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**Association of Serum Amylase Levels With Mortality in Critically Ill Patients With Coronavirus Disease 2019**

**To the Editor:**

Progress in the understanding of coronavirus 2019 (COVID-19) has been substantial but incomplete. Infection of the gastrointestinal system with the SARS-CoV-2 virus may result in systemic inflammation and increased bowel permeability. Serum amylase released from the pancreas into the gut may exit the bowel and enter the systemic circulation in critical illness. Increases in serum amylase may thus serve as a surrogate marker for impaired bowel integrity and severity of illness in COVID-19. 

We sought to test the hypothesis that pancreatic inflammation as assessed by serum amylase is associated with poor outcomes in critically ill patients with COVID-19.

**MATERIAL AND METHODS**

The University of California, San Diego institutional review board approved waiver of consent for this prospective observational cohort study. All adult patients with COVID-19 admitted to the intensive care unit (ICU) for more than 1 day between May 1, 2020, and October 31, 2020, were enrolled. A random sample of COVID-19 “rule-out” patients admitted to the ICU and subsequently found to be COVID-19 negative were enrolled as comparators.

Serum amylase levels were recorded on all patients as part of usual care on ICU days 1, 4, 7, 10, and 20 as available. Maximum amylase levels in survivor and nonsurvivor cohorts during ICU stay were recorded as the primary outcome, with amylase 150 U/L or greater defined as “elevated.” Demographics, laboratory values, and outcomes were gathered as part of routine (Table 1).

Amylase values, demographics, interventions, laboratory values, and comorbidities between survivor and nonsurvivor cohorts were compared using Welch 2-sample t test and Pearson χ² test for continuous and categorical variables, respectively. We performed univariate logistic regression for each covariate to determine its association with mortality. Covariates with P < 0.2 on univariate logistic regression were included in the initial model for the multivariable logistic regression. The final model was built via a combination of forward selection and backward elimination based on the Akaike information criterion. From the final model, the association of elevated amylase to mortality was reported. Odds ratios (OR) and their 95% confidence intervals (CI) were reported for covariates. P < 0.05 was considered to be significant.

We assessed discrimination of a predictive model using variables available upon ICU admission and reported the area under the receiver operating characteristics curve (AUC). The model was tested via 10-fold cross validation, and the mean AUC with 95% CI was reported.

**RESULTS**

Of the 137 patients with COVID-19 enrolled, there were 29 (21.2%) patients with a maximum amylase level during their ICU stay of 150 U/L or greater. Overall, 38 of the 137 patients with COVID-19 (27.8%) died. There was a statistically significantly greater proportion of patients who died in the elevated amylase cohort (44.8%) compared with the nonelevated amylase cohort (23.1%, P = 0.037) (Table 1). In contrast, mortality in COVID-19 rule-out patients (n = 23) was significantly less (17% vs 27.8%, P = 0.015) than for COVID-19–positive patients.

On univariate logistic regression analysis modeling covariates to mortality, maximum amylase 150 U/L or greater (OR, 2.70; 95% CI, 1.14–6.36; P = 0.02), endotracheal intubation (OR, 6.38; 95% CI, 1.83–22.24; P = 0.004), use of extracorporeal membrane oxygenation (OR, 3.18; 95% CI, 1.20–8.0; P = 0.02), use of continuous renal replacement therapy (OR, 15.5; 95% CI, 5.47–43.94; P = 0.0001), pressor requirement (OR, 9.74; 95% CI, 3.50–27.1; P < 0.0001), and presence of a concomitant infection (OR, 2.61; 95% CI, 1.20–5.69; P = 0.02) were associated with increased mortality.

We performed multivariable logistic regression to determine the association of maximum amylase values during ICU stay with mortality. The final model included maximum amylase, age, extracorporeal membrane oxygenation, continuous renal replacement therapy, need for pressors, tracheostomy, elevated white blood cell count, elevated D-dimer, and elevated C-reactive protein. When controlling for confounders, patients with COVID-19 with elevated maximum amylase levels had significantly increased odds of mortality (OR, 4.64; 95% CI, 1.07–20.23; P = 0.04) (Fig. 1).

From our predictive model for mortality using only covariates known at time of ICU admission, an initial amylase of 150 U/L or greater was predictive of mortality (OR, 6.17; 95% CI, 1.61–23.63; P = 0.008). The mean AUC was 0.769 (95% CI, 0.676–0.854) calculated on 10-fold cross-validation.

**DISCUSSION**

Results from this study demonstrate a strong association between elevated serum amylase and mortality in ICU patients with COVID-19. This finding is consistent with the notion that bowel permeability may be causally important in the pathophysiology of COVID-19. In theory, amylase elevations in ICU patients with COVID-19 reflect pancreatic inflammation (ie, amylase production) rather than changes in small bowel permeability, and elevated amylase has not consistently been associated with worsened outcomes in retrospective analyses of general (non–critically ill) hospitalized populations. Nonetheless, results from this study implicate bowel and perhaps pancreatic dysfunction in the propagation of severe disease in patients with COVID-19. Our design does not allow definitive mechanistic conclusions, and we welcome further data to corroborate or refute our findings. We believe our findings may be clinically important and hope that they stimulate further research.

