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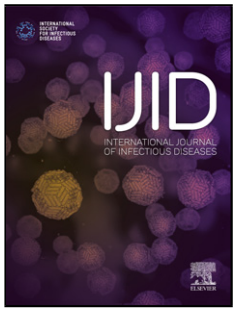

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Analysis of the predictive factors for critical illness of COVID-19 during treatment

− Relationship between serum zinc level and critical illness of COVID-19 –

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Highlights

Close relationship is found between low serum zinc and severe states of COVID-19.

Hypozincemia critically contributes to aggravation of COVID-19.

Serum zinc level can be predictive factor for critical illness of COVID-19.

We would recommend oral medication of zinc salts to patients with COVID-19.

Abstract

Objectives: Because most severely ill patients with COVID-19 in our hospital showed zinc deficiency, we aimed to examine the relationship between the patient's serum zinc level and severe cases of COVID-19.

Methods: Serum zinc <70 μg/dL was defined as the criterion for hypozincemia, and patients continuously with serum zinc <70 μg/dL were classified in the hypozincemia cohort. To evaluate whether hypozincemia could be a predictive factor for critical illness of COVID-19, we performed the multivariate analysis by employing logistic regression analysis.

Results: Prolonged hypozincemia was found to be a risk factor for a severe case of COVID-19. In evaluating the relationship between the serum zinc level and severity of patients with COVID-19 by multivariate logistic regression analysis, critical illness can be predicted through the sensitivity and false specificity of an ROC curve with an error rate of 10.3% and AUC of 94.2% by only two factors: serum zinc value (P=0.020) and LDH value (P=0.026).

Conclusions: Proper management of the prediction results in this study can contribute to the establishment and maintenance of a safe medical system, taking the arrival of the second wave and spread of COVID-19 in the future into consideration.

Keywords: COVID-19, Serum Zinc, Predictive Factors, Logistic Regression Analysis, Critical Illness, Japan

Introduction

A study has reported that COVID-19 in a specific portion of inpatients has become severe (Wu et al., 2020a). Other studies have indicated that among those severe cases, the mortality rate of patients who require ICU care is substantially high at rate of 32% to 72% (Huang et al., 2020; Wu et al., 2020b; Yang et al., 2020; Zhou et al., 2020). Considering the expected next wave and spread of infection in the future, having the ability to predict the influx of inpatients with COVID-19 using multiple basic factors will have a significant impact in terms of securing ICU availability and subsequent decisions required in medical institutions who treat patients with COVID-19. When this becomes feasible, it will enable medical institutions to preliminarily categorize patients whose symptoms may potentially progress to severe and focus on the treatment from an early stage. Therefore, it is considered essential to examine highly predictive clinical factors to forecast immediately after hospitalization if patients will be cured while remaining within the mild to moderate range or become critically ill during treatment.

Based on the previous reports (Zhang et al., 2020; Li et al., 2020; Jordan et al., 2020), the Sakai City Medical Center has been treating patients using the following risk factors for critical illness: age≥65 years, obesity (BMI≥25), history of diseases such as hypertension, respiratory disease, diabetes, cardiovascular disorders, and LDH≥445 IU/L (Li et al., 2020). Additionally, levels of

ferritin, KL-6, CRP, and D-dimer have been used as observation items since the beginning. As it has been reported that more than a certain number of cases of dysosmia and dysgeusia were observed in addition to fever, malaise, cough, etc. as the initial symptoms of COVID-19, it was assumed that hypozincemia—a condition deeply associated with the onset of dysgeusia—has occurred in patients (Kodama et al., 2016). Since it had been reported that immunocompromised and inflammatory states are caused by hypozincemia (Kodama et al., 2016), serum zinc concentration (serum zinc) was added as an item in COVID-19 patients from the middle of the pandemic in Japan.

Zinc is one of the “essential minerals” in the human body. Zinc deficiency in the body due to various causes is called hypozincemia. Symptoms of hypozincemia include dysgeusia, loss of appetite, dermatitis, hair loss, anemia, and stomatitis. The Japanese Society of Clinical Nutrition suggests that a serum zinc level of 80-130 μg/dL is defined as normal, a level of 60-80 μg/dL is defined as subclinical zinc deficiency, and a level of <60 μg/dL is defined as clinical zinc deficiency in Japan (Kodama et al., 2016). In contrast, in the Harrison’s Principles of Internal Medicine, used as an international standard indicator, clinical zinc deficiency is defined <70 μg/dL (10.7 μmol/L) (Russell et al., 2018). In this study, the zinc deficiency state was set at <70 μg/dL to enable direct comparison with the domestic and international information.

Based on the measurement results of serum zinc levels in patients with COVID-19 in our hospital, almost all severe cases showed subclinical or clinical zinc deficiency, and prolonged hypozincemia is additionally considered a risk factor for critical illness of COVID-19. In response to the above results, we aimed to examine the relationship between the patient's serum zinc level and severe cases of COVID-19 in detail and to evaluate whether hypozincemia could be a predictive factor for critical illness of COVID-19.

