Evaluation of functional humoral immune responses in COVID-19 patients in the ICU

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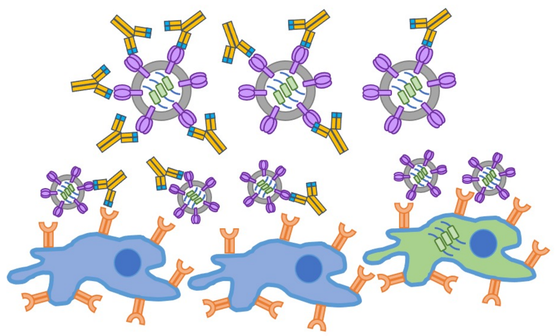
Introduction

Background and Significance

COVID-19 is an infectious disease of the respiratory system caused by SARS-CoV-2 and transmitted through air droplets. It presents as a clinically diverse manifestation ranging from asymptomatic through to critical illness with severe pneumonia, acute respiratory distress syndrome, and respiratory or multiple organ failure. Abnormal immunological indicators associated with disease severity and mortality in patients with COVID-19 have been described, however limited data are available concerning neutralizing antibody (nAb) responses against SARS-CoV-2 in this cohort. We were, therefore, interested in evaluating functional antibody responses in ICU patients with severe disease. Our hypothesis was that functional antibody responses against SARS-CoV-2 are inhibited in severe COVID-19.

Experimental Approach

Plasma samples were collected from patients in the ICU at UMC-New Orleans who were severely ill with COVID-19 in the early stages of the pandemic (LSUHSC IRB Protocol #641). ICU patient samples (n=24) had high levels of D-dimers (3,929 ± 1142 ng/mL; normal range = 0-500 ng/mL) and C-reactive protein (41.3 ± 15.3 mg/dL; normal <0.9 mg/dL) that are prognostic indicators of severe COVID-19. The mean interval ± SEM between COVID-19 diagnosis and sample collection was 15.7 ± 9.9 days. Samples were also collected from healthy donors (n=16) to establish baseline data. IgG, IgG subclass, and IgA antibody responses against the receptor binding domain (RBD) of the Spike (S) glycoprotein of SARS-CoV-2 were measured by ELISA. Neutralizing antibody responses against the virus were evaluated in SARS-CoV-2 Spike pseudotyped virus inhibition assays.



Use of pseudovirus technology to detect neutralizing antibody responses in ICU patients. Donor plasma were pre-incubated with SARS-CoV02 Spike pseudotyped virus to measure inhibition of pseudovirus entry into 293T-ACE2 permissive cells.

RBD-reactive IgG and IgA titers

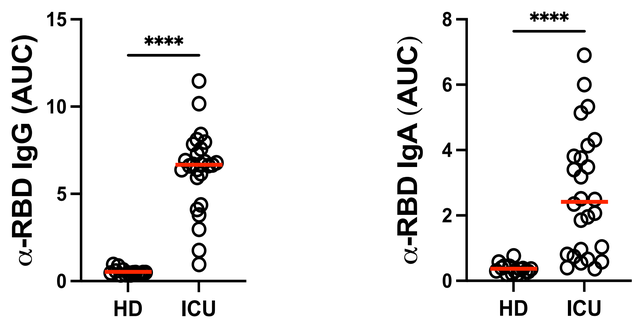


Figure 1. RBD-reactive IgG and IgA titers are readily detectable in ICU patient cohort. Plasma RBD-reactive (Wuhan-Hu-1) IgG and IgA titers are detected in ICU patient plasma by ELISA. Statistical significance was determined using an unpaired t-test of mean area under the curve (AUC) values (red bars) between ICU patient and healthy donor (HD) plasma samples: ****p<0.0001.

ICU patients have potent nAb responses

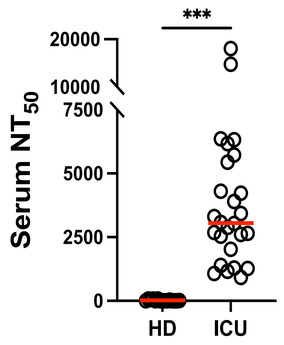


Figure 2. ICU patients have potent neutralizing antibody responses in plasma. ICU patients' plasma potently inhibited SARS-CoV-2 Spike (Wuhan-Hu-1) pseudotyped virus. Statistical significance was determined using an unpaired t-test of mean 50% plasma neutralization titers (NT_{50}) values (red bars) between ICU patient and healthy donor (HD) plasma samples: ****p<0.0001.

