Salivary CD8+ T cells are elevated in COVID-19+ emergency room patients compared to COVID-19- controls.

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BACKGROUND

Early during the COVID-19 pandemic, saliva testing became one of the fastest diagnostic options to identify whether a person was infected with SARS-CoV2. Saliva is a relatively easy biologic sample to collect from patients as it is a non-invasive procedure performed by simply asking a patient to "spit in a cup" once consented. The respiratory tract extends up from the alveoli, bronchioles, to the major bronchus, up to the trachea and esophagus. At this convergence between the lungs and the GI tract, the ciliary machinery on epithelial cells mechanically moves respiratory secretions (containing mucus, foreign debris, and viral particles) up out of the lungs and over into the esophagus or GI tract. We thought that this large interconnected mucosal surface could be examined via saliva collection in individuals infected with SARS-CoV2 to determine aspects of immune function. As such we hypothesized that we would detect increased amounts of CD45+ cell populations originating from the respiratory tract in SARS-CoV2 infected individuals in comparison to non-infected controls. More detailed flow cytometric analysis revealed that CD45+ CD8+ T cells were specifically increased in salivary secretions of COVID-19+ male patients compared to females, and even at relatively small amounts, statistical associations were drawn between lymphocyte numbers in the saliva and clinical outcomes, such as oxygen saturation and respiration rate.

MATERIALS & METHODS

- Patient Population: Male and female emergency room patients were approached at random to determine eligibility for the study. Individuals over the age of 18+ were enrolled in the study, relevant demographic data is presented in Table 1.
- SARS-CoV2 was inactivated by incubating saliva samples 1:1 with Streck Preservative for 12 hours prior to flow cytometry testing.
- Saliva was washed with FACs stain buffer using previous protocols, and incubated with Fc Block followed by a panel of anti-human antibodies.
- A 22-parameter flow cytometry data was acquired on the Cytek Aurora operated by the University of Utah Flow Cytometry Core.
- A randomized selection of saliva samples were applied to glass slides to perform cell differentials. These were air dried, fixed with methanol and stained with Giemsa Stain according to standard protocols. Images are shown at 10-20x to demonstrate the cell differentials present in salivary secretion of COVID-19+ individuals in comparison to controls (Figure 1).
- Statistical differences were determined using ANOVAs with appropriate post tests, and correlations were determined using Pearson’s correlation matrix. All statistics were performed using the analysis software that accompanies GraphPad Prism, version 10.

RESULTS

We have shown that flow cytometry may be an effective strategy to identify immune populations associated with poor clinical outcomes and need for hospitalization. The limitation of the study is that saliva provides only a glimpse into the respiratory tract, and therefore, more studies are necessary to understand the role of these cells, whether pathologic or protective, in SARS-CoV2 immunity.

One of the more striking findings from this study was the association between male gender and lower oxygen saturation in the COVID-19+ individuals. This highlights the need for understanding biological sex, and differences in immune and pulmonary function, in SARS-CoV2 infection. Future studies potentially should include those that are critically ill to determine whether CD8- and CKCR3-positivity are associated with worse outcomes compared to outpatients. The relative ease by which these flow cytometric data were collected warrants consideration as a diagnostic test to identify these biomarkers of SARS-CoV2 based on salivary cell content.

CONCLUSION

The last analysis determined whether clinical outcomes (hospitalization, O2 blood saturation [O2 sat, or respiration rate [rr]) in COVID-19+ patients correlated with immune outcomes. Figure 3A shows the total number of hospitalization by sex in the COVID-19+ ER cohort. Figure 3B. Shows that COVID-19+ males had a decrease in O2 saturation in comparison to female COVID-19+, however, rr was not different between male and female COVID-19+ individuals (3C). For the next analysis a Pearson correlation matrix (3D & 3E), with male and female cases in separate analysis. A green box indicates a significant association (p < 0.05) between a clinical outcome and an immune outcome (e.g CD45+ cells vs hospitalization; 3D). O2 sat and rr correlated with hospitalization in both males and females. CD45+ cell numbers correlated with hospitalization, O2 sat and rr in females, whereas only CD3+ T cells correlated with hospitalization in males. Interestingly, salivary CD3+ T cells, CD8+ and CD8+CKCR3- T cells subsets, negatively correlated (p <0.05) with rr in male patients.