DeCOmPRESS: Defining the Disease Course and Immune Profile of COVID-19 in the Immunosuppressed Patient

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Abstract

The ongoing coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Current advisory guidelines for high-risk groups - including people with autoimmune disease currently taking immunosuppressive therapies - encourage increased precautions. The aim of the DeCOmPRESS study is to define the disease course and immune profile of COVID-19 in immunosuppressed patients. We will clinically phenotype patients with ANCA-Associated Vasculitis (AAV) who develop COVID-19 using a customized REDCap data collection instrument embedded within the Rare Kidney Disease (RKD) Biobank that is interoperable with the rheum-COVID, Global Rheumatology Alliance and SPRINT-SARI datasets, facilitating international data linkage. Acute and convalescent blood samples will be analysed by flow cytometry and ELISA to define the immunophenotype and cytokine profile. Patients will track COVID-19 and AAV symptoms through a bespoke smartphone app. Serology assessments will determine the antibody response in this vulnerable population. DeCOmPRESS study findings will rapidly inform management of immunosuppressed patients who contract COVID-19 by defining the natural history and immunological manifestations of the disease in these patients. We will also determine whether pre-existing immunosuppressant therapy lessens the cytokine storm associated with COVID-19, thereby paradoxically improving rather than worsening clinical outcome. Here we also present preliminary 3-month study data relating to recruits from the first COVID-19 wave.

Keywords

COVID-19, coronavirus, immunosuppression, autoimmunity, vasculitis, ANCA, cytokines, immunophenotype

Introduction

Coronavirus disease 2019 (COVID-19) is a novel, infectious, multi-system disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease presents in a range of clinical severity, with disease characteristics ranging from no symptoms, a mild cough or severe respiratory failure and death. The COVID-19 pandemic is particularly concerning for people taking immunosuppressive medications for diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Anti-Neutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis (AAV). The course of SARS-CoV-2 infection in patients with systemic autoimmune diseases who take immunosuppressive medication is poorly defined. Official guidance on strategies to prevent severe COVID-19 in patients taking immunosuppression is speculative, modelling of disease natural history is ill-informed, and treatment approaches rely upon empirically reducing immunosuppression and providing “stress dose” glucocorticoids. If concurrent immunosuppressive therapy is viewed as an additional vulnerability factor, rather than as protective, then a ward-based ceiling of care might be erroneously applied.

We are addressing by analysing the impact of immunosuppression on the natural history of, and immune response to, SARS-CoV-2 infection. We will primarily use systemic vasculitis as a prototypical chronic autoimmune disease requiring long-term immunosuppression; however, findings will be broadly revealing about other chronic autoimmune diseases and will inform immunosuppressive medication management during this pandemic. The overarching goal of the DeCOmPRESS study is to determine whether immunosuppressant therapy for chronic autoimmune disease protects against the cytokine storm associated with COVID-19 and reduces the severity of the clinical syndrome. To achieve this goal, we will examine the following specific aims:

- Characterise immune cell subsets altered in COVID infection, comparing immunosuppressed to non-immunosuppressed persons;
- Profile serum cytokines in COVID infection, comparing immunosuppressed to non-immunosuppressed persons;
- Compare differences in clinical characteristics and outcomes between those who are versus those who are not immunosuppressed at the onset of COVID-19;

Study Design

The DeCOmPRESS study will use AAV as a chronic autoimmune disease model and take advantage of the existing Irish clinical research infrastructures built around this rare disease. This is a prospective multi-site cohort sub-study of the Rare Kidney Disease (RKD) Biobank, with collection of clinical data and biospecimens as defined in the primary RKD protocol (accessible at: https://www.tcd.ie/medicine/thkc/research/rare.php). This bioresource includes approximately 70% of all known Irish AAV cases, has already supported many published studies. All recruits have provided biological samples during periods of remission (pre-pandemic) and have consented to further sampling and data collection as envisaged for this project. All patients with systemic vasculitis enrolled in the RKD registry who develop probable or confirmed COVID-19 over the next 12-18 months will be included in the DeCOmPRESS study. We will also collaborate with patient cohorts from rheumatology and dermatology to expands the scope of our findings.

Our key outcome measure is severe COVID-19 infection, defined as death, ICU admission and/or need for high-flow (FiO2>30%) oxygen/non-invasive ventilation/invasive ventilation. Patients are grouped by immunosuppressant exposure, defined as current use of >5mg prednisolone, azathioprine, mycophenolate or methotrexate; or rituximab and/or cyclophosphamide use within the previous 6 months. The control group will comprise patients on minimal immunosuppression, defined as ≤5mg daily prednisolone and no other immunosuppressant exposure (including cyclophosphamide or rituximab within the last 6 months). Review of the REDCAP eCRF clinical records is used to determine type, use, dosage, and frequency of immunosuppressive medications at the time of COVID-19 onset. The start date for this project was June 1st, 2020 with an estimated timeframe of 24 months. Existing infrastructure supports the immediate implementation of this work.

