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ORIGINAL ARTICLE

Peripheral granular lymphocytopenia and dysmorphic leukocytosis as simple prognostic markers in COVID-19

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Abstract

Introduction: Developing prognostic markers can be useful for clinical decision-making. Peripheral blood (PB) examination is simple and basic that can be performed in any facility. We aimed to investigate whether PB examination can predict prognosis in coronavirus disease (COVID-19).

Methods: Complete blood count (CBC) and PB cell morphology were examined in 38 healthy controls (HCs) and 40 patients with COVID-19. Patients with COVID-19, including 26 mild and 14 severe cases, were hospitalized in Juntendo University Hospital (Tokyo, Japan) between April 1 and August 6, 2020. PB examinations were performed using Sysmex XN-3000 automated hematology analyzer and Sysmex DI-60 employing the convolutional neural network-based automatic image-recognition system.

Results: Compared with mild cases, severe cases showed a significantly higher incidence of anemia, lymphopenia, and leukocytosis ($P < .001$). Granular lymphocyte counts were normal or higher in mild cases and persistently decreased in fatal cases. Temporary increase in granular lymphocytes was associated with survival of patients with severe infection. Red cell distribution width was significantly higher in severe cases than in mild cases ($P < .001$). Neutrophil dysplasia was consistently observed in COVID-19 cases, but not in HCs. Levels of giant neutrophils and toxic granulation/Döhle bodies were increased in severe cases.

Conclusion: Basic PB examination can be useful to predict the prognosis of COVID-19, by detecting SARS-CoV-2 infection-induced multi-lineage changes in blood cell counts and morphological anomalies. These changes were dynamically correlated with disease severity and may be associated with disruption of hematopoiesis and the immunological system due to bone marrow stress in severe infection.

KEYWORDS

blood cell counts, COVID-19, granular lymphocytes, morphological anomalies, peripheral blood

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects various cells, including alveolar macrophages, via angiotensin-converting enzyme 2 (ACE2) receptors, activating monocytes and macrophages, and resulting in a cytokine storm, including chemokine ligand 7 (CCL7).[1] Although most patients with coronavirus disease (COVID-19) have mild symptoms, up to 15% of patients develop severe pneumonia and approximately 5% of patients develop acute respiratory distress syndrome and/or multiple organ failure.[2] Despite the fact that there are no proven remedies, except for supportive treatment, developing prognostic markers can be useful for clinical decision-making, such as the timing of intubation and the introduction of extracorporeal membrane oxygenation.

Over recent months, hundreds of clinicians and researchers have reported various prognostic markers for SARS-CoV-2, such as leukocytosis and C-reactive protein (CRP),[3] serum pro-inflammatory cytokines,[4,5] and blood urea nitrogen combined with D-dimer.[6] Furthermore, abnormal hematological findings, such as lymphopenia, monocytosis, and thrombocytopenia, besides leukocytosis, have been described.[7] In addition, morphological examinations have revealed reactive lymphocytes, overreacted T cells,[8] acquired Pelger-Huët anomaly (APHA), leukoerythroblastosis, and abnormal platelets in patients with COVID-19.[9,10] However, these markers were mostly reported as case series.

To investigate whether peripheral blood (PB) examination can be used as a prognostic marker in COVID-19, we compared complete blood count (CBC) and PB cell morphology using convolutional neural network (CNN)-based image diagnostic systems[19] between patients with mild-to-severe COVID-19 and healthy controls. We also examined changes in PB cell morphology during the clinical course of the disease.

2 | MATERIALS AND METHODS

2.1 | Patient cohorts

Forty patients with confirmed COVID-19 hospitalized in our institution between April 1 and August 6, 2020, were enrolled in this study. COVID-19 was diagnosed using PCR-based nasopharyngeal swab testing with the LightMix Modular SARS-CoV-2 (COVID-19) N-gene and E-gene assay (Roche Diagnostics, Tokyo, Japan) or the 2019 Novel Coronavirus Detection Kit (Shimadzu). Patients with COVID-19 were classified into "mild" or "severe" subgroups according to the WHO Guideline (Clinical management of COVID-19: https://www.who.int/publications/i/item/clinical-management-of-covid-19). Severe cases were defined as having any of the following features: (i) respiratory rate ≥30 breaths/minutes, (ii) oxygen saturation ≤93% at rest; (iii) ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2) ≤300; and (iv) pulmonary imaging showing that lesions increased >50% within 24-48 hours. Cases that did not meet the criteria for a "severe" case were classified as "mild." Thirty-eight age-matched healthy subjects who undertook regular checks in our hospital between February 2017 and December 2018 were enrolled as controls.

