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Title
Relationship of Heterologous Virus Responses and Outcomes in Hospitalized COVID-19 Patients.

Permalink
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Journal
The Journal of Immunology, 211(8)

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Publication Date
2023-10-15

DOI
10.4049/jimmunol.2300391

Peer reviewed

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HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2024 October 15.

Published in final edited form as:

J Immunol. 2023 October 15; 211(8): 1224–1231. doi:10.4049/jimmunol.2300391.

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Relationship of Heterologous Virus Responses and Outcomes in Hospitalized COVID-19 Patients

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Abstract

The clinical trajectory of COVID-19 may be influenced by previous responses to heterologous viruses. We examined the relationship of antibodies against different viruses to clinical trajectory groups from the NIH IMPACC (IMunoPhenotyping of A COVID-19 Cohort) study of hospitalized COVID-19 patients. While initial antibody titers to SARS-CoV-2 tended to be higher with increasing severity (excluding fatal disease), those to seasonal coronaviruses trended in the opposite direction. Initial antibody titers to influenza and parainfluenza viruses also tended

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to be lower with increasing severity. However, no significant relationship was observed for antibodies to other viruses, including measles, CMV, EBV, and RSV. We hypothesize that some individuals may produce lower or less durable antibody responses to respiratory viruses generally (reflected in lower baseline titers in our study); and that this may carry over into poorer outcomes for COVID-19 (despite high initial SARS-CoV-2 titers). We further looked at longitudinal changes in antibody responses to heterologous viruses, but found little change over the course of acute COVID-19 infection. We saw significant trends with age for antibody levels to many of these viruses, but no difference in longitudinal SARS-CoV-2 titers for those with high vs. low seasonal coronavirus titers. We detected no difference in longitudinal SARS-CoV-2 titers for CMV seropositive vs. seronegative patients, though there was an over-representation of CMV seropositives among the IMPACC cohort, compared to expected frequencies in the U.S. population. Our results both reinforce findings from other studies and suggest new relationships between the response to SARS-CoV-2 and antibodies to heterologous viruses.

Introduction

The relationship of COVID-19 outcomes and antibody responses to other viruses has been incompletely examined. While T cell responses to seasonal coronaviruses may be protective against severe COVID-19 disease (1), a conclusive effect of antibody responses to seasonal coronaviruses has not been shown. For influenza, vaccination was found to be slightly protective against severe COVID-19 in children (2), but more detailed relationships have yet to be uncovered. For cytomegalovirus (CMV), there is evidence that seropositivity may predispose to more severe disease (3), though the mechanism is unclear.

The NIH IMPACC study (IMunoPhenotyping of A COVID-19 Cohort) enrolled over 1000 hospitalized COVID-19 patients with follow up for up to one year post-admission, with frequent sampling during the first 28 days of hospitalization and quarterly thereafter (4). Multiple immunological and virological assays were performed over time, yielding correlates of disease severity. To quantify disease severity, patients were grouped into five clinical trajectory groups, from most mild to fatal (5).

To assess antibody responses to a wide variety of viruses, we created a Luminex-based serology assay to measure antibodies to several SARS-CoV-2 antigens as well as antigens from seasonal coronaviruses, influenza, parainfluenza, RSV, CMV, EBV, and measles (Table 1). We then applied this assay to sera from a large subset of IMPACC patients (498 patients assayed at Stanford via Olink). Samples were taken during acute illness (up to 28 days post-admission) and convalescence (up to one year later). We sought to answer the following questions: (1) How do initial antibody responses to heterologous viruses correlate with the clinical response to COVID-19? (2) How do responses to heterologous viruses change over the course of COVID-19? And (3) What trends do these antibody responses show with age and sex? Our findings demonstrate new relationships between heterologous virus responses and COVID-19, and reinforce some findings from previous studies.

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Methods

IMPACC Patients and Samples.

1699 serum samples were drawn from 498 patients in the IMPACC study (4), which corresponded to all samples sent to the Stanford Olink core. Clinical characteristics are summarized in Supplemental Table 1. Samples were stored at −80C after an initial thaw for Olink immunoassay. They were later thawed and diluted for Luminex as described below.

Luminex Assay of Anti-Viral Antibodies.

Antigens of interest (Table 1) were coupled/conjugated to barcoded beads as recommended by the manufacturer (Luminex Corporation, Austin, Texas).

Assay Chex Control beads by Radix BioSolutions, Georgetown, Texas., were added to the panel of beads and included in each well. Samples were diluted 1:400 in PBS&0.5%TritonX100 and 25 μl of diluted serum or plasma was added to assay plate containing the Antigen-coupled beads and incubated for 2 hours at room temperature, shaking on an orbital shaker at 500–600rpm. This step supports the binding of antibodies to their antigens creating multiple unique sandwich assays on beads.

The plate was washed in a Bio-Tek Magnetic washer (ELX-405, BioTek, Winooski, VT) to remove serum/plasma from previous step. A secondary antibody Goat-anti Human IgG (Fc fragment) or Anti-IgM Fc coupled to PE (Phycoerythrin) were diluted and added to the plate. Incubation was performed for 30 minutes at room temperature with shaking as above. (Anti IgG-Cat# NC9822979; Anti-IgM Cat# 501941614; Anti-IgA Cat# NC0631234). The plate was washed in a Bio-Tek Magnetic washer (ELX-405, Bio-Tek, Winooski, VT) to remove excess secondary antibody.

Wash buffer (130ul) was added to wells and read on a Luminex Flex 3D instrument with lower bound of 50 beads per target antigen. Output csv file was analyzed with MasterPlex QT Software by MiraiBio, Hitachi. Data are presented as MFI (Median Fluorescence Intensity). The PBS-T buffer was measured for background and a control serum sample was included as a negative control (Sigma-Aldrich, serum purchased prior to COVID-19 pandemic).