Methods

Patients information

The research was conducted by identifying 62 patients with COVID-19 who were admitted to the Sakai City Medical Center since March 24 to May 24, 2020, and then forming a sub-group of 29 patients who underwent a determination of the serum zinc concentration. According to the follow-up observation after hospital admission this time, all patients (62 patients) were classified into two groups: mild/moderate and severe. The classification criteria were based on the nine-score ordinal scale described in COVID-19 therapeutic trial synopsis: (0) No clinical or virological evidence of infection; (1) No limitation of activities; (2) Limitation of activities; (3) Hospitalized, no oxygen therapy; (4) Hospitalized, oxygen by mask or nasal prongs; (5)

Hospitalized, non-invasive ventilation or high-flow oxygen; (6) Hospitalized, intubation and mechanical ventilation; (7) Hospitalized, ventilation + additional organ support—pressors, RRT, ECMO; (8) Death. Patients (0) to (5) were classified into the mild/moderate group, and those who fall within (6) to (8) were classified into the severe group. Further, death was not observed in all the hospitalized patients this time.

Blood sampling and measurement of serum zinc

Serum zinc was measured several times at the time of blood sampling on the first day of hospitalization and two to three days later. When measuring serum zinc, it is required to pay attention to the influence of diurnal variation and diet. It can also be affected by stress and hormone conditions. Therefore, the blood sampling and measurement were conducted on multiple days on an empty stomach early in the morning after hospitalization, and centrifugation of blood was performed as early as possible after blood collection. To determine serum zinc, we used Accuras Auto Zn (Shino-Test Corporation, Tokyo), a test kit for pharmaceuticals for external diagnosis, developed to measure zinc concentration in serum (Higurashi, 2015), using the automatic analyzer (Automatic Analyzer AU5800, Beckman Coulter, Inc., Tokyo, Japan) at the Sakai City Medical Center. We defined the concentration of serum zinc <70 μg/dL (10.7 μmol/L)

as the criterion for hypozincemia, and patients with serum zinc <70 μg/dL continuously after the first and second measurements were classified in the hypozincemia cohort.

Estimates of other clinical laboratory data

LDH and ferritin levels, and KL-6 in patients' serum were measured using the automatic analyzer (AU5800, Beckman Coulter, Inc.) as well. White blood cell, neutrophil, and lymphocyte counts were also measured using a blood cell count device (Multiple Automated Blood Cell Counter XN-3000, Sysmex Corporation); D-dimer was measured using a blood-coagulation-ability measurement device (CS-5100, Sysmex Corporation). From the medical record data, the patients' sex, age, obesity (BMI≥25), history of previous disease (diabetes, chronic renal disorder, liver disease, dyslipidemia, cardiovascular disease, hypertension, respiratory disease, taste abnormality), as well as HbA1c were verified at the time of admission.

Statistical analysis

We examined each of 21 clinical factors, including the items mentioned above and serum zinc level in the laboratory data, to identify whether the factors associated with the critical illness of COVID-19 were statistically significant. Regarding the statistical analysis between the two groups,

the chi-square test was conducted for the categorical data items, and the Student-*t*-test was conducted for the numerical data items, and $P<0.05$ was determined as significant. The 29 subgroup patients with serum zinc data were divided into two groups of mild/moderate and severe, and the same statistical analysis was performed. Next, to confirm if each factor found to be significant between the two groups were confounding factors, an independence test using the chi-square value and correlation evaluation using univariate analysis were performed.

In order to examine to what extent clinical factors—recognized not as confounding but independent factors—had an impact on the critical illness of COVID-19 and if they can be used as predictive factors for aggravation, we performed the multivariate analysis by employing logistic regression analysis using clinical factors as explanatory variables and critical illness of COVID-19 as objective variables. Regarding the analysis results, the C-statistic by the area under the curve (AUC) of the ROC curve by logistic regression, the contribution rate by the square of the correlation coefficient, and the error rate were simultaneously evaluated; then, variable selection was adopted in the case where the *P*-value of covariance for an explanatory variable is to be <0.2. In reporting the calculation results, the regression coefficient of the covariate employed in the multivariate analysis, its standard deviation, the Wald χ^2 value, the *P*-value, the odds ratio, and the 95% confidence interval of the odds ratio were used. All statistical analysis of the data was

performed using Excel statistics (BellCurve for Excel, version 3.20, Stock Social Information Service, Tokyo, Japan).

