

Original Article



The Association of Serum Zinc, Copper and Selenium Levels on Mortality, Need for Mechanical Ventilation and Duration of ICU Stay in Critically Ill Patients with COVID-19: A Cross-Sectional Study

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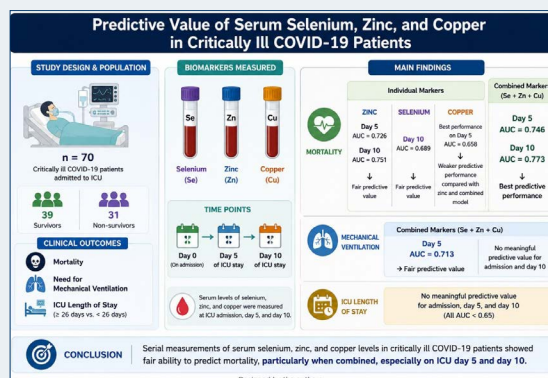
Abstract

Introduction: The objective of the study was to evaluate the effect of serum zinc (Zn), copper (Cu), and selenium (Se) levels on outcomes of patients with Coronavirus disease 2019 (COVID-19) admitted to the intensive care unit (ICU).

Methods: This cross-sectional study was conducted on COVID-19 patients. Serum concentrations of Zn, Cu, and Se on admission, on the 5th and 10th days in the ICU were measured in all subjects, and their association with patients' outcomes were evaluated. The area under curve (AUC) of the receiver operating characteristic (ROC) curve and adjusted binary logistic regression analysis were used to explore the association between Zn, Cu, and Se levels on mortality as the primary outcome.

Results: Seventy patients, including 33 (47.1%) men and 37 (52.9%) women, were enrolled. Lower serum levels of Zn (85.93 ± 14.20 vs. 92.55 ± 8.25 , $P=0.017$) and Se (97.29 ± 11.74 vs. 104.74 ± 9.17 , $P=0.004$) were observed in the non-survivors group compared to survivors of COVID-19. In adjusted analysis, higher Zn levels were associated with lower odds of mortality on 5th (OR:0.909, 95%CI:0.839-0.985, $P=0.020$) and 10th (OR:0.911, 95%CI:0.85-0.975, $P=0.007$) day of ICU and increased with higher levels of Cu on 5th day of ICU (OR:2.852, 95%CI:1.487-7.666, $P=0.034$).

Conclusion: This study suggests a fair predictive value of Zn and a poor predictive value of Se and Cu, as well as a fair predictive value of their cumulative effect on mortality. However, these micronutrients did not predict the need for mechanical ventilation and ICU Length of stay. Except for the combination of serum Se, Zn, and Cu on the 5th day of ICU, which had fair predictive value for mechanical ventilation.



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Introduction

Following the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in Wuhan, China between late 2019 and February 2022, more than 388 million cases of coronavirus disease 2019 (COVID-19) have been reported globally, and more than 5.7 million people have died as a result.¹⁻³ In addition, the emergence of new variants of SARS-CoV-2 with altered traits effecting the virus characteristics, such as transmissible and virulent, has made it even more difficult to try to end the pandemic.^{4,5} Even though over half of the world's population has received recommended vaccines, vaccination alone was not sufficient enough to stop the disease from spreading further,⁶ and it remains important to study the underlying mechanism of the COVID-19 pathogen.⁷ Growing evidence suggests that host immune dysregulation is a major contributor to disease progression and adverse outcomes in COVID-19.^{8,9} Excessive immune activation following viral replication may trigger an exaggerated inflammatory response characterized by increased cytokine production.^{10,11} This may result in coagulopathy, thromboembolic consequences, acute inflammation, and hyperimmune response.¹²

The immune system needs enough micronutrients to perform its essential monitoring and defensive functions.¹³ Research indicates that a micronutrient deficit may weakens the body's defenses against COVID-19 and accelerates the development of serious illness.¹⁴ Serum Zinc (Zn), Copper (Cu), and Selenium (Se) levels are suggested as potential predictors of poor outcomes in critically ill patients. Essential metals, also known as biometals, are micronutrients that have signaling, catalytic, cofactor, and structural functions in the functioning of organisms. Zn, Cu, and Se are essential elements that regulate immune system function, including antiviral immunity and inflammatory response.¹⁵⁻¹⁸ Zn contributes to multiple immune and metabolic pathways and is involved in maintaining normal antiviral defense mechanisms. In association with COVID-19, Inadequate Zn status may impair both innate and adaptive immune mechanisms, thereby increasing susceptibility to severe viral infections,¹⁹⁻²² lymphopenia,²³ and activation of Nuclear factor kappa B (NF- κ B) pathway that produces interleukin 6 (IL-6) and other cytokines involved in "cytokine storm".²⁴⁻²⁶ Furthermore, studies have indicated that Zn can directly combat viruses by hindering the replication of SARS-CoV in laboratory settings.²⁷ Cu participates in several biological processes including antioxidant defense, cellular metabolism, and immune regulation.^{17,28} Cu can have an active antioxidant response during inflammation, same as Zn. Because Cu deficiency reduces T-cell proliferation, it can cause immunosuppression and neutropenia.²⁹ Cu can also potentially have antiviral properties by stimulating autophagy.³⁰ Twenty-five different selenoproteins play a role in maintaining redox balance, managing stress in the endoplasmic reticulum, and regulating inflammatory responses.³¹ In general, Se can be a minor determinant of viral severity.³² Similar to Zn, Se is recognized for its

inhibitory effects on NF- κ B. Research suggests that it may reduce apoptosis triggered by NF- κ B activation and potentially contribute to cytokine storms observed in severe cases of COVID-19.³³ Se deficiency causes oxidative stress in the host, which not only disrupts the host's immune system but also rapidly mutates benign RNA viruses into pathogens.³⁴ Studies conducted in laboratory settings indicated that Se supplementation can hinder the reproduction of the circovirus.³⁵

Although several studies have explored the association between trace elements and COVID-19 outcomes, their findings remain inconsistent.^{28,29,36,37} Thus, we conducted this study to evaluate the association of serum Zn, Cu, and Se levels in COVID-19 patients admitted to the intensive care units (ICU)s.