Data Covariate Detrending.

Data were detrended by regressing log median fluorescence intensity (MFI) for each viral antigen and isotype on covariates using ordinary least squares regression (6). Regression residuals (unexplained variation) were retained and added back to the regression intercept. All continuous covariates were mean-centered for detrending.

Analysis of Seasonal Coronavirus (HCoV) Antigens.

Titers for each HCoV antigen (HCov-NL63 S1, HCov-OC43 HE, HCoV-229E S1, HCoV-HKU1 S1) were split at baseline visit detrended (6) median. Then two groups of participants were selected, those who fall below all four medians and those who fall above all four medians. For each SARS-CoV-2 antigen (S-M, RBD, NP), temporal trends in covariate

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detrended (6) data were plotted for these two groups within same graph. Detrended (6) MFI was regressed on a b spline (7) over elapsed time, baseline seasonal HCoV status, and their interaction using generalized linear mixed models (8). A random intercept per participant was included to account for longitudinal structure. Agreement of residuals and random effects with normal distribution was marginal to fair as assessed by quantile-quantile plots.

Baseline Primary Hypotheses for SARS-CoV-2 and HCoV.

The logarithm of MFI for each antigen was regressed on age, gender, logarithm of MFI of nonspecific binding, plate/batch, and trajectory group using ordinary least squares (6) with allowance for variance heterogeneity (9). All p-values were adjusted for multiple comparisons using the method of Holm (10), separately for each combination of antigen type (SARS-CoV-2, HCoV) and antibody isotype. All means comparisons are against trajectory group 1. Data in plots are detrended (6) for covariates.

Baseline Additional Antigen Hypotheses.

The logarithm of MFI for each antigen was regressed on age, gender, logarithm of MFI of nonspecific binding, plate/batch, and trajectory group using ordinary least squares (6) with allowance for variance heterogeneity (9). All p-values were adjusted (11, 12) to control the false discovery rate at 5%, separately for each antibody isotype and trajectory group. All means comparisons are against trajectory group 1. Data in plots are detrended (6) for covariates.

Baseline IgG CMV Seropositivity.

IgG CMV seropositivity status (0 = negative, 1 = positive) was regressed on age, gender, logarithm of MFI of nonspecific binding, plate/batch, and trajectory group using binomial generalized linear models (13). All means comparisons are against trajectory group 1.

Baseline Age and Gender Hypotheses.

Logarithm of MFI for each antigen and antibody isotype was regressed on age, gender, logarithm of MFI of nonspecific binding, plate/batch, and trajectory group using ordinary least squares (6) with allowance for variance heterogeneity (9). All p-values were adjusted (11, 12) to control the false discovery rate at 5%, by each combination of age or gender and isotype. Data in plots are detrended (6) for covariates.

Longitudinal Hypotheses in SARS-CoV-2 and HCoV.

Logarithm of MFI of each antigen was regressed on age, gender, logarithm of MFI of nonspecific binding, batch/plate, trajectory group, square root of elapsed days, the interaction of trajectory group and square root of elapsed days, and a quadratic (curvilinear) term for square root of elapsed days using linear quantile mixed models (LQMMs; (14)). LQMMs included a random intercept per participant to account for longitudinal structure. All p-values were adjusted for multiple comparisons using Holm (10), separately for each combination of antigen type (SARS-CoV-2, HCoV), antibody isotype, and trajectory group. All comparisons of slopes over time are against the slope in trajectory group 1. Data in plots are detrended (6) for covariates.

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Trends Following CMV and EBV Seropositivity at Baseline.

Patients were classified as CMV or EBV seropositive at the first visit based on empiric cutoffs. IgG MFI data detrended (6) for nonspecific binding and plate/batch were regressed on a b-spline basis (7) for elapsed days, initial CMV or EBV seropositivity, and their interaction using generalized linear mixed models (8). A random intercept per participant was included to account for longitudinal structure. The interaction term was tested to assess if longitudinal trends varied with initial CMV or EBV seropositivity. Agreement of residuals and random effects with normal distribution was poor to good as assessed by quantile-quantile plots.

Correlations of titers with serum cytokine levels.

Olink serum cytokine data have been previously reported for the IMPACC study (15). All records in the cytokine data that did not pass quality control or were missing observations for all cytokines were excluded from analyses. Remaining missing cytokine values were imputed using the knn.impute function in R package impute (16). Cytokines were excluded from the analyses if their dynamic range (maximum minus minimum) fell at or below the 10th percentile of all cytokines' dynamic ranges. Cytokine data were detrended for plate effects and antibody data were detrended for plate/batch and nonspecific binding effects using ordinary least squares (6). All antibody median fluorescence intensity data were logarithmically transformed for analyses. Kendall's correlation (17) was calculated for all pairs of antigens and proteins. All p-values were adjusted (11, 12) to control the false discovery rate at 5%.

Software.

Analysis and graphing were performed in SAS® (SAS® Institute, Cary, North Carolina, USA) and R (www.r-project.org) under R Studio (www.rstudio.com) with R packages dplyr (18), impute (16), ggplot2 (19), mutoss (20), Kendall (21), sandwich (22), and lqmm (23).

Results

SARS-CoV-2 titers.

