

# Decreased Copper/Selenium Ratio Among Non-Responder Healthcare Workers to SARS-CoV-2: An Evidence of High Copper/Selenium Ratio Effects on the Immune Response to COVID-19 and Symptoms

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## Research

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# Abstract

**Background:** The relationship between strong immune response to infections and trace elements such as selenium (Se) and copper (Cu) is well documented. Furthermore, Se and Cu behave as negative and positive acute phase reactants under infectious conditions, respectively. Since SARS-CoV-2 causes systemic inflammation, this study was conducted to evaluate the association of Se and Cu serum levels with symptoms and immune response to SARS-CoV-2, and then assess the Cu/Se ratio in this matter.

**Methods:** Blood samples and nasopharyngeal swabs were obtained from 126 SARS-CoV-2 participants with mild and severe clinical symptoms. The SARS-CoV-2 infection and immune response to the virus were confirmed by RT-PCR and anti-SARS-CoV-2 IgG, respectively. The measurement of Se and Cu serum levels were analyzed by atomic absorption spectrophotometry and colorimetric assay, respectively. Finally, data were analyzed and a *P-value* < 0.05 was considered statistically significant.

**Results:** The mean Se levels were higher in patients with mild symptoms ( $108.73 \pm 5.38 \mu\text{g/L}$ , *P-value* = 0.0012) and IgG non-responders ( $110.33 \pm 3.38 \mu\text{g/L}$ , *P-value* < 0.001), whereas, the mean Cu was higher in participants with severe symptoms ( $111.055 \pm 11.98 \mu\text{g/dL}$ , *P-value* = 0.045) and IgG responders ( $112 \pm 9.98 \mu\text{g/dL}$ , *P-value* = 0.0058). The Cu/Se ratio was lower (ratio < 1) in participants with no immune responses to infection and mild symptoms versus immune responder patients with severe symptoms (*P-value* < 0.001).

**Conclusion:** Our results suggest that Cu/Se ratio may be considered as a nutritional biomarker of severity and immune response in SARS-CoV-2-infected patients.

## 1. Background

Coronavirus disease 2019 (COVID-19) is a condition caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that was first identified in Wuhan, China, in December 2019. The COVID-19 has spread rapidly all over the world and consequently COVID-19 pandemic has seriously affected human health and social life, and the World Health Organization (WHO) declared a global pandemic for COVID-19 on March 11, 2020 [1]. The SARS-CoV-2 can cause a spectrum of clinical features from mild signs and symptoms to acute respiratory distress syndrome, severe pneumonia, and death [2]. There is growing evidence that points to the relationship between inflammation and immune response to the SARS-CoV-2 virus [3].

Sufficient amounts of micronutrients are required for immune system development, maintenance, functions, surveillance and finally defense against infections such as SARS-CoV-2. It is therefore believed that trace elements and vitamins including selenium (Se), copper (Cu), zinc (Zn), vitamin C, and vitamin D play an important role in an effective immune response to SARS-CoV-2 infection [4–6]. These micronutrients have an impact especially on cell- and humoral-mediated immune response against infections [7]. Deficiencies of trace elements cause susceptibility to infections [8], and also infections such as SARS-CoV-2 or systemic inflammation status such as COVID-19 could induce a reduction of Se

serum level concentration during the acute phase response [9–11]. This event may be due to the redistribution of the serum Se into the damaged tissues [12]. Besides, acute infections or inflammation status lead to an increase of Cu serum level concentration [13]. Interaction of this process results in an increased serum Cu/Se ratio.

The importance of trace element balance such as Cu/Zn ratio in autoimmune diseases [14], inflammation [15], infections such as SARS-CoV-2 [16], and cancers [17, 18] is well documented. In this regard, the high Cu/Se ratio has also been reported in recurrent aphthous stomatitis, as an inflammatory condition [19]. To the best of our knowledge, no studies were reported the possible relation between Cu and Se balance (Cu/Se ratio) in SARS-CoV-2-infected patients with immune response. Therefore, we aimed to evaluate the relationship between the levels of Cu, Se, and Cu/Se ratio and immune response to the SARS-CoV-2 infection in healthcare workers (HCWs) at Rafsanjan University of Medical Sciences (RUMS), Rafsanjan, Iran in the third quarter of 2021.

