Supplemental information

Nucleocapsid-specific antibody function is associated with therapeutic benefits from COVID-19 convalescent plasma therapy

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Supplementary Materials
Herman et al. “Nucleocapsid-specific antibody function is associated with therapeutic benefit from Covid-19 Convalescent plasma therapy”
**Supplementary Figure 1**: Supplement to Figure 2 (A) This histogram shows the distribution of Local Inverse Simpson's Index (LISI) scores for treatment group in UMAP visualization of all patient samples profiled in this paper shown in Fig. 2A. LISI measures the degree of mixing in an embedding ranging from 1 to the number of categories (here, will be two), where larger LISI scores indicate less separation and more homogenous mixing. (B) Univariate box plots of Day 1 (pre-existing) Spike specific antibody features of CCP-treated and control patients. Each box represents the median (central line) and IQR (25% and 75% percentiles) and the two whiskers represent 1.5 * IQR. (C) The histogram shows the distribution of days of symptomatic COVID-19 prior to enrollment for all participants. (D) The polar plots depict the min-max proportion of each Spike-specific antibody features at each sample day across the control (top) and CCP-treated (bottom) participants. Antibody features are colored by type of feature with ab-directed functions in light blue, Fc receptor binding in medium blue, and Ab titer in dark blue. (E) The bar plot depicts the delta-AIC of the best model compared with the model without differences of the four parameters. An abbreviated form of just the top 30 features is included in Figure 2E. The higher the delta-AIC, the better the model can explain the trajectory difference. The sign of delta-AIC represents the
AUC difference between the CCP-treated and control curves, thereby showing whether the antibody feature is enriched in the CCP-treated model (negative) or the control model (positive). The bars are colored according to whether the feature is enriched in the CCP-treated model (pink) or control model (blue). (F) This heatmap shows the Akaike Information Criterion (AIC) weighted average parameter differences between the two groups. Each column shows a parameter, which is normalized across the features. The color intensity indicates whether the parameter is higher in the CCP-treated (blue) or control (orange) model. An abbreviated form of this heatmap is included in Figure 2D. (G) To validate the PLS-R model in Figure 2H of Clinical Severity based on the Top 30 features, we calculated the correlation between the predicted clinical severity score from five-folder cross-validation and the actual clinical severity score. (H) To further demonstrate the performance and robustness of the PLS-R model, we compared the cross-validation result with a randomly permuted dataset. The violin plots show the distribution of repeated five-fold cross validation $R^2$ values using the actual data (model) or shuffled labels (permuted labels). Black dot indicates the median values and whiskers represent the one standard variance. All Ab measurements were taken in technical duplicate (Ab level and FcR-binding assays) and biologic duplicate (ADCP, ADNP, ADNKA) and used as an average of the two for the analysis in this figure.
Supplementary Figure 2: Supplement to Figure 2. Fitted temporal evolutionary curves of antibody features by our regression model depicted in Figure 2 D-G. For each antibody feature, the optimal model fit is shown for each group. Dots indicate the average antibody value for an individual patient (technical duplicate for Ab titers and Fc-R binding, biological replicate for ADCP, ADNP, ADNKA), diamonds indicate the binned median, the curves indicate the optimal fitted model, and the color shows the group. The parameters which are different for the displayed model are indicated in the top left corner and color-coded according to the group for which the parameter is higher.
Supplementary Figure 3: Supplement to Figure 3. (A) Univariate box plots on Nucleocapsid specific measurements of CCP-treated and Control patients on Day 1 (Pre-existing). Each box represents the median (central line) and IQR (25% and 75% percentiles) and the two whiskers represent 1.5 * IQR. All Ab measurements were taken in technical duplicate (Ab level and FcR-binding assays) and biologic duplicate (ADCP, ADNP, ADNKA) and used as an average of the two for the analysis in this figure.
Supplementary Figure 4: Supplement to Figure 4. (A, B) Average Silhouette values in Spearman correlation-based community detection algorithm were used to select the number of clusters. (A) Point plot of the average silhouette values for 1 through 10 clusters using the community detection algorithm to separate participants by their pre-existing SARS-CoV-2 antibody profile. (B) Network plot of individual participants arranged into the four clusters selected by Silhouette analysis. Each dot represents each sample, and each colored region indicates each cluster defined by community detection algorithm. Clusters are indicated by color. (C) Boxplot of Day of Symptom Onset prior to enrollment in the clinical trial of CCP-treated and Control patients in Cluster 4 and Cluster 1, 2, 3. A two-sided Wilcoxon test was performed to compare age between treatment arms. (D) Participants were re-clustered by their CCP benefit signature, identified in Figure 4D, and we used silhouette analysis to choose the number of clusters. This point plot shows the calculated average silhouette values for 1 through 15 clusters using the community detection algorithm to separate participants by their CCP benefit signature identified in Figure 4D. (E) Network plot of individual participants arranged into two clusters. Each dot represents each sample, and each colored region indicates each cluster defined by community detection algorithm. Clusters are indicated by color. (F-H) Three separate linear regression models were used to assess which type of pre-existing antibody features best predicted clinical severity in CCP-treated individuals. The bar plots show the percentage of explained variance (%) by all 12 antibody titers (F), all 12 antibody functions (G), or all 12 IgG1 titer-corrected antibody functions (H) in the three separate models. The bars are colored by the SARS-CoV-2 antigen for which they correspond, and the asterisks represents Ab features with a statistically significant relationship with clinical severity score. For the boxplots in C, each box represents the median (central line) and IQR (25% and 75% percentiles) and the two whiskers represent 1.5 * IQR. * represents a p-value < 0.05.
Supplementary Figure 5: Supplement to Figure 4. Univariate box plots for all antigen-specific antibody measurements of CCP-treated and control patients in Cluster 4 (in) and Cluster 1,2,3 (out), described in Figure 4. Each box represents the median (central line) and IQR (25% and 75% percentiles) and the two whiskers represent 1.5 * IQR. The difference antigen-specific antibody features between CCP-treated and control patients was tested by a two-sided Wilcoxon test. * represents a p-value < 0.05. ** represents a p-value < 0.01. *** represents a p-value <0.001 . **** represents a p-value < 0.0001.
**Supplementary Figure 6**: Supplement to Figure 4. Evaluating the effect of Pre-treatment Viral Load on CCP-Associated Clinical Improvement