1699 serum samples from 498 IMPACC patients (4) were collected at admission (initial samples) and at 1–28 days later (longitudinal samples). These were assessed via Luminex assay for their responses to SARS-CoV-2 antigens and antigens from seasonal coronaviruses as well as other respiratory and non-respiratory viruses. IgM responses were analyzed in a subset (192 samples from 85 patients). We first asked whether initial responses to SARS-CoV-2 antigens varied by clinical trajectory group (5). As seen in Figure 1, the first visit titers for four SARS-CoV-2 antigens were quite heterogeneous, but tended to increase slightly from the mildest cases (group 1) to the most severe non-fatal cases (group 4), with fatal cases (group 5) being more similar to group 1. The difference between group 4 and group 1 reached statistical significance for RBD and S-M.We further asked whether longitudinal trends in SARS-CoV-2 antibody titers over the acute and convalescent period (up to 1 year post-admission) differed by clinical trajectory group. As seen in Figure 1B, trajectory group 4 showed a significant difference from group 1 for the NP antigen of SARS-

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CoV-2. Longitudinal trends for the other SARS-CoV-2 antigens showed similar patterns, but did not reach statistical significance (not shown).

Seasonal coronavirus titers.

We performed a similar analysis for initial titers to the four seasonal coronaviruses, 229E, HKU1, OC43, and NL63. Figure 2A shows that these titers tended to be lower with increasing disease severity, reaching statistical significance for groups 4 and/or 5 depending on the seasonal coronavirus strain.

When examining longitudinal trends for seasonal coronaviruses, we found little change over the course of 28 days post-admission, except for several cases of spiking IgM responses (Figure 2B). We interpreted these to be due to cross-reactivity of SARS-CoV-2 antigens with seasonal coronaviruses, since it's unlikely that these patients experienced concomitant seasonal coronavirus infections.

Responses to SARS-CoV-2 in patients with high vs. low initial titers to seasonal coronaviruses.

Given the suspected cross-reactivity mentioned above, we sought to ask whether pre-existing seasonal coronavirus titers might cross-react with SARS-CoV-2 to influence the outcome of COVID-19. However, we found no difference in outcome groups for those with high vs. low initial titers to the seasonal coronaviruses (Figure 2C).

Influenza titers.

We next asked whether initial titers to influenza and parainfluenza antigens varied by COVID-19 trajectory group. We assessed responses to a common influenza A antigen as well as to hemagglutinins from the 2019 influenza vaccine strains, plus parainfluenza 2 and parainfluenza 3. As seen in Figure 3, virtually all of these showed a similar trend as the seasonal coronavirus titers, with lower average titers as the COVID-19 trajectory group increased. The differences relative to group 1 reached statistical significance for groups 2, 3, 4, and/or 5 depending upon the antigen. The only exception was the influenza B strain, B/Phuket/3073/2013, which had no significant differences between trajectory group 1 and other groups, but for which titers were overall relatively low.

Relation to serum cytokines.

We next asked whether decreasing titers for the above viruses with COVID-19 severity were related to a serum cytokine signature. To examine this, we tested for correlations between antibody titers for these viruses and serum cytokines as determined by Olink proximity extension assay. We found a number of significant correlations (Table 2), both positive and negative. Many of these were significant for multiple viruses, e.g., positive correlations of CCL8, CCL25, DNER, IL-12β, and KITLG; and negative correlations of CCL4, OSM, S100A12, and TNFSF14 with titers for multiple viruses.

Other unrelated viral titers.

Finally, we assessed initial titers to other unrelated viruses (CMV, EBV, measles, and RSV) by COVID-19 trajectory group. As seen in Figure 4A, there were no significant differences

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relative to trajectory group 1 for any of these viruses. Furthermore, the longitudinal plots of titers were generally flat, with the exception of a few patients who showed a spike in IgM for CMV or EBV during the course of COVID-19 infection (Figure 4B).

Effects of CMV and EBV seropositivity.

Since CMV has been associated with an increased risk of hospitalization for COVID-19 (3), we asked whether our hospitalized cohort had an over-representation of CMV or EBV seropositive individuals. To determine this, we calculated an expected rate of CMV or EBV positivity based upon published studies in healthy adults. This was aided for CMV by a large study of CMV seropositivity rates by age in U.S. adults (24). From this, we calculated an age-adjusted expected frequency of CMV seropositives of 0.752, which compared to an observed frequency of 0.844 in our cohort (Supplemental Figure 1A). For EBV, the observed rate of 0.965 in our cohort was very similar to the expected rate of 0.94–0.96 from other studies (25, 26). We determined seropositivity for CMV and EBV using an empiric cutoff as shown in Supplemental Figure 1B.

CMV seropositivity was not associated with trajectory group (data not shown). We did not see a difference in CMV (or any other virus) titers between males and females (Supplemental Figure 1C). We also did not see a difference in longitudinal trend of titers to SARS-CoV-2 antigens in those who were CMV or EBV seropositive vs. seronegative (Supplemental Figure 1D–E).

Age associations.

We also checked our cohort for associations of age with viral titers. We saw a downward trend with age for SARS-CoV-2 titers, which was significant for all three SARS-CoV-2 antigens tested ($p=0.001–0.038$; Supplemental Figure 2A). Interestingly, all other significant age associations were upward trends with age. These included one of the seasonal coronaviruses (OC43), as well as measles, parainfluenza 2 and 3, and several influenza antigens (Supplemental Figure 2B and C). We did not see significant age associations for CMV or EBV.

Between-antigen titer correlations.

Finally, we looked for correlations between titers for different antigens tested. Not surprisingly, we saw strong correlations between titers for the three SARS-CoV-2 antigens tested, with the strongest being between S-M and RBD (the latter being a subunit of the former) ($r=0.97$, $p<0.01$; Supplemental Figure 3A). The correlations in titers for the four seasonal coronaviruses were more modest but still significant ($r=0.19–0.51$, $p<0.01$; Supplemental Figure 3B). For all other virus titers, there was low or no correlation (Supplemental Figure 3C), except for a good alignment between titers for the two influenza B strains (B/Colorado/06/2017 and B/Phuket/3073/2013; $r=0.84$, $p<0.01$).

Discussion

Here we described the relationships of viral titers for different viruses in a subset of approximately half of the IMPACC cohort of hospitalized patients with COVID-19 (4).