Sample and Data Collection

Patients with vasculitis previously recruited to the RKD Biobank are identified, where possible, at the time of COVID-19 diagnosis. Patients will be treated at the local inpatient site and clinical data recorded in the RKD database. Blood samples will be obtained for immunophenotyping, serum cytokine measurements, and serologic analysis. Convalescent samples will be obtained at 3-9 months during follow-up clinic appointments. Clinical data will be recorded at each encounter in REDCAP. A simplified workflow for the DeCOmPRESS study is described in Figure 1. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/) and in our recently published Study Protocol on HRB Open[1]. These protocols are continuously being updated and optimised as new research interests emerge.

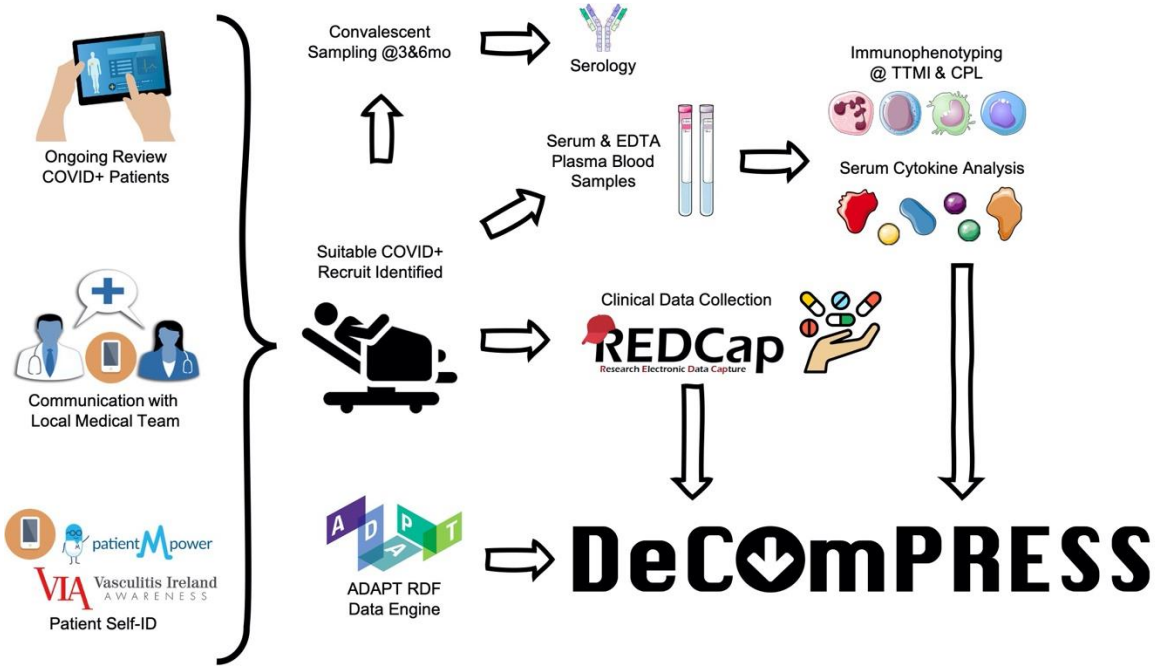

Figure 1: A simplified workflow of the DeCOmPRESS Study. Patients from designated cohorts are recruited at local hospital sites and clinical data recorded in REDCap. Serum and EDTA plasma samples are taken for immunophenotyping and serum cytokine analysis at the Central Pathology Laboratory in St. James's Hospital. Repeat sampling at 3 and 6 months for convalescent antibody testing. Data will be incorporated into the ADAPT RDF data engine for subsequent analysis.

Interim Results

Study Population

From the inception of this project to June 2020 (“Wave One”) 8 AAV patients/RKD recruits were infected with SARS-CoV-2. This included 2 new diagnoses of AAV who developed COVID-19 during their inpatient stay for investigation and management of their new diagnosis, and a further patient who developed COVID-19 while being investigated for a vasculitis flare. The mean age was 59.6 (SD 17.3, Table 1) and 6 (75%) were female. All but 1 patient was ANCA positive (5 anti-MPO+, 2 anti-PR3+) and MPA was the dominant disease phenotype (50% MPA, 25% EGPA, 25% GPA). Half of the patients had active AAV at the time of COVID-19 diagnosis. According to our classification five of the eight recruits were defined as “immunosuppressed/exposed”.

