed into two defined groups based

on appropriate follow up visits.

Group 1: Discharged within 48 hours (mild disease) – follow up visits on day seven and week 12 for blood sample and clinical data collection, and a validated questionnaire at the 12-week visit.

Group 2: Discharged after 48 hours (moderate to severe disease) – follow up visits on day 4, 7, 14 for blood and nasal swab samples and clinical data collection. A 12 week follow up visit for blood sample and clinical data collection and validated questionnaire.

The analysis included looking at immune cells from blood samples of patients who either had oxygen administered on admission and those who did not. Immune cells within blood samples were labelled with fluorescent dyes and data acquired using a specific machine that is able to detect single cells and measure the level of fluorescent dyes. In brief, this technique enabled us to determine which immune cell populations were present and in what quantities, and the level of cellular activation. This allowed us to determine what differences, if any, existed between those with mild disease and those who went on to develop severe COVID pneumonia.

By matching up information about how the disease progresses (mild, moderate severe progression and outcomes) and how the patient's immune system responds over time in response to any treatments, we can build up a signature of different biomarkers, which could allow clinicians to distinguish patients into groups which predict disease.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Within the COLLECT study we evaluated both clinical and immunological outcomes and discovered the following.

Hypertension:

Severe COVID-19 was found to be strongly linked to cardiovascular disease, including ischaemic heart disease, left ventricular failure as well as hypertension (high blood pressure, which is linked to pre-existing cardiovascular disease). Hypertension was the most common cardiovascular co-morbidity, and notably it was present in all of patients who required supplemental oxygen.

Obesity and Higher Baseline Glucose Levels:

Although there was no significant difference in the numbers of those with a formal diabetic diagnosis between the groups, there was a strong association between obesity, high blood sugar levels, and severe COVID-19. Patients who required supplemental oxygen had significantly higher baseline glucose levels compared to those who did not. The associations shown here between severe COVID, obesity and hypertension imply a metabolic syndrome component to the differing outcomes seen in COVID infection. Metabolic syndrome is a collection of conditions that often occur together and increase a person's risk of diabetes, stroke, and heart disease. The main components include obesity, high blood pressure, high blood triglycerides, low levels of high-density lipoprotein (HDL) cholesterol, and insulin resistance.

Immune Cell Populations:

Significant differences were observed in the immune cell populations of patients with severe versus mild COVID-19. Those who needed supplemental oxygen had lower levels of lymphocytes (e.g. B cells) and higher levels of monocytes. The lymphocyte/monocyte ratio might be a useful biomarker to predict disease severity. (Lymphocytes and monocytes are both types of white blood cells that help the body fight infection, but they have different functions as explained above.)

Immune Signalling:

Patients who required oxygen had significantly lower activation of STAT5, a protein essential for immune cell signalling. STAT5 plays an important role in the maintenance of normal immune function. Following STAT5 signalling, certain types of cells and mediators of the immune system are activated which in turn keeps the immune system in balance. Reduced activation of STAT5 in severe cases offers new insights into the complex immune mechanisms against COVID-19. Understanding STAT5's involvement in COVID-19 severity may help to develop new therapeutic strategies.

Reduced STAT5 Activation in All Types of Lymphocytes:

The reduced activation of STAT5 observed in patients requiring oxygen was not limited to a specific lymphocyte subtype but was found in all types of lymphocytes. This, combined with lower levels of lymphocytes, suggests a globally impaired immune response in severe disease.

WHAT IMPACT COULD THE FINDINGS HAVE?

- **Patients** - Further work into the changes in STAT5 and other cytokine markers seen in the COVID immune response, may result in their use as prognostic biomarkers to aid in the prediction of outcome for COVID. For STAT5, patients identified by this prognostic marker could be given oxygen earlier in the course of the disease and this could result in a better and quicker outcome for the patient.

- **Policy** – If future work shows that STAT5 and possibly other immune markers can be used as prognostic markers of disease, or identifies suitable drug targets, then healthcare policies for clinical practice may be modified to include these prognostic marker tests and use of any targeted drug therapy.
- **Practice** - in the future, using these prognostic biomarkers to stratify patients could provide targeted interventions quicker, resulting in a better outcome for the patient and a reduced burden on healthcare services.

HOW WILL THE OUTCOMES BE DISSEMINATED?

- The results of this study will be published in high quality peer reviewed medical journals (manuscript in preparation) and presented at national and international conferences.
- The results of this study will be used to support applications for future funding.
- The results may be used in future patient and public involvement opportunities to highlight the research into COVID, the potential for prognostic markers and potential drug targets for COVID, and hear peoples lived experience of having COVID and its treatment.

CONCLUSION

In summary, the current study is the first, to our knowledge, to describe a difference in individual STAT5 response to COVID. We report a novel finding of lower activation of STAT5 in those with severe COVID requiring oxygen than in those with mild disease. Additionally, we found that in this cohort (as has been widely reported in previous studies) severe COVID was significantly associated with obesity, hypertension, and lymphopenia (a condition where there are fewer than normal levels of lymphocytes, a type of white blood cell that helps protect the body from infection). As a result, this study provides new insights into the factors contributing to severe COVID-19 and improves our understanding of the immune mechanisms underlying COVID-19 severity, thus providing a foundation for future research and therapeutic developments.

The findings reported above are from preliminary work based on a small cohort of just 49 people from one centre, therefore future work is still required with a larger sample size to assess these findings further.

The results described here represent the immune response at one point in time – the day of hospital admission. This may be important as it has been suggested that the immune response to COVID likely changes throughout the course of the disease. Further work is therefore needed to assess if this finding of a decreased level of activation of STAT5 in those with severe disease is present and maintained throughout the course of the disease. In our cohort, our baseline samples were taken on average at almost day nine of disease and therefore it is impossible to know from our data when

this change in STAT5 activation happens or indeed if it is maintained throughout the disease course.

One factor which may also be important to look at in future work, is the change in the level and activity of other immune signalling molecules (other than STAT5) over time. Despite the previously mentioned cytokine storm, no one cytokine has been established as a driver of this aberrant reaction. One reason for this could be changes in the levels of cytokines over the course of the disease and that a blanket use of drugs targeted against specific immune components is therefore unlikely to produce beneficial effects in everyone at all points of their disease course.

Given there is still much we do not know about the immune response to COVID, there are many reasons to continue to improve our understanding. One such reason is the potential for better patient selection into clinical trials or even the discovery of new therapeutic targets. For example, this increased understanding of a potential role of STAT5 in severe COVID cases could make it or one of its downstream signalling molecules a possible focus for drug development.

Another potential consequence of an improved understanding of the immune response to COVID would be the discovery of a prognostic biomarker to aid further in the prediction of outcome from COVID.

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ADDITIONAL INFORMATION

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