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These titers were quite heterogeneous, possibly due in part to differing time since symptom onset, which we did not account for. Nevertheless, we showed upward trends with increasing clinical trajectory group for baseline SARS-CoV-2 titers (except for group 5, which was fatal cases). Conversely, we found downward trends with increasing clinical trajectory group for other respiratory virus baseline titers, including seasonal coronaviruses, influenza, and parainfluenza. No significant differences with trajectory group were seen for titers to CMV, EBV, measles, or RSV. With the exception of RSV, these latter are all non-respiratory viruses; while those with significant downward trends with increasing clinical trajectory group (seasonal coronaviruses, influenza, and parainfluenza) are all respiratory viruses. A possible hypothesis for this finding is that some individuals intrinsically mount poor responses to respiratory viruses, and these individuals then fare more poorly when they contract COVID-19. For influenza, there is also data to suggest a positive benefit of vaccination for COVID-19 outcome, but this was in children rather than adults (2).

A parallel study on the full IMPACC Cohort (Diray-Arce et al., 2023) found no such associations of SARS-CoV-2 titers and trajectory group, using a highly comprehensive Virscan assay. However, more severe disease (group 5) was associated with increased seroreactivity to the N terminal domain (NTD) of S and decreased antibody seroreactivity to the LINK domain of N (adj.p =0.023) (Figure S2D of that manuscript). We could not corroborate the domain-specific findings, as our study used only whole protein antigens or subunits (RBD). It is possible that our Luminex assay was more quantitative than the Virscan assay, and thus able to distinguish differences using the whole protein antigens that were not apparent in the latter.

Many factors may contribute to the strength of antibody responses to heterologous viruses, including age of exposure, number of exposures, and exposure through infection vs. vaccination. While we do not know all of these variables for the IMPACC participants, we can make some inferences. For example, measles immunity is presumably almost all vaccine-derived, while CMV and EBV are entirely infection-derived; yet all of these show no trend with COVID-19 severity. Thus, the difference between viruses, whose titers trend with COVID-19 severity and those that do not, cannot be due simply to the variable of whether immunity was derived from infection vs. vaccination.

It is also unlikely that immunosuppressive therapies caused the decreasing titers with severity seen for most of the respiratory viruses, since (a) the lower titers were seen on admission (visit 1), and (b) only respiratory viruses were affected. Similarly, conditions such as immune deficiency or cancer would not be expected to affect respiratory viruses preferentially. In fact, we tested for association of viral titers with malignant neoplasm and found none (all adjusted p=1, data not shown).

We tracked titers to SARS-CoV-1 as a negative control, but instead saw positive responses in a subset of IMPACC patients (data not shown). We assume these reflected cross-reactivity with SARS-CoV-2 epitopes, and that only some COVID-19 patients mounted antibodies to the cross-reactive epitopes.

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Patients with high titers to seasonal coronaviruses did not show a significant longitudinal difference in their titers to SARS-CoV-2 antigens compared to those with low seasonal coronavirus titers (Figure 2C). This suggests no advantage in the development of humoral immunity to SARS-CoV-2 based on previous exposure to seasonal coronaviruses. While other studies have suggested potential benefits for COVID-19 patients from cross-reactive immunity to seasonal coronaviruses, this has mostly been on the side of cellular immunity (1, 27), so our results for cross-reactive antibody are not too surprising.

A previous study suggested cross-reactivity of T cell responses to CMV with SARS-CoV-2 (28). However, we did not find differences in the longitudinal development of antibodies to SARS-CoV-2 in those who were CMV seropositive vs. not. We did, however, see an over-representation of CMV seropositives in our hospitalized COVID-19 cohort compared to the expected frequency in an age-adjusted healthy population (24). This is consistent with other studies suggesting that CMV seropositivity may be associated with increased risk of hospitalization for COVID-19 (3, 29).

We saw suggestions of CMV and EBV reactivation during COVID-19, in the form of spikes for IgM against CMV or EBV during acute COVID-19. Reactivation of these viruses in the setting of COVID-19 has been previously described, and may be either an epiphenomenon or a driver of more severe inflammatory disease (30). In any case, it is interesting that we only saw IgM spikes in a small minority of COVID-19 patients, despite the fact that these patients were all severe cases necessitating hospitalization.

We tested IgM and IgA responses in a subset (about 17%) of participants, and did not find significant differences in titer by severity group. This could be because of the smaller sample size, resulting in lower statistical power; or it could be related to the higher prevalence and longer half-life of serum IgG relative to IgM and IgA.

We wondered whether the decreasing titers with COVID-19 severity for most respiratory viruses might be associated with a particular serum cytokine signature. Indeed, there were repeated correlations found between certain serum cytokine levels and multiple of these respiratory viruses (Table 2). While the pattern of positive and negative correlations was consistent, it did not suggest a simple conclusion, such as an increased proinflammatory environment associated with higher titers.

In examining the relationship of age and viral titers, we were surprised to see so many viruses for which there were significantly increased titers with age. These included one seasonal coronavirus (OC43), influenza, parainfluenza 2 and 3, and measles. Most of these are explainable by repeated exposure and/or vaccination over the course of the lifespan. However, measles vaccine is generally only given in early childhood, and circulating virus is currently rare in the U.S. The high titers for measles in older patients is thus likely a reflection of natural immunity rather than vaccine-acquired immunity seen in young patients.

In summary, our study both reinforces other studies and adds new hypotheses about the relationship of responses to heterologous viruses and the course of SARS-CoV-2 infection. Perhaps most intriguingly, we find a trend, for many respiratory viruses, of

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decreasing titers with increasing COVID-19 severity, suggesting possible common defects in response to multiple respiratory pathogens. These could include defects in cell trafficking or differentiation in respiratory mucosa, or other unknown differences when encountering respiratory pathogens.