The majority of patients in our cohort (6/8) were required to be admitted to hospital during the course of their COVID-19 infection. The median length of hospital stay was 8.5 days (IQR 5-33). Three (38%) experienced our defined composite critical outcome, including one death. This finding may indicate selection bias via increased testing of hospital inpatients during the peak of the first wave of the COVID-19 outbreak in Ireland.

Preliminary results from immunophenotyping and serum cytokine profiling of DeCOmPRESS recruits at the acute and convalescent encounters are shown in Figures 2-8. There is greater interpersonal variation between readouts than between the acute vs. convalescent encounter or the immunosuppressed vs. non-immunosuppressed patients. Given the

In October 2020 convalescent DeCOmPRESS patient samples were tested for the presence anti-RBD IgG, IgM and IgA (Figure 9). A selection of samples from the RKD biobank were also collected during routine clinic visits in March-September 2020, and matched to historical/archived samples prior to September 2019. All COVID-era and samples and matched historic samples were negative on each serological assay. Of the six convalescent DeCOmPRESS samples analysed, five were positive for anti-RBD IgG and one for anti-RBD IgA. The one recruit who failed to mount an antibody response had been treated with rituximab (Figure 9).

Table 1. Baseline patient characteristics at time of COVID-19 (C19) diagnosis

				Immunosuppression			
				NO		YES	
		All	%	N	%	N	%
Total		8	100%	3	37.50%	5	62.50%
Gender	Female	6	75%	3	100%	3	60%
	Male	2	25%	0	0%	2	40%
Age	Mean, SD	59.6	17.3	51.6	15.5	73.0	11.5
Ethnicity	W2 - White Irish	8	100%	3	100%	5	100%
Smoking	Never	7	88%	3	100%	4	80%
	Previous	1	13%	0	0%	1	20%
Vasculitis type	EGPA	2	25%	0	0%	2	40%
	GPA	2	25%	1	33%	1	20%
	MPA	4	50%	2	67%	2	40%
End-stage Kidney Disease	No	7	88%	3	100%	4	80%
	Yes	1	13%	0	0%	1	20%
Disease activity since last return	Active	4	50%	2	67%	2	40%
	Remission	4	50%	1	33%	3	60%
N days between C-19 symptom onset and diagnosis	median, IQR	2.5	0.75-9.75	1.0	1-4	12.0	6-17.5
Admission to hospital required	No	2	25%	2	67%	0	0%
	Yes	6	75%	1	33%	5	100%
Admission to Intensive Care Unit	No	8	100%	3	100%	5	100%
C-19 Outcome (worst recorded)	Not hospitalized, limitation on activities	2	25%	2	67%	0	0%
	Hospitalized, not requiring supplemental O_2	3	38%	1	33%	2	40%
	Hospitalized, requiring supplemental O_2	2	25%	0	0%	2	40%
	Death	1	13%	0	0%	1	20%
Laboratory data*							
N sampled		7		5		2	
CRP	median, IQR	84	32.5-123.5	43	22-84	124.1	107.56-140.69
Creatinine	median, IQR	97	60.5-128.5	97	60-114	102	81.5-122.5
Total white cell count (x10^9/L)	median, IQR	5.7	4.53-11.6	5.2	3.9-8.4	10.3	7.98-12.53
Neutrophil count (x10^9/L)	median, IQR	3.7	2.5-8.79	3.7	2-5.2	7.9	5.45-10.35
Lymphocyte count (x10^9/L)	median, IQR	1.4	1.21-2.05	1.4	1.31-2.2	1.5	1.3-1.7
Neutrophil Lymphocyte ratio	median, IQR	2.4	1.53-7.5	2.4	1.47-3.36	6.6	4.09-9.12
Platelet count (x10^9/L)	median, IQR	362	197-472	203	191-496	405	383.5-426.5

Immunosuppressed defined as currently receiving corticosteroids at a dose >=10mg or currently receiving any other form of immunosuppressive therapy (including exposure to rituximab or cyclophosphamide within the prior 6 months) at time of COVID-19 symptom onset. Non-immunosuppressed includes patients receiving <10mg corticosteroids and no other immunosuppression or those receiving no immunosuppression at time of COVID-19 symptom onset. *Not available for 1 recruit. IS, immunosuppression other than corticosteroids.