(A) Box plots of nasopharyngeal swab viral load represented as log10 copies/mL of CCP-treated group in clusters A and B. The difference between Cluster A and Cluster B in the CCP-treated group was tested by the two-sided Wilcoxon test (p-value: 0.0261). (B) Heatmap showing the Spearman correlation coefficients between S and N antibody features and viral load in CCP-treated participants. Orange indicates a positive correlation, whereas Blue indicates a negative correlation. Statistical significance is indicated by gray asterisks with Holm-Bonferroni correction for multiple hypothesis testing (* p<0.05, ** p < 0.01, *** p < 0.001). (C-D) A linear regression model was used to assess which type of pre-treatment feature – antibody functions, antibody titer, or viral load - best predicted clinical severity in CCP-treated individuals. The bar plots show the percentage of explained variance (%) by antibody titers, antibody functions, and viral load in the model. (C) Bar plots show the explained variance of three groups of features. (D) Bar plots show the explained variance per each measurement in the model. We used the top 12 features that differed between Cluster 4 and Cluster 1, 2, 3 for the linear regression model of each antibody feature category.
Supplementary Figure 7: Supplement to Figure 5. (A) The PLS-DA model in Figure 5 C, D was found to have an accuracy of 63.8% and under a five-fold cross-validation framework run 100 times and preformed significantly better than both negative models. The violin plots show the distribution of repeated cross validation R^2 values using the actual data (model), randomly selected antibody features (random features) or shuffled participant labels (permuted labels). The whiskers represent the one standard variance and * represents a p-value < 0.0001 by two-tailed Mann Whitey U testing.
Supplementary Table 1: Table of the Clinical Characteristics of Pre-existing Ab Cluster 4 vs. Clusters 1,2,3