Our study is limited by only measuring antibody (not T cell) responses, and by only sampling hospitalized (and thus more severe) COVID-19 patients. We also analyzed data using pre-defined trajectory groups as the major outcome; the choice of ordinal scale alone could have impacted our results. Still, the inclusion of around 500 patients from across the U.S., with multiple time point sampling, is a strength. Further dissection of the role of immunity to heterologous viruses, especially CMV and influenza, is encouraged by these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the IMPACC clinical sites for recruiting patients and processing and banking samples, and the IMPACC patients for contributing to the study.

This work was supported by grant 2U19AI057229 from the U.S. National Institutes of Health.

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Key points:

1. Baseline antibodies to respiratory viruses are lower with higher COVID-19 severity.
2. Antibodies to non-respiratory viruses are not different between severity groups.
3. Titers to seasonal coronaviruses don’t predict longitudinal SARS-CoV-2 titers.

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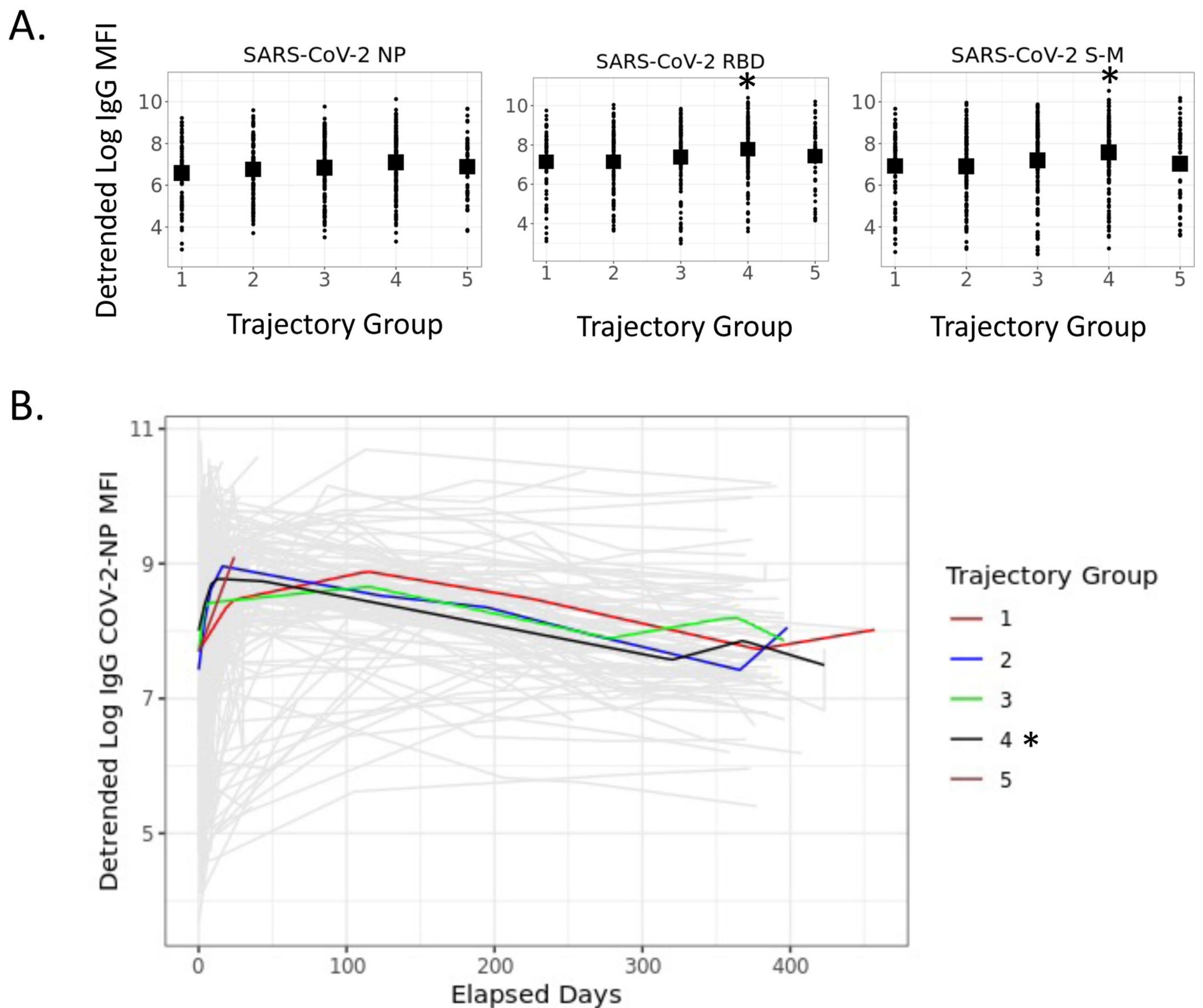

Figure 1.

Serum IgG levels specific for SARS-CoV-2 antigens in COVID-19 patients, by clinical trajectory group (1–5, where 1=most mild and 5=fatal). All data are detrended for covariates. MFI = median fluorescence intensity. **A.** Visit 1 SARS-CoV-2-specific IgG levels tend to increase with severity, from trajectory group 1 to 4, reaching significance for RBD and S-M antigens for trajectory group 4 (vs. group 1). Squares are means. **B.** Longitudinal trend for NP-specific serum IgG differs significantly for trajectory group 4 (vs. group 1; P=0.003). Longitudinal trends for the RBD- and S-M-specific serum IgG were not significantly different from trajectory group 1 (not shown).