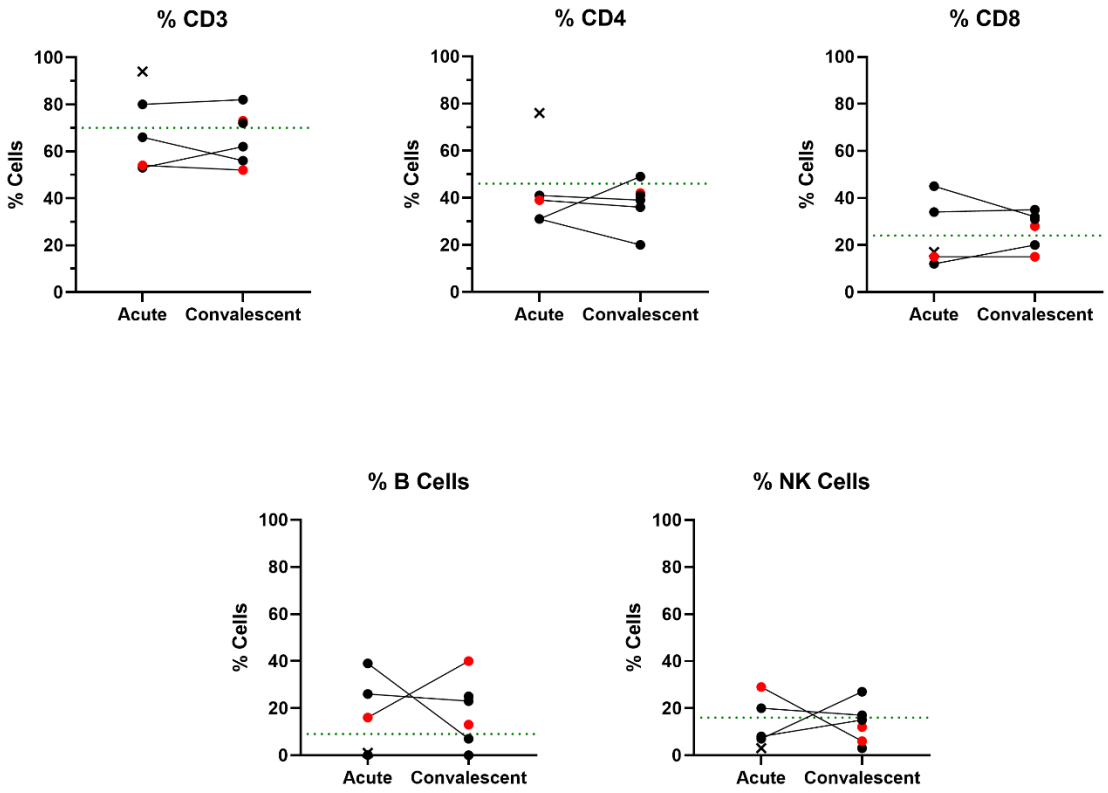

Figure 2: Percentages Of T, B and NK Cells in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Green dotted line represents the healthy control median value. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

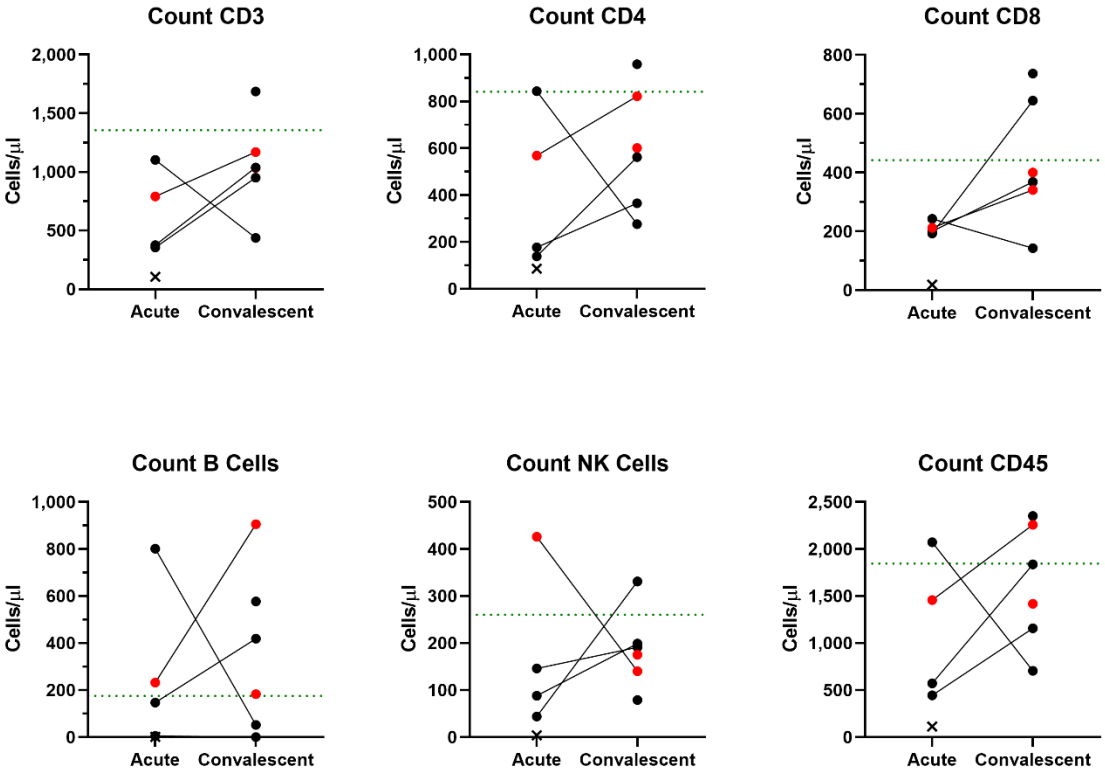

Figure 3: Absolute Cell Subset Counts Expressed as Number of Cells per Microlitre of blood of T, B and NK Cells in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Green dotted line represents the healthy control median value. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