The research related to human use has complied with all relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and was approved by the institutional review board (IRB) at our institute (IRB #20-036). Informed consent from individual participants was waived because all samples were de-identified in line with the Declaration of Helsinki.

2.2 | Blood cell counts and morphological differential

One hundred and ninety-four PB samples, containing the potassium salt of ethylenediaminetetraacetic acid (K_2-EDTA), as an anticoagulant, were obtained from 40 patients for diagnostic and/or follow-up evaluation. CBCs were determined using Sysmex XN-3000 and XE-5000 automated hematology analyzer (Sysmex; Kobe, Hyogo). PB smear slides were prepared with May Grunwald-Giemsa stain using the Sysmex SP-10 autostainer. Preparation of blood smears was performed within two hours after collecting the blood samples. Morphological examination was performed utilizing digital blood cell images collected by a Sysmex DI-60, which were subsequently automatically analyzed by the recently developed CNN-based image-recognition system that we have previously reported.[11] Briefly, the deep learning system powered by CNNs, which has been trained using 700 000 cell images collected using the DI-60, automatically classifies cells into 17 subtypes and detects 97 abnormal morphological features.[11] It also records the differential counts and rates of morphological abnormalities in WBCs and PLTs. Two hundred cell images per slide were collected using the DI-60 for the CNN-based automatic image analysis. The accuracy of the CNN-based image-recognition system was assessed by determining the concordance and reclassification rate between the pre- and reclassified results for each cell subtype. To detect morphological abnormalities in RBCs, 1500-2000 cells were counted per slide. Advanced RBC software (CellaVision, Inc, Sweden) was utilized for quantitative analysis of erythrocyte morphological abnormalities.

Two laboratory technologists, board-certified in hematology, reviewed all images separately and accepted or reclassified the preclassification provided by the CNN-based analysis. In cases of disagreement, one senior hematopathologist re-verified the images and a decision was made by consensus. Standardized nomenclature and scoring of cellular features were applied according to the recommendations of the International Council for Standardization in Haematology (ICSH).[12] The definitions of the representative morphologically abnormal cell subtypes are shown in Table S2.

2.3 | Statistical analysis

We used the Wilcoxon rank sum test to evaluate the significance of differences in blood test results and morphological abnormalities between patients and HCs and among patients with different disease severity (mild vs severe). Data were analyzed using JMP15 software (SAS Institute, Inc,), and P values <.05 were considered significant.

3 | RESULTS

3.1 | Peripheral blood cell quantity and morphological alterations

Table 1 shows the baseline characteristics of 40 patients with COVID-19 and 38 healthy controls (HCs). The 14 patients with severe disease were older than the 26 patients with mild disease ($P = .0016$), and most patients with COVID-19 were male ($P = .0194$), which was consistent with previous reports.[13] All patients underwent multiple blood examinations on and after admission. Based on the patients' clinical condition upon collection of the blood specimens, a total of 194 samples were divided into two groups and analyzed: 101 samples from patients in mild-moderate conditions (mild) and 93 from patients in a severe and critical condition (severe). Three patients received treatments (two mild cases and one severe case) including ciclesonide, favipiravir, and/or steroids (Table 2). Thirty-seven patients were not taking any medications to treat COVID-19 on admission.

Table 3 summarizes the laboratory data. Compared with HCs, patients with COVID-19 had significantly higher white blood cell (WBC) count ($P = .0091$), absolute neutrophil count ($P = .0004$), neutrophil-lymphocyte ratio (NLR) ($P < .0001$), and RBC distribution width-standard deviation (RDW-SD) ($P = .0071$). Moreover, the COVID-19 group had significantly lower absolute lymphocyte count and hemoglobin (Hb) levels than the HC group ($P < .0001$). There was no significant difference in the absolute count of platelets (PLT) and reactive lymphocyte% between the two groups.