## **2. Methods**

### **2.1. Population, setting and procedures**

This study was approved by the ethics committee of RUMS, Rafsanjan, Iran (Ethical code: IR.RUMS.REC.1400.024). Also, this trial procedure was conducted under the ethical standards of the Iranian Ministry of Health and Medical Education on human experimentation and in concordance with the Helsinki Declaration. 126 HCWs were participated in this study. All participants completed a questionnaire containing sociodemographic data (i.e., age, sex, height, weight), clinical information such as exposure to confirmed COVID-19 cases, professional information (i.e., shift, hospital department, and occupation), and self-report of COVID-19 related symptoms (i.e., fever, dry cough, fatigue and weakness, sore throat, the impaired sense of smell and taste) after obtaining written informed consent. Five milliliters (ml) vein blood samples and nasopharyngeal swabs were collected from participants (HCWs in medical centers and HCWs in administrative departments) with clinical symptoms, under aseptic and standard conditions. The exclusion criteria were: taking any form of dietary trace elements or multivitamins/supplements, pregnancy, history of cancer, autoimmune disease and taking immunosuppressive medication. All collected bloods were immediately transferred to the laboratory for the next steps. To select participants, we predicted exposure to SARS-CoV-2 according to clinical symptoms. For this aim, based on participants' self-report of clinical symptoms, two clinical criteria (major and minor) were considered [20], and then the participants were divided into two subgroups, high and medium to low possibility to COVID-19, according to clinical criteria (Table 1).

Table 1  
Categories of participants for prediction of exposure to SARS-CoV-2 according to the clinical criteria.

Clinical criteria	
Major signs	Minor signs
- Fever or chills	- Sore throat
- Dry cough or shortness of breath	- Body aches or headaches
- Fatigue and weakness	- The impaired sense of smell and taste
- Close contact with a COVID-19 patient	- Gastrointestinal symptoms
Categories of participants	
High possibility to COVID-19 (Severe symptoms)	Medium or low possibility to COVID-19 (Mild symptoms)
≥ 3 major signs or	≥ 2 major signs or
≥ 2 major signs and ≥ 1 minor sign	≥ 1 major sign and ≥ 2 minor signs

## 2.2. Confirmation of SARS-CoV-2 infection

3-4 days after the onset of clinical symptoms, a standard molecular method (RT-PCR) was performed for the confirmation of SARS-CoV-2 infection. The RNA extraction, cDNA synthesis, and RT-PCR were conducted by ROJE (Roje Co., Tehran, Iran), YEKTA TAJHIZ (Yekta-tajhiz Co., Tehran, Iran), and One-step RT-PCR Kit (Pishtazteb Co., Tehran, Iran), respectively. The RT-PCR results were analyzed according to the manufacturer's instructions.

## 2.3 Measurement of Se and Cu serum levels

After centrifugation of the bloods for 5 min at 5000 rpm, serum was obtained and stored at -20 °C until Se and Cu assessment. The Se and Cu serum levels were measured by atomic absorption spectrophotometry and colorimetric assay, respectively. According to the instruction manual of the kit, the reference range for serum Se was 46-143 µg/L and for Cu was 60-153 µg/dL. The remaining serum was used for IgG antibody measurement.

## 2.4. Assessment of immune response to the SARS-CoV-2 infection

About one month after clinical symptoms and confirmed infection, the immune response to SARS-CoV-2 infection was evaluated by measuring serum anti- SARS-CoV-2 IgG antibody. For this purpose, serum IgG antibody to SARSCoV2 was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Pishtazteb Co., Tehran, Iran) according to the manufacturer's instructions. The cutoff index 0.9, 1.1 and

0.9-1.1 was considered as negative, positive and borderline result, respectively. The ELISA diagnostic kit sensitivity and specificity for IgG was 94.1% and 98.3%, respectively.

## 2.5. Data Analysis

Data analysis was performed using the IBM SPSS 22 statistical software. Data was reported as mean  $\pm$  standard deviation (SD). The difference in serum Se and Cu levels between subgroups was analyzed using independent sample t-tests. Pearson correlation test was used to analyze the mutual relationships between elements. A *P-value*  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Demographic data

The flow diagram of study selection and outcomes are shown in Figure 1. 126 HCWs in medical centers and administrative departments with a mean range of age  $37.7 \pm 5.0$  (76 female (60%) and 50 male (40%)) were enrolled in this study. There were statistical age difference (*P-value* = 0.026) and no statistical gender difference (*P-value* = 0.42) among participants. The results of the COVID-19 prediction based on clinical symptoms according to sex, age, and other demographic data are shown in Table 2. 96 of the participants (76%) were HCWs in medical centers, and the others (24%) worked in the administrative departments. The HCWs who worked in medical centers had more contact with COVID-19 patients (*P-value*  $< 0.5$ ), which results in a high possibility of COVID-19.

Table 2  
Demographic data of participants.