Materials and Methods

Study design and ethics statement

This single-center, cross-sectional study was conducted on COVID-19 patients admitted to the ICU of Emam Khomeini Hospital, Ardabil University of Medical Sciences, Ardabil, Iran. This medical facility in Ardabil Province serves as both an educational and healthcare institution, providing 551 beds (340 active beds) for the treatment of various adult health issues. The protocol study was reviewed and approved by the Research Council of Ardabil University of Medical Sciences under the ethics code IR.ARUMS.REC.1399.056 in accordance with the Declaration of Helsinki of the World Medical Association.³⁸ In addition, the study was conducted and reported in accordance with the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.³⁹

Study population

This research recruited adult patients with severe COVID-19 who were hospitalized to ICUs. The study did not include patients who were pregnant or who were using any kind of nutritional supplements, multivitamins, or trace elements. An infectious disease specialist used the Iranian National Committee on COVID-19's recommendations and the World Health Organization's (WHO) interim guidelines to identify the subjects. Positive results from a chest computed tomography (CT) and real-time polymerase chain reaction (RT-PCR) of the respiratory tract samples were used to diagnose COVID-19.

Data collection and outcomes

This study gathered data on the demographic and clinical characteristics such as comorbidities, severity of illness based on Acute Physiology and Chronic Health Evaluation (APACHE) IV, and laboratory data including hemoglobin, white blood cells (WBCs) and platelet; as well as comorbidities including hypertension, chronic kidney disease, malignancies, lung diseases, ischemic heart disease, neurological comorbidities, and diabetes mellitus. APACHE score is used for in-patient mortality prediction based on physiological and laboratory variables. This score

utilizes multivariate linear regression to predict hospital mortality and ICU length of stay (LOS). Vandenbrande et al. aimed that APACHE IV in critically ill COVID-19 patients presents the best scoring system for discrimination and calibration.⁴⁰ The primary outcome was mortality rate and the secondary outcomes were undergoing mechanical ventilation (MV) and LOS.

Measurement the Serum levels of Zn, Cu, and Se

Fasting blood samples (3 cc) were collected from all patients at the time of ICU admission, on the 5th day, and on the 10th day of ICU stay and were centrifuged within 30 min of collection. The serum samples were stored at -20°C. All specimens were handled with appropriate care, following standard laboratory protocols that accounted for potential COVID-19 complications. Serum Zn and Cu levels were measured using Randox colorimetric assays (Randox Laboratories Ltd., Crumlin, UK). According to the kit's instructions, the established reference ranges were 70-140 µg/dL for serum Cu, and for serum Zn, they were 72.6-127 µg/dL for men and 77.0-114 µg/dL for women. Serum Se concentrations were quantified using graphite furnace atomic absorption spectrophotometry (GFAAS).⁴¹ Regarding Se, normal reference values were considered to be 70 to 150 µg/L.

Statistical analysis

The Shapiro-Wilk test was performed to assess the data's normal distribution. For continuous variables, normally distributed data are expressed as mean and standard deviation (SD), and for categorical traits, as percentage (%). However, the median and Interquartile Range (IQR) are used to represent data that are not normally distributed. The t-test or Chi-square (Fisher's exact test) for continuous and categorical variables, respectively, were used to compare clinical factors and demographic traits between the non-survivor and survivor groups. Two-way analysis of variance with repeated measurements (RMANOVA), adjusted, and non-adjusted models were used to evaluate changes in blood levels of Se, Zn, and Cu according to time trends (on admission, fifth day, and tenth day of ICU). Age, gender, comorbidities, and APACHE IV score were among the baseline data that were taken into consideration while creating the adjusted RMANOVA model. Binary logistic regression, both unadjusted and adjusted (adjusting for age, gender, comorbidities, and APACHE IV), was used to determine the predictive effect of Se, Zn, and Cu on outcomes (mortality, MV, and ICU length of stay). The results were presented as odds ratios (OR) with 95% confidence intervals (CI). For Cu, Zn, and Se, the receiver operating characteristic (ROC) the curve was created at various points in time. Area under curve (AUC) between (0.9–1.0), (0.8–0.9), (0.7–0.8), and (0.6–0.7) was regarded as outstanding, good, fair, and bad, respectively, according to the general recommendation for the discriminative power of a test based on ROC. SPSS (ver. 21) was used for statistical analysis (IBM Corp. Released 2012).

Results

Patient's characteristics

Demographic and clinical characteristics of participants according to survivor and non-survivor groups are presented in Table 1. A total of 70 patients with COVID-19 were enrolled, including 33 men (47.1%) and 37 women (52.9%). COVID-19 survivors and non-survivors showed similar baseline characteristics, except for significantly higher age (61.71 ± 14.66 vs. 58.33 ± 14.46 , $P=0.009$) and APACHE IV scores (31.61 ± 8.61 vs. 27.85 ± 5.90 , $P=0.034$) in non-survivors compared with survivors.^{3,2}

Time trends of micronutrients changes

Time trend changes of Se, Zn, and Cu levels on admission, 5th day, and 10th day of ICU between survival and non-survival groups are presented in Table 2. Comparison of the mean serum levels of Se on admission, 5th day, and 10th day of ICU in the non-survival group showed a significant decrease (106.7 ± 13.60 to 97.29 ± 11.74 , $P<0.001$). However, no significant change in Se levels was observed in the survival group at the three times intervals (104.1 ± 10.85 to 104.74 ± 9.17 , $P=0.426$). The mean level of serum Se on the 10th day of ICU in non-survival group was significantly lower than the survivor group (97.29 ± 11.74 vs. 104.74 ± 9.17 , $P=0.004$) (Figure 1A). The results of the ANOVA repeated measure on Se showed a significant time and group interaction in adjusted analyses according to age, gender, comorbidities, and APACHE IV score ($P<0.001$) (Supplementary File, Figure S1).