Results

Table 1 (total patients) and Table 2 (patients with serum zinc data) show the results of the statistical analysis of clinical factors, which were obtained by dividing inpatients into two groups, respectively. Eight categorical factors have been determined as statistically significant between the two groups for all hospitalized patients (Table 1). The following factors are associated with critical illness: age≥65 years, diabetes, HbA1c≥6.0%, liver disease, cardiovascular disease, hypertension, LDH≥445 IU/L, and high ferritin (male: ≥300 ng/mL, female: ≥200 mg/mL, cutoff value). The significant numerical variables are actual age, HbA1c, LDH, and ferritin levels. The clinical factors reported in these results were similar to the ones that have already been reported in China, Europe, and the United States (Gandhi et al., 2020; Jordan et al., 2020; Li et al., 2020; Zhang et al., 2020; Zhu et al., 2020). In contrast, of the categorical factors shown in Table 2, the following four items have been determined as statistically significant and are associated with critical illness: HbA1c≥6.0%, cardiovascular disease, LDH≥445 IU/L, and serum zinc<70 μg/dL. The significant numerical factors are LDH, ferritin, and serum zinc levels. Because of the smaller

size of the sub-group with serum zinc level, compared to the whole group, there was no difference in the factors associated with critical illness, and fewer factors were identified.

The serum zinc levels in the subgroups are shown in Figure 1. There were three patients (14%) in the mild/moderate group and six patients (86%) in the severe group whose zinc level was below the cutoff value of zinc deficiency (<70 μg/dL), indicating that the serum zinc level in the patients of the severe group was remarkably low. With the above results, we decided to perform an analysis to predict the critical illness of inpatients with COVID-19 for the next step based on the multivariate analysis of the factors that showed significant differences between the two groups. First, to narrow down clinical factors used as covariates that have been determined as not confounding but independent factors, mutual confounding was evaluated by independence test using the chi-square value and correlation analysis using the univariate analysis. As a result, the following confounding factors have identified: "age≥65 years vs. cardiovascular disease (χ^2=5.47, P=0.02)," "age≥65 years vs. hypertension (χ^2=4.59, P=0.03)," "diabetes vs. HbA1c≥6.0% (χ^2 =14.33, P=0.0002)," "diabetes vs. liver disease (χ^2=3.97, P=0.046)," "LDH≥445 IU/L vs. high ferritin (χ^2=9.82, P=0.002)," "LDH value vs. ferritin value (r=0.633, P<0.001)," and the factors strongly associated with critical illness had been narrowed down to three: "age≥65 years (or actual age), diabetes/HbA1c≥6.0% (or HbA1c level), and LDH≥445 IU/L (or LDH value) (Liang et al.,

2020; Yan et al., 2020; Zhu et al., 2020).” Figure 2 shows a collinear relationship between the LDH and serum ferritin values that has been determined significantly by univariate analysis as an example of confounding factors. Moreover, the identified confounding factors among the subgroup with the serum zinc was measured were as follows: “serum zinc <70 μg/dL vs. cardiovascular disease (χ^2=4.77, *P*=0.03),” “diabetes vs. HbA1c≥6.0% (χ^2=7.06, *P*=0.008),” “LDH≥445 IU/L vs. high ferritin (χ^2=4.21, *P*=0.04),” “LDH value vs. ferritin value (r=0.650, *P*<0.001).” The same three factors were found to be associated with critical illness in the sub-group: “HbA1c≥6.0%, hypozincemia (or serum zinc level), LDH≥445 IU/L (or LDH level).”

As such, we conducted a logistic regression analysis for all inpatients using three continuous variable data of “actual age, HbA1c value, LDH value” as explanatory variables in the multivariate analysis and critical illness was set for objective variables. Akaike’s Information Criteria (AIC), which is widely used when selecting the optimum result from multiple models based on statistical judgment, was applied to select the optimum model. A combination of the minimum AIC and a P-value of 0.2 or less for the covariate, including the constant term, was adopted. The results of the logistic regression analysis performed at that time are shown in Table 3, and the ROC curve generated using the logistic regression is shown in Figure 3. In the adopted model, HbA1c value was rejected as an explanatory variable, and it was found that critical illness

is predicted through the sensitivity and false specificity of an ROC curve with its error rate of 14.8% and AUC of 84.9% by two factors alone: actual age (P=0.088) and LDH value (P=0.003). Our predictive analysis for the overall patients in this study showed very similar results to those recently reported in China for predicting morbidity and mortality of patients with COVID-19 (Liang et al., 2020; Yan et al., 2020).

Conversely, for the patients in the sub-group with serum zinc level, logistic regression analysis was conducted using three continuous variable data of "serum zinc value, HbA1c value, LDH value" as explanatory variables and critical illness as the objective variables. The selection criteria for the optimum model has remained the same, and the results of the logistic regression analysis of the adopted model (the constant term is judged to be zero from AIC) are shown in Table 4, and the ROC curve is shown in Figure 4. Similarly, with the adopted model, HbA1c value was rejected as an explanatory variable, and critical illness can be predicted through the sensitivity and false specificity of an ROC curve with an error rate of 10.3% and AUC of 94.2% by two factors alone: serum zinc value (P=0.020) and LDH value (P=0.026). The predictive results of the sub-group using serum zinc level was found to remarkably exceed those of overall patients.

