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LETTER TO THE EDITOR

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Development of an evaluation model to determine disease severity in COVID-19 using basic laboratory markers

Dear Editors

Since late 2019, the COVID-19 pandemic, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), has had an enormous impact on the medical field and society across the world.[1] To date, quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) has been used as a gold standard to detect the RNA of SARS-CoV-2 in patient samples. Though RT-qPCR can show viral loads, association between viral loads and disease severity remains unelucidated. Therefore, many researchers have been investigating various laboratory markers to link disease severity and prognosis.[2] For example, procalcitonin (PCT)[3] and interleukin 6 (IL-6)[4] were found to be associated with disease severity. However, these tests are expensive and not always available in daily clinical practices. The aim of this study was to examine the feasibility and usefulness of basic laboratory markers to determine the severity of COVID-19.

Thirty-three patients, whose RT-qPCR tests were positive, were included in this study. They were admitted to Juntendo University Hospital (Tokyo, Japan) from April 1 to August 6, 2020. This study was approved by the Institutional Ethics Committee of Juntendo University Hospital (IRB #20-036) and was performed in accordance with the Declaration of Helsinki. All data were anonymous and written informed consents were waived. Data were analysed with JMP15 software (SAS Institute, Inc., Tokyo, Japan). $p < .05$ was considered as statistically significant.

A total of 120 blood samples were collected from 33 patients for analysis of complete blood count (CBC) and blood biochemistry. CBCs were measured by the Sysmex XE-5000 automated hematological analyzer (Sysmex, Hyogo, Japan), and biochemical tests were performed using the Labospect008 (Hitachi High-Tech, Tokyo, Japan). The samples were analysed within 2 h after blood collection. The samples were classified into two groups based on the clinical conditions according to the World Health Organization (WHO) at the time of collection.[5] Mild COVID-19 was defined as respiratory symptoms without evidence of pneumonia or hypoxia, while moderate or severe infection was defined as presence of clinical and radiological evidence of pneumonia. In moderate cases, saturation of percutaneous oxygen (SpO_2) must be ≥90% in room air while one of the following was required to define the severe cases: respiratory rate >30 breaths/min or SpO_2 < 90% in room air. Critical cases include individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.[5]

Table 1 summarizes the clinical characteristics and laboratory data of the 33 patients on admission. Twenty patients showed mild–moderate severity (MILD), and 13 patients showed severe-critical severity (SEVERE), and four patients died. The SEVERE patients were older than the MILD patients, but there was no difference in gender compositions and observation periods. Regarding the laboratory data on admission, neutrophil cells, neutrophil-to-lymphocyte ratio (NLR), red blood cell distribution width-standard deviation (RDW-SD), blood urea nitrogen (BUN), ferritin (FERR), and C-reactive protein (CRP) were higher in SEVERE patients than MILD patients while lymphocyte % and counts (LYMPH), protein (TP), and albumin (ALB) were lower in SEVERE patients than MILD patients.

Next, we analysed the 120 samples collected from the 33 patients. Sixty-five samples obtained from 20 patients whose conditions were equivalent to MILD, and 55 samples obtained from 13 patients whose conditions were equivalent to SEVERE. We first analysed the diagnostic values of a single parameter with Wilcoxon rank sum test and the receiver operating characteristic (ROC) analysis (Figure 1 and Table 2). Figure 1A shows the ROC curves using a single parameter. The standard errors (SE) and 95% confidence intervals of AUCs were calculated. Cutoff points were identified following Youden's index of ROC. The comparison of the results between SEVERE and MILD on admission (Table 1) were somewhat similar to the original analysis between the two groups using multiple samples per patient (Table 2).

When the data was analysed using a single parameter of CBCs, LYMPH% and NLR showed the top two highest AUCs (0.9501 and 0.9454, respectively). For biochemical parameters, TP and ALB showed the top two highest AUCs (0.9287 and 0.9117, respectively).

We then performed multivariate logistic regression analysis to construct a diagnostic system to evaluate disease severity using CBC and biochemical parameters. We implemented a forward stepwise approach to identify risk factors for SEVERE cases. Three CBC parameters (haemoglobin, HGB; RDW-coefficient of variation, RDW-CV; and LYMPH%) and three biochemical parameters (BUN; TP; and FERR) were used for multivariable analysis due to relatively high AUCs among the combinations of these parameters. Three models were generated based on the multivariate analyses. These models were validated by fourfold cross-validation (75% training/25% test random splits), and were then evaluated by ROC and AUC to confirm their performances (Figure 1B and Table 2).