Among patients with COVID-19, the severe group exhibited significantly higher WBC, neutrophil count, NLR, immature granulocyte %, RDW-SD, and CRP levels. Furthermore, lymphocyte count, reactive lymphocyte%, Hb, and PLT levels were significantly lower in the severe group than in the mild group ($P < .0001$).

We next investigated morphological changes in the PB of patients with COVID-19 compared these with changes in HCs. Figure 1A shows dot plots of morphological changes in each group (please see Table S2 for the definitions): neutrophils: APHA (A), chromatin abnormality (B), degranulation/hypogranulation (C), giant neutrophils (D), toxic changes (E), Döhle bodies (F), and vacuoles (G); lymphocytes: nucleus atypia (H), granular lymphocytes (I), reactive lymphocytes (J), and vacuoles (K); and red blood cells (RBCs): polychromatic RBCs (L), hypochromic RBCs (M), schistocytes (N), and giant platelets (O). Figure 1B shows pictures of blood cells detected by Sysmex DI-60. The concordance and reclassification rates of the CNN-based automatic image recognition of each abnormal cell subtype are shown in Table S1.

PB smears revealed neutrophil dysplasia, including increased APHA and monolobated neutrophils %, degranulation/hypogranulation %, and chromatin abnormality % in both mild and severe cases compared with HCs ($P < .05$). However, no significant differences in these dysplastic anomalies were observed between mild and severe cases of COVID-19. Neutrophil dysplasia, including APHA, chromatin abnormality, and degranulation, was observed in 71% of severe patients and 54% of mild patients.

The toxic changes %, Döhle body %, vacuoles %, and giant neutrophils % were significantly higher in patients with COVID-19 than in HCs, with higher percentages in the severe group than in the mild group ($P < .01$). These changes may reflect the fact that severe infections with SARS-CoV-2 induce the systemic inflammatory response and were observed in 57% of severe patients and 15% of mild patients. Vacuoles of neutrophils were observed in 95% of all patients.

TABLE 1 Baseline characteristics of the patients with COVID-19 and healthy controls

				Patients with COVID-19		
	Healthy Controls (n = 38)	Patients with COVID-19	*P* value*	Mild (n = 26)	Severe (n = 14)	*P* value**
age, years	63 (55-70)	61 (46-67)	.4870	50.5 (37-64)	71.5 (63-80)	.0016
sex			.0194			.4457
men	18 (47.4%)	30 (75.0%)		18 (69.2%)	12 (85.7%)	
women	20 (52.6%)	10 (25.0%)		8 (30.8%)	2 (14.3%)	
Time from onset to admission, days		9 (6-11)		7.5 (6-15)	10 (7-14)	
observation period, days	1	7 (3-14)	NA	3.5 (1-14)	9.5 (5-34)	.0411
number of collected parameters per case	1	3 (2-4)	NA	2.5 (1-3)	4 (3-15)	.0076

Note: Data are presented as median (IQR).

*P values comparing healthy controls and patients with COVID-19.