Variables	Prediction to COVID-19		Total (n=126 [100%])	P-value	
	High possibility (Severe symptoms) (n=101 [80%])	Medium or low possibility (Mild symptoms) (n=25 [20%])			
Age	> 25	8 [80%]	2 [20%]	37.7±5.0 <sup>a</sup>	0.026 <sup>b</sup>
	25-34	41 [93%]	3 [7%]		
	35-44	29 [76.3%]	9 [23.7%]		
	45-54	22 [71%]	9 [29%]		
	< 54	1 [33%]	2 [67%]		
Sex	Female	61 [80%]	15 [20%]	76 [60%]	0.42
	Male	40 [80%]	10 [20%]		
Occupation	HCWs <sup>c</sup>	76 [79%]	20 [21%]	96 [76%]	< 0.05 <sup>b</sup>
	ADSs <sup>d</sup>	25 [83%]	5 [17%]		
Close contact with <sup>a</sup> COVID-19 patient	Yes	90 [91%]	9 [9%]	99 [79%]	< 0.05 <sup>b</sup>
	No	11 [41%]	16 [59%]		
Anti-SARS-CoV-2 IgG	Pos.	26 [100%]	0 [0%]	26 [21%]	< 0.05 <sup>b</sup>
	Neg.	75 [75%]	25 [25%]		
RT-PCR result	Pos.	71 [100%]	0 [0%]	71 [56.3%]	< 0.05 <sup>b</sup>
	Neg.	30 [55%]	25 [45%]		
Mean Se serum levels (µg/L)		104.66 ± 2.84	108.73 ± 5.38	106.7 ± 4.11	0.0012 <sup>b</sup>
Minimum		89.9	73.9	73.9	N/A <sup>e</sup>
Maximum		115.2	124	124	N/A <sup>e</sup>

<sup>a</sup> Mean (SD) age, <sup>b</sup> Significant difference, <sup>c</sup> Health care workers, <sup>d</sup> Administrative Department Staff, <sup>e</sup> Not Applicable

Variables	Prediction to COVID-19		Total (n=126 [100%])	P-value
	High possibility (Severe symptoms) (n=101 [80%])	Medium or low possibility (Mild symptoms) (n=25 [20%])		
Mean Cu serum levels (µg/dL)	111.055 ± 11.98	106.167 ± 10.4	108.6 ± 11.19	0.045 <sup>b</sup>
Minimum	58.8	57.3	57.3	N/A <sup>e</sup>
Maximum	169.1	153.6	169.1	N/A <sup>e</sup>
Cu/Se ratio	1.06 ± 0.05	0.97 ± 0.04	1.0 ± 0.1	< 0.001 <sup>b</sup>
Minimum	0.65	0.75	0.77	
Maximum	1.4	1.2	1.3	N/A <sup>e</sup>
				N/A <sup>e</sup>

<sup>a</sup> Mean (SD) age, <sup>b</sup> Significant difference, <sup>c</sup> Health care workers, <sup>d</sup> Administrative Department Staff, <sup>e</sup> Not Applicable

## 3.2. Prediction of COVID-19 based on clinical symptoms

We predicted a high possibility of COVID-19 in 101 (80%) of HCWs based on clinical symptoms and the others (20%) were with a medium or low possibility of infection. The RT-PCR test was positive only in 71 (56.3%) individuals with severe symptoms and negative in the others (43.7%). All negative RT-PCR individuals were also negative for anti-SARS-CoV-2 IgG (Figure 1). Interestingly, evaluation of immune response to infection by anti-SARS-CoV-2 IgG indicated that only 26 (21%) of participants with positive RT-PCR had a history of COVID-19 (Figure 1 and Table 2). The outcome results of the molecular and serological tests were statistically different between the two subgroups ( $P$ -value < 0.5) (Table 2).

## 3.3. Cu/Se serum levels and COVID-19 symptoms

The associations between Cu, Se and Cu/Se ratio serum levels with COVID-19 symptoms were estimated. The Se serum levels were in the normal range among all participants (Up to 124 µg/L), while, some individuals had high Cu serum levels (up to 169.1 µg/dL). The mean Se serum level was significantly higher in HCWs with mild symptoms ( $108.73 \pm 5.38$  µg/L,  $P$ -value = 0.0012), whereas, the mean Cu serum level was statistically higher in individuals with severe symptoms ( $111.055 \pm 11.98$  µg/dL,  $P$ -value = 0.045) (Figure 2, Table 2). In terms of the Cu/Se ratio, a higher statistical difference in the ratio was found in participants with severe symptoms ( $P$ -value < 0.001) (Figure 2).