Discussion

Recent research was reported from China on the severe disease and mortality of patients with COVID-19, in which 575 hospitals in China participated to develop risk prediction scores of the patients with critical illness (ICU admission, invasive ventilation and death) (Liang et al., 2020). Patients are categorized into the development cohort and the confirmation cohort comprising 1,590 and 710 patients, respectively. In the actual calculation, the following ten items from 72 potential factors were finally adopted as independent predictors (numbers in parentheses are odds ratios). They include chest X-ray abnormality at admission (3.39), age (1.03), hemoptysis (4.53), dyspnea (1.88), consciousness disorder (4.71), number of comorbidities (1.60), history of cancer (4.00), neutrophil/lymphocyte ratio (1.06), LDH (1.002), direct bilirubin (1.15). Of these, test values for only three factors from the blood samples were available: neutrophil/lymphocyte ratio, LDH, and direct bilirubin (Liang et al., 2020).

Similarly, it has been reported that a study by Chinese researchers on predicting mortality from blood samples attempted machine learning with AI to identify biomarkers predicting mortality using blood samples from 485 patients in Wuhan. The research findings demonstrated that (values in parentheses indicate cutoff values), using three factors of high LDH (365 IU/L), high level of high-sensitive CRP (41.2 mg/L), and reduced lymphopenia (14.7%), predicting patient mortality has become feasible more than ten days earlier than prior readings, with an accuracy of less than

10% error rate (AUC 97.8% and 95.1% in the development and confirmation cohorts, respectively.) However, in practice, it has been reported that the use of LDH alone has made it possible to identify many patients who require quick medical intervention (AUC 92.3%-94.2%) (Yan et al., 2020).

In summary of the above report, LDH level and white blood cell count (including neutrophil/lymphocyte ratio) have been identified as the most critical factors in blood sampling for predicting the severe disease states and mortality of patients with COVID-19 based on a large number of patients in China, which supported the results of this study. Actually, we found precisely the opposite relationships (data not shown) of LDH level with the number of neutrophil or that of lymphocyte where the former and latter were positive and negative, respectively, suggesting that activation of cellular immunity as the innate immune system might contribute to rapid and non-specific increase of inflammation in early phase of infection of COVID-19, rather than activation of killer and helper T-lymphocytes as the acquired immunity. White blood cells are the compartmental location site where zinc is distributed and stored at the maximum concentration (380-450 μg/mL) in circulating blood (Kodama et al., 2016); and in patients with zinc deficiency or hypozincemia, a decrease in the white blood cell count can easily occur after COVID-19, or it is assumed that it has already occurred before the infection.

In this study, no association between dysgeusia and hypozincemia was found in patients with COVID-19. In contrast, results of an *in vitro* study in which zinc ion (Zn^{2+}) and ionophore were used in combination, has originally reported its antiviral effect against SARS coronavirus (Velthuis et al., 2010), and it has been proved that zinc ions directly inhibit the activity of the RNA-dependent RNA polymerase of SARS-CoV-1 to suppress the replication of viral genomic RNA and block the replication of viral particles in the mechanism of action (Velthuis et al., 2010). Compared with the target molecule for the mechanism of action of Remdesivir or Favipiravir against SARS-CoV-2, whose pharmacological effects are being evaluated in observational studies and clinical trials as potential therapeutic agents for COVID-19 (Guy et al., 2020; Sanders et al., 2020), the target molecule for the mechanism of action of zinc ion is the same molecule that is present in coronavirus; as a result, the effects of zinc ion using ionophore (Xue et al., 2014) and complexation (Sakurai et al., 2002; Sakurai et al. 2008) on SARS-CoV-2 is expected to be evaluated in the future. Besides, while the therapeutic effect of hydroxychloroquine on COVID-19 has remained uncertain (Geleris et al., 2020), it is possible to establish a working hypothesis in which hydroxychloroquine can demonstrate synergistic effects with zinc ion (Liu et al., 2020; Wang et al., 2020). However, it is necessary to prove the synergistic effect in an *in vivo* experimental system before determining the clinical therapeutic effects.

Conversely, zinc ion is known for its mechanism involving the activation of immune cells that regulate the promotion or suppression of cell-mediated immunity (Hirano et al., 2008). Intracellular zinc ion is reported to inhibit activation of phosphorylation of STAT3, which is a crucial molecule of intracellular signal transduction by IL-6 stimulation, resulting in the suppression of cytokine storm (Kitabayashi et al., 2010); zinc ion supplementation regulates the helper T2 lymphocyte-M2 macrophage activation pathway to promote anti-inflammatory effects (Kido et al., 2019). Although immunocompromised state caused by hypozincemia is a well-known fact (Kodama et al., 2016), this state is revealed by the findings that intracellular zinc ion is involved in the activation of T cells in cell-mediated immunity, as described above. Therefore, hypozincemia may be closely related to events that have a significant impact on the prognosis of COVID-19; for example, patients with hypozincemia whose natural immunity is low are vulnerable to viral infection. At the same time, once infected, excessive immune reactions can easily cause a cytokine storm or the imbalance between M1 and M2 macrophages that regulate the degree of inflammatory reactions.

