Supplement

Haemodialysis patients make long-lived antibodies against SARS-CoV-2 that may be associated with reduced re-infection

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Serological testing

Antibodies (combined IgG, IgA and IgM (IgGAM)) against the SARS-CoV-2 spike glycoprotein were measured using a CE marked, validated, commercially available ELISA (Product code: MK654, The Binding Site (TBS), Birmingham), as previously described (1), as per manufacturer's instructions. Prior validation of this assay has shown it demonstrates 100% sensitivity in individuals with PCR-proven disease 7 days post symptom onset (n=59 hospitalised, n=31 community) and 97.8% specificity based on 270 individual negative pre-2019 samples from commercial sources (2).

Statistical analysis

Demographic data was summarised as means and standard deviations for normally distributed data, medians and (interquartile) ranges for non-parametric data and counts and percentages for categorical data.

Due to the variable number of samples and sampling times for each patient, we used generalised estimating equations (GEE) with an exchangeable correlation structure to model mean antibody responses over time. Predicted mean serological responses were calculated from the complete dataset - 650 samples from 256 antibody positive patients. We hypothesised that the mean antibody ratio would rise to a peak and then fall. We proposed the simplest model that could be used to reflect our hypothesis - a line representing the initial rise in antibody ratio (the upslope) and a second line representing the fall (the downslope).

Our model included time as a continuous independent variable, an indicator variable to denote whether each time was before or after the turning point and an interaction between

these two variables to allow for the upslope and downslope. The position of the turning point was varied in candidate models and the lowest quasi-likelihood under independence model criterion (QIC) was used to select the final model. This final model was used for the prediction of the mean antibody ratio at any given timepoint. The data were log transformed to achieve normality (as assessed by Q-Q plots of the generated residuals). The resulting predicted mean appears as two curves when represented on the original linear scale.

For comparisons within the cohort, the GEE model was extended to include the grouping variable and its interactions with the other variables in the model. The significance of the grouping variable was assessed via Wald Chi-Squared tests in terms of the main effect, the two-way interactions and the three-way interaction. Where interactions were found to be non-significant, they were removed from the model in a stepwise fashion to generate the simplest model.

Comparisons were made using Wilcoxon Rank tests for non-parametric data; Chi-squared tests for categorical data; Wald Chi-squared tests for variables within GEE models. P-value <0.05 were deemed statistically significant.

Generalised estimating equations were performed using SPSS Version 26 (IBM). All other analyses were performed using STATA Version 16 (StataCorp).

References:

1. Shields A, Faustini SE, Perez-Toledo M, Jossi S, Aldera E, Allen JD, et al.: SARS-CoV-2 seroprevalence and asymptomatic viral carriage in healthcare workers: a cross-sectional study. *Thorax*, 2020 10.1136/thoraxjnl-2020-215414

2. Cook AM, Faustini SE, Williams LJ, Cunningham AF, Drayson MT, Shields AM, et al.: Validation of a combined ELISA to detect IgG, IgA and IgM antibody responses to SARS-CoV-2 in mild or moderate non-hospitalised patients. *J Immunol Methods,* 494**:** 113046, 2021 10.1016/j.jim.2021.113046