**P values comparing mild cases and severe cases.

TABLE 2 Clinical characteristics of patients with COVID-19

Pt No.	Disease severity*	Outcome	Sex	Past medical history		Therapy
1	critical	cure/discharge	M	HT, DM, Pancreatic cancer, Hepatic metastasis	Ventilation, O_2	Favipiravir
2	severe	cure/discharge	M	IGT, Alcoholic liver disease, Cholelithiasis	O_2	Ciclesonide, Favipiravir
3	severe	cure/discharge	M	HT	O_2	Ciclesonide, Favipiravir
4	severe	cure/discharge	M	DM, RA, Dementia, Parkinson disease, Pneumonia	O_2	None
5	severe	cure/discharge	M	None	O_2	Ciclesonide, Favipiravir, Steroid
6	severe	cure/discharge	M	Parkinson disease	O_2	Heparin
7	severe	cure/discharge	M	HT	O_2	Heparin
8	severe	cure/discharge	M	Stiff-person syndrome, DM, HL, Coronary vasospasm	O_2	Ciclesonide, Favipiravir, Steroid
9	critical	dead	F	HL, Breast cancer	Ventilation	CHDF, Plasmapheresis, FFP, Ciclesonide, Favipiravir
10	critical	dead	M	HT, DM, Prostate cancer	Ventilation	CHDF, Plasmapheresis, Ciclesonide, Favipiravir
11	critical	dead	M	HT, RF	Ventilation	CHDF, Plasmapheresis, Favipiravir, Nafamostat
12	severe	dead	M	CRF, HT, Hepatic cancer	O_2	Plasmapheresis, Ciclesonide, Favipiravir, Remdesivir, Steroid, Heparin
13	severe	cure/discharge	M	None	O_2	Ciclesonide
14	severe	cure/discharge	M	HT,DM, Cardiovascular disease	O_2	Favipiravir
15	moderate	cure/discharge	M	HT	N/A	Ciclesonide
16	mild	cure/discharge	F	Lymphoma	N/A	Ciclesonide, Favipiravir
17	mild	cure/discharge	F	None	N/A	None
18	mild	cure/discharge	M	None	N/A	None
19	moderate	cure/discharge	M	Sarcoidosis	N/A	Ciclesonide, Favipiravir
20	moderate	cure/discharge	M	None	N/A	Favipiravir
21	moderate	cure/discharge	F	None	N/A	Ciclesonide
22	moderate	cure/discharge	M	None	N/A	Ciclesonide, Favipiravir
23	moderate	cure/discharge	F	DM	N/A	Favipiravir, Steroid
24	moderate	cure/discharge	M	HT	N/A	Ciclesonide, Favipiravir
25	moderate	cure/discharge	F	HT, Cardiovascular disease	N/A	Ciclesonide, Favipiravir
26	mild	cure/discharge	M	None	N/A	Ciclesonide, Favipiravir
27	moderate	cure/discharge	F	Pancreatic cancer	N/A	Ciclesonide, Favipiravir
28	mild	cure/discharge	M	Hepatic cancer	N/A	Steroid
29	moderate	cure/discharge	M	None	N/A	Ciclesonide, Favipiravir, Steroid
30	moderate	cure/discharge	M	None	N/A	None
31	mild	cure/discharge	M	None	N/A	None
32	moderate	cure/discharge	M	HT, RF	N/A	Ciclesonide, Favipiravir, Steroid
33	mild	cure/discharge	M	None	N/A	None
34	moderate	cure/discharge	F	None	N/A	None

(Continues)

TABLE 2 (Continued)

Pt No.	Disease severity*	Outcome	Sex	Past medical history		Therapy
35	mild	cure/discharge	M	HT, cholangiocarcinoma	N/A	Favipiravir
36	moderate	cure/discharge	M	Pancreatic cancer	N/A	None
37	moderate	cure/discharge	M	None	N/A	None
38	mild	cure/discharge	F	Neuromyelitis optica	N/A	Steroid
39	mild	cure/discharge	M	None	N/A	None
40	mild	cure/discharge	M	None	N/A	None

Note: Disease severity refers to severity at hospitalization.

Abbreviations: CHDF, Continuous hemodiafiltration; CRF, Chronic renal failure, DM, Diabetes mellitus; FFP, Fresh frozen plasma; HL, Hyperlipidemia; HT, Hypertension; IGT, Impaired glucose tolerance; O2, Oxygen inhalation; RA, Rheumatoid arthritis; RF, Renal failure.

TABLE 3 Complete blood cell counts of patients with COVID-19 and healthy controls