We observed that the combination of HGB, RDW-CV, and LYMPH% showed the highest AUC (0.9580) in the CBC parameters, and the combination of BUN, TP, and FERR demonstrated the highest AUC (0.9717) in the biochemical parameters. The combination of RDW-CV, LYMPH%, BUN, TP, and FERR also increased AUCs in the

TABLE 1 Demographics of patients with severe and mild COVID-19

	Patients with COVID-19			
	Total ($n = 33$)	MILD ($n = 20$)	SEVERE ($n = 13$)	p value*
Characteristics				
Age, years	63 (46–73)	58 (37.8–68.8)	66 (59.5–78.5)	.0192*
Sex				.4311
Men	25 (75.8%)	14 (70.0%)	11 (84.6%)	
Women	8 (24.2%)	6 (30.0%)	2 (15.4%)	
Observation period, days	7 (1–12.5)	6.5 (1–12)	7 (2–22.5)	.4212
Number of collected data per case	2 (1–3)	2 (1–3)	3 (1.5–7.5)	.1159
Data on admission				
White blood cells	5.50 (4.65–7.00)	4.95 (4.05–7.15)	5.80 (5.10–7.40)	.2935
Neutrophils	70.2 (55.6–81.0)	58.4 (49.3–73.1)	76.8 (68.9–85.3)	.0043*
Lymphocytes	20.1 (11.1–32.3)	25.0 (16.4–33.1)	11.7 (6.1–18.2)	.0019*
Monocytes	6.7 (4.3–9.2)	8.7 (4.5–9.2)	5.7 (4.1–8.2)	.2238
Eosinophils	1.5 (0.3–3.0)	1.6 (0.6–3.1)	0.8 (0.0–2.6)	.4035
Basophils	0.5 (0.0–0.9)	0.5 (0.1–1.0)	0.0 (0.0–0.5)	.0702
Blastoid cells	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	.0824
Immature granulocytes	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–1.3)	.0103*
Reactive lymphocytes	0.7 (0.2–1.0)	0.9 (0.4–1.3)	0.5 (0.0–1.0)	.4233
Neutrophils	3.66 (2.55–4.68)	3.20 (2.34–4.10)	4.39 (3.59–6.52)	.0193*
Lymphocytes	1.01 (0.62–1.62)	1.37 (0.83–1.80)	0.67 (0.32–1.15)	.0129*
Monocytes	0.37 (0.24–0.56)	0.38 (0.26–0.58)	0.37 (0.23–0.55)	.7823
Eosinophils	0.07 (0.01–0.18)	0.08 (0.02–0.21)	0.05 (0.00–0.15)	.5403
Basophils	0.03 (0.00–0.04)	0.03 (0.00–0.04)	0.00 (0.00–0.03)	.1512
NLR	3.22 (1.90–4.36)	2.20 (1.68–3.80)	4.41 (3.05–9.90)	.0061*
Haemoglobin	132 (118–146)	140 (126–148)	126 (110–141)	.1403
RDW-SD	42.1 (39.4–44.8)	41.6 (38.6–43.9)	43.6 (41.8–49.0)	.0284*
RDW-CV	12.8 (12.4–13.8)	12.8 (12.2–13.6)	12.8 (12.7–14.0)	.2930
Platelets	244 (170–316)	251 (159–322)	224 (170–293)	.8538
AST	25 (18–34)	22 (17–30)	33 (19–37)	.1837
ALT	28 (17–38)	28 (16–38)	29 (17–42)	.9266
LD	201 (182–275)	199 (179–220)	277 (194–317)	.0530
BUN	4.6 (3.7–6.2)	3.9 (3.6–5.0)	6.1 (4.3–7.5)	.0261*
CRE	61 (52–72)	62 (51–72)	60 (52–74)	.8973
TP	66 (60–71)	70 (65–71)	59 (57–65)	.0018*
ALB	33 (27–40)	38 (32–40)	27 (24–30)	.0004*
FERR	582 (368–815)	503 (315–647)	885 (567–1493)	.0034*
CRP	8.4 (1.9–58.6)	3.6 (0.9–8.4)	57.4 (20.3–101.8)	.0005*

Note: Data are median (IQR).