According to a recent literature in South Korea, the relationships between “general severely ill patients and ICU transfer rate” and “serum trace element levels in patients and their changes over time elapsed”, have been investigated in detail. Statistically, a significant number of cases have

been confirmed in which severely ill patients, who died after ICU transfer, had hypozincemia and whose serum zinc concentration did not increase after treatment (Lee et al., 2019). The University of Melbourne in Australia has announced on their website that they will begin clinical trials of intravenous injection of zinc chloride for patients with COVID-19 (World-first trial, 2020). The group of organizers of this prospective clinical study has reported actual cases of inhibiting organ disorders in several organs (lungs, liver, and kidneys) in their previous fundamental researches and clinical applications, in which reperfusion disorders in the organs where depleted oxygen supply is caused by ischemia can be treated with intravenous injection of zinc chloride—in other words, organ disorders can be inhibited with oxygenation therapy by intravascular zinc supplementation into the vessels before ischemia reperfusion (Ischia et al., 2019). Based on these results, they seem to have initiated clinical tests to observe if zinc chloride can bring positive effects on the treatment of COVID-19 (World-first trial, 2020). Although it is estimated to take approximately one year to report the results of this study, it is expected to determine the effects of zinc supplementation in formal clinical tests.

This study suggested that hypozincemia could be a predictor for critical illness of COVID-19. However, trials with an increased number of patients should be evaluated; and at the same time, whether zinc salts medication can inhibit viral replication of SARS-CoV-2 and it potentially can

be a therapeutic agent should be verified in prospective clinical trials as described above (World-first trial, 2020). In Japan, zinc acetate (brand name Nobelzin) as a pharmaceutical drug has been applied to treat Wilson's disease and hypozincemia, which can be prescribed based on the diagnosis of medical doctors. If the relationship between hypozincemia and the severe cases of COVID-19 in a larger number of patients is identified like this study, it may be useful for the future medication of COVID-19 with oral zinc salt therapy. In the context and perspective of prevention and treatment of COVID-19, we would like to provide the additional result for time-dependent profiles of zinc levels of 4 critically ill patients in this study who were admitted into ICU, delivered by enteral nutrition from the tube inserted through the nose, observable continuously for one month after pathogenesis, gradually recovered and finally discharged from the hospital, as shown in Figure 5. From this data, it was indicated that after supplying adequate enteral nutrition in ICU, an aggressive and enteral nutrition support for 10-12 days would produce a recovery of serum zinc level over the normal range in critically ill patients. Additionally, there were not especially any adverse effects in these patients. Therefore, we considered it possible to use both zinc supplementation to uninfected people for prevention and zinc medication to patients for treatment who might exhibit lower serum zinc level than normal range. However, as the conceivable adverse effect of zinc administration, excess zinc supply could induce lower copper

level because of each other's opposing absorption and metabolism in the body, and thereby additional monitoring of both zinc and copper levels may be needed simultaneously in those case.

In conclusion, according to the results of measuring serum zinc in hospitalized patients with COVID-19 at this hospital, most severely ill patients showed subclinical or real zinc deficiency, and prolonged hypozincemia can be a risk factor for a severe case of COVID-19. In evaluating the relationship between the serum zinc level and severity of patients with COVID-19 by multivariate logistic regression analysis, it was suggested that the ROC curve with an error rate of 10.3% and AUC of 94.2% by only two factors, serum zinc and LDH levels, could predict severe cases. Therefore, we would like to emphasize the advantage of measuring the serum zinc level as one of the routine and standard physiological blood testing. Proper management of the prediction results in this study can contribute to the establishment and maintenance of a safe medical system, taking the arrival of the second wave and spread of COVID-19 in the future into consideration.

Declarations of interest

Funding source: This study did not receive any funding.

Ethical approval: This study involved human subjects, and then institutional review board (IRB) approval on an opt-out in the clinical observation study was obtained for experimentation with

human subjects before starting the research. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Conflict of interest: We declare no conflict of interests, competing financial interests, and personal relationships that could have appeared to influence this study.

We have read the current "Instructions to Authors" and understood compliance with those instructions and acceptance of the conditions posed. Therefore, we make a declarative statement that all authors have seen and agreed to the submitted version of the paper, and have been acknowledged as contributors, the material is original, and it has been neither published elsewhere nor submitted for publication simultaneously. In addition, we make a declaration that if accepted, the paper will not be published elsewhere in the same form, in English or in any other language, without written consent of the copyright holder.

We declare no conflict of interests, competing financial interests, and personal relationships that could have appeared to influence this study.

Declaration of interests

The authors 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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Figure captions

Figure 1. Comparison of serum zinc concentrations in patients with COVID-19 (n = 29) between the mild/moderate and severe (intubation) conditions.

Statistical significance: $p < 0.01$ by Student-t test.