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The authors declare no conflict of interest.

The views and results presented here are entirely those of the authors and do not necessarily represent those of the Department of Defense or its components.

Ethics approval and consent to participate—included in text. The University of California, San Diego Institutional Review Board (IRB) provided approval of the study conduct (IRB approval #2007222); subject informed consent was waived by the IRB because of the observational nature of the study and deidentified use of the data.

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

M.K. helped with data collection and analysis, manuscript review, and approval.
R.A.G. helped with statistical analysis and interpretation, manuscript preparation, review, and approval; A.M. helped to prepare, review, and approve the manuscript; and E.B.K. helped to design, prepare, review, and approve the manuscript.

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TABLE 1. Characteristics of the Amylase Cohorts

| Covariate                        | Maximum Amylase ≤150 U/L, n (%) | Maximum Amylase >150 U/L, n (%) | P     |
|---------------------------------|---------------------------------|---------------------------------|-------|
| Total, n                        | 108                             | 29                              |       |
| Inpatient mortality             | 25 (23.1)                       | 13 (44.8)                       | 0.037 |
| Age, mean (SD), y               | 58.9 (15.05)                    | 57.0 (14.3)                     | 0.56  |
| Sex, male, n                    | 70                              | 12                              | 0.04  |
| Ethnicity                       |                                  |                                 | 0.99  |
| Non-Hispanic                    | 28 (25.9)                       | 5 (17.2)                        |       |
| Hispanic                        | 79 (73.1)                       | 24 (82.8)                       |       |
| Unknown                         | 1 (0.9)                         | 0                               |       |
| Required endotracheal intubation| 74 (68.5)                       | 25 (86.2)                       | 0.098 |
| ECMO                            | 16 (14.8)                       | 4 (13.8)                        | 0.99  |
| CRRT                            | 15 (13.9)                       | 10 (34.5)                       | 0.02  |
| Required pressors               | 54 (50.0)                       | 19 (65.5)                       | 0.2   |
| Had another infection           | 47 (43.5)                       | 20 (69.0)                       | 0.03  |
| Received tracheostomy           | 20 (18.5)                       | 7 (24.1)                        | 0.68  |
| Comorbidities                   |                                  |                                 |       |
| COPD                            | 6 (5.6)                         | 0                               | 0.43  |
| Chronic kidney disease          | 13 (12.0)                       | 2 (6.9)                         | 0.65  |
| End-stage renal disease         | 4 (3.7)                         | 0                               | 0.67  |
| Coronary artery disease         | 10 (9.3)                        | 2 (6.9)                         | 0.98  |
| Hypertension                    | 58 (53.7)                       | 11 (37.9)                       | 0.19  |
| Congestive heart failure        | 11 (10.2)                       | 2 (6.9)                         | 0.86  |
| Cardiac arrhythmia              | 7 (6.5)                         | 2 (6.9)                         | 0.99  |
| Diabetes mellitus               | 44 (40.7)                       | 10 (34.5)                       | 0.69  |
| Obesity                         | 21 (19.4)                       | 7 (24.1)                        | 0.77  |
| Liver cirrhosis                 | 3 (2.8)                         | 0                               | 0.85  |
| Maximum laboratory values, mean (SD) |                      |                                 |       |
| White blood count, 10^9/L       | 16.4 (8.7)                      | 19.7 (10.8)                     | 0.14  |
| International normalized ratio  | 1.5 (0.58)                      | 1.3 (0.19)                      | 0.03  |
| D-dimer, ng/mL                  | 6812 (12.370)                   | 4407 (8766)                     | 0.35  |
| Fibrinogen, mg/dL               | 656.3 (241.3)                   | 645.5 (237.4)                   | 0.88  |
| Ferritin, mcg/L                 | 3052.1 (6702.8)                 | 4784 (14,784.6)                 | 0.66  |
| C-reactive protein, mg/L        | 20.4 (13.4)                     | 17.3 (12.6)                     | 0.37  |
| Lactate dehydrogenase, U/L      | 584.5 (353.1)                   | 599.9 (515.8)                   | 0.91  |
| Procalcitonin, ng/mL            | 6.5 (33.3)                      | 4.0 (11.0)                      | 0.56  |
| Lactate, mmol/L                 | 3.7 (5.0)                       | 4.4 (4.6)                       | 0.49  |

P was calculated by Welch 2-sample t test and Pearson χ² test for continuous and categorical variables, respectively.

COPD indicates chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; CRRT, continuous renal replacement therapy; SD, standard deviation.