## 3.4. Cu/Se serum levels and response to SARS-CoV-2

The associations between Cu, Se and Cu/Se ratio serum levels and immune response to SARS-CoV-2 were also evaluated. For this aim, the individuals with a cut-off index of anti-SARS-CoV-2 IgG  $\geq 1.1$  were considered as a COVID-19 immune responder, while, the participants with a cut-off index of anti-SARS-CoV-2 IgG  $\leq 0.9$  were considered as non-responders to the COVID-19. The results indicated that the mean Se serum level was statistically higher in COVID-19 immune non-responders ( $110.33 \pm 3.38 \mu\text{g/L}$ ,  $P\text{-value} < 0.001$ ), whereas in responders participants, the Cu serum level was statistically higher ( $112 \pm 9.98 \mu\text{g/dL}$ ,  $P\text{-value} = 0.0058$ ) (Figure 3, Table 3). The Cu/Se ratio was higher in HCWs with IgG response to the infection with statistically different ( $P\text{-value} < 0.001$ ) (Figure 3, Table 3). For the determination of correlation between Se and Cu with response/non-response to SARS-CoV-2 infection, the Pearson correlation analysis was performed. The results showed that a negative significant correlation of Se with non-responders ( $r = -0.774$ ,  $P\text{-value} = 0.014$ ), whereas, the correlation of Cu was positive with COVID-19 immune responders ( $r = 0.601$ ) but not statistically difference ( $P\text{-value} = 0.05$ ) (Table 4).

Table 3  
The results of Cu, Se, and Cu/Se ratio along with IgG response to SARS-CoV-2.

Trace elements	RT-PCR positive		Total (n=71)	P-value
	Anti-SARS-CoV-2 IgG			
	IgG Responders (n=26)	IgG Non-responders (n=45)		
Mean Se serum levels ( $\mu\text{g/L}$ )	$102.42 \pm 1.83$	$110.33 \pm 3.38$	$106.37 \pm 2.6$	$< 0.001^a$
Minimum	89.9	98	89.9	N/A <sup>b</sup>
Maximum	112.3	124	124	N/A <sup>b</sup>
Mean Cu serum levels ( $\mu\text{g/dL}$ )	$112 \pm 9.98$	$105.1 \pm 9.4$	$108.55 \pm 9.7$	$0.0058^a$
Minimum	83.9	74.8	74.8	N/A <sup>b</sup>
Maximum	157.5	153.6	157.5	N/A <sup>b</sup>
Cu/Se ratio	$1.09 \pm 0.08$	$0.95 \pm 0.05$	$1.0 \pm 0.2$	$< 0.001^a$
Minimum	0.93	0.76	0.83	N/A <sup>b</sup>
Maximum	1.4	1.2	1.2	N/A <sup>b</sup>

<sup>a</sup> Significant difference, <sup>b</sup> Not Applicable

Table 4  
Pearson correlation analysis of Cu and Se with IgG response to SARS-CoV-2.

Variables	Pearson correlation (P-value)			
	IgG Responders	Cu in IgG Responders	IgG Non-responder	Cu in IgG Non-responder
Se	0.110 (0.7)	-0.377 (0.318)	-0.774 (0.014 <sup>a</sup> )	0.451 (0.223)
Cu	0.601 (0.05)	N/A <sup>b</sup>	-0.234 (0.5)	N/A <sup>b</sup>
<sup>a</sup> Significant difference, <sup>b</sup> Not Applicable				

## 4. Discussion

Our results indicate that HCWs with severe symptoms may have shown a better immune response to SARS-CoV-2 infection. Inflammation caused by severe symptoms such as fever, chills, dry cough or shortness of breath, fatigue, and weakness causes a decrease and increase of Se and Cu, respectively, resulting in an imbalance in serum Cu/Se levels. Interestingly, decreased Cu/Se ratios in non-responders to SARS-CoV-2 infection indicate mild inflammation and weak immune responses. Therefore, Cu/Se ratio could represent an inflammatory and immune status in SARS-CoV-2-infected HCWs.

Despite the normal levels of Se in all HCWs, the Se serum levels in patients with severe symptoms were found to be lower than in patients with mild symptoms. In complication status such as COVID-19 disease, the severe symptoms indicate inflammation and involvement of the immune system in the body. Inflammation, pro-inflammatory cytokines, hypoxia, and many stress signals can affect the Se serum level, which led to aggravating and accelerating Se decline [21, 22]. The COVID-19 infection is characterized by systemic inflammation especially in the lung, which resulting hypoxia [1]. In a recent study by Sun *et al.*, reduced Se status was reported in patients with systemic sclerosis, as an autoimmune disease with systemic inflammation, vasculopathy, fibrosis, and hypoxia [23]. Also, in another study by Younesian *et al.*, for assessment of Se serum levels in COVID-19 patients with severe and mild symptoms, it was shown that patients with severe conditions had lower Se serum levels compared with patients with mild symptoms [10]. It seems that a decline in Se serum level is occurred in an inflammatory condition and considered as a negative phase reactant in these situations [24, 25].