IgG1 and IgG3 subclasses predominate

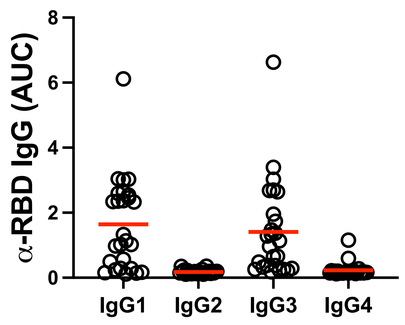


Figure 3. RBD-specific IgG1 and IgG3 subclasses predominate in ICU cohort plasma. α-RBD IgG1 and IgG3 titers are readily detectable in ICU patient plasma by ELISA. RBD-reactive IgG2 and IgG4 levels were very low or not detected in this patient cohort.

No correlation between RBD titers and nAb

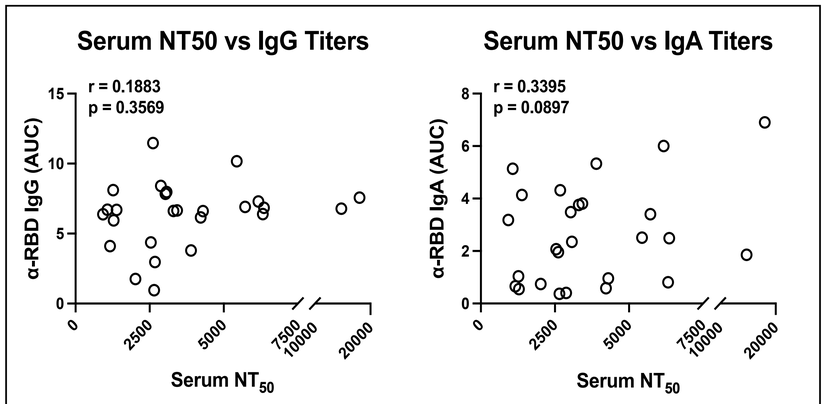


Figure 4. No correlation between RBD-reactive titers and neutralizing antibody responses in plasma. No significant correlation between α-RBD IgG and IgA levels and plasma NT_{50} values. The Pearson coefficient of correlation (r) is listed for these analyses.

Results Summary

Plasma RBD-specific IgG titers were readily detected in ICU patients (mean AUC 6.36) but not in HD controls (mean AUC 0.52). Anti-RBD IgA antibodies were also detected in ICU plasma (mean AUC 2.64) but not HD patients (mean AUC 0.36).

ICU donor RBD-specific IgG responses were predominantly of the IgG1 and IgG3 subclasses, with levels of anti-RBD IgG2 and IgG4 very low or not detected.

ICU patient plasma potently neutralized Spike pseudovirus, with mean 50% plasma neutralization titer (NT50) estimated at 1:4253 (range = 1:923–1:18038). No correlation between plasma NT50 values and anti-RBD IgG or IgA titers was found.

Conclusions

This study was designed to evaluate binding and neutralizing antibody responses against SARS-CoV-2 in a cohort of seriously ill COVID-19 patients in the ICU. Plasma analyses confirmed high levels of both nAb activity against SARS-CoV-2 and RBD-specific IgG and IgA antibody titers. There was, however, no correlation between the neutralization and binding activities, nor evidence of abnormal IgG subclass distribution. Our findings indicate that severe COVID-19 developed in these patients in the face of potent nAb responses against SARS-CoV-2, suggesting that other factors influenced the course of disease. Caveats to note are that longitudinal plasma samples were not available and that the samples tested were taken at different intervals after diagnosis. Future work will involve assay of inflammatory cytokines and chemokines in these samples to investigate any correlations with severe COVID-19 in this cohort.

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