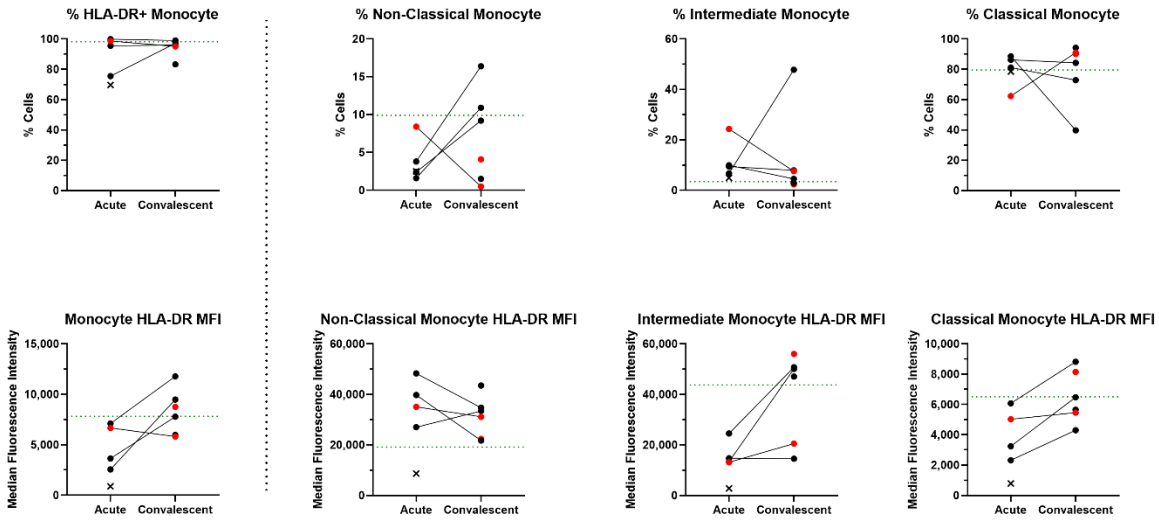

Figure 4: Percentages of Monocyte Subsets and HLA-DR Expression in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Green dotted line represents the healthy control median value**.** Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

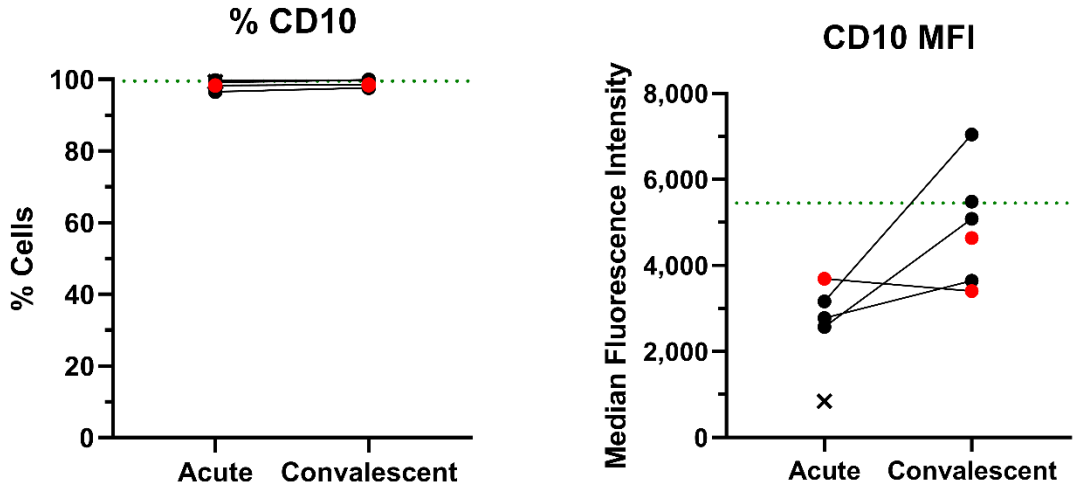

Figure 5: Neutrophil CD10 Expression in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Green dotted line represents the healthy control median value. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