In the current study, increased Cu serum level was also shown in HCWs with severe symptoms, such that some individuals had higher Cu serum levels versus normal range (up to 169.1 µg/dL). Unlike selenium, studies were shows that Cu is increased in infections, such that progressed rising in Cu serum level is the hallmark of infections [26, 27]. In another hand, the inflammatory conditions significantly affect Cu serum level, such that increased Cu is known as a positive phase reactant [28, 29]. Since the pathophysiology of SARS-CoV-2 is characterized by systemic inflammation, so the elevated Cu serum level in patients with severe symptoms is reasonable. In two studies by Bagher Pour *et al.*, and Skalny *et al.*, for estimate

association of trace elements with severity and clinical outcomes of COVID-19 patients, it was shown that high Cu serum level is associated with severe symptoms, hospitalization, and mortality [16, 30].

Our results also indicated that HCWs with severe symptoms had a high Cu/Se ratio compared with mild symptoms subjects. Normal levels of trace elements cause maintain general health, but the imbalance between them could have serious consequences. Some trace elements are antagonists, which means they may compete against each other. In this regard, the association of Cu and Zn balance with inflammation is well known. Several studies have shown a correlation of high Cu/Zn ratio with an inflammatory condition such as oxidative stress [14], autoimmune diseases [31], infections such as SARS-CoV-2 [16], and cancers [17, 18]. In another hand, a low Cu/Zn ratio is associated with an increased risk of incident infections [32]. In terms of Cu and Se balance, Cu has also been shown to be a Se antagonist [33]. Thus, elevated Cu levels in inflammatory conditions can affect Se and lead to an imbalance in the Cu/Se ratio. The high Cu/Se ratio has also been reported in inflammatory conditions such as recurrent aphthous stomatitis [19], pulmonary arterial hypertension in systemic sclerosis patients [23], acute ischemic stroke [34]. So, it seems that a high Cu/Se ratio may be considered as an inflammatory situation such as SARS-CoV-2 infection.

Our results also demonstrate that HCWs with an IgG response to SARS-CoV-2 belonged to the group with severe symptoms. Positive and negative correlations were found between Cu and IgG responders, and Se and IgG non-responders. Also, the Cu/Se ratio was higher in IgG responders compared with IgG non-responder subjects. The inflammation during infections such as SARS-CoV-2 is caused by the strong activation of the innate immune system. SARS-CoV-2 activates innate immune cells, which led to the production and release of pro-inflammatory and inflammatory cytokines named cytokine storm. Although cytokine storm leads to severe symptoms, hospitalization, and mortality in patients with COVID-19, it can also activate the efficient adaptive immune responses [35, 36]. The link between innate and adaptive immunity is well known, and numerous studies have indicated strong an innate response lead to the full activation of adaptive immunity [37]. In a study by Hackler *et al.*, it was reported that surviving COVID-19 patients showed higher Cu and Se serum levels in comparison to COVID-19-dead patients, and Cu and Se serum levels are contributed to a good prediction of survival [38]. It seems that the Cu/Se ratio could be considered as the status of immune response activation and survival in patients with COVID-19 disease.

The limitation of the current study was the small population, and authors suggest this finding estimate in a large population with severe symptoms and strong immune response against SARS-CoV-2 infection. As well as, we measured only anti-SARS-CoV-2 IgG, so other antibodies, such as long-term IgM and IgA, may be involved in the long-term immune response to COVID-19 and protect HCWs against re-infection. In another hand, we did not estimate cellular immunity in this matter, and individuals with severe symptoms, and without humoral response, may have efficient cellular immunity against infection, and further research is needed.

## 5. Conclusion

Our results suggest that the normality of Se and Cu serum levels are important in inflammatory conditions such as response to SARS-CoV-2 infection. Besides, the balance between them, in the inflammatory condition such as COVID-19, is also more important. Hence, the Cu/Se ratio may be considered as a nutritional biomarker for inflammation and immune response in SARS-CoV-2-infected patients.

## Abbreviations

cDNA  
complementary DNA  
COVID-19  
Coronavirus disease 2019  
Cu  
Copper  
ELISA  
Enzyme-Linked Immunosorbent Assay  
HCW  
Health care worker  
IgG  
Immunoglobulin-G  
°C  
Centigrade  
RNA  
Ribonucleic Acid  
rpm  
Revolutions Per Minute  
RT-PCR  
Reverse Transcription-Polymerase Chain Reaction  
SARS-CoV-2  
Severe Acute Respiratory Syndrome-Coronavirus-2  
Se  
Selenium  
WHO  
World Health Organization  
Zn  
Zinc  
µg/dL  
Microgram/deciliter  
µg/L  
Microgram/liter

# Declarations

## 6. Confirmation statement

All authors confirmed that this research is supported by RUMS, Rafsanjan, Iran that is primarily and involved in education and research.

