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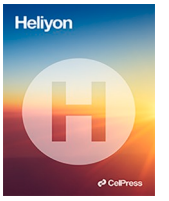

Research article

The close association of micronutrients with COVID-19

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ABSTRACT

Objectives: The present study was conducted to explore the performance of micronutrients in the prediction and prevention of coronavirus disease 2019 (COVID-19).
Methods: This is an observational case-control study. 149 normal controls (NCs) and 214 COVID-19 patients were included in this study. Fat-soluble and water-soluble vitamins were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, and inorganic elements were detected by inductively coupled plasma-mass spectrometry (ICP-MS) analysis. A logistic regression model based on six micronutrients were constructed using DxAI platform.
Results: Many micronutrients were dysregulated in COVID-19 compared to normal control (NC). 25-Hydroxyvitamin D3 [25(OH)D3], magnesium (Mg), copper (Cu), calcium (Ca) and vitamin B6 (pyridoxic acid, PA) were significantly independent risk factors for COVID-19. The logistic regression model consisted of 25(OH)D3, Mg, Cu, Ca, vitamin B5 (VB5) and PA was developed, and displayed a strong discriminative capability to differentiate COVID-19 patients from NC individuals [area under the receiver operating characteristic curve (AUROC) = 0.901]. In addition, the model had great predictive ability in discriminating mild/normal COVID-19 patients from NC individuals (AUROC = 0.883).
Conclusions: Our study showed that micronutrients were associated with COVID-19, and our logistic regression model based on six micronutrients has potential in clinical management of COVID-19, and will be useful for prediction of COVID-19 and screening of high-risk population.

1. Introduction

The coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global infectious disease. Due to its rapid spread and high fatality rate, it is extremely harmful to human health [1]. The number of people infected with COVID-19 increased rapidly around the world from early 2020 to early 2023. According to statistics, up to now, over 760 million people around the world have been infected with COVID-19, and over 6 million people have died of COVID-19 [2]. Most

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studies focused on the increase in the number of cases and deaths, the clinical characteristics and pathogenesis of COVID-19, and the treatment of infected patients [3,4]. However, why are some people infected with COVID-19 and others not? Why do some people have severe illness while others have mild illness? Are there any factors in the body that affect COVID-19 infection and disease progression? These issues deserve further study.

Like protein, carbohydrate and lipid, micronutrients are also essential nutrients for the human body, and they participate in various physiological activities in the body. Micronutrients mainly include fat-soluble vitamins, water-soluble vitamins and inorganic metal or non-metal elements such as iron, copper, zinc and selenium [5]. Micronutrients have a significant impact on human health, and deficiency or excess can lead to pathological reactions in the human body. Previous studies have shown that micronutrient deficiencies are common among populations and have become a global public health problem [6]. The lack of micronutrients is closely related to diseases such as tumors and infections [7,8]. This may be because micronutrients can participate in regulating the immune response in the body, and their deficiency will cause immune imbalance in the body, and then induce various diseases [9,10]. It has been reported that vitamin D insufficiency was closely related to the risk and severity of COVID-19 infection [11–16]. However, whether other micronutrients have an impact on COVID-19 infection is largely unknown.

In this study, we detected various micronutrients in serum samples of COVID-19 patients and normal controls, including fat-soluble vitamins [vitamin A (VA), 25-hydroxyvitamin D2 (25(OH)D2), 25-hydroxyvitamin D3 (25(OH)D3), vitamin E (VE) and vitamin K1 (VK1)], water-soluble vitamins [vitamin B1 (VB1), vitamin B2 (VB2), vitamin B3 (VB3), vitamin B5 (VB5), vitamin B6 (VB6, including pyridoxal phosphate (PLP) and pyridoxic acid(PA)), vitamin B9 (5-methyltetrahydrofolic acid, 5MTHF)] and inorganic elements [magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), plumbum (Pb)], to determine the differences in various micronutrients between COVID-19 patients and normal people, and evaluate whether micronutrient deficiency is a risk factor for COVID-19 infection and whether micronutrients could be used to screen high-risk groups for COVID-19.

