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

Background: Coronavirus disease 2019 (COVID-19) is an infectious disease of the respiratory system caused by SARS-CoV-2 and transmitted through air droplets. COVID-19 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. For this reason, we were interested in evaluating functional antibody responses in ICU patients with severe COVID-19.

Methods: Plasma samples were collected in the early stages of the pandemic from patients in the ICU at University Medical Center New Orleans who were severely ill with COVID-19. ICU patient samples (n=24) were assayed for levels of D-dimers and C-reactive protein (CRP), both prognostic indicators for severe COVID-19. Levels (mean $\pm$ SEM) of D-dimers were 3,929 $\pm$ 1142 ng/mL (normal range = 0-500 ng/mL) and CRP were 41.3 $\pm$ 15.3 mg/dL (normal <0.9 mg/dL). The mean interval $\pm$ SEM between COVID-19 diagnosis and sample collection was 15.7 $\pm$ 9.9 days. Samples were also collected from healthy donors (n=16) to establish baseline data. IgG and IgA antibody responses in plasma against the receptor binding domain (RBD) of the Spike (S) glycoprotein of SARS-CoV-2 were measured by ELISA. Levels of individual IgG1-4 subclass antibodies against RBD were also measured by ELISA. NAb responses against the virus in plasma were evaluated in SARS-CoV-2 Spike pseudotyped virus inhibition assays.

Results: Plasma RBD-specific IgG titers were readily detected in these ICU patients (mean AUC of 6.36) but not, as expected, in the HD control samples (mean AUC of 0.52 $p<0.0001$). Similarly, anti-RBD IgA antibodies were detected in ICU patient plasma (mean AUC of 2.64 AUC units) but not in HD plasma (mean AUC of 0.36, $p<0.0001$). ICU donor RBD-specific IgG responses were predominantly of the IgG1 and IgG3 subclasses, with nearly undetectable levels of anti-RBD IgG2 and IgG4. ICU patient plasma potently neutralized Spike pseudovirus, with the mean 50% plasma neutralization titer (NT_{50}) estimated to be 1:4253.3 (range = 1:923-1:18038). No correlation between plasma NT_{50} values and anti-RBD IgG or IgA titers was found.

Conclusions: This study was designed to evaluate binding and nAb responses against SARS-CoV-2 in a cohort of seriously ill COVID-19 patients in the ICU. We were interested in the level of these responses in the face of severe disease. 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 neutralization and binding activities, nor 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 cytokine and chemokine levels in these plasma samples to investigate any correlations with severe COVID-19.