## Ethics approval and consent to participate

This study was approved by the ethics committee of RUMS, Rafsanjan, Iran (Ethical code: IR.RUMS.REC.1400.024). Also, this trial procedure was conducted under the ethical standards of the Iranian Ministry of Health and Medical Education on human experimentation and in concordance with the Helsinki Declaration. All participants signed a consent form.

## Consent for publication

The submitted paper is an original and unpublished work in which the listed authors have all made important contributions explaining the contributions in detail. Also, the authors guarantee that, once their material has been accepted for publication by the journal, they will not make submission of the same material or portions thereof to another journal.

## Availability of data and materials

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

## Competing interests

The authors declared no conflicts of interest with regard to the research, authorship, and publication of this article.

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## Authors' contributions

MT contributed to the design and implementation of the research, and the writing of the manuscript. AJ and MN-G contributed to organized sampling from participants. MH and AY-A contributed to the analysis of the results. FM-M and MR contributed to the final version of the manuscript. KM contributed to the final version of the manuscript, analyzed the data, and supervised the project. All authors approved the final version of the manuscript for submission

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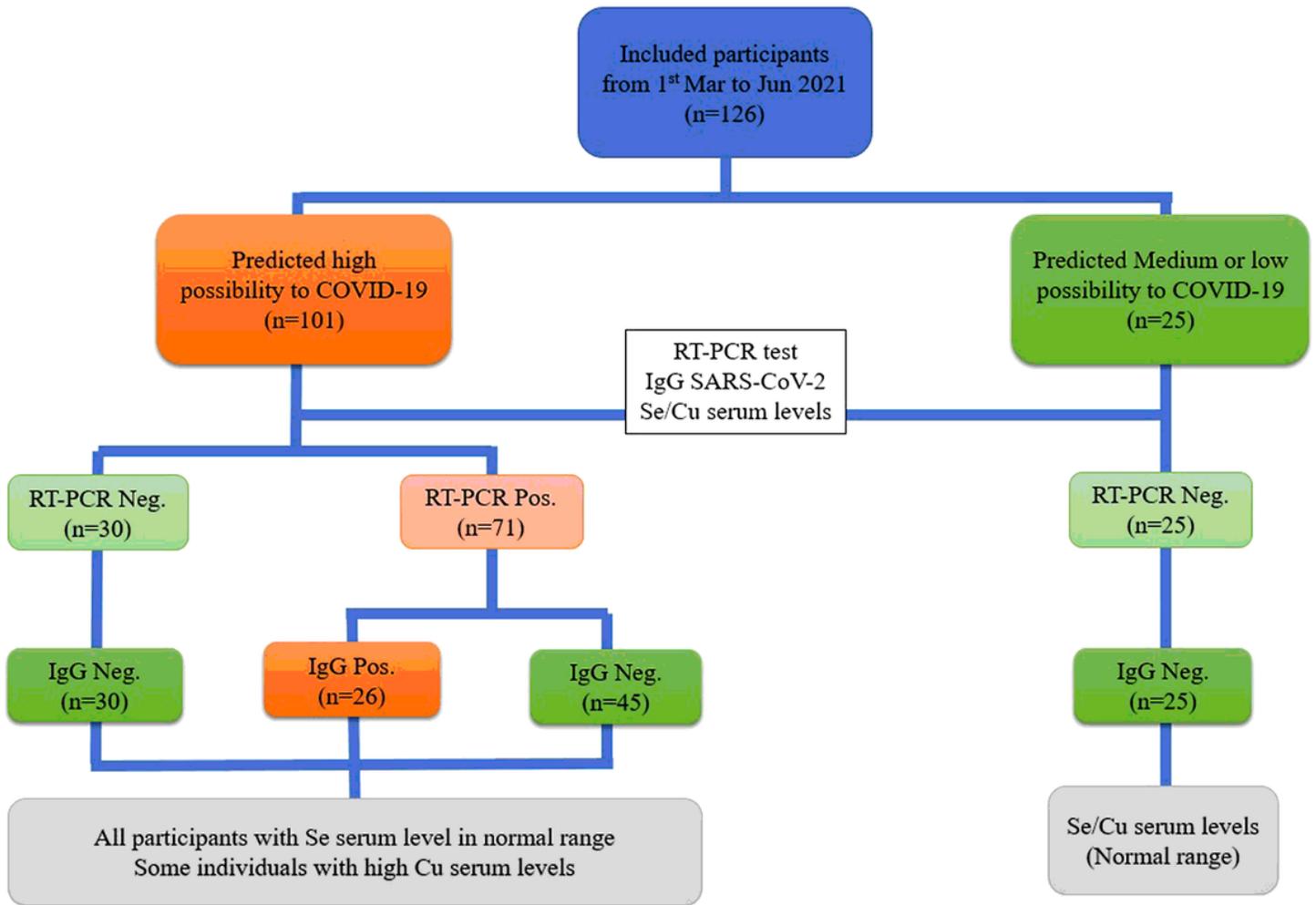
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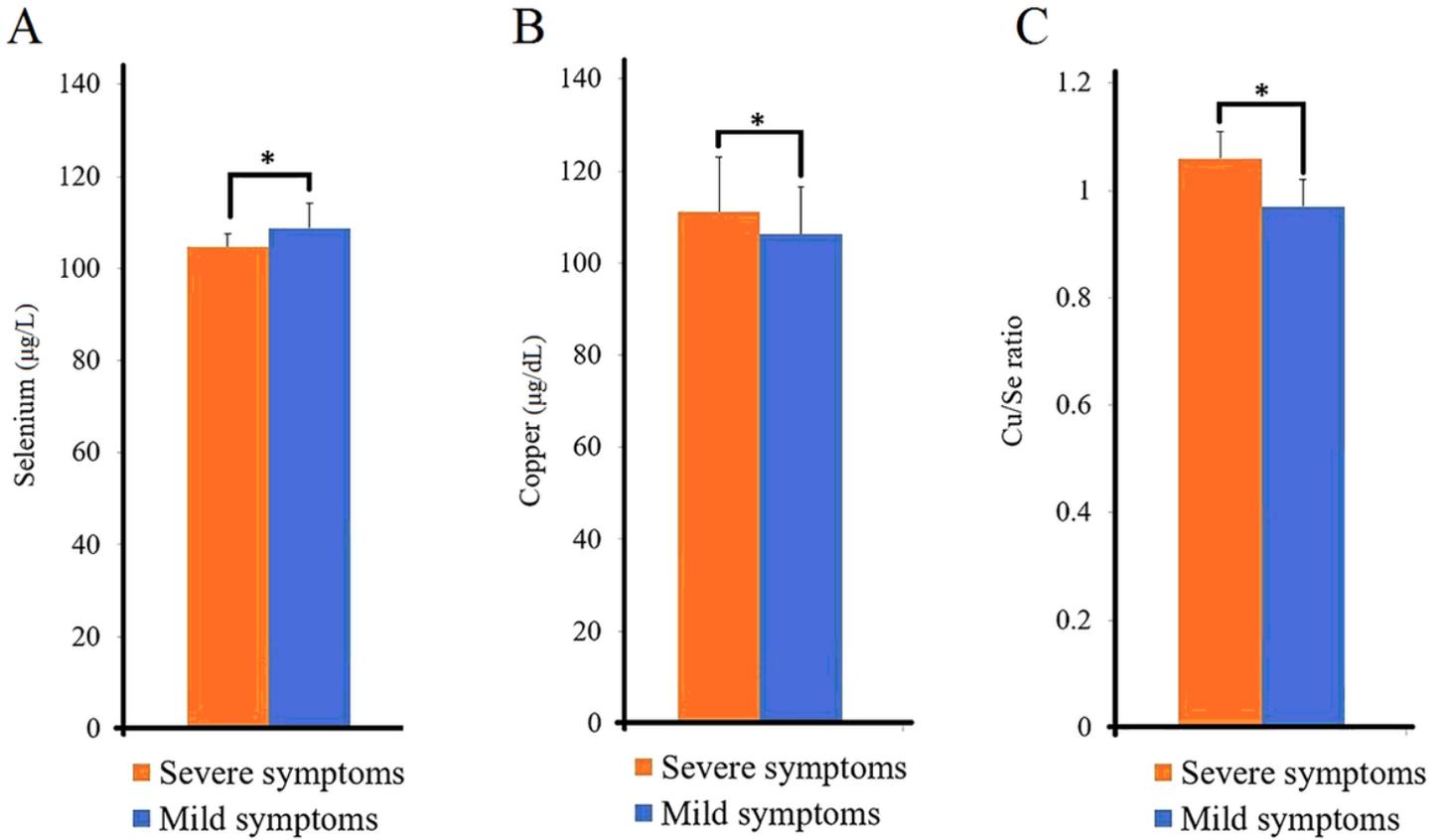
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## Figures