2. Material and methods

2.1. Study population

This is an observational case-control study. A total of 363 individuals were recruited in this study, including 149 normal controls (NCs) and 214 COVID-19 patients. The diagnosis of COVID-19 patients was in accordance with the Guidelines on the COVID-19 Diagnosis and Treatment (9th edition) [17]. The inclusion criteria were patients with one of the following etiological or serological evidences: (1) The nucleic acid test of SARS-CoV-2 was positive; (2) The specific IgM and IgG antibody of SARS-CoV-2 were positive in those who were not vaccinated with SARS-CoV-2 vaccine. The exclusion criteria were: (1) Patients under 18 years old; (2) Pregnant women or individuals who were supplementing micronutrients for other reasons; (3) Patients who did not have enough blood samples for testing. NCs were apparently healthy individuals who underwent physical examinations, and people with respiratory symptoms or any other diseases were excluded.

The peripheral blood of the COVID-19 patients was collected when they first visited the hospital and were diagnosed with COVID-19, and that of NCs was collected when they visited the hospital for routine examination during the same period of time. The serum was separated after centrifugation and stored at −80 °C for detection. All samples were collected during the outbreak of COVID-19 Omicron from October 2022 to March 2023 in Peking University People's Hospital, Beijing, China. Almost all of the included patients and healthy participants have received COVID-19 vaccines. This study was conducted according to the principles of the Helsinki Declaration and approved by the Ethical Review Committee of Peking University People's Hospital, and informed consent was obtained from each participant prior to participating in this study.

2.2. Chemicals and reagents

High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were from Fisher Chemical, and HPLC-grade formic acid and ammonium acetate were from Aladdin. Distilled water was from Watsons. The kit for fat-soluble vitamins was from GBI Biotechnology, the kit for water-soluble vitamins was from Haosi Biotechnology, and the kit for detecting 9 inorganic elements was from Bioyong Technologies Inc. All testing processes were carried out according to the manufacturer's instructions, and the concentrations of each indicator in the serum samples were calculated when the calibration curve and quality control were qualified.

2.3. Detection of fat-soluble vitamins

Fat-soluble vitamins, including VA, 25(OH)D2, 25(OH)D3, VE and VK1, were detected as follows. The serum samples stored at −80 °C were thawed completely and vortexed for 10 s 300 μL of sample and 200 μL of precipitant solution containing internal standard mixture were added into a 96-well plate. The sample was vortexed for 5 min and then centrifuged at 4000 rpm for 10 min (4 °C). 800 μL of n-hexane (GBI Biotechnology) were added into the each well, and vortex twice for 10 min each time. Then the 96-well plate was centrifuged at 4000 rpm for 15 min. Transfer 600 μL of the uppermost n-hexane into a new 96-well plate and blow dry with nitrogen. Add 120 μL of reconstituted solution (H_2O: acetonitrile = 1:4), shake for 10 min to reconstitute, and then transfer into fresh glass vials for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

All samples were acquired by a Jasper HPLC coupled to a Triple Quadrupole 4500MD mass spectrometer (SCIEX). Waters ACQUITY UPLC BEH C18 (1.7 μm, 2.1 mm × 100 mm) column was used for analyte separation. The HPLC conditions were as follows: column

oven temperature, 45 °C; flow rate, 0.4 mL/min; injection volume, 20 μL. The mobile phase A was H_2O (0.1% formic acid), and mobile phase B was methanol (0.1% formic acid). The gradient of the mobile phase was as follows: 0–0.2 min, 80% B; 0.2–2 min, 80%–100% B; 2–4 min, 100% B; 4–5 min, 100%–80% B; 5–6 min, 80% B. The conditions of the mass spectrum were: ionspray voltage, 5500 V, ion source gas1, 40 psi; ion source gas2, 50 psi; curtain gas, 35 psi; capillary temperature, 400 °C.

2.4. Detection of water-soluble vitamins

Water-soluble vitamins, including VB1, VB2, VB3, VB5, VB6(including PLP and PA) and 5MTHF were detected as follows. 100 μL of sample and 160 μL of extraction solution containing internal standard mixture were added into a 96-well plate. The sample was vortexed for 5 min and then centrifuged at 4000 rpm for 10 min (4 °C). Transfer 100 μL of supernatant into another 96-well plate for LC-MS/MS analysis.

All samples were acquired by CalQuant-S (CALIBRA) platform. WELCH XB-C18 (5 μm, 4.6 mm × 50 mm) column was used for analyte separation. The HPLC conditions were as follows: column oven temperature, 40 °C; flow rate, 0.7 mL/min; injection volume, 20 μL. The mobile phase A was H_2O (5 mM ammonium acetate), and mobile phase B was methanol (5 mM ammonium acetate, 0.1% formic acid). The gradient of the mobile phase was as follows: 0–0.7 min, 1% B; 0.7–1.9 min, 1%–80% B; 1.9–3.0 min, 80% B; 3.0–3.1 min, 80%–99% B; 3.1–3.6 min, 99% B; 3.6–3.7 min, 99%–1% B; 3.7–4.5 min, 1% B. The conditions of the mass spectrum were: ionspray voltage, 5500 V, ion source gas1, 45 psi; ion source gas2, 45 psi; curtain gas, 20 psi; capillary temperature, 550 °C.