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; CRP, C-reactive protein; FERR, ferritin; LD, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; RDW-CV, red cell volume distribution width-coefficient of variation; RDW-SD, red cell volume distribution width-standard deviation; TP, total protein.

*p value <.05.

CBC and biochemical combination models to distinguish between SEVERE and MILD group (AUC = 0.9852).

Our study showed that LYMPH% and NLR were found to be significantly higher in SEVERE than MILD group. While previous studies proposed that NLR alone can be a useful marker to evaluate disease severity,[3] our data indicate that white blood cell (WBC) differentiation cannot be mutually exclusive and should not be evaluated separately. Notably, BUN was found to be related to the severity of COVID-19

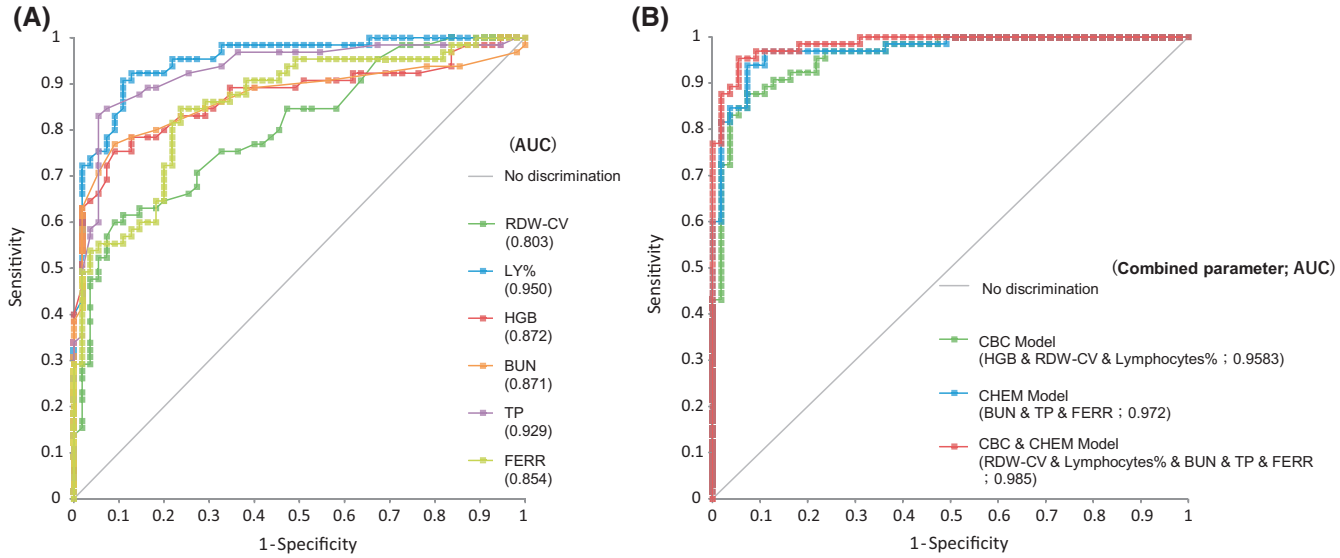

FIGURE 1 Receiver operating characteristic (ROC) curves using a single parameter (A) and combined parameters (B). Area under the ROC curve (AUC) values are shown in insets

TABLE 2 Complete blood cell counts and biochemical findings of 120 samples from COVID-19 patients