	Healthy controls (n = 38)	Patients with COVID-19, total (n = 194)	*P* value*	Patients with COVID-19		
				Mild (n = 101)	Severe (n = 93)	*P* value**
White blood cells (x 10^9/L)	5.22 (4.21-5.82)	5.96 (4.22-9.75)	.0091	4.76 (3.61-5.94)	9.32 (6.12-12.00)	<.0001
Neutrophils (%)	61.0 (52.6-65.7)	78.1 (61.5-88.4)	<.0001	62.8 (76.0-52.8)	87.8 (79.0-93.2)	<.0001
Lymphocytes (%)	30.2 (23.9-35.4)	10.4 (4.0-24.0)	<.0001	23.1 (12.3-33.5)	4.2 (1.5-9.8)	<.0001
Monocytes (%)	6.0 (4.4-7.7)	5.7 (3.6-8.4)	.9789	6.8 (4.6-9.2)	4.3 (2.8-6.8)	<.0001
Eosinophils (%)	2.6 (1.1-3.7)	1.4 (0.0-2.9)	.0068	2.2 (0.5-3.4)	0.0 (0.0-1.9)	<.0001
Basophils (%)	1.0 (0.6-1.6)	0.4 (0.0-0.9)	<.0001	0.6 (0.0-1.5)	0.0 (0.0-0.5)	<.0001
Blastoid cells (%)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.178	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.2325
Immature granulocytes (%)	0.0 (0.0-0.0)	0.0 (0.0-0.6)	<.0001	0.0 (0.0-0.0)	0.5 (0.0-1.2)	<.0001
Reactive lymphocytes (%)	0.0 (0.0-0.5)	0.4 (0.0-0.9)	.0527	0.5 (0.0-1.4)	0.0 (0.0-0.5)	<.0001
Neutrophils (x 10^9/L)	3.06 (2.43-3.61)	4.12 (2.64-8.31)	.0004	2.81 (2.20-3.85)	8.04 (4.92-10.95)	<.0001
Lymphocytes (x 10^9/L)	1.39 (1.25-1.82)	0.60 (0.29-1.29)	<.0001	1.21 (0.54-1.63)	0.39 (0.16-0.61)	<.0001
Monocytes (x 10^9/L)	0.28 (0.18-0.43)	0.35 (0.22-0.57)	.0409	0.35 (0.24-0.53)	0.37 (0.21-0.74)	.1988
Eosinophils (x 10^9/L)	0.11 (0.06-0.21)	0.06 (0.00-0.18)	.0203	0.08 (0.03-0.20)	0.00 (0.00-0.12)	.0016
Basophils (x 10^9/L)	0.06 (0.03-0.08)	0.01 (0.00-0.05)	<.0001	0.03 (0.00-0.07)	0.00 (0.00-0.02)	<.0001
NLR (ratio)	2.09 (1.53-2.58)	4.87 (2.25-9.81)	<.0001	2.45 (1.56-4.12)	9.59 (5.94-19.31)	<.0001
Hemoglobin (g/L)	138 (127-145)	103 (88-131)	<.0001	125 (103-142)	89 (83-102)	<.0001
RDW-SD (fL)	44.6 (42.4-46.4)	47.5 (42.0-52.6)	.0071	43.4 (40-4-48.9)	51.5 (46.5-55.3)	<.0001
Platelets (x 10^9/L)	232 (207-264)	200 (119-293)	.0552	226 (171-312)	153 (80-259)	<.0001
CRP (mg/L)	N/A	27.4 (6.5-72.4)	N/A	11.5 (1.9-44.5)	55.3 (20.5-100.9)	<.0001

Note: Data are presented as median (IQR).

Abbreviations: CRP, C-reactive protein, NLR, Neutrophil-lymphocyte ratio; RDW-SD, Red blood cell distribution width-standard deviation.

**P* values comparing healthy controls and patients with COVID-19.

***P* values comparing mild cases and severe cases.

Morphological changes in lymphocytes were observed in all COVID-19 patients. Vacuole % in lymphocytes was also significantly higher in patients with COVID-19 than in HCs, with a higher percentage in the severe group than in the mild group, also suggestive of the inflammatory response. Granular lymphocyte % in lymphocytes was significantly increased in the mild group compared with the HC ($P < .001$) and severe groups ($P < .05$), and a similar trend was observed for reactive lymphocyte % in total lymphocytes ($P < .001$). Granular lymphocytes were observed in all patients. We observed elevated RDW-SD in patients with COVID-19, particularly in the severe group. Elevated RDW can be associated with an increased mortality in patients with COVID-19.[14] Therefore, morphological changes in RBCs were further examined. Compared with the HCs, COVID-19 cases had a significantly higher polychromatic RBC %

($P < .0001$), indicating that abnormal amounts of immature reticulocytes may have been released from bone marrow.[15] However, we did not observe any significant difference in polychromatic RBC % between mild and severe groups. In contrast, the percentage of schistocytes, fragmented RBCs, was moderately increased in the mild group compared with HCs ($P < .05$) and increased further in the severe group compared with the mild group ($P < .001$). Hypochromic RBC % was moderately increased after SARS-CoV-2 infection, and further increased with disease progression ($P < .001$).