The central red lines correspond to the mean values, and the upper and lower red lines correspond to the means + standard deviation (SDs) and means – SDs, respectively. The dotted line denotes the threshold concentration of hypozincemia as 70 μg/dL.

Figure 2. Colinear relationship between the serum ferritin and LDH values in all patients (n = 62) with moderate correlation (r = 0.633, P<0.001).

Collinearity between the data of serum ferritin and LDH was statistically recognized because both parameters would be increased by the infection and inflammation.

Figure 3. Receiver operating characteristic (ROC) curve for all patients with COVID-19 (n = 62) by LDH and ages.

Figure 4. Receiver operating characteristic (ROC) curve for patient subgroups with COVID-19 (n

= 29) by LDH and serum Zn.

Figure 5. Time-dependent changing profiles of serum zinc concentrations in four critically ill (severe) patients after pathogenesis of COVID-19.

These patients were able to be monitored continuously for one month after pathogenesis, treated with enteral nutrition delivered from the tube inserted through the nose.

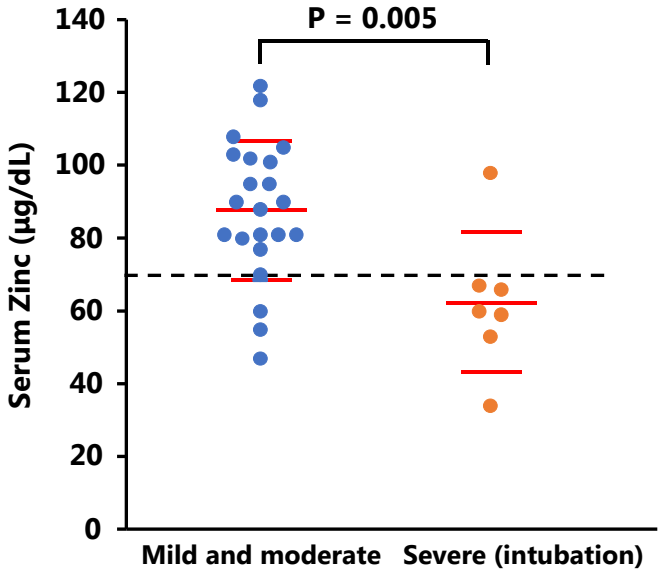
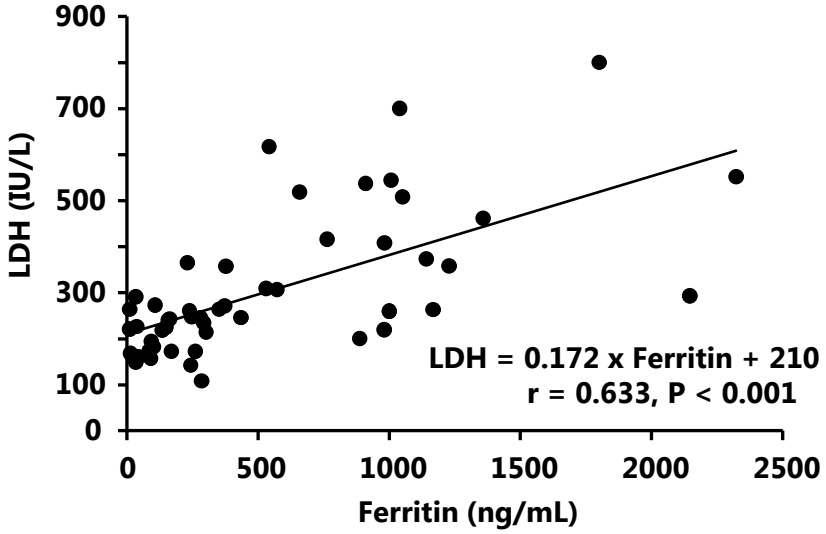


Figure 1 by Yukako Yasui et al.



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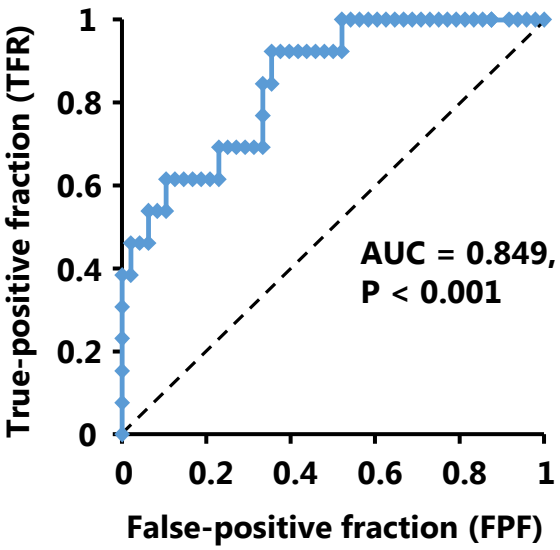

Figure 3 by Yukako Yasui et al.