2.5. Detection of inorganic elements

400 μL of serum sample were diluted 20 times with the prepared diluent containing nitric acid, Triton X-100 and isopropanol. The blank sample, standards and quality control used in the test process are all prepared according to the same procedures. Then the diluted samples and internal standard solution were injected simultaneously for detection. Bioyong Clin-ICP-QMS-I inductively coupled plasma-mass spectrometry (ICP-MS) instrument was used to detect the 9 inorganic elements, including Mg, Ca, Mn, Fe, Cu, Zn, As, Se and Pb. The temperature of spray chamber was 2 °C, the carrier gas flow rate was 1 L/min, and compensator flow rate was 0.11 L/min.

Table 1
The clinical characteristics of the included individuals.

Variables		NC N = 149	COVID-19 N = 214
Age		55.76 ± 14.60	77.98 ± 12.45****
Gender	Male	89	149^{ns}
	Female	60	65
Smoke	Yes	40	55^{ns}
	No	109	159
Alcohol	Yes	30	38^{ns}
	No	119	176
BMI		23.03 ± 2.20	24.39 ± 4.07^{ns}
Blood Oxygen Saturation (%)		–	94.09 ± 6.36
Fever	Yes	–	174
	No	–	40
Cough	Yes	–	144
	No	–	70
Expectoration	Yes	–	51
	No	–	163
Fatigue	Yes	–	40
	No	–	174
Diarrhea	Yes	–	5
	No	–	209
Any Comorbidity	Yes	–	174
	No	–	40
Antiviral Therapy	Yes	–	73
	No	–	141
Use of Corticosteroid	Yes	–	162
	No	–	52
Oxygen Support	Yes	–	179
	No	–	35
Invasive Mechanical Ventilation	Yes	–	43
	No	–	171
Clinical Classification	Mild/Normal	–	98
	Severe	–	92
	Critical	–	24
ICU	Yes	–	36
	No	–	178
Death	Yes	–	40
	No	–	174

BMI, body mass index; ICU, intensive care unit; NS, not significant; ****, $p < 0.0001$.

As the collision reaction cell was obviously helpful to reduce interference and improve the detection limit of elements, helium, hydrogen or oxygen mode were selectively used for some elements. The permissible limits have been described before [18,19]. The method validation of ICP-MS was performed according to the consensus on the application of mass spectrometry technology in the detection of clinical trace element [20]. The standard reference materials used in the detection of inorganic elements were national first-class and second-class reference materials and standard samples according to Chinese National Standard GB/T 21415-2008/ISO 17511: 2003 [21].

2.6. Statistical analysis

The absolute concentrations of each micronutrient in serum samples were quantified using standard curves. The test results were displayed as mean ± standard deviation (SD) or median with interquartile range (IQR). If the values of the micronutrient followed a Gaussian distribution, student's *t*-test was used to compare the values between groups. Mann-Whitney *U* test was used for the variables that did not follow a Gaussian distribution. The correlations of clinical characteristics and the concentrations of micronutrients were assessed by Spearman's correlation analysis. The diagnostic performances of the micronutrients were evaluated using receiver operating characteristic (ROC) analysis. All statistical analyses were performed using GraphPad Prime 5.01 (GraphPad Software), SPSS 20.0 software (IBM) and DxAI platform (https://www.xsmartanalysis.com/beckman/login/) (Beckman Coulter). A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. COVID-19 patients had significantly dysregulated micronutrient pattern compared to NC

The clinical characteristics of the included individuals, including NC and COVID-19 patients, were displayed in Table 1. The gender of the individuals in NC and COVID-19 group was well matched ($p > 0.05$), while the average age of COVID-19 patients was higher than that of NC ($p < 0.0001$). LC-MS/MS analysis of fat-soluble and water-soluble vitamins, as well as ICP-MS analysis of inorganic elements were performed, and the methodology validation for the quantification of serum micronutrients and the detection results were shown in Table 2 and Table 3. We found that some micronutrients, such as PA, VB5, VB3, Se, As, Zn, Cu, Ca, Mg and 25(OH)D3, were significantly dysregulated in COVID-19 patients, while other micronutrients, including 5MTHF, PLP, VB2, VB1, Pb, Fe, Mn, VK1, VE, 25(OH)D2 and VA, were not significantly altered in COVID-19 patients compared to NC. These results indicated that COVID-19 patients had significantly dysregulated micronutrient pattern compared to NC.