| Demographics | Cluster 4 (n=18) | Cluster 1,2,3 (n=61) | p-value (Fisher's Exact test) |
|--------------|----------------|---------------------|------------------------------|
| Age - Median (IQR) | 66.5(32.75) | 62(20) | 0.35 |
| Sex | | | 0.79 |
| Male | 9 | 27 |
| Female | 9 | 34 |
| Race | | | 0.033 |
| African-American | 5 | 37 |
| Asian | 1 | 3 |
| Caucasian | 10 | 20 |
| Unknown | 2 | 1 |
| Ethnicity | | | 0.13 |
| Hispanic | 2 | 1 |
| Non-hispanic | 16 | 60 |
| Blood Group | | | 0.58 |
| A | 5 | 23 |
| B | 1 | 7 |
| O | 12 | 31 |
| Study Characteristics | | | 0.021 |
| Enrollment Quarter | | | |
| May - June 2020 | 2 | 17 |
| July-August 2020 | 1 | 18 |
| September-October 2020 | 4 | 6 |
| November-January2021 | 11 | 20 |
| COVID-19 Co-morbidities | | | 0.16 |
| Cardiovascular disease - no. | 9 | 18 |
| Cancer - no. | 7 | 14 |
| Obesity - no. | 4 | 32 |
| Chronic Kidney Disease - no. | 10 | 16 |
| Hypertension - no. | 11 | 42 |
| Diabetes - no. | 6 | 26 |
| Treatments | | | |
| Immodulatory Treatment - no. | 4 | 7 |
| Remdesivir - no. | 14 | 50 |
| Corticosteroids - no. | 14 | 52 |

Supplementary Table 1: Supplement to Figure 4. Table of the Clinical Characteristics of Pre-existing Ab Cluster 4 vs. Clusters 1,2,3
**Supplementary Table 2**: Table of the Clinical Characteristics of Pre-existing Ab Cluster where CCP provides Benefit: Cluster 4 CCP-treated vs. Control Patients

| Demographics | CCP-treated (n=7) | Control (n=11) | (Fisher’s Exact test) |
|--------------|------------------|---------------|----------------------|
| Age - Median (IQR) | 86 [14] | 64 [30.5] | 0.0184 |
| Sex |  |  |  |
| Male | 3 | 6 | 1 |
| Female | 4 | 5 |  |
| Race |  |  | 0.25 |
| African-American | 1 | 4 |  |
| Asian | 0 | 1 |  |
| Caucasian | 6 | 4 |  |
| Unknown | 0 | 2 |  |
| Ethnicity |  |  |  |
| Hispanic | 1 | 1 |  |
| Non-hispanic | 6 | 10 |  |
| Blood Group |  |  | 0.75 |
| A | 3 | 2 |  |
| B | 0 | 1 |  |
| O | 4 | 8 |  |
| Study Characteristics |  |  | 0.88 |
| Enrollment Quarter |  |  |  |
| May - June 2020 | 1 | 1 |  |
| July-August 2020 | 0 | 1 |  |
| September-October 2020 | 1 | 3 |  |
| November-January 2021 | 5 | 6 |  |
| COVID-19 Co-morbidities |  |  |  |
| Cardiovascular disease - no. | 5 | 4 | 0.33 |
| Cancer - no. | 4 | 3 | 0.33 |
| Obesity - no. | 1 | 3 | 1 |
| Chronic Kidney Disease - no. | 6 | 4 | 0.066 |
| Hypertension - no. | 6 | 5 | 0.15 |
| Diabetes - no. | 3 | 3 | 0.63 |

| Treatments |  |  |  |
| Immodulatory Treatment - no. | 1 | 3 | 1 |
| Remdesivir - no. | 5 | 9 | 1 |
| Corticosteroids - no. | 6 | 8 | 1 |