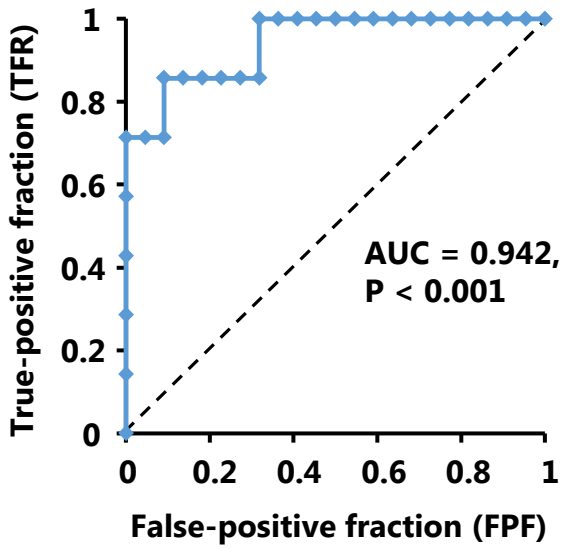

Figure 4 by Yukako Yasui et al.

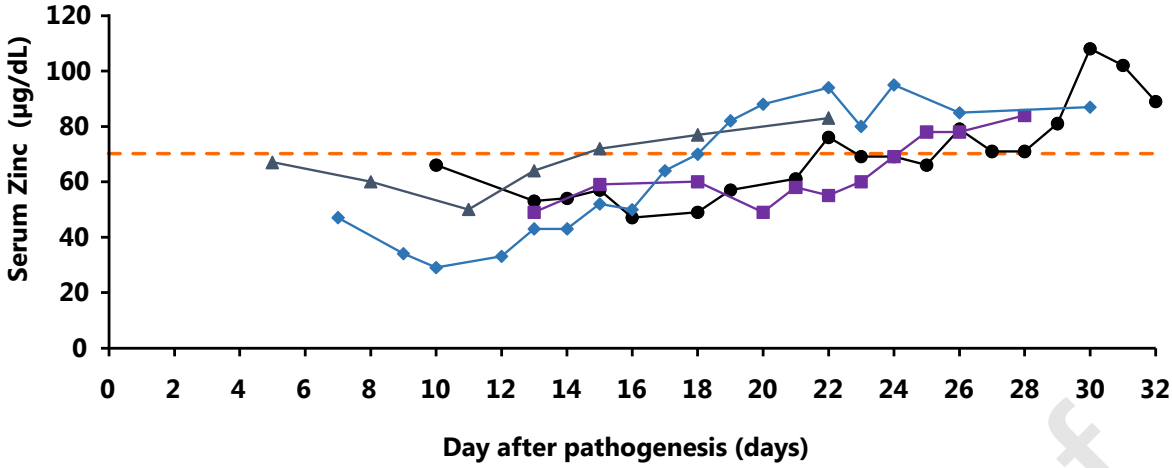

Figure 5 by Yukako Yasui et al.

Table 1. Demographic, clinical, and laboratory characteristics of all patients with COVID-19

Characteristics or Condition	Total patients	Mild and Moderate	Severe (Intubation)	χ^2	P value	
	(n = 62)	(n = 49, 79%)	(n = 13, 21%)			
Male sex	34	26 (53%)	8 (62%)	0.30	0.585	
Age >= 65 years	17	10 (20%)	7 (54%)	5.77	0.016	*
Obesity (BMI >= 25)	25	21 (43%)	4 (31%)	0.62	0.430	
Diabetes mellitus	13	7 (14%)	6 (46%)	6.30	0.012	*
HbA1c >= 6.0%	28/61	17 (35%)	11 (85%)	9.97	0.002	**
Chronic kidney disease	6	4 (8.2%)	2 (15%)	0.61	0.434	
Liver disease	3	1 (2.0%)	2 (15%)	3.97	0.046	*
Hyperlipidemia	7	4 (8.2%)	3 (23%)	2.28	0.131	
Cardiovascular disease	2	0 (0%)	2 (15%)	7.79	0.005	**
Hypertension	20	12 (24%)	8 (62%)	6.45	0.011	*
Respiratory disease	8	8 (89%)	0 (0%)	2.44	0.119	
Palate disorder	12/32	10 (45%)	2 (20%)	1.90	0.168	
LDH >= 445 IU/L	11/61	5 (10%)	6 (46%)	8.84	0.003	**
Ferritin >= ♂300 (♀200) ng/mL	30/51	19 (49%)	11 (92%)	6.99	0.008	**

Labortory Parameters	Total patients	Mild and Moderate	Severe (Intubation)	t-statistics	P value	
Age (year)	62 (49, 13)	49.7 ± 16.1	63.2 ± 14.1	2.77	0.008	**
BMI (kg/m^2)	62 (49, 13)	24.6 ± 4.5	26.6 ± 6.9	1.24	0.218	
HbA1c (%)	61 (48, 13)	6.0 ± 1.0	6.7 ± 1.1	2.20	0.032	*
LDH (IU/L)	61 (48, 13)	250 ± 111	442 ± 191	4.67	0.00002	**
Ferritin (ng/mL)	51 (39, 12)	386 ± 393	1117 ± 654	4.77	0.00002	**

Statistical significance: *; p < 0.05, **; p < 0.01.