		Samples of patients with COVID-19					95% CI of AUC	
Parameters	**Unit**	**MILD ($n = 65$)**	**SEVERE ($n = 55$)**	***p* value**	**AUC**	**SE†**	**LL**	**UL**
White blood cells	$\times 10^9$/L	5.20 (4.00–6.10)	10.10 (6.05–13.00)	<.0001*	0.8092	0.0391	0.7326	0.8858
Neutrophils	%	56.5 (47.3–70.4)	87.1 (78.3–92.6)	<.0001*	0.9270	0.0244	0.8793	0.9747
Lymphocytes	%	27.4 (19.0–39.2)	4.2 (1.5–8.4)	<.0001*	0.9501	0.0201	0.9108	0.9894
Monocytes	%	6.6 (4.7–9.2)	4.6 (3.1–7.2)	.0004*	0.6877	0.0479	0.5938	0.7816
Eosinophils	%	2.8 (1.4–5.3)	0.0 (0.0–2.4)	<.0001*	0.7309	0.0453	0.6420	0.8198
Basophils	%	0.5 (0.4–1.4)	0.0 (0.0–0.5)	<.0001*	0.7524	0.0438	0.6665	0.8383
Blastoid cells	%	0.0 (0.0–0.0)	0.0 (0.0–0.0)	.5752	0.5130	0.0530	0.4090	0.6170
Immature granulocytes	%	0.0 (0.0–0.0)	0.6 (0.0–1.6)	<.0001*	0.7264	0.0456	0.6369	0.8159
Reactive lymphocytes	%	0.8 (0.4–1.5)	0.0 (0.0–0.6)	<.0001*	0.7313	0.0453	0.6425	0.8201
Neutrophils	$\times 10^9$/L	2.81 (2.20–3.86)	8.04 (5.15–11.4)	<.0001*	0.8871	0.0303	0.8276	0.9466
Lymphocytes	$\times 10^9$/L	1.37 (0.92–1.94)	0.39 (0.17–0.59)	<.0001*	0.8807	0.0312	0.8196	0.9418
Monocytes	$\times 10^9$/L	0.36 (0.27–0.51)	0.42 (0.24–0.87)	.1410	0.5783	0.0521	0.4762	0.6804
Eosinophils	$\times 10^9$/L	0.14 (0.05–0.31)	0.00 (0.00–0.16)	.0005*	0.6799	0.0483	0.5852	0.7746
Basophils	$\times 10^9$/L	0.03 (0.01–0.07)	0.00 (0.00–0.03)	<.0001*	0.7222	0.0459	0.6322	0.8122
NLR	(ratio)	1.87 (1.29–3.56)	9.53 (6.22–16.06)	<.0001*	0.9454	0.0210	0.9042	0.9866
Haemoglobin	g/L	132 (119–143)	88 (83–104)	<.0001*	0.8722	0.0323	0.8089	0.9355
RDW-SD	fl	41.8 (39.4–45.3)	51.7 (46.5–56.6)	<.0001*	0.8646	0.0332	0.7995	0.9297
RDW-CV	%	13.1 (12.4–14.2)	15.7 (13.8–17.3)	<.0001*	0.8032	0.0397	0.7255	0.8809
Platelets	$\times 10^9$/L	252 (191–321)	196 (102–276)	.001*	0.6752	0.0486	0.5800	0.7704
AST	U/L	26 (19–35)	35 (26–60)	.0002*	0.6966	0.0474	0.6036	0.7896
ALT	U/L	31 (20–52)	32 (17–75)	.6148	0.5269	0.0529	0.4231	0.6307
LD	U/L	201 (179–225)	327 (278–457)	<.0001*	0.8807	0.0312	0.8196	0.9418
BUN	mmol/L	4.3 (3.9–5.0)	9.3 (6.1–19.5)	<.0001*	0.8706	0.0325	0.8070	0.9342
CRE	μmol/L	62 (50–70)	66 (55–211)	.0248*	0.6193	0.0509	0.5195	0.7191
TP	g/L	69 (64–71)	54 (49–59)	<.0001*	0.9287	0.0241	0.8815	0.9759

(Continues)

TABLE 2 (Continued)

Parameters	Unit	Samples of patients with COVID-19		p value	AUC	SE†	95% CI of AUC	
		MILD ($n = 65$)	SEVERE ($n = 55$)				LL	UL
ALB	g/L	35 (32–39)	25 (23–28)	<.0001*	0.9117	0.0268	0.8591	0.9643
FERR	μg/L	516 (326–707)	1472 (891–3008)	<.0001*	0.8543	0.0344	0.7868	0.9218
CRP	mg/L	3.5 (0.9–11.5)	57.2 (20.4–101.5)	<.0001*	0.8761	0.0318	0.8138	0.9384
(Combined parameter; CBC) HGB & RDW-CV & Lymphocytes %				<.0001*	0.9583	0.0183	0.9224	0.9942
(Combined parameter; CHEM) BUN & TP & FERR				<.0001*	0.9717	0.0150	0.9422	1.0000
(Combined parameter; CBC & CHEM) RDW-CV & Lymphocytes % & BUN & TP & FERR		–	–	<.0001*	0.9852	0.0108	0.9640	1.0000