3.2. The diagnostic value of the dysregulated micronutrients in predicting COVID-19

Then we evaluated the diagnostic value of the dysregulated micronutrients, including PA, VB5, VB3, Se, As, Zn, Cu, Ca, Mg and 25(OH)D3. ROC curves were drawn, and the area under ROC curves (AUCs) were calculated separately. As shown in Table 4 and Fig. 1A and 25(OH)D3 had an AUC of 0.835 in discriminating COVID-19 patients from NC individuals, and the optimal sensitivity and

Table 2
The results of methodology validation for the quantification of serum micronutrients.

	Precisions (%)				Linear Range	Regression Coefficient (R^2)	Recoveries (%)	
	intra-day		inter-day					
	Low	High	Low	High			Low	High
5MTHF	6.4	5.5	8.4	6.7	2–100 ng/mL	>0.990	111.5	106.1
PA	4.2	2.9	6.9	3.5	1–50 ng/mL	>0.990	91.5	92.3
PLP	6.0	6.0	9.4	5.5	4–200 ng/mL	>0.990	101.5	98.3
VB5	7.8	6.1	6.6	7.3	5–250 ng/mL	>0.990	95.3	92.1
VB3	12.1	4.7	13.9	6.1	2–100 ng/mL	>0.990	96.0	94.3
VB2	5.7	5.3	4.9	4.7	2–100 ng/mL	>0.990	100.4	99.4
VB1	1.3	4.9	11.2	3.8	1–50 ng/mL	>0.990	102.3	98.1
Se	4.7	6.7	5.9	5.2	0.28–32 μg/L	>0.990	111.24	110.77
Pb	1.9	1.8	2.9	3.8	0.01–32 μg/L	>0.990	88.27	88.58
As	5.3	6.5	3.6	3.4	0.03–16 μg/L	>0.990	91.29	92.81
Zn	0.8	2.1	2.7	3.4	0.26–1200 μg/L	>0.990	99.69	96.08
Cu	0.9	4.8	2.7	5.7	0.19–240 μg/L	>0.990	113.21	104.13
Fe	0.8	1.9	2.8	5.4	0.01–80 μg/L	>0.990	92.81	93.67
Mn	2.4	3.7	1.9	6.3	0.07–16 μg/L	>0.990	95.88	97.20
Ca	2.8	3.4	2.2	4.1	0.23–16 mg/L	>0.990	110.23	116.04
Mg	1.9	3.9	1.1	2.5	0.02–8 mg/L	>0.990	90.25	98.92
VK1	3.0	2.0	2.9	2.8	0.4–50 ng/mL	>0.990	103.87	107.25
VE	3.8	3.8	6.1	6.0	200–30000 ng/mL	>0.990	90.16	87.56
25(OH)D3	5.5	5.1	5.6	5.1	2–200 ng/mL	>0.990	93.20	102.55
25(OH)D2	5.0	4.3	4.3	4.2	0.7–75 ng/mL	>0.990	98.85	99.07
VA	2.1	3.2	3.3	2.8	20–2000 ng/mL	>0.990	87.90	92.38

Table 3
The values of the micronutrients of the included individuals.