Comparison of the mean serum levels of Zn three times on admission, 5th day and 10th day of ICU showed a significant reduction in the non-survivor group (90.28 ± 6.53 to 85.82 ± 7.61 , $P=0.002$). However, in three different periods, no significant changes in selenium levels were observed in the surviving group (91.88 ± 9.31 vs. 92.82 ± 8.76 , $P=0.156$). According to the results, the mean level of Zn on the 5th day (85.93 ± 14.20 vs. 92.55 ± 8.25 , $P=0.017$) and 10th day (85.82 ± 7.61 vs. 92.82 ± 8.76 , $P<0.001$) of ICU in the patients of the non-survival group was remarkably lower than the survival group (Figure 1B). The results of the Analysis of variance (ANOVA) repeated measure on Zn showed a significant time and group interaction in adjusted and unadjusted analyses between two groups ($P<0.001$) (Supplementary File, Figure S2).

Comparison of the mean serum levels of Cu at three times on admission, 5th day, and 10th day of ICU showed a significant reduction in both survival (1.15 ± 0.22 to 0.964 ± 0.121 , $P<0.001$) and non-survivor (1.21 ± 0.32 to 0.977 ± 0.163 , $P<0.001$) groups. The mean level of serum Cu on the 5th day (1.07 ± 0.140 vs. 1.01 ± 0.106 , $P=0.0125$) of ICU in the patients of the non-survive group was remarkably higher than the survival group (Figure 1C). The results of ANOVA repeated measure on Cu showed no significant time and group interaction in both adjusted ($P=0.556$) and unadjusted ($P=0.486$) analyses between the two groups (Supplementary File, Figure S3).

Table 1. Demographic and clinical characteristics of participants according to survivor and non-survivor groups

Variables		Total Participants (n = 70)	Survivors (n = 39)	Non-survivors (n = 31)	P-value
Age (years)	Mean $\pm$ SD (range)	62.49 $\pm$ 15.19 (27-93)	58.33 $\pm$ 14.46 (27-38)	61.71 $\pm$ 14.66 (41-93)	0.009*
	Median (IQR)	64 (50.75-72.25)	59 (25-32)	70 (57-80)	
Gender	Male (%)	33 (47.1)	18 (46.2)	15 (48.4)	0.853
	Female (%)	37 (52.9)	21 (53.8)	16 (51.6)	
Comorbidities	Yes (%)	49 (70)	29 (74.4)	20 (64.5)	0.372
	No (%)	21 (30)	10 (25.6)	11 (35.5)	
Type of	HTN (%)	25 (35.7)	17 (43.6)	8 (25.8)	0.123
comorbidities	CKD (%)	6 (8.6)	2 (5.1)	4 (12.9)	0.395
	Cancer (%)	2 (2.9)	0	2 (6.5)	0.193
	Lung disease (%)	3 (4.3)	3 (7.7)	0	0.249
	IHD (%)	19 (27.1)	11 (28.2)	8 (25.8)	0.823
	Neurologic (%)	2 (2.9)	2 (5.1)	0	0.499
	DM (%)	20 (28.6)	14 (35.9)	6 (19.4)	0.128
APACHE IV	Mean $\pm$ SD (range)	29.51 $\pm$ 7.41 (15-60)	27.85 $\pm$ 5.901 (15-40)	31.61 $\pm$ 8.605 (16-60)	0.034*
	Median (IQR)	30 (25-34)	28 (25-32)	30 (27-35)	
Hb	Mean $\pm$ SD (range)	13.09 $\pm$ 2.32 (7.4-20)	12.89 $\pm$ 2.311 (8.4-20)	13.34 $\pm$ 2.35 (7.4-19)	0.423
	Median (IQR)	13.2 (11.55-14.7)	13.2 (11-13.9)	13.2 (11.9-15)	
Platelet	Mean $\pm$ SD (range)	191014.3 $\pm$ 82440.7 (53000-525000)	200666.6 $\pm$ 85439.5 (81000-525000)	178870.9 $\pm$ 78178.3 (53000-395000)	0.275
	Median (IQR)	173500 (236000-130000)	187000 (151000-263000)	169000 (117000-233000)	
WBC	Mean $\pm$ SD (range)	8477.14 $\pm$ 3593.92 (3000-18000)	8025.64 $\pm$ 3375.3 (3000-17000)	9045.16 $\pm$ 3831.3 (3300-18000)	0.241
	Median (IQR)	7950 (5575-10425)	7500 (5500-9900)	8400 (6200-10600)	
ICU LOS	Mean $\pm$ SD (range)	27.43 $\pm$ 9.95 (15-62)	25.95 $\pm$ 10.48 (15-62)	29.29 $\pm$ 9.07 (15-55)	0.165
	Median (IQR)	26 (20-32.5)	23 (18-30)	28 (22-35)	
MV	Yes (%)	61 (78.1)	32 (82.1)	29 (93.5)	0.153
	No (%)	9 (12.9)	7 (17.9)	2 (6.5)	