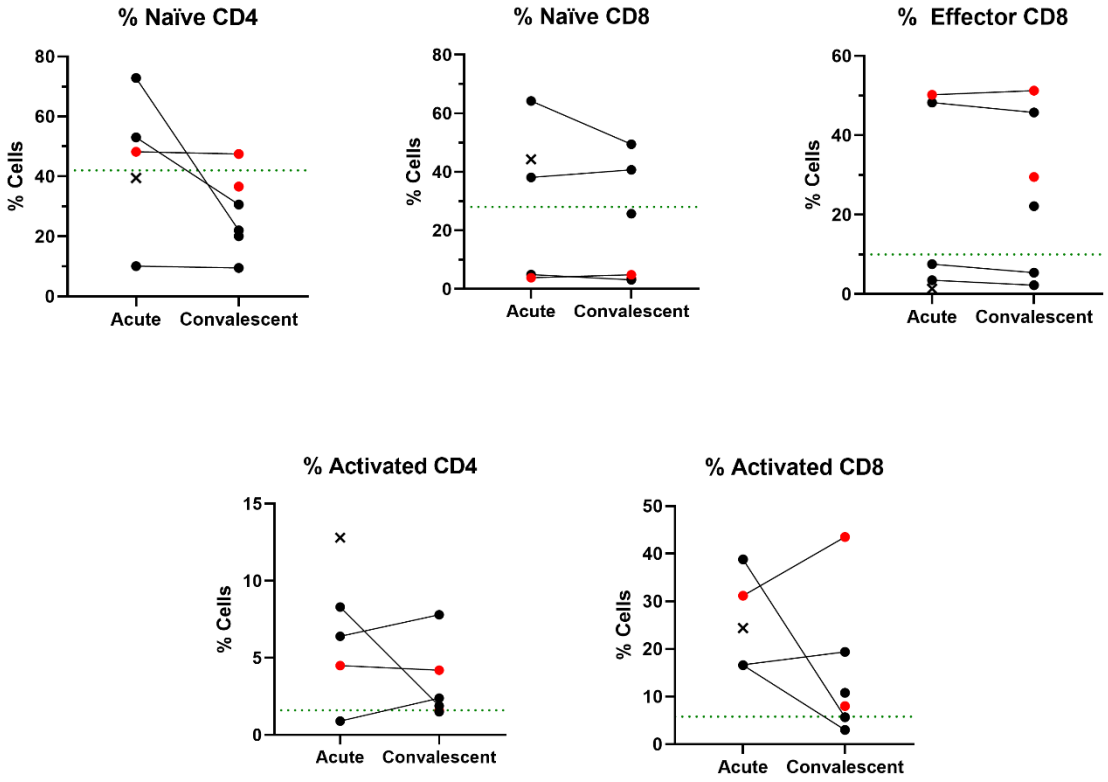

Figure 6: Percentages of T Cell Subsets in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an “X”. Green dotted line represents the healthy control median value. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