Variables	All Mean ± SD	NC Mean ± SD	COVID-19 Mean ± SD	Statistic	p
5MTHF	7.991 ± 32.499	5.592 ± 6.342	5.217 ± 10.16	−1.151	0.69^{ns}
PA	6.178 ± 7.399	4.267 ± 5.590	7.497 ± 8.168	−4.442	<0.001***
PLP	10.944 ± 57.707	12.410 ± 12.221	9.933 ± 74.316	0.399	0.69^{ns}
VB5	51.884 ± 35.744	43.955 ± 20.128	57.356 ± 42.502	−3.987	<0.001***
VB3	15.485 ± 10.082	14.220 ± 8.566	16.358 ± 10.922	−2.072	0.039*
VB2	11.883 ± 18.907	13.546 ± 25.240	10.735 ± 12.700	1.387	0.166^{ns}
VB1	1.707 ± 1.780	1.920 ± 1.337	1.561 ± 2.017	1.886	0.06^{ns}
Se	114.032 ± 52.984	102.728 ± 25.856	121.902 ± 64.386	−3.916	<0.001***
Pb	0.580 ± 0.875	0.569 ± 1.103	0.588 ± 0.673	−0.186	0.853^{ns}
As	0.176 ± 0.858	0.056 ± 0.403	0.259 ± 1.057	−2.555	0.011*
Zn	792.996 ± 264.964	735.365 ± 156.331	833.121 ± 313.288	−3.907	<0.001***
Cu	1144.589 ± 381.788	968.385 ± 216.782	1267.272 ± 421.737	−8.804	<0.001***
Fe	2.039 ± 9.344	1.327 ± 0.496	2.535 ± 12.137	−1.211	0.227^{ns}
Mn	4.979 ± 2.335	4.805 ± 3.102	5.100 ± 1.586	−1.18	0.239^{ns}
Ca	128.017 ± 33.551	117.745 ± 21.914	135.168 ± 38.085	−5.495	<0.001***
Mg	29.263 ± 8.601	24.686 ± 4.196	32.450 ± 9.407	−10.621	<0.001***
VK1	3.182 ± 20.014	1.412 ± 0.943	4.413 ± 25.983	−1.405	0.161^{ns}
VE	15312.828 ± 45844.544	11598.232 ± 3339.533	17899.158 ± 59506.382	−1.288	0.199^{ns}
25(OH)D3	12.210 ± 8.916	16.044 ± 5.877	9.540 ± 9.666	7.305	<0.001***
25(OH)D2	2.181 ± 8.661	1.771 ± 4.105	2.465 ± 10.738	−0.75	0.454^{ns}
VA	480.736 ± 1038.256	442.406 ± 102.549	507.424 ± 1348.877	−0.586	0.559^{ns}

NS, not significant; *, $p < 0.05$; ***, $p < 0.001$.

specificity according to the youden index were 77.6% and 79.8%, respectively. In addition, serum Mg exhibited an AUC of 0.779 in differentiating COVID-19 patients from NC individuals, and the optimal sensitivity and specificity according to the youden index were 57.3% and 87.8%, respectively. Cu (AUC = 0.728), VB5 (AUC = 0.612), PA (AUC = 0.68) and Ca (AUC = 0.636) showed slightly lower diagnostic values than 25(OH)D3 and Mg. However, the diagnostic performances of other micronutrients (AUC: VB3, 0.552; As, 0,547; Zn, 0.583; Se, 0.595) were poor. These results indicated that 25(OH)D3, Mg, Cu, VB5, PA and Ca might be associated with the development of COVID-19.

3.3. Establishment of prediction model for COVID-19

Then we used these clinical parameters, including 25(OH)D3, Mg, Cu, VB5, PA and Ca, to construct a prediction model for COVID-19. At first, univariate regression analyses of these parameters indicated that each of the six parameters was statistically significant for COVID-19 ($p < 0.05$) (Supplementary Table 1). Then a logistic regression model was constructed based on 25(OH)D3, Mg, Cu, VB5, PA and Ca. The logistic regression model showed that 25(OH)D3, Mg, PA, Cu and Ca, but not VB5, were significantly independent predictors for COVID-19 (Fig. 1B). The equation for the detection of COVID-19 was constructed as follows: logit [p = COVID-19] = −4.165 + 0.086 × [PA] + 0.008 × [VB5] + 0.308 × [Mg] + 0.002 × [Cu]– 0.049 × [Ca] – 0.105 × [25(OH)D3] (Supplementary Table 2). As shown in Fig. 1C, the logistic regression model had an AUC of 0.901, which was much higher than any single parameter. The optimal sensitivity and specificity were 87.8% and 80.3%, respectively (Supplementary Table 3). The nomograms combining six clinical parameters, including 25(OH)D3, Mg, Cu, VB5, PA and Ca to predict the probability of COVID-19 was shown in Fig. 1D. These results indicate that the logistic regression model was suitable for diagnosing COVID-19 patients from NC individuals.

3.4. The performance of the logistic regression model in predicting mild/normal COVID-19 patients

It is well known that mild/normal COVID-19 patients are usually not easy to diagnose in clinical practice. Next, we explored the

Table 4
The diagnostic performances of the dysregulated micronutrients.