Abbreviations; Chronic kidney disease (CKD), Ischemic heart disease (IHD), Hypertension (HTN), Diabetes mellitus (DM), Hemoglobin (Hb), White Blood Cells (WBCs), length of stay (LOS), Mechanical ventilation (MV), Acute Physiology and Chronic Health Evaluation IV (APACHE IV), * $P < 0.05$ considered as significant

Table 2. Time trend changes of Selenium, Zinc, and Copper, on admission, 5th day, and 10th day of ICU stay according to survivor and non-survivor groups

Groups	Time trends			P-value ^a	Time interaction		Time * group interaction	
	On admission	5 th day	10 th day		Unadjusted (P-value) ^b	Adjusted (P-value) ^c	Unadjusted (P-value) ^d	Adjusted (P-value) ^e
Selenium (Se)								
Survivors	104.1±10.85	102.9±9.67	104.74±9.17	0.426	<0.001*	0.461	0.635	<0.001*
Non-survivors	106.7±13.60	104.2±8.76	97.29±11.74	<0.001*				
P-value ^f	0.357	0.579	0.004*					
Zinc (Zn) (µg/L)								
Survivors	91.88±9.31	92.55±8.25	92.82±8.76	0.156	0.080	0.008*	0.014*	0.036*
Non-survivors	90.28±6.53	85.93±14.20	85.82±7.61	0.002*				
P-value ^f	0.419	0.017*	0.001*					
Copper (Cu)								
Survivors	1.15±0.22	1.01±0.106	0.964±0.121	<0.001*	<0.001*	0.872	0.486	0.556
Non-survivors	1.21±0.32	1.07±0.140	0.977±0.163	<0.001*				
P-value ^f	0.361	0.025*	0.704					

* $P < 0.05$ considered as significant, ^a P-value based on paired t-test within the group, ^b Unadjusted P-value based on time interaction in repeated measures ANOVA, ^c Adjusted P-value (adjusting for age, gender, comorbidities and APACHE IV) based on time interaction in repeated measures ANOVA, ^d Unadjusted P-value based on time*group interaction in repeated measures ANOVA, ^e Adjusted P-value (adjusting for age, gender, comorbidities and APACHE IV) based on time*group interaction in repeated measures ANOVA, ^f P-value based on t-test between groups

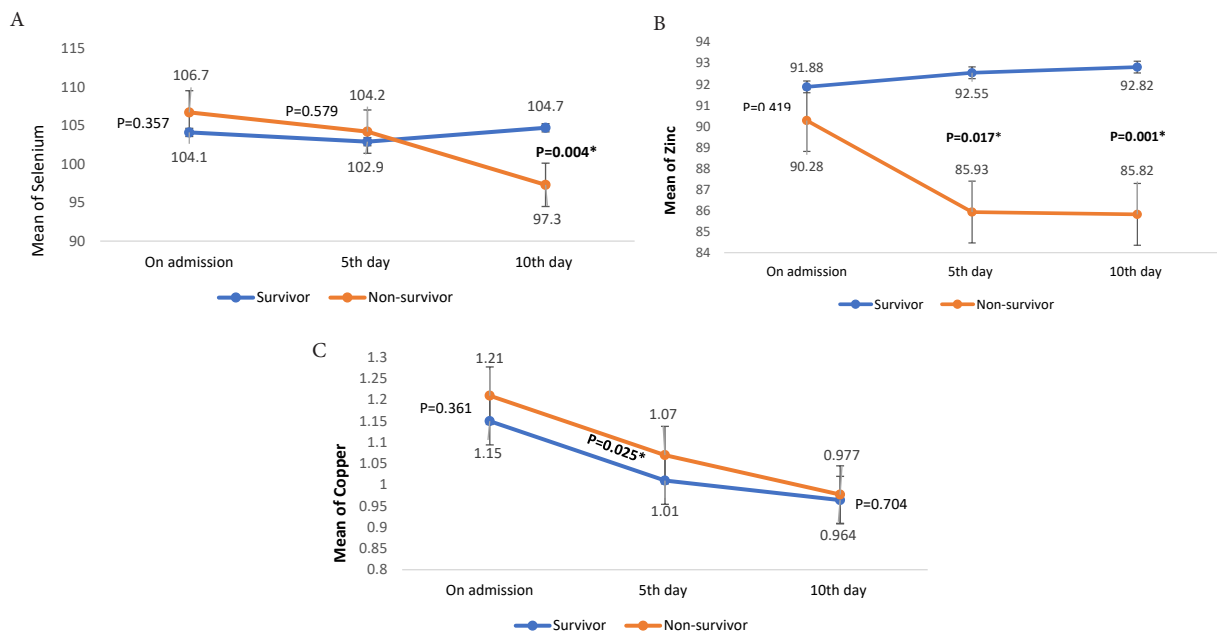


Figure 1. Time trend changes of (A) Selenium, (B) Zinc, and (C) Copper, on admission, the 5th day, and the 10th day of ICU

Logistic regression analysis

Unadjusted and adjusted binary logistic regression analysis to compare the predictive effect of Se, Zn, and Cu on outcomes including mortality, needed MV, and ICU length of stay are presented in Table 3. According to the unadjusted analysis, minimal evidence of reduction of odds ratio (OR) for mortality was obtained from higher levels of Se on 10th day of ICU (OR: 0.929, 95%CI: 0.88-0.981, $P=0.008$), Zn on 5th day of ICU (OR: 0.921, 95%CI: 0.858-0.987, $P=0.020$) and Zn on 10th day of ICU (OR: 0.904, 95%CI: 0.847-0.964, $P=0.002$). However, the OR of mortality was remarkably increased with higher levels of Cu on the 5th day of ICU (OR: 1.283, 95%CI: 1.498-8.22, $P=0.032$). In adjusted analysis, OR of mortality was decreased with higher levels of Zn on 5th (OR: 0.909, 95%CI: 0.839-0.985, $P=0.020$) and 10th (OR: 0.911, 95%CI: 0.85-0.975, $P=0.007$) day of ICU and increased with higher levels of Cu on 5th day of ICU (OR: 2.852, 95%CI: 1.487-7.666, $P=0.034$). Whereas, no predictive effect of Se, Zn, and Cu was observed on MV and LOS ICU at different measurement times. Except the OR of ICU LOS was significantly increased with the level of Se on the 10th day of ICU in both unadjusted (OR: 1.058, 95%CI: 1.00-1.12, $P=0.049$) and adjusted (OR: 1.081, 95%CI: 1.014-1.153, $P=0.017$) analysis.