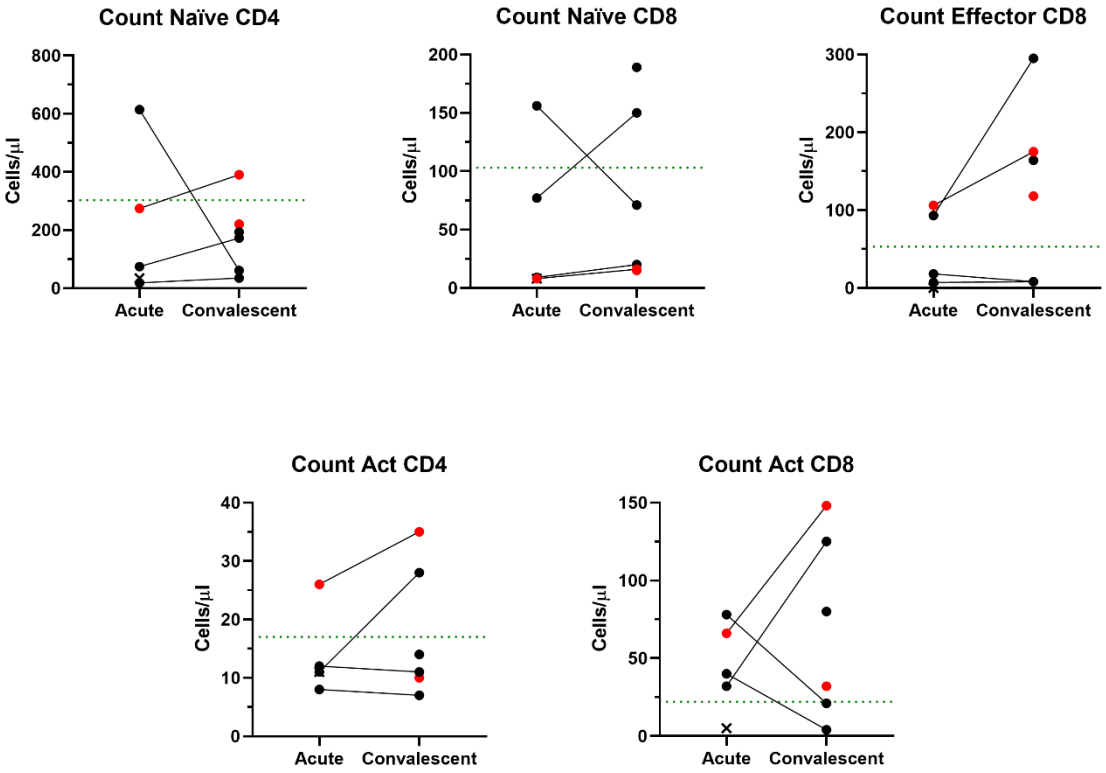

Figure 7: Absolute T Cell Subset Counts Expressed as Number of Cells per Microlitre of blood in Patient Blood Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Green dotted line represents the healthy control median value. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

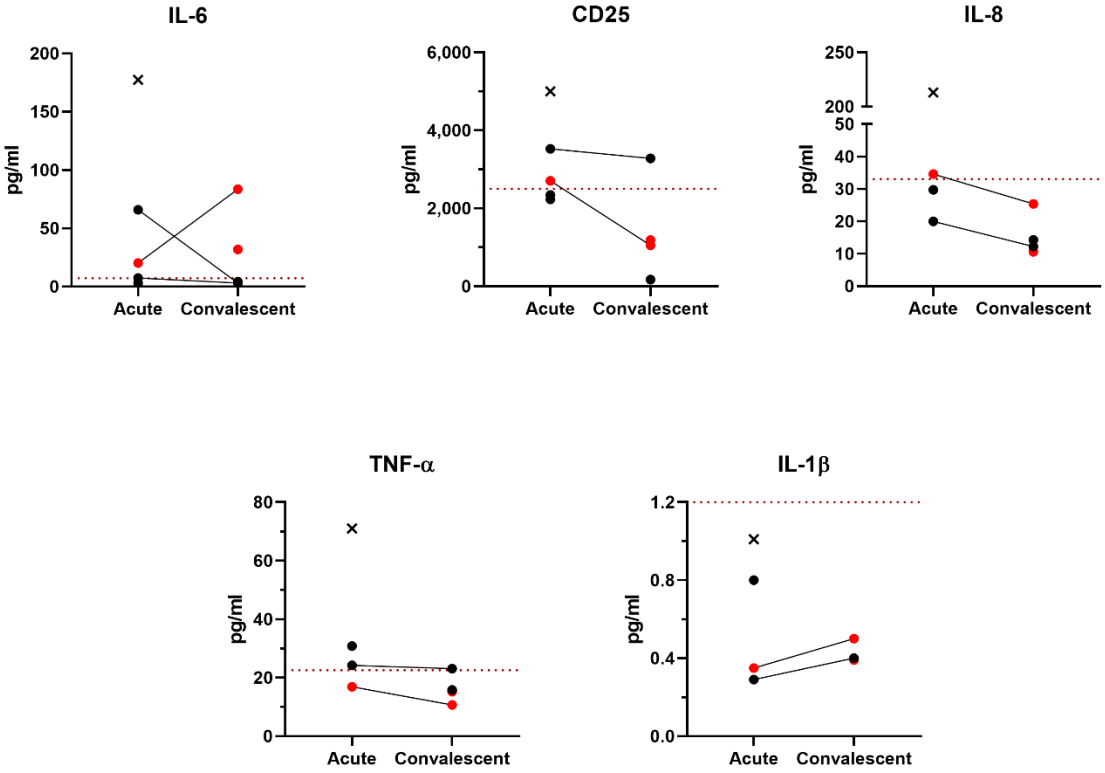

Figure 8: Serum Cytokine Levels in Patient Samples at Acute and 3-Month Convalescent Encounter. Non-immunosuppressed patients are plotted in red and patients with a critical outcome are plotted with an "X". Red dotted line represents a "HIGH" threshold reading based on healthy control reference values. Full details of clinical and immunophenotyping analysis workflows are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/).