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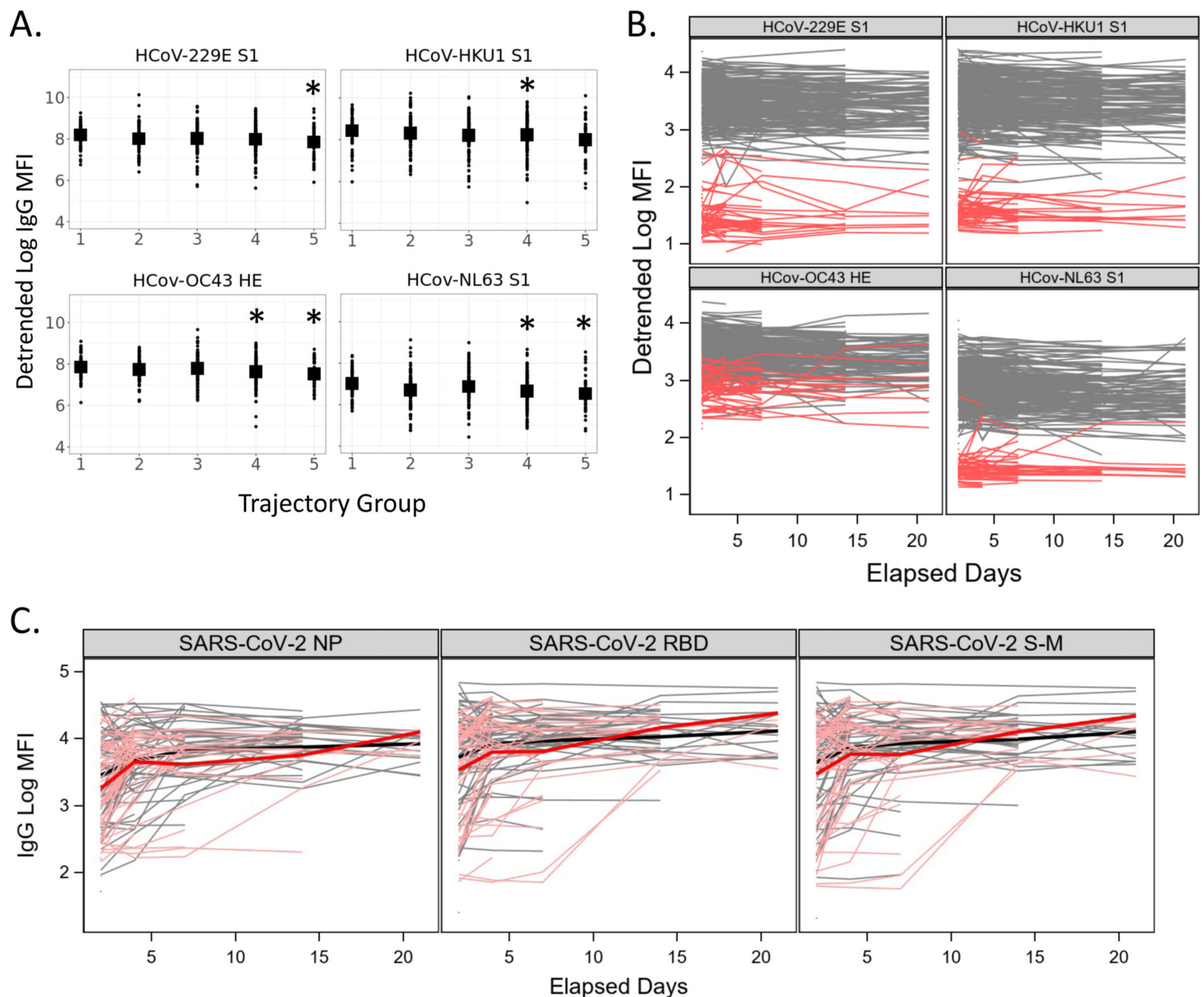

Figure 2.

Serum antibody levels specific for seasonal coronavirus antigens in COVID-19 patients. All data are detrended for covariates. MFI = median fluorescence intensity. **A.** Visit 1 serum IgG levels for seasonal coronavirus antigens decrease on average from clinical trajectory group 1 to 5 (1=most mild; 5=fatal), with significant differences (vs. group 1) as marked. There were no significant differences by trajectory group for IgM or IgA for any of these antigens (not shown). Squares are means. **B.** Longitudinal plots of serum IgG (gray) and IgM (red) for these antigens over the course of 28 days show little change with time, although there are spikes for a minority of individuals, especially for IgM. These likely represent cross-reactive responses to SARS-CoV-2. **C.** Longitudinal trends in serum IgG response to SARS-CoV-2 antigens (NP, RBD, and S-M) in patients with low (black lines) vs. high (red lines) levels of IgG to seasonal coronaviruses at first visit. While there is a trend towards slightly higher starting levels of anti-SARS-CoV-2 IgG in those with low seasonal coronavirus IgG, the

overall differences are not significant ($P>0.09$ for all antigens). All data are detrended for covariates. MFI = median fluorescence intensity.