ROC curve results of micronutrients for predicting outcomes

Table 4 shows the predictive value of Se, Zn, and Cu for outcomes with cutoff points in different measurement times. According to the results, Se, Zn, and Cu had fair predictive values for mortality with AUC between 0.658 and 0.751. Best performing predictive value for mortality was related to the level of Zn on 10th day of ICU (AUC: 0.751, 95% CI: 0.633-0.846, $P<0.0001$), and the best cut-off value (≤ 88) had a value sensitivity 67.74%, specificity

82.05%, (LR+) 3.77, (LR-) 0.39, PPV 75%, NPV 76.2%, and 0.497% of Yuden index. Lower performing predictive value for mortality was related to the level of Zn on 5th day of ICU (AUC: 0.658, 95% CI: 0.534-0.767, $P<0.017$), and the best cut-off value (> 1.02) had a value sensitivity 61.29%, specificity 69.23%, (LR+) 1.99, (LR-) 0.56, PPV 61.3%, NPV 69.2%, and 0.305% of Yuden index. However, the micronutrients had no significant predictive value for MV and ICU LOS with AUCs from 0.515 to 0.687 and 0.509 to 0.623, respectively. ROC curves of Se, Zn, and Cu to predict mortality, needed MV, and ICU LOS, on admission, the 5th day, and the 10th day of ICU are available in the Supplementary File, Figure S4).

Cumulative effect of micronutrients

To have a cumulative effect of micronutrients, a new marker in a prediction regression model was introduced in three different time intervals to predict the outcomes of COVID-19 (Table 5). Combined markers on the 5th day and 10th day of ICU had significant fair predictive value for mortality with AUCs (0.746, 95%CI: 0.628-0.843, $P<0.0001$) and (0.773, 95%CI: 0.658-0.865, $P<0.001$), respectively. According to a comparison of the AUCs using the Delongi test, the cumulative effect of micronutrients on the 5th day of ICU was comparable as a predictor of mortality with Se on the 5th day of ICU (AUC: 0.746 vs. 0.552, $P=0.0248$). In terms of the cumulative effect of micronutrients on the 10th day of ICU to predict mortality, it was comparable with Cu on the 10th day of ICU (AUC: 0.773 vs. 0.541, $P=0.0102$). However, there was no remarkable difference between the AUCs of the combined marker and Se on the 10th day of ICU (AUC: 0.773 vs. 0.689, $P=0.140$), Zn on the 5th day of ICU (AUC: 0.746 vs. 0.726, $P=0.626$) and Zn on 10th day of ICU (AUC: 0.773 vs. 0.751, $P=0.581$) for predicting mortality. In terms of the cumulative effect of micronutrients on the 5th day of

Table 3. Unadjusted and adjusted binary logistic regression analysis to compare the predictive effect of Selenium, Zinc, and Copper on mortality, need for mechanical ventilation, and duration of ICU stay

Variables	Unadjusted		Adjusted	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Mortality vs. survival				
Selenium on admission	1.019 (0.979-1.06)	0.353	1.035 (0.989-1.083)	0.137
Selenium on 5 th day	1.015 (0.964-1.068)	0.574	1.042 (0.98-1.107)	0.187
Selenium on 10 th day	0.929 (0.88-0.981)	0.008*	0.944 (0.889-1.001)	0.055
Zinc on admission	0.976 (0.92-1.035)	0.414	0.972 (0.909-1.038)	0.398
Zinc on 5 th day	0.921 (0.858-0.987)	0.020*	0.909 (0.839-0.985)	0.020*
Zinc on 10 th day	0.904 (0.847-0.964)	0.002*	0.911 (0.85-0.975)	0.007*
Cu on admission	2.293 (0.389-4.502)	0.359	2.269 (0.355-4.496)	0.386
Cu on 5 th day	1.283 (1.498-8.22)	0.032*	2.852 (1.487-7.666)	0.034*
Cu on 10 th day	1.949 (0.066-7.48)	0.699	2.393 (0.054-9.192)	0.651
Need for MV vs. no need for MV				
Selenium on admission	1.022 (0.962-1.086)	0.482	1.081 (0.973-1.201)	0.147
Selenium on 5 th day	1.042 (0.953-1.138)	0.366	1.144 (0.982-1.334)	0.084
Selenium on 10 th day	1.006 (0.944-1.073)	0.844	1.067 (0.946-1.205)	0.289
Zinc on admission	0.956 (0.873-1.047)	0.333	0.915 (0.801-1.045)	0.189
Zinc on 5 th day	0.921 (0.822-1.032)	0.158	0.894 (0.765-1.046)	0.162
Zinc on 10 th day	0.925 (0.843-1.014)	0.097	0.872 (0.749-1.016)	0.078
Cu on admission	0.806 (0.064-1.096)	0.867	0.718 (0.017-2.657)	0.861
Cu on 5 th day	0.215 (0.001-4.315)	0.573	0.681 (0.021-6.671)	0.944
Cu on 10 th day	2.859 (0.018-4.227)	0.683	3.124 (0.067-8.982)	0.189
ICU length of stay (≥ 26 days vs. < 26 days)				
Selenium on admission	1.033 (0.991-1.077)	0.126	1.047 (1-1.095)	0.051
Selenium on 5 th day	1.058 (1.00-1.12)	0.049*	1.081 (1.014-1.153)	0.017*
Selenium on 10 th day	1.019 (0.975-1.064)	0.411	1.042 (0.99-1.096)	0.114
Zinc on admission	1.017 (0.96-1.078)	0.563	1.017 (0.957-1.081)	0.583
Zinc on 5 th day	0.999 (0.959-1.04)	0.951	1.01 (0.964-1.059)	0.668
Zinc on 10 th day	1.006 (0.954-1.06)	0.832	1.017 (0.961-1.076)	0.569
Cu on admission	1.718 (0.296-3.973)	0.546	1.595 (0.27-3.412)	0.606
Cu on 5 th day	1.072 (0.026-4.998)	0.971	0.555 (0.011-7.453)	0.768
Cu on 10 th day	0.145 (0.004-4.723)	0.278	0.08 (0.002-3.508)	0.19