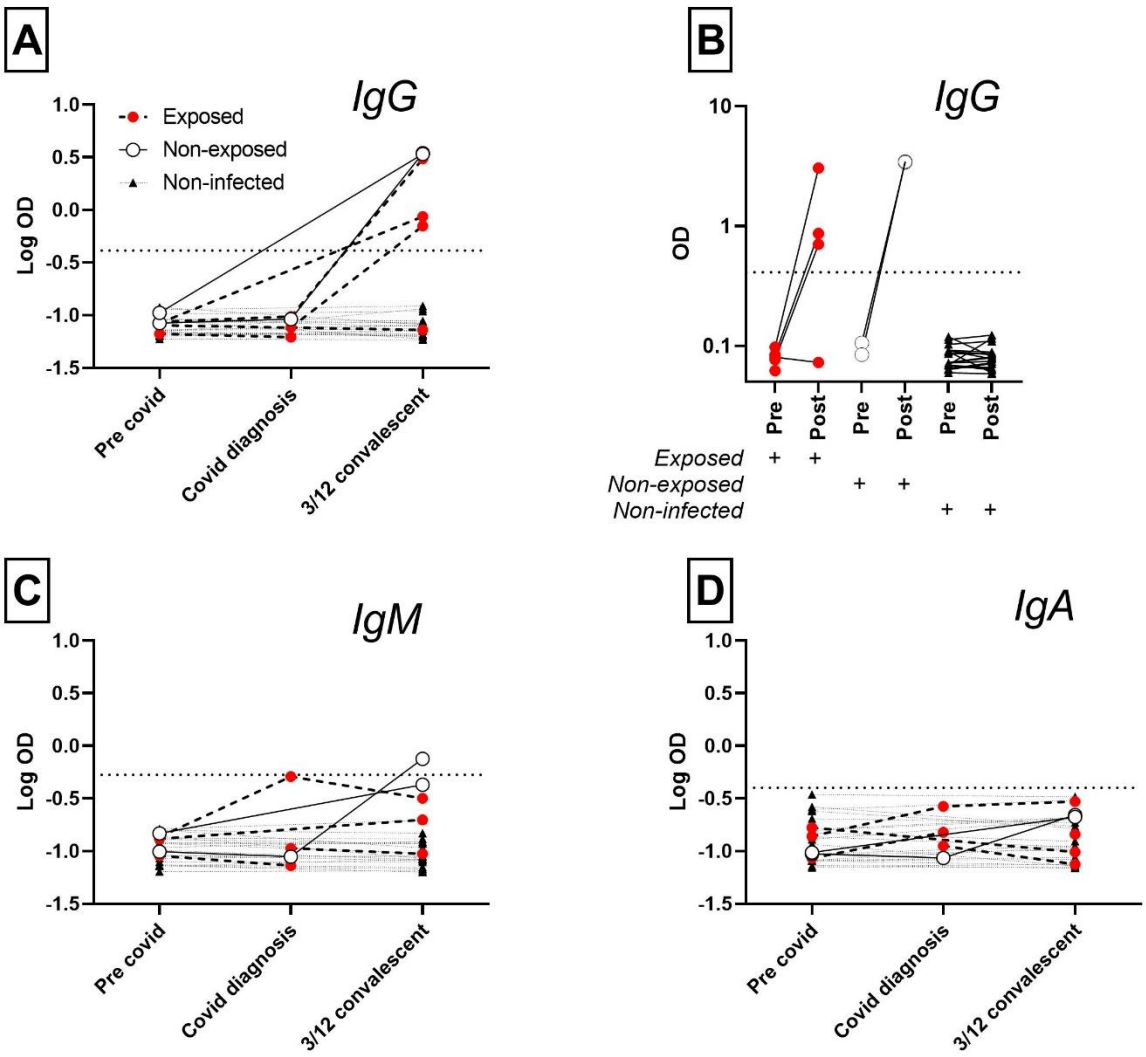

Figure 9: Sars-CoV2 Receptor binding domain serology. A. Trend in anti-RBD IgG in immunosuppressed (exposed), non-immunosuppressed and non-infected patients with vasculitis. B. Paired anti-RBD IgG levels. C. Trend in anti-RBD IgM antibodies. D. Trend in anti-RBD IgA antibodies. Full details of serology assay protocols are available in the DeCOmPRESS study manual (accessible at: https://www.tcd.ie/medicine/thkc/decompress/.

Discussion

A key challenge of this study is our recruitment target. Based on epidemic modelling of COVID-19 incidence rates from April 2020, we aim to recruit 75% (n=306) of potential cases. However, since the design of this study, vulnerable patients, such as those targeted in our study, have been advised to take additional precautions to prevent them acquiring COVID-19; accordingly, in order to achieve this recruitment target we are exploring the addition of patients with rheumatic and dermatological diseases (including those receiving immunosuppressive therapy as defined in this protocol). Further, we are planning more in-depth immunophenotyping to examine in detail the myeloid cell (neutrophil and monocyte) and innate T-cell response to SARS-CoV-2 that could be addressed with smaller patient numbers, should our original target not be reached. We are also expanding the scope of our study to profile responses to COVID-19 vaccination in immunocompromised people

References

1. Leacy, E., et al., *Study Protocol for DeCOmPRESS: Defining the Disease Course and Immune Profile of COVID-19 in the Immunosuppressed Patient.* HRB Open Research, 2021. **4**.