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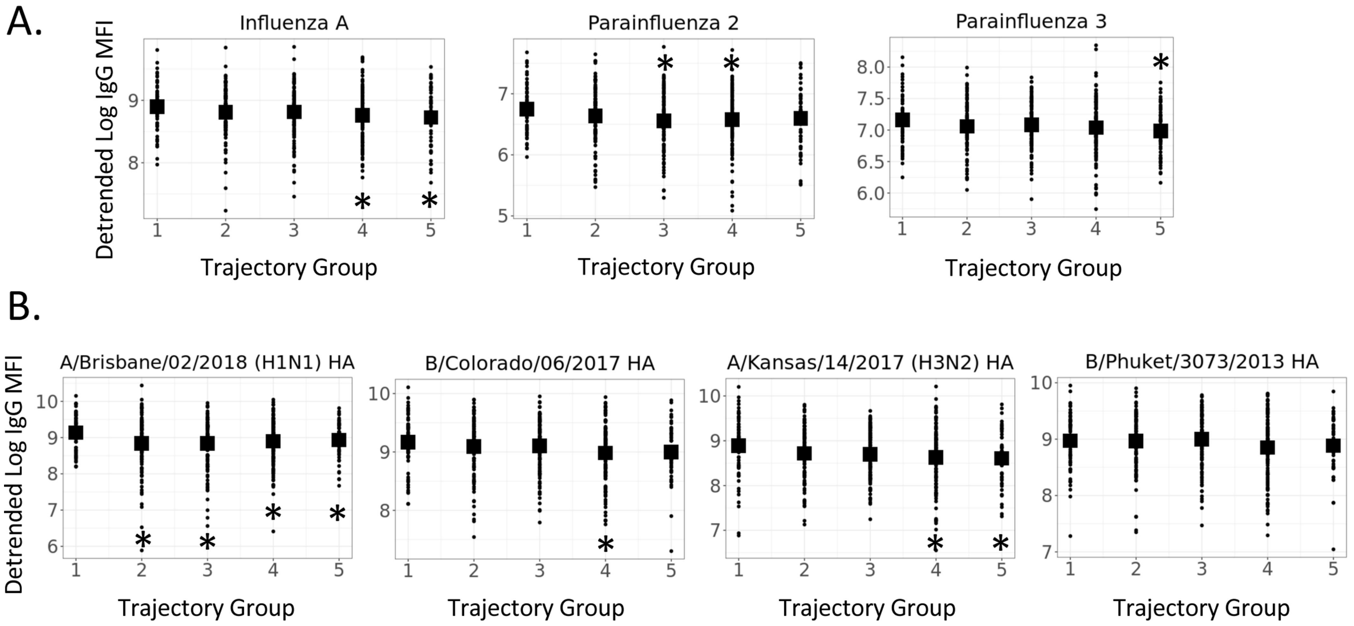

Figure 3.

Visit 1 serum IgG levels for other respiratory viruses in COVID-19 patients, by clinical trajectory group (1=most mild; 5=fatal). All data are detrended for covariates. MFI = median fluorescence intensity. Squares are means. **A.** Serum IgG levels for influenza A, parainfluenza 2, and parainfluenza 3 tend to decrease from trajectory group 1 to 5, with statistical significance for the groups marked (vs. group 1). **B.** Serum IgG levels for influenza strain-specific hemagglutinins also tend to decrease from trajectory group 1 to 5, with statistical significance for the groups marked (vs. group 1). There were no significant differences by trajectory group for IgM or IgA for any of these antigens (not shown).

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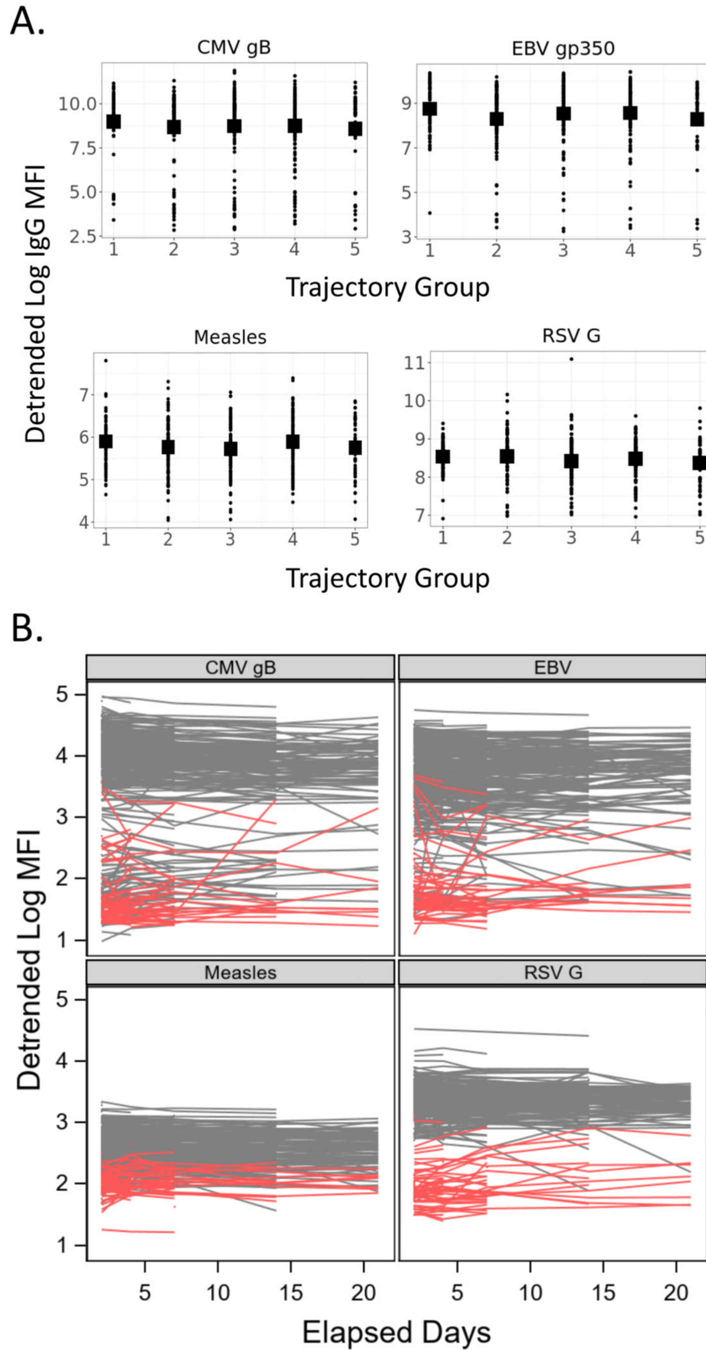

Figure 4.

