

Supplemental Material for Turner-Stokes et al.,
“Serological screening for COVID-19 in patients with glomerular disease”

Supplemental Material Table of Contents

Methods

Figure S1: Clinical characteristics of COVID-19 and outcomes in patients with PCR proven disease

Figure S2: COVID-19 symptoms and timing of onset

Figure S3: SARS-CoV-2 IgG titres measured by Abbott assay

Figure S4: Effect of age, gender and testing interval on performance of Abbott IgG SARS-CoV-2 assay

Supplementary references for main manuscript¹⁻⁸

METHODS

Patient Selection and Data Collection

This study was approved by the Renal Quality and Safety (Governance) Committee of Imperial College Healthcare NHS Trust (ICHNT) as a part of a service evaluation following the COVID-19 outbreak in the UK and implementation of Public Health England (PHE) approved serology testing for COVID-19 at ICHNT.

During the UK COVID-19 outbreak, the specialist GN clinic at ICHNT (which cares for approximately 1500 patients with primary GN, vasculitis and systemic lupus erythematosus) continued to provide follow-up for essential clinical and safety monitoring. To reduce risk of exposure to SARS-CoV-2 infection, most patients attended for blood tests only, with parallel teleconsultations with their physician. At each consultation, patients were evaluated for evidence of current or prior COVID-19 by structured symptoms review, which assessed whether they had (i) experienced symptoms compatible with COVID-19; (ii) ever had a PCR test for SARS-CoV-2; (iii) attended other healthcare facilities where they might have been tested for SARS-CoV-2.

From the 19th May 2020, when PHE-approved antibody tests for SARS-CoV-2 were introduced at ICHNT, patients were also offered serological screening for prior infection. Serum samples were taken after informed consent, at the same time as clinically indicated blood tests. The serology analysis includes all patients tested between 19th May and 21st July 2020, and excludes those who did not consent for testing, and those receiving renal replacement therapy (HD, PD or renal transplant).

In addition, we retrospectively identified all patients under care of the clinic who had PCR-proven disease from hospital records, including those who did not survive COVID-19 infection or who did not have scheduled follow-up during the study period.

COVID-19 severity was graded as: asymptomatic; mild (symptomatic, managed at home); moderate (symptomatic, required admission to hospital); severe (symptomatic, required non-invasive ventilatory support/admission to Intensive Care); or fatal (death directly attributable to COVID-19 disease).

Serology testing for SARS-CoV-2

Samples were tested in the Infection and Immunity Laboratory at North West London Pathology (within ICHNT) using the Abbott SARS-CoV-2 IgG assay on an Architect system. This is an automatic immunoassay that measures IgG to the nucleocapsid protein (N-protein) of SARS-CoV-2 and has been evaluated for clinical use by Public Health England⁷. Samples were interpreted as positive or negative for IgG to SARS-CoV-2 according to the relative light units (RLU) in the sample, compared to the calibrator, according to the manufacturer's instructions: positive RLU ≥ 1.4 , negative RLU < 0.25 ⁷.

As part of internal validation work within the Infection and Immunity Laboratory comparing immunoassays for SARS-CoV-2, samples were classified as indeterminate using the Abbott IgG SARS-CoV-2 assay if the RLU was within the range 0.25-1.3. Indeterminate samples were reprocessed by the UKAS-accredited Molecular Diagnostics Unit (MDU) at Imperial College London, using a 'spike-' (S-) protein/RBD hybrid dual antigen binding assay (DABA), which

measures total IgG⁸. Samples with a binding ratio equal to or higher than one are considered positive.

Statistical analysis

All data were analysed using the GraphPad Prism 7.0 software package. Data were tested for normality of distribution using the Shapiro-Wilk normality test. Statistical differences between groups were analysed using Student's t-test for parametric data, and Mann-Whitney or Kruskal-Wallis test (with Dunn's multiple comparisons test) for non-parametric data. P values of <0.05 were considered significant.