Adjusting for age, gender, comorbidities, and APACHE IV * $P < 0.05$ considered as significant, Abbreviations: Odds ratio (OR), Confidence Interval (CI)

ICU for predicting MV, results showed the increase in AUC (AUC: 0.713, 95%CI: 0.593-0.815, $P = 0.032$) to compare AUCs for each micronutrient. However, these differences were not remarkably ($P > 0.05$). Comparison ROC curves of Se, Zn, and Cu with combined markers to predict mortality, MV, and ICU LOS, on admission, 5th day, and 10th day of ICU are presented in [Figure 2](#).

Discussion

In this single-center, cross-sectional study, we assessed serum levels of Zn, Cu, and Se in patients with severe COVID-19 and compared the predictive ability of these micronutrients on outcomes such as mortality, MV, and ICU LOS. In addition, to evaluate and compare the cumulative effect of micronutrients, the combination of Se, Zn, and Cu in three different time intervals was used as a new marker in the predictive regression to predict the consequences of COVID-19. Lower serum levels of Zn and

Se were observed in the non-survival group compared to survivors of COVID-19, which may indicate the association of the micronutrients with virus control and mortality. However, in adjusted regression analysis, results showed little evidence of association between micronutrients and COVID-19 outcomes. We found fair predictive value of Zn (5th and 10th day of ICU) and poor predictive value of Se (10th day of ICU) and Cu (5th day of ICU), as well as fair predictive value of their cumulative effect (5th and 10th day) for mortality. However, these micronutrients were not predicted to be needed for MV and ICU LOS. Except the combined serum Se, Zn, and Cu on the 5th day of ICU had a fair predictive value for MV with AUC (0.713).

Considering to antiviral, immunomodulatory, and anti-inflammatory functions of Zn, Cu, and Se, there has been a great deal of interest in their potential use in the management of the disease since the beginning of the COVID-19 pandemic.^{29,36,37,42} Numerous investigations

Table 4. ROC curve results of Selenium, Zinc, and Copper on admission, 5th day, and 10th day of ICU to predict mortality, need for mechanical ventilation, and duration of ICU stay

	Variables	AUC (95% CI)	P-value	SN	SP	LR+	LR-	PPV	NPV	Youden Index	Cut-point
Mortality	Selenium on admission	0.586 (0.462-0.703)	0.224	54.84	66.67	1.65	0.68	56.7	65.0	0.2151	>105
	Selenium on 5 th day	0.552 (0.428-0.671)	0.460	64.52	51.28	1.32	0.69	51.3	64.5	0.1580	>99
	Selenium on 10 th day	0.689 (0.567-0.794)	0.005*	41.94	100.0	16.35	0.58	100.0	68.4	0.4194	≤91
	Zinc on admission	0.593 (0.469-0.709)	0.183	80.65	48.72	1.57	0.40	55.6	76.0	0.2936	≤93.9
	Zinc on 5 th day	0.726 (0.606-0.826)	0.0004*	77.42	69.23	2.52	0.33	66.7	79.4	0.4665	≤90
	Zinc on 10 th day	0.751 (0.633-0.846)	<0.0001*	67.74	82.05	3.77	0.39	75.0	76.2	0.4979	≤88
	Cu on admission	0.560 (0.437-0.679)	0.408	51.61	71.79	1.83	0.67	59.3	65.1	0.2341	>1.2
	Cu on 5 th day	0.658 (0.534-0.767)	0.017*	61.29	69.23	1.99	0.56	61.3	69.2	0.3052	>1.02
	Cu on 10 th day	0.541 (0.418-0.661)	0.574	35.48	84.62	2.31	0.76	64.7	62.3	0.2010	>1.02
MV	Selenium on admission	0.546 (0.422-0.665)	0.621	29.51	100.0	2.80	0.77	95.0	16.1	0.2951	>111
	Selenium on 5 th day	0.584 (0.460-0.701)	0.403	16.39	100.0	2.07	0.87	93.3	14.6	0.1639	>112
	Selenium on 10 th day	0.515 (0.393-0.637)	0.851	44.26	77.78	1.99	0.72	93.1	17.1	0.2204	>102
	Zinc on admission	0.598 (0.474-0.714)	0.456	96.72	33.33	1.45	0.098	90.8	60.0	0.3005	≤100
	Zinc on 5 th day	0.687 (0.565-0.792)	0.074	44.26	88.89	3.98	0.63	96.4	19.1	0.3315	≤89.03
	Zinc on 10 th day	0.674 (0.551-0.781)	0.102	62.30	77.78	2.80	0.48	95.0	23.3	0.4007	≤91
	Cu on admission	0.529 (0.406-0.650)	0.743	26.23	100.0	2.36	0.74	100.0	16.7	0.2623	≤0.95
	Cu on 5 th day	0.551 (0.427-0.670)	0.611	26.23	88.89	2.36	0.83	94.1	15.1	0.1512	≤0.97
	Cu on 10 th day	0.536 (0.412-0.656)	0.728	24.59	88.89	2.21	0.85	93.7	14.8	0.1348	>1.03
ICU LOS	Selenium on admission	0.604 (0.480-0.719)	0.124	24.32	93.94	4.01	0.81	81.8	52.5	0.1826	>118
	Selenium on 5 th day	0.623 (0.499-0.736)	0.067	48.65	75.76	2.01	0.68	74.4	50.4	0.2441	>103
	Selenium on 10 th day	0.569 (0.445-0.687)	0.321	35.14	81.82	1.93	0.79	73.7	46.5	0.1695	>109
	Zinc on admission	0.532 (0.408-0.652)	0.655	37.84	75.76	1.56	0.82	63.7	52.0	0.1360	>95.2
	Zinc on 5 th day	0.550 (0.426-0.669)	0.473	45.95	66.67	1.38	0.81	60.8	52.3	0.1261	>94
	Zinc on 10 th day	0.514 (0.392-0.636)	0.837	18.92	90.91	2.08	0.89	70.0	50.0	0.0982	>98
	Cu on admission	0.539 (0.416-0.659)	0.576	62.16	54.55	1.37	0.69	60.6	56.2	0.1671	>1.1
	Cu on 5 th day	0.509 (0.386-0.630)	0.901	13.51	93.94	2.23	0.92	71.5	49.2	0.0745	≤0.88
	Cu on 10 th day	0.554 (0.431-0.673)	0.433	51.35	63.64	1.41	0.76	61.3	53.8	0.1499	≤0.93