Variables	AUC	Sensitivity	Specificity	Youden Index	Optimal Cutoff
PA	0.68	0.563	0.803	0.366	4.23
VB5	0.612	0.413	0.83	0.243	52.6
VB3	0.552	0.474	0.68	0.154	15.6
Se	0.595	0.488	0.748	0.237	117.953
As	0.547	0.122	0.973	0.095	0.194
Zn	0.583	0.385	0.871	0.256	891.975
Cu	0.728	0.535	0.918	0.454	1228.974
Ca	0.636	0.512	0.741	0.253	130.842
Mg	0.779	0.573	0.878	0.45	29.522
25(OH)D3	0.835	0.776	0.798	0.574	11.799

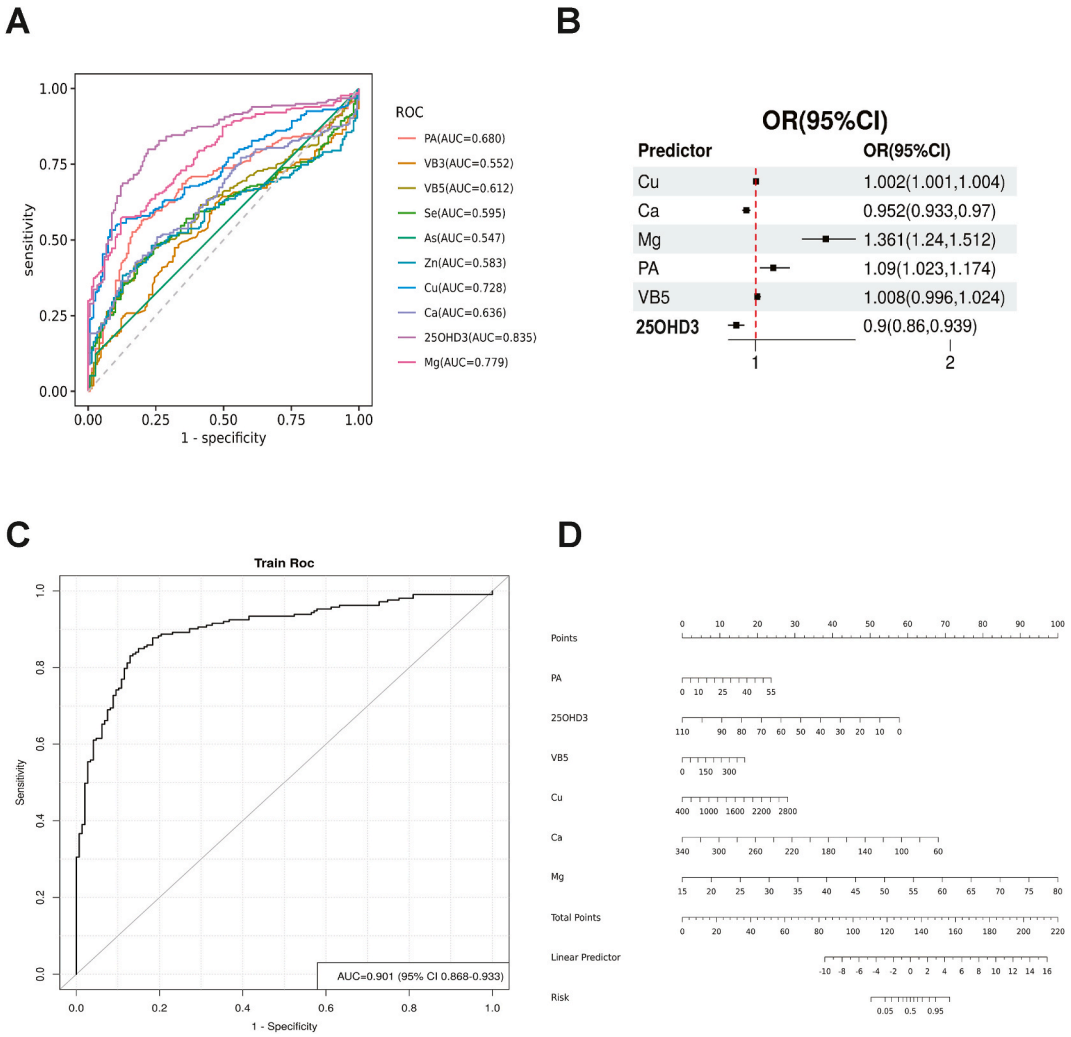

Fig. 1. The diagnostic performances of the micronutrients and the combined logistic regression model. (A) ROC curves for the dysregulated micronutrients. (B) Forest plot showed results of logistic regression analysis with 25(OH)D3, Mg, Cu, VB5, PA and Ca. (C) ROC curve of the logistic regression model based on 25(OH)D3, Mg, Cu, VB5, PA and Ca. (D) Nomograms combining 25(OH)D3, Mg, Cu, VB5, PA and Ca to predict the probability of COVID-19 patients.