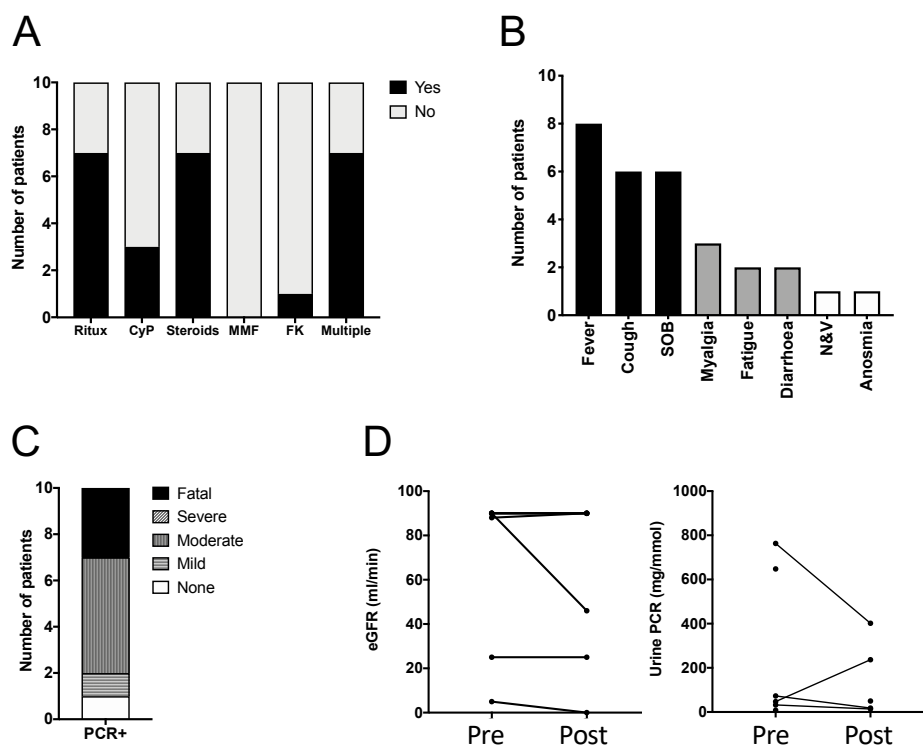


Figure S1: Clinical characteristics of COVID-19 and outcomes in patients with PCR-proven disease
 (A) Immunosuppression at the time of COVID-19 diagnosis. Patients who were still B-cell deplete following treatment with rituximab were included in the Rituximab treatment group.
 (B) Reported symptoms associated with COVID-19 infection.
 (C) COVID-19 disease severity
 (D) eGFR and urine protein:creatinine ratio pre- and post-COVID-19 infection.

Ritux=rituximab, CyP=cyclophosphamide, Steroids=corticosteroids, i.e. oral prednisolone or IV methylprednisolone, MMF=mycophenolate mofetil, FK=tacrolimus, multiple=receiving immunosuppression with two or more agents, N&V=nausea and vomiting

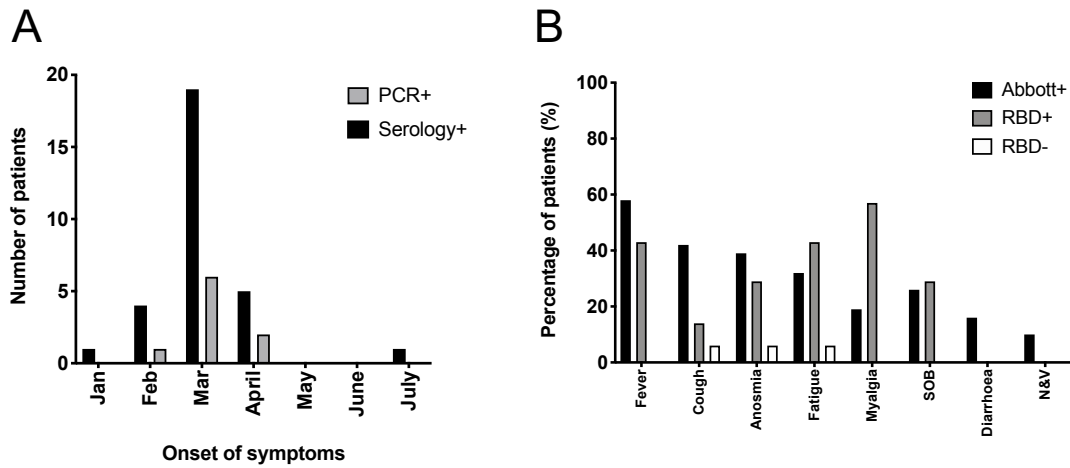


Figure S2: COVID-19 symptoms and timing of onset

(A) Timing of onset of symptoms in patients with evidence of previous SARS-CoV-2 infection. Patients with PCR proven SARS-CoV-2 infection (PCR+), or positive serology using the SARS-CoV-2 IgG Abbott assay, or the S-protein/RBD hybrid DABA assay included.