Note: MILD, equivalent to mild and moderate; SEVERE, equivalent to severe and critical; based on the clinical conditions according to the World Health Organization (WHO) at the time of collection.[5] Data are median (IQR). *p* value: Wilcoxon rank sum test was used to analyse the parameters with significant differences between the two groups. Multivariate logistic regression analysis was used to establish the prediction model below: HGB & RDW-SD & Lymphocytes % = 4.01886915 × ln(HGB) + 8.08983234 × ln(RDW-CV) + 3.47274052 × ln(Lymphocytes % + 1) − 49.438051. BUN & TP & FERR = −3.8055462 × ln(BUN) + 18.9819676 × ln(TP) − 0.9311549 × ln(FERR) − 65.594697. RDW-CV & Lymphocytes % & BUN & TP & FERR = 18.894407 × ln(RDW-CV) + 2.80991529 × ln(Lymphocytes % + 1) − 5.5972457 × ln(BUN) + 23.7711027 × ln(TP) − 1.6050739 × ln (FERR) − 135.23566.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve (AUC) of ROC analysis between the two groups; BUN, blood urea nitrogen; CRE, creatinine; CRP, C-reactive protein; FERR, ferritin; LD, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; RDW-CV, red cell volume distribution width-coefficient of variation; RDW-SD, red cell volume distribution width-standard deviation; TP, total protein.

*p value <.05. †Standard error of AUC.

patients. However, creatinine (CRE) values were comparable between the two groups. A previous study showed that elevated BUN but not CRE was associated with adverse outcomes in severe COVID-19 patients.[6] These findings indicate that the increase in BUN observed in severely ill patients may be due to increased protein catabolism associated with deterioration of nutritional status rather than due to renal damage. The low TP level might have been caused, at least in part, by protein consumption with the same mechanism.

Another finding of this study was that RDW-CV levels were increased in SEVERE cases than MILD cases. It has been demonstrated that RDW-CV strongly correlates with conventional inflammatory biomarkers. Inflammations may, in fact, induce an isocytosis due to the disruption of iron metabolism and response of bone marrows to erythropoietin. These delays in maturation of erythrocytes allow the immature erythrocytes to appear in the blood flow. Inflammations can also shorten the life-expectancy of erythrocytes, leading to mixed erythrocyte populations with various volumes in the vessels.[7] While RDW-CV is known to be also affected by underlying haematological conditions,[8] patients with pre-existing severe iron deficiency anaemia or hemoglobinopathies/thalassemia was not included in this study. We further observed that the levels of FERR, a mediator of immune dysregulation in acute inflammation,[9] were higher in SEVERE cases than MILD cases. SARS-CoV-2 infection can disrupt bone marrow erythropoiesis, leading to phagocytosis of both erythroblasts and mature erythrocytes by macrophages.[10] This might partially account for the increase of FERR along with the progression of inflammatory responses.

For the clinical classification of COVID-19, several models using systemic inflammatory index have been proposed.[11] For example, the NLR has been shown to be an independent biomarker for poor clinical outcomes.[12] Conversely, Linssen et al.[12] has demonstrated that the scoring system using multiple haemocytometoric parameters obtained from automated haematology analyzers can be more suitable than standard parameters, such as NLR, in early recognition of severe COVID-19.

This study has several limitations: (1) this was a retrospective observational study performed at a single centre with a limited number of patients, and (2) a validation study of the developed model could not be conducted.

These CBC and biochemical parameters can be quickly measured by automated analyzers with relatively low costs in basic clinical laboratories. Our study demonstrated that the combination of RDW-CV, LYMPH%, BUN, TP, and FERR can be a useful combinational marker to classify the severity of COVID-19.

AUTHOR CONTRIBUTIONS

Ryosuke Maki, Yuki Horiuchi, Fumiaki Hayashi, Yosuke Iwasaki, and Yoko Tabe designed the study. Fumiaki Hayashi, Ikki Takehara, Shuko Nojiri, and Yosuke Iwasaki performed data analysis. Ryosuke Maki, Yuki Horiuchi, Fumiaki Hayashi, Tomohiko Ai, and Yoko Tabe wrote the manuscript. Kazunori Miyake and Tomohiko Ai provided the idea for material preparation.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated in the current study are available from the corresponding author on reasonable request.

EMPLOYMENT OR LEADERSHIP

Fumiaki Hayashi, Ikki Takehara, Yosuke Iwasaki are employees of SYSMEX Corporation.

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