3.2 | Association of granular lymphocytes with severity of COVID-19

We next investigated chronological changes in granular lymphocyte count and severity. Figure 1C shows the absolute count of total lymphocytes, and granular and reactive lymphocytes. Comparing the mild group with HCs, the total lymphocytes count was decreased ($P < .01$), granular lymphocyte count remained stable ($P = .1481$), and reactive lymphocyte count was increased in the mild group

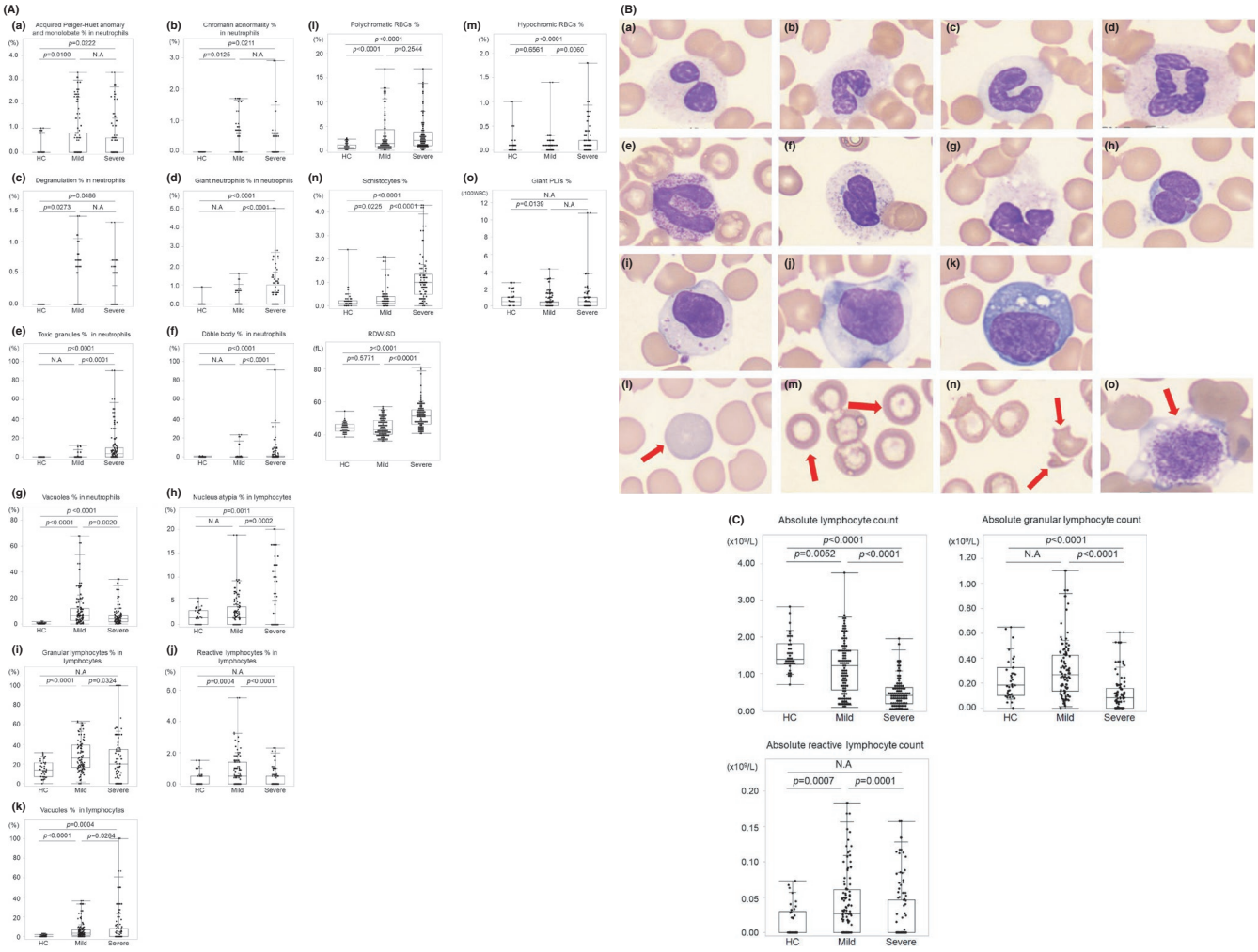

FIGURE 1 Morphological changes in blood cells in patients with COVID-19 and healthy controls. (A) Morphological abnormalities with a significant difference between the patients with COVID-19 and the HCs, or between the mild and severe cases. A total of 194 specimens were tested as described in the Materials and Methods. The severity was classified at the time of measurement. Data are median (IQR). Wilcoxon rank sum test was conducted. HC, healthy control; N.A, not applicable, a-g; neutrophils, h-k; lymphocytes, l-n; red blood cells, o; platelets, a, Acquired Pelger-Huët anomaly (APHA). b, Chromatin abnormality. c, Degranulation/hypogranulation. d, Giant neutrophils. e, Toxic changes. f, Döhle bodies. g, Vacuoles. h, Nucleus atypia. i, Granular lymphocytes. j, Reactive lymphocytes. k, Vacuoles. l, Polychromatic RBCs. m, Hypochromic RBCs. n, Schistocytes. o, Giant platelets. (B) Representative images of PB cells from patients with COVID-19. Morphological image findings in peripheral smears in patients with COVID-19 (May-Gürunwald's-Giemsa stain, ×100, ×1000). a-g; neutrophils, h-k; lymphocytes, l-n; red blood cells, o; platelets, a, Acquired Pelger-Huët anomaly (APHA). b, Chromatin abnormality. c, Degranulation/hypogranulation. d, Giant neutrophils. e, Toxic changes. f, Döhle bodies. g, Vacuoles. h, Nucleus atypia. i, Granular lymphocytes. j, Reactive lymphocytes. k, Vacuoles. l, Polychromatic RBCs. m, Hypochromic RBCs. n, Schistocytes. o, Giant platelets. Red arrows show each cell. (C) Absolute cell count of peripheral lymphocytes, granular lymphocytes, and reactive lymphocytes in patients with COVID-19 and healthy controls. Data are median (IQR). The Wilcoxon rank sum test was conducted. NA; not applicable