(B) Symptoms suggestive of COVID-19 reported in patients in each of the 3 serological groups: Abbott+, RBD+ and RBD-

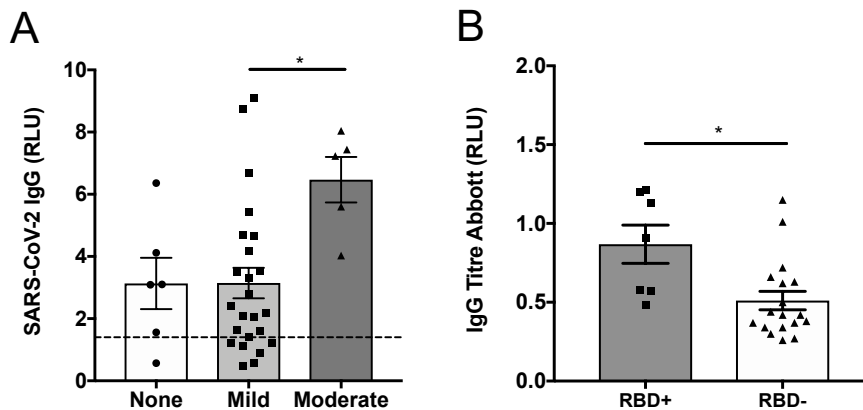


Figure S3: SARS-CoV-2 IgG titres measured by Abbott assay

IgG titres to SARS-CoV-2 measured by the Abbott assay in, (A) patients with different COVID-19 disease severity (none, mild and moderate) and, (B) patients with positive (RBD+) and negative (RBD-) results on the S-protein/RBD hybrid DABA assay.

Scatter plots show mean±SEM. *p<0.05 by, (A) Kruskal-Wallis test with Dunn's multiple comparisons, and (B) Mann-Whitney U test.

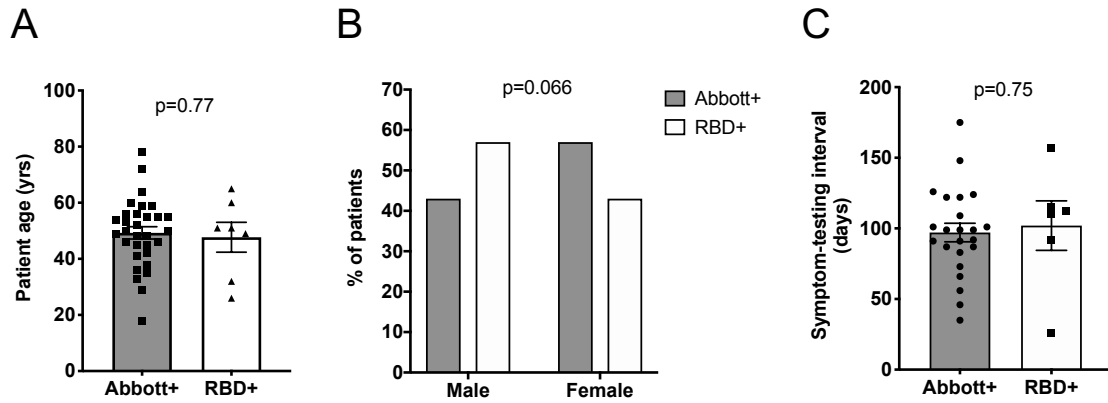


Figure S4: Effect of age, gender and testing interval on performance of Abbott IgG SARS-CoV-2 assay

The Abbott+ and RBD+ serological groups were compared with respect to:

- (A) age of patients in each group (students t-test confirmed no significant difference between the two groups),
 (B) the proportion of male and female patients in each group (contingency analysis using Fisher's exact test confirmed no significant difference between the two groups), and (C) the interval between symptom onset and serological testing (student's t-test confirmed no significant difference between the two groups)

Graphs show mean±SEM

References

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