R.A.G. helped with statistical analysis and interpretation, manuscript preparation, review, and approval; A.M. helped to prepare, review, and approve the manuscript; and E.B.K. helped to design, prepare, review, and approve the manuscript.

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Letters to the Editor
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Recent studies have investigated accounting for 1% to 2% of all pancreatic epithelial-derived pancreatic neoplasms, tumors.1

To the Editor:

Pancreatic neuroendocrine tumors (PanNETs) constitute a heterogeneous group of epithelial-derived pancreatic neoplasms, accounting for 1% to 2% of all pancreatic tumors.2 Recent studies have investigated various immunotherapy options for neuroendocrine tumors (NETs), including checkpoint inhibitors, chimeric antigen receptor–T-cell therapy, and neoantigen-based therapeutic cancer vaccines.2–4 Nevertheless, such approaches may have only modest to low clinical utility. Significant heterogeneities of tumor immune microenvironments (TIMEs) in primary and metastatic tumors may exhibit different responses to immunotherapy,5 and most well-differentiated NETs were considered “immunologically cold” tumors.2,6 Personalized neoantigens arising from somatic mutations may be targets of tumor-specific immune responses via T-cell expansion. Previous studies have demonstrated the correlation of tumor mutation burden with the number of neoantigens and immune status.7 However, in PanNETs, whether T cells that recognize tumor neoantigens can be elicited from the patient’s peripheral blood has yet to be investigated, and the potential of neoantigen-based immunotherapy remains unexplored.

We have recently reported that a patient with a well-differentiated PanNET (G2) developed multiple metastases in the liver and local lymph nodes (LNs) over ~13 years.6 Following 2 years after his last surgery, the patient refused chemotherapy and remained on oral everolimus. Computed tomography scans (once 1 to 6 months) showed additional metastatic nodules in the LNs, lungs, and liver, progressing steadily. Given a probable resistance to everolimus,6 we investigated neoantigen-based therapeutic potential in vitro in this case. The patient was in good mental health and approved our study for the potential of neoantigen-based immunotherapy with informed consent.

We first characterized the extent of heterogeneity of TIMEs among primary and metastatic tumor tissues using immune-repertoire sequencing. The materials and methods are provided in detail in the Supplemental Digital Content (http://links.lww.com/MPA/A967). Compared with metastatic tissues, the hypermutated primary tumor showed relatively lower T-cell receptor repertoires (ie, the normalized Shannon–Wiener index) (Fig. 1A) because of a relatively limited cancer cell fraction.6 Thus, the corresponding immunogenicity in the primary tumor is limited. The complementarity determining region 3 (CDR3) clonotype showed homogeneity in anatomical locations, in which samples from the liver and LNs were separately clustered (Fig. 1B). According to the 50 most frequent clonotypes from each tumor sample, the predominantly unique clonotypes in the primary tumor suggested an exclusive TIME; however, the clonotype was longitudinally and spatially homogeneous in metastases from the same tissue (Fig. 1C).

The immunological state (eg, T-cell infiltration) in metastatic sites may have important implications for immunotherapy. The number of helper CD4+ and cytotoxic CD8+ T cells reduced significantly in the liver metastatic samples compared with their adjacent noncancerous tissues (P = 0.0005 and 0.0007, respectively), but not FOXP3+ T cells (P = 0.3), corresponding to a significant increased FOXP3/CD4 ratio (Fig. 1D). Immunohistochemistry staining for CD4+, CD8+, and FOXP3+ T cells confirmed a reduced T-cell infiltration (Fig. 1E). The T-cell receptor diversity was positively correlated with the number of CD4+ but not with CD8+ or FOXP3+ T cells (Fig. 1F). These results suggested an “immunologically cold” sign in PanNET metastases.

We finally investigated the immunotherapeutic potential of tumor neoantigen by analyzing antigen-specific T-cell populations with peptide-major histocompatibility complex (pMHC) tetramers. Tumor neoantigens were prioritized based on an in silico prediction and an evolutionary principle (see clonal analysis)6 (Fig. 1G). Then, we synthesized 3 candidate neoantigen peptides (Fig. 1H) to assess their effects in inducing neoantigen-reactive T cells from the patient’s peripheral blood in vitro. Finally, the presence of antigen-specific CD8+ T cells with pMHC was counted by flow cytometry. Unfortunately, we did not note a significant CD8+ T-cell expansion stimulated by neoantigens pMHC-tetramers (Fig. 1I). A complete delineation of the immune landscape of PanNETs may pave the way for developing precision immunotherapy for this rare disease. Although an “immunologically cold” state in pancreatic ductal carcinoma has been characterized previously, an increased tumor mutational burden and CD8+ T cell were associated with long-term survival.8 In the present case, multiple metastatic sites demonstrated a lower tumor mutational burden than a “hypermutator” phenotype noted in the primary tumor.8 The concordant decrease in the number of neoantigens