($P < .001$). The absolute count of total lymphocytes, granular lymphocytes, and reactive lymphocytes was significantly lower in the severe group than in the mild group ($P < .0001$).

Figure 2 shows chronological changes in granular lymphocytes in the 12 patients with severe infection; two severe cases who underwent less than two samplings were excluded. Table 2 shows the clinical characteristics of the 12 patients with severe infection (Pt#1-12). Patients 1 to 8 are cured cases, and patients 9 to 12 are deceased cases. In most of the cured cases, granular lymphocyte counts were greater than 0.200×10^9/L throughout the clinical course (Figure 2A).

Reported normal granular lymphocyte values are approximately 0.2×10^9/L (ie, $0.223 \pm 0.099 \times 10^9$/L[16] or $0.198 \pm 0.112 \times 10^9$/L[17]). Between days 10 and 20, granular lymphocyte counts of patient 1 transiently increased above 0.2×10^9/L despite his critical condition. The granular lymphocyte counts then gradually increased and recovered reaching levels above 0.2×10^9/L on day 40 while the patient was recovering clinically (Figure 2A, Pt 1). Unlike this case, there was no recovery of granular lymphocytes in the remaining deceased cases and granular lymphocyte counts remained lower than $<0.200 \times 10^9$/L throughout the clinical course (Figure 2B). This suggests that granular lymphocyte count can be a potential indicator of prognosis. All fatal cases developed disseminated intravascular coagulation (DIC). Of these, three patients also had cancer and the remaining patient developed pancreatitis and thrombosis with leukoerythroblastosis (>1% blasts).

4 | DISCUSSION

This study investigated the role of PB examination as a prognostic marker of COVID-19. Our data demonstrated that SARS-CoV-2