diagnostic performance of the logistic regression model for mild/normal COVID-19. As shown in Fig. 2A, the logit(p) of the mild/normal, severe and critical COVID-19 patients were statistically comparable, indicating that there was no significant difference according to the logistic regression model among the three groups. The AUC of the logistic regression model for predicting mild/normal COVID-19 patients was 0.883, with the optimal sensitivity 82.65% and specificity 85.91% (Fig. 2B), indicating that the logistic regression model based on 25(OH)D3, Mg, Cu, VB5, PA and Ca exhibited great performance for predicting mild/normal COVID-19 patients from NC individuals.

4. Discussion

In this study, serum micronutrients, including fat-soluble vitamins, water-soluble vitamins and inorganic elements, were found to be dysregulated in COVID-19 patients compared with healthy control. Based on some dysregulated micronutrients, including 25(OH)D3, Mg, Cu, VB5, PA and Ca, we constructed a logistic regression model to predict the risk of COVID-19. The model not only exhibited great diagnostic performance in differentiating COVID-19 patients from NC individuals, but also showed good predictive capability for mild/normal COVID-19, suggesting its potential for the identification of susceptible population of COVID-19 and early detection of COVID-19 in clinical practice.

It is really important to investigate the potential correlation between serum micronutrients and COVID-19, and explore new disease biomarker for COVID-19 due to the rapid spread and highly pathogenic nature of SARS-CoV-2 [22,23]. Up to now, the relationship between micronutrients and COVID-19, and their role in the disease process of COVID-19 are very unclear. In this study, we found that

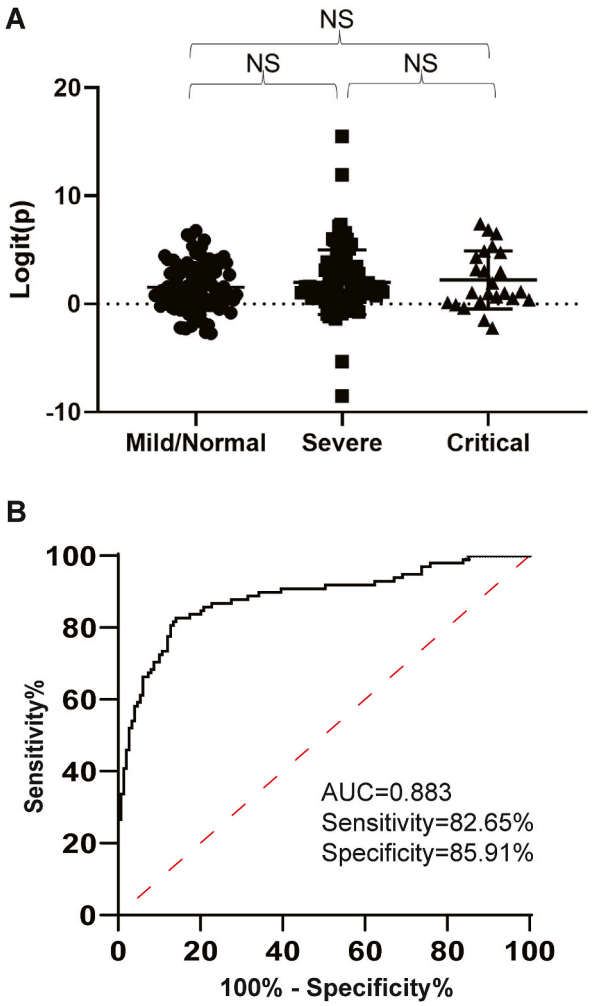

Fig. 2. The diagnostic performance of the combined logistic regression model for mild/normal COVID-19. (A) The logit(p) values of the mild/normal, severe and critical COVID-19 patients. (B) ROC curve of the logistic regression model for predicting mild/normal COVID-19. NS, not significant.

25(OH)D3, Mg, Cu, PA and Ca were independent risk factors of COVID-19, and the logistic regression model based on 25(OH)D3, Mg, Cu, VB5, PA and Ca was demonstrated to effectively discriminate COVID-19 patients from NC individuals, and its performance to diagnose mild/normal COVID-19 from NC was great as well. Our study suggested the clinical association of micronutrients with COVID-19 and the diagnostic potential of the logistic regression model for COVID-19 patients, which will be of great significance for preventing COVID-19 infection and predicting the high-risk population of COVID-19.