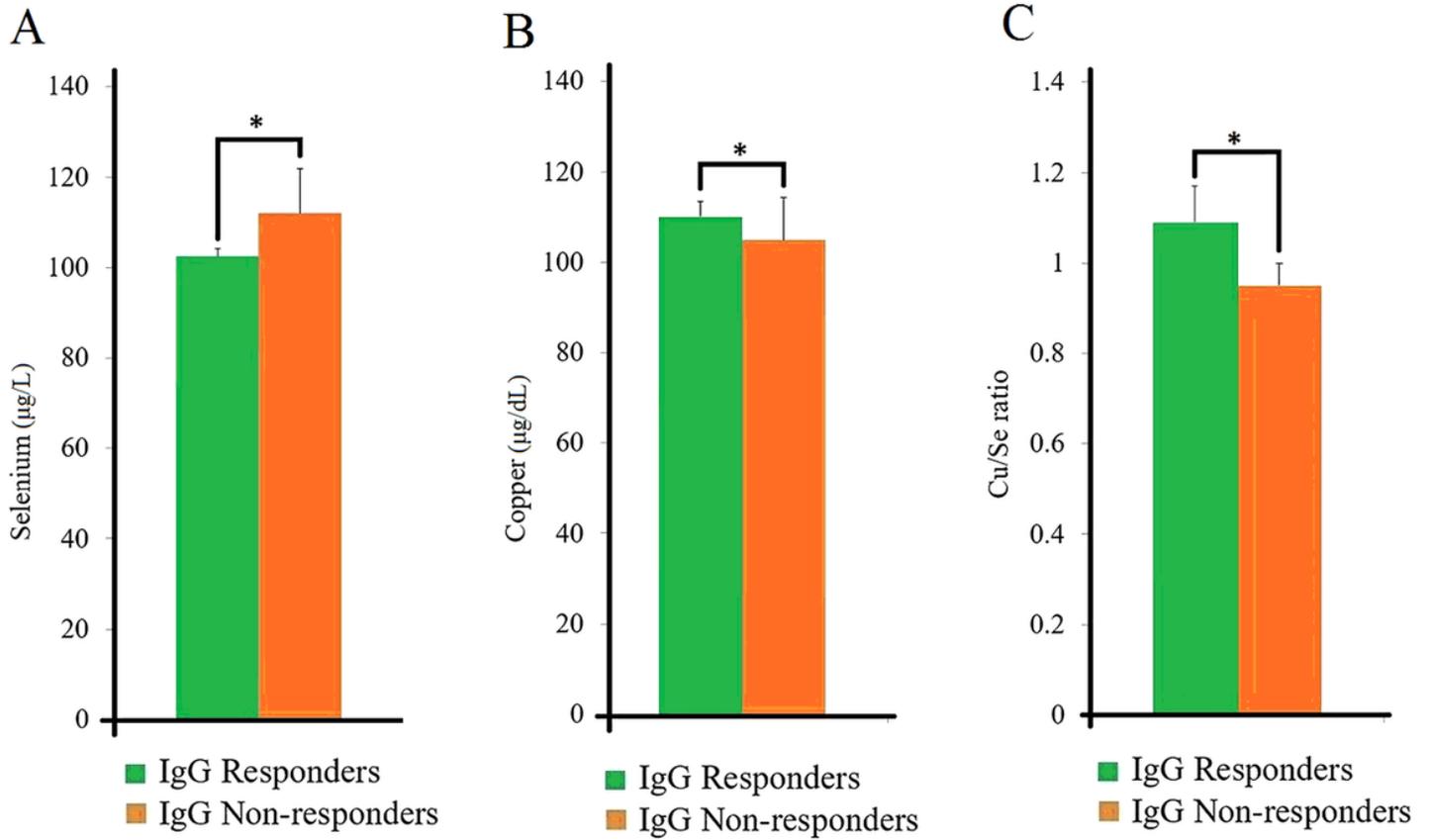
**Figure 1**

Flow diagram of study selection and outcomes. The levels of Se and Cu were in the normal range among participants with mild symptoms, while, HCWs with severe symptoms had normal and high serum levels of Se and Cu, respectively (Pos: Positive, Neg: Negative).



**Figure 2**

The results of Se, Cu, and Se/Cu ratio in HCWs with severe and mild symptoms. Se and Cu serum levels were statistically difference between two group with  $P\text{-value} = 0.0012$  (A) and  $P\text{-value} = 0.045$  (B), respectively. The Se/Cu ratio was also statistically different between the two groups with severe and mild symptoms with  $P\text{-value} < 0.001$  (C).



**Figure 3**

The results of Se, Cu, and Se/Cu ratio in HCWs with the response and non-response to SARS-CoV-2 infection. The Se and Cu were statistically different between the two groups with *P-value* < 0.001 (A) and *P-value* = 0.0058 (B), respectively. The Se/Cu ratio was also statistically different between the two groups with *P-value* < 0.001 (C).