Serum antibody levels to other viruses in COVID-19 patients, by clinical trajectory group (1=most mild; 5=fatal). All data are detrended for covariates. MFI = median fluorescence intensity. **A.** Visit 1 serum IgG levels specific to CMV, EBV, measles, and RSV antigens are not significantly different for any clinical trajectory group (vs. group 1). There were also no significant differences by trajectory group for IgM or IgA for any of these antigens (not shown). Squares are means. **B.** Longitudinal plots of serum IgG (gray) and IgM (red) for

these antigens over the course of 28 days show little change with time, although there are IgM spikes for CMV and EBV for a few individuals.

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Table 1.

Antigens included in the Luminex Viral Antibody assay.

Group	Viral Protein	Abbreviation for Figures	Source
SARS-CoV-2 antigens	Spike membrane protein	SARS-CoV-2 S-M	Origene
	Receptor binding domain	SARS-CoV-2 RBD	RayBiotech
	Nucleocapsid protein	SARS-CoV-2 NP	RayBiotech
SARS-CoV-1 antigens	Spike glycoprotein (S1) *	SARS S1	Native Antigen
Seasonal Coronavirus antigens	OC43 Hemagglutinin esterase	HCov-OC43 HE	Sino Biological
	229E Spike/S1 protein	HCov-229E S1	Sino Biological
	HKU1 Spike glycoprotein S1	HCov-HKU1 S1	Sino Biological
	NL63 Spike protein S1	HCov-NL63 S1	Sino Biological
Influenza 2019 vaccine strains	B/Colorado/06/2017 HA (aa 16–546)	B/Colorado/06/2017 HA	eEnxyme
	A/Brisbane/02/2018 (H1N1) HA (aa 18–529)	A/Brisbane/02/2018 HA	eEnzyme
	A/Kansas/14/2017 (H3N2) HA (aa 17–529)	A/Kansas/14/2017 HA	eEnzyme
	B/Phuket/3073/2013 HA (aa 16–546)	B/Phuket/3073/2013 HA	eEnzyme
Pan-influenza and parainfluenza	Influenza A lysate	Influenza A	Fitzgerald
	Parainfluenza Type 3 strain C243 Ag prep	Parainfluenza 3	Ray Biotech
	Parainfluenza Type 2 strain GREER Ag prep	Parainfluenza 2	Ray Biotech
Other viruses	Measles virus lysate	Measles	MyBioSource
	Cytomegalovirus glycoprotein B	CMV gB	Sino Biological
	Epstein-Barr virus glycoprotein 350	EBV gp350	Sino Biological
	Respiratory syncytial virus (B1) glycoprotein G	RSV G	Sino Biological

*Responses to SARS-CoV-1 are not presented here, as there was apparent cross-reactivity to SARS-CoV-2 in a subset of participants.

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Table 2.

Significant associations of serum cytokine levels with titers of respiratory viruses that had a negative association of titer with COVID-19 severity

Antigen	Cytokine	Tau	Pvalue	Adjusted_p
HCov-NL63 S1	KITLG	0.112	0.000	0.022
HCov-NL63 S1	DNER	0.119	0.000	0.014
HCov-NL63 S1	CCL8	0.106	0.001	0.030
HCov-OC43 HE	CD6	0.118	0.000	0.015
HCov-229E S1	TNFRSF11B	−0.096	0.002	0.052
HCov-229E S1	IL15RA	−0.099	0.002	0.043
HCov-229E S1	DNER	0.103	0.001	0.034
Influenza A	CCL4	−0.098	0.002	0.045
Influenza A	KITLG	0.152	0.000	0.001
Influenza A	IL12B	0.106	0.001	0.030
Influenza A	S100A12	−0.109	0.001	0.028
Influenza A	CCL8	0.099	0.002	0.043
Influenza A	CCL25	0.104	0.001	0.034
Influenza A	CX3CL1	0.113	0.000	0.022
B/Colorado/06/2017 HA	OSM	−0.099	0.002	0.043
B/Colorado/06/2017 HA	TNFSF14	−0.104	0.001	0.034
B/Colorado/06/2017 HA	HGF	−0.103	0.001	0.034
B/Colorado/06/2017 HA	IL12B	0.103	0.001	0.034
B/Colorado/06/2017 HA	IL24	−0.096	0.002	0.052
B/Colorado/06/2017 HA	S100A12	−0.131	0.000	0.005
A/Brisbane/02/2018 HA	CCL4	−0.102	0.001	0.035
A/Brisbane/02/2018 HA	KITLG	0.122	0.000	0.013
A/Brisbane/02/2018 HA	S100A12	−0.100	0.002	0.040
A/Brisbane/02/2018 HA	CCL8	0.103	0.001	0.034
A/Kansas/14/2017 HA	AXIN1	−0.103	0.001	0.034
A/Kansas/14/2017 HA	OSM	−0.108	0.001	0.028
A/Kansas/14/2017 HA	CCL4	−0.149	0.000	0.001
A/Kansas/14/2017 HA	KITLG	0.110	0.001	0.026
A/Kansas/14/2017 HA	TGFA	−0.121	0.000	0.013
A/Kansas/14/2017 HA	TNFSF14	−0.110	0.001	0.026
A/Kansas/14/2017 HA	CXCL5	−0.096	0.002	0.052
A/Kansas/14/2017 HA	IL12B	0.119	0.000	0.014
A/Kansas/14/2017 HA	CCL3	−0.101	0.001	0.037
A/Kansas/14/2017 HA	S100A12	−0.142	0.000	0.001
Parainfluenza 3	CST5	0.114	0.000	0.021
Parainfluenza 3	KITLG	0.127	0.000	0.008

Antigen	Cytokine	Tau	Pvalue	Adjusted_p
Parainfluenza 3	CCL25	0.108	0.001	0.028
Parainfluenza 2	LIF	−0.096	0.002	0.053

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