**Supplementary Table 2**: Supplement to Figure 4. Table of the Clinical Characteristics of Pre-existing Ab Cluster where CCP provides Benefit: Cluster 4 CCP-treated vs. Control Patients
Supplementary Table 3: Table of the Clinical Characteristics of CCP Benefit Signature Clustered Groups: Cluster A vs. Cluster B

| Demographics | Cluster A (n=57) | Cluster B (n=22) | p-value (Fisher’s Exact test) |
|--------------|-----------------|-----------------|-----------------------------|
| **Age - Median (IQR)** | 64(22) | 60.5(20.75) | 0.336 |
| **Sex** | | | 0.626 |
| Male | 27 | 9 | | 
| Female | 30 | 13 | | 
| **Race** | | | 0.498 |
| African-American | 29 | 13 | | 
| Asian | 2 | 2 | | 
| Caucasian | 23 | 7 | | 
| Unknown | 3 | 0 | | 
| **Ethnicity** | | | 1 |
| Hispanic | 2 | 1 | | 
| Non-hispanic | 55 | 21 | | 
| **Blood Group** | | | 0.579 |
| A | 22 | 6 | | 
| B | 5 | 3 | | 
| O | 30 | 13 | | 
| **Study Characteristics** | | | 0.871 |
| Enrollment Quarter | | | |
| May - June 2020 | 14 | 5 | | 
| July-August 2020 | 15 | 4 | | 
| September-October 2020 | 7 | 3 | | 
| November-January 2021 | 21 | 10 | | 
| **COVID-19 Co-morbidities** | | | 0.797 |
| Cardiovascular disease - no. | 19 | 8 | | 
| Cancer - no. | 14 | 7 | | 
| Obesity - no. | 25 | 11 | | 
| Chronic Kidney Diseae - no. | 20 | 6 | | 
| Hypertension - no. | 39 | 14 | | 
| Diabetes - no. | 22 | 10 | | 
| **Treatments** | | | 0.616 |
| Immodulatory Treatment - no. | 9 | 2 | | 
| Remdesivir - no. | 45 | 19 | | 
| Corticosteroids - no. | 47 | 19 | | 

**Supplementary Table 3**: Supplement to Figure 4. Table of the Clinical Characteristics of CCP Benefit Signature Clustered Groups: Cluster A vs. Cluster B
Supplementary Table 4: Table of Clinical Characteristics of CCP Benefit Signature Clustered Group where CCP provides benefit: Cluster A CCP-treated vs. Control

| Demographics | Control (n=29) | CCP-treated (n=28) | p-value (Fisher’s Exact test) |
|--------------|---------------|--------------------|-------------------------------|
| Age - Median (IQR) | 62(19) | 65(31.75) | 0.615 |
| Sex |                      |                    |                               |
| Male | 11 | 16 | 0.189 |
| Female | 18 | 12 | 0.611 |
| Race |                      |                    |                               |
| African-American | 16 | 13 | . |
| Asian | 0 | 2 | . |
| Caucasian | 12 | 11 | . |
| Unknown | 1 | 2 | . |
| Ethnicity |                      |                    |                               |
| Hispanic | 1 | 1 | 1 |
| Non-hispanic | 28 | 27 | 1 |
| Blood Group |                      |                    |                               |
| A | 11 | 11 | . |
| B | 3 | 2 | . |
| O | 15 | 15 | . |
| Study Characteristics | Enroll Quarter |                      | 0.946 |
| May - June 2020 | 8 | 6 | . |
| July-August 2020 | 7 | 7 | . |
| September-October 2020 | 4 | 3 | . |
| November-January 2021 | 10 | 11 | . |
| COVID-19 Co-morbidities | Cardiovascular disease - no. | 7 | 12 | 0.167 |
| Cancer - no. | 8 | 6 | 0.76 |
| Obesity - no. | 15 | 10 | 0.289 |
| Chronic Kidney Disease - no. | 12 | 8 | 0.408 |
| Hypertension - no. | 23 | 16 | 0.092 |
| Diabetes - no. | 14 | 8 | 0.175 |
| Treatments | Immodulatory Treatment - no. | 5 | 4 | 1 |
| Remdesivir - no. | 23 | 22 | 1 |
| Corticosteroids - no. | 26 | 21 | 0.179 |

Supplementary Table 4: Supplement to Figure 4. Table of Clinical Characteristics of CCP Benefit Signature Clustered Group where CCP provides benefit: Cluster A CCP-treated vs. Control