Abbreviations; ROC: Receiver operating characteristic; LOS: Length of stay, MV: Mechanical ventilation, CI: Confidence interval, SN: Sensitivity; SP: Specificity; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; PPV: Positive Predictive value; NPV: Negative Predictive value; AUC: Area under curve, * $P < 0.05$ considered as significant

found a negative correlation between COVID-19 severity and mortality and blood levels of Zn and Se.^{43,44} Skalny et al,³⁷ reported that serum Se levels inversely associated with lung damage in COVID-19. According to Jothimani et al, a considerable proportion of COVID-19 patients had Zn deficiency in comparison to healthy controls.⁴⁴ Moreover, 13% lower Se levels in Indian COVID-19 patients compared to healthy controls is reported.⁴⁵ A large population-based study in China reported that the Se concentration in hair is associated with a higher COVID-19 cure rate.⁴⁶ A study by Heller et al,⁴³ demonstrated that the combination of serum SELENOP, Zn, and age is a reliable predictor of COVID-19 survival. In contrast, evidence showed that the increase in serum Cu was associated with the intensity of COVID-19,^{28, 37} which confirms the findings of this study that showed a higher mean level of Cu on the 5th day of ICU in the non-survivor group than in the survivor group.

In line with the present study, some studies found little evidence of the effect of Zn, Se, or Cu levels for preventing infection, and disease progression.²⁹ Zn, Cu, and Se levels were not linked to COVID-19 severity, but Zn levels

were highly linked to patient outcomes, according to a prospective study by Bagher Pour et al. that evaluated blood trace element status in both severe and non-severe COVID-19 patients.⁴⁷ An observational study by Razeghi Jahromi et al,⁴² reported that the correlation between Se and Zn with COVID-19 severity lost its significance after adjusting for confounding factors such as age, urea, and C-reactive protein. It can be concluded that the effect of confounding variables on the effect of Se, Zn, and Cu levels in predicting the severity of COVID-19 and its associated consequences is very high. Therefore, this can be one of the reasons for the difference between the results of the studies which can related to the study population, research setting, and severity of diseases.

limitations of the study

The small sample size of this study as a single-center observational study was the main limitation which indicates the need for future studies on this topic. Furthermore, due to the cross-sectional design of our study, examination of changes over time was not possible, and the long length of

Table 5. Cumulative effect of Selenium, Zinc, and Copper on admission, 5th day, and 10th day of ICU to predicting mortality, need for mechanical ventilation, and duration of ICU stay

Markers	AUC (95% CI)	P-value	SN	SP	LR +	LR-	PPV	NPV	Youden index	Cut-point
Mortality (Yes vs. No)										
On admission	0.594 (0.470-0.710)	0.172	38.71	79.49	1.89	0.77	60.0	62.0	0.1820	>0.47648
5 th day	0.746 (0.628-0.843)	<0.0001*	96.77	56.41	2.22	0.057	63.8	95.6	0.5318	>0.33927
10 th day	0.773 (0.658-0.865)	<0.0001*	61.29	84.62	3.98	0.46	76.0	73.3	0.4591	>0.50992
MV (Yes vs. No)										
On admission	0.632 (0.508-0.744)	0.231	62.30	66.67	1.87	0.57	92.7	20.7	0.2896	>0.87828
5 th day	0.713 (0.593-0.815)	0.032*	57.38	88.89	5.16	0.48	97.2	23.5	0.4627	>0.89618
10 th day	0.694 (0.572-0.799)	0.065	68.85	77.78	3.10	0.40	95.5	26.9	0.4663	>0.86083
ICU length of stay (≥26 days vs. <26 days)										
On admission	0.609 (0.485-0.724)	0.109	75.68	48.48	1.47	0.50	62.3	64.0	0.2416	>0.48028
5 th day	0.627 (0.503-0.740)	0.059	51.35	75.76	2.12	0.64	70.4	58.1	0.2711	>0.52286
10 th day	0.595 (0.471-0.711)	0.164	51.35	72.73	1.88	0.67	67.9	57.1	0.2408	>0.55276

Abbreviations; LOS: Length of stay, MV: Mechanical ventilation, CI: Confidence interval, SN: Sensitivity; SP: Specificity; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; PPV: Positive Predictive value; NPV: Negative Predictive value, AUC: Area under curve; *P<0.05 considered as significant

patients' follow-up should be considered in future studies.