Several studies have indicated that serum micronutrients were associated with the development of COVID-19. For example, it has been reported that the lower the level of vitamin D in the body, the greater the risk of severe disease or death of COVID-19, suggesting that vitamin D might be a very important prognostic factor of COVID-19 [24–26]. In addition, vitamin C has antioxidant and antiviral effects, and may also play an important role in the prevention and treatment of COVID-19 [27–29]. Another study indicated that a combination therapy based on vitamin D, magnesium, and vitamin B12 in older COVID-19 patients was correlated with a significant reduction of clinical deterioration, such as requiring oxygen support and intensive care support [30]. Moreover, it is reported that the levels of vitamin D, vitamin B12 and zinc in patients who died in COVID-19 were significantly lower than those in patients who survived, but the levels of these micronutrients had little relationship with the length of hospital stay [31]. The intrinsic mechanism of the roles of these micronutrients in COVID-19 might lie in their involvement in the recruitment of immune responses to viral infections, and dysregulation of micronutrients contributes to immune response dysfunction [32,33]. These studies have shown that micronutrients might play an important role in the prevention and treatment of COVID-19. However, there are many kinds of micronutrients, and the above research only focuses on one or a few micronutrients. In this study, we detected the levels of 20 micronutrients, and found that many micronutrients were significantly dysregulated in the serum of patients with COVID-19. We further developed a logistic regression model to predict the possibility of COVID-19, which will be helpful for screening high-risk population.

In this study, we found that the levels of some indicators decreased significantly in COVID-19 patients, but some indicators increased in COVID-19 patients. The increase of these indicators might be due to the following three reasons. At first, some indicators, such as Pb and As, are hazardous elements to human body, and their elevation in COVID-19 patients has negative effects on the human respiratory system [34]. In addition, although some indicators increased in COVID-19 patients, they did not necessarily indicate an increase in active vitamin, such as PA. PA is metabolized from PLP by oxidation in vivo, and PLP is the biologically active form of VB6, which can better reflect the nutritional status of VB6. The increase of PA might be related to the metabolic abnormalities in vivo, and did not reflect the increase of active VB6 [35]. Moreover, many COVID-19 patients were complicated with basic chronic illness, such as diabetes, hypertension, nephropathy and other diseases. These patients may take drugs for a long time, and their diet structure may be different from that of healthy individuals, which might lead to the increase of individual micronutrients in COVID-19 patients.

This study still has some limitations. At first, our study is a single-center retrospective study with limited sample size. In addition, due to the differences in diet structure, environment and prevalent strains in different regions, the levels of micronutrients in the population of different regions may be different, and the impact on the risk of COVID-19 infection may also be different. However, our research only focused on the population in northern China, and large-scale multi-center prospective studies are still needed in the future to further explain the impact of micronutrient levels on the risk of COVID-19.

5. Conclusion

In conclusion, using LC-MS/MS and ICP-MS analyses, many micronutrients were identified to be dysregulated in COVID-19 patients compared to NC population. A novel logistic regression model was developed based on six micronutrients, including 25(OH)D3, Mg, Cu, VB5, PA and Ca, and this model showed great predictive ability for COVID-19, which might be useful for screening COVID-19 high-risk groups. In the future, our model requires further clinical verification and might be applied in clinical practice.

Ethics declarations

This study was reviewed and approved by the Ethical Review Committee of Peking University People's Hospital, with the approval number: 2023PHB218-001 and 2023PHB156-001. Informed consent was obtained from all subjects involved in the study.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Aimin Zhang: Methodology, Investigation. **Yue Yin:** Methodology, Investigation. **Jiashu Tian:** Investigation. **Xialin Wang:** Software, Methodology. **Zhihong Yue:** Formal analysis, Data curation. **Lin Pei:** Formal analysis, Data curation. **Li Liu:** Investigation. **Li Qin:** Investigation. **Mei Jia:** Supervision, Project administration. **Hui Wang:** Supervision, Project administration. **Qingwei Ma:** Methodology, Investigation. **Wei-bo Gao:** Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation. **Lin-Lin Cao:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hui Wang reports financial support was provided by Beijing Major Epidemic Prevention and Control Key Specialty Project-Medical Laboratory Excellence Project (2022). Wei-bo Gao reports financial support was provided by Beijing Natural Science Foundation (L222118). Lin-Lin Cao reports financial support was provided by Peking University People's Hospital Scientific Research Development Funds (RDJP2022-57). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28629.

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