Laboratory data are expressed as the mean ± SD.

Ferritin ≥ 300 or 200 (ng/mL) corresponds to the male or female cases, respectively.

Laboratory data are expressed as the means ± standard deviations (SDs).

Statistical significance: *; $P < 0.05$, **; $P < 0.01$. Categorical and variable data analyses were performed by the chi-squared and Student-t-tests,

respectively.

Table 2 Demographic, clinical, and laboratory characteristics of the subgroups of patient with COVID-19

Characteristics or Condition	Total patients	Mild and Moderate	Severe (Intubation)	χ^2	P value	
	(n = 29)	(n = 22, 76%)	(n = 7, 24%)			
Male sex	16	12 (55%)	4 (57%)	0.01	0.904	
Age >= 65 years	11	7 (32%)	4 (57%)	1.45	0.229	
Obesity (BMI >= 25)	12	10 (45%)	2 (29%)	0.62	0.430	
Diabetes mellitus	6	3 (14%)	3 (43%)	2.76	0.097	
HbA1c >= 6.0%	15	9 (41%)	6 (86%)	4.27	0.039	*
Chronic kidney disease	3	2 (9.1%)	1 (14%)	0.15	0.694	
Liver disease	1	1 (4.5%)	0 (0%)	0.33	0.566	
Hyperlipidemia	4	3 (14%)	1 (14%)	0.00	0.965	
Cardiovascular disease	2	0 (0%)	2 (29%)	6.75	0.009	**
Hypertension	12	7 (32%)	5 (71%)	3.43	0.064	
Respiratory disease	2	2 (9.1%)	0 (0%)	0.68	0.408	
Palate disorder	12	10 (45%)	2 (29%)	0.62	0.430	
LDH >= 445 IU/L	7	2 (9.1%)	5 (71%)	11.27	0.0008	**
Ferritin >= ♂300 (♀200) ng/mL	21	15 (68%)	6 (86%)	0.82	0.366	
Zinc < 70 μg/dL	9	3 (14%)	6 (86%)	12.89	0.0003	**

Labortory Parameters	Total patients	Mild and Moderate	Severe (Intubation)	t-statistics	P value	
Age (year)	29 (22, 7)	54.3 ± 17.5	62.7 ± 15.3	1.14	0.264	
BMI (kg/m^2)	29 (22, 7)	25.0 ± 3.8	26.0 ± 5.4	0.55	0.587	
HbA1c (%)	29 (22, 7)	6.1 ± 1.0	6.7 ± 1.3	1.41	0.170	
LDH (IU/L)	29 (22, 7)	283 ± 99	495 ± 165	4.19	0.0003	**
Ferritin (ng/mL)	29 (22, 7)	458 ± 399	956 ± 689	2.40	0.024	*
Zinc (μg/dL)	29 (22, 7)	87.7 ± 19.1	62.4 ± 19.2	3.04	0.005	**

Statistical significance: *; p < 0.05, **; p < 0.01.

Laboratory data are expressed as the mean ± SD.

Ferritin ≥ 300 or 200 (ng/mL) corresponds to the male or female cases, respectively.

Laboratory data are expressed as the means ± standard deviations (SDs).

Statistical significance: *; $P < 0.05$, **; $P < 0.01$. Categorical and variable data analyses were performed by the chi-squared and Student-t-tests, respectively.

Table 3 Estimated parameters based on the multivariable logistic analysis for predicting severe cases (intubation) using two factors, LDH and age, among all patients (n = 61)

Factor	Coefficient	SE	Wald χ^2	P value	Odds ratio	95% CI
LDH	0.008	0.003	8.529	0.003	1.008	1.003 - 1.013
Age	0.047	0.028	2.909	0.088	1.049	0.993 - 1.107
intercept	-6.617	2.006	10.877	< 0.001	0.001	0.000 - 0.068

AUC = 0.849, R^2 = 0.284, correlation coefficient = 0.551, and error rate = 0.148

AUC = 0.849, R^2 = 0.284, correlation coefficient = 0.551, and error rate = 0.148.

Table 4 Estimated parameters based on the multivariable logistic analysis for predicting severe cases (intubation) using two factors, LDH and serum Zn concentrations, among zinc-determined patients (n = 29)

Factor	Coefficient	SE	Wald χ^2	P value	Odds ratio	95% CI
Serum Zn	-0.106	0.046	5.421	0.020	0.899	0.823 - 0.983
LDH	0.016	0.007	4.950	0.026	1.016	1.002 - 1.031
intercept	0.000					

AUC = 0.942, R^2 = 0.663, correlation coefficient = 0.782, and error rate = 0.103

AUC = 0.942, R^2 = 0.663, correlation coefficient = 0.782, and error rate = 0.103.