Conclusion

Our findings revealed a fair predictive value of Zn, a poor predictive value of Se and Cu, and a fair predictive value of their cumulative effect on mortality. However, these micronutrients were not predicted to be needed for MV and ICU LOS. On the fifth day of stay in the ICU, the combined serum levels of Se, Zn, and Cu were found to have a fair predictive value for MV.

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Competing Interests

The authors have no conflicts of interest to disclose related to this study.

Consent for Publication

Not applicable.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The protocol study was reviewed and approved by the Research Council of Ardabil University of Medical Sciences under the ethics code IR.ARUMS.REC.1399.056 in accordance with the Declaration of Helsinki of the World Medical Association. Informed consent was obtained from all subjects and/or their legal guardian(s).

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Supplementary Files

Supplementary file 1 contains Figures S1-S4.

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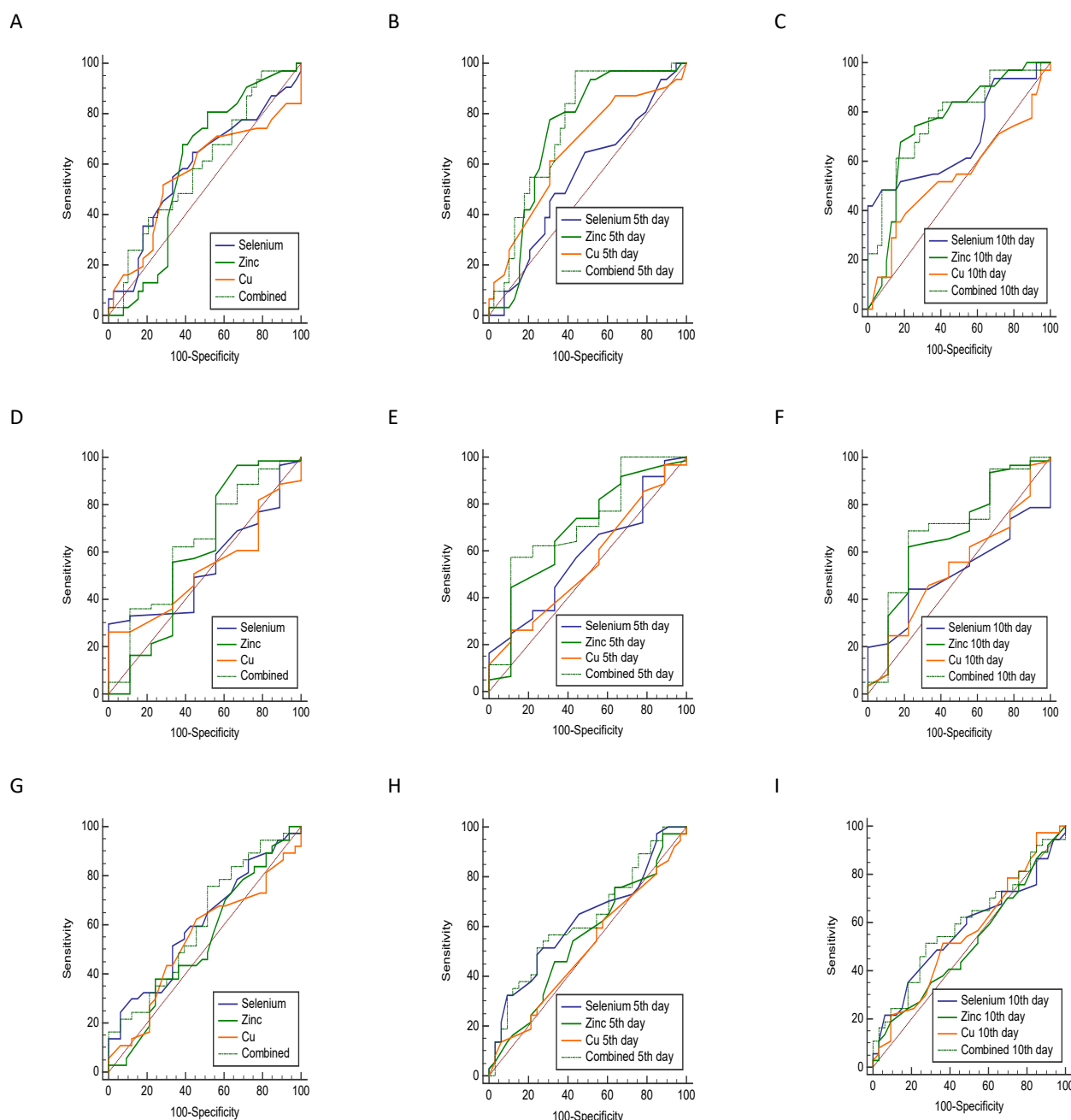


Figure 2. Comparison of ROC curves for serum selenium, zinc, copper, and their combined marker for predicting mortality, mechanical ventilation, and ICU length of stay on admission, the 5th day, and the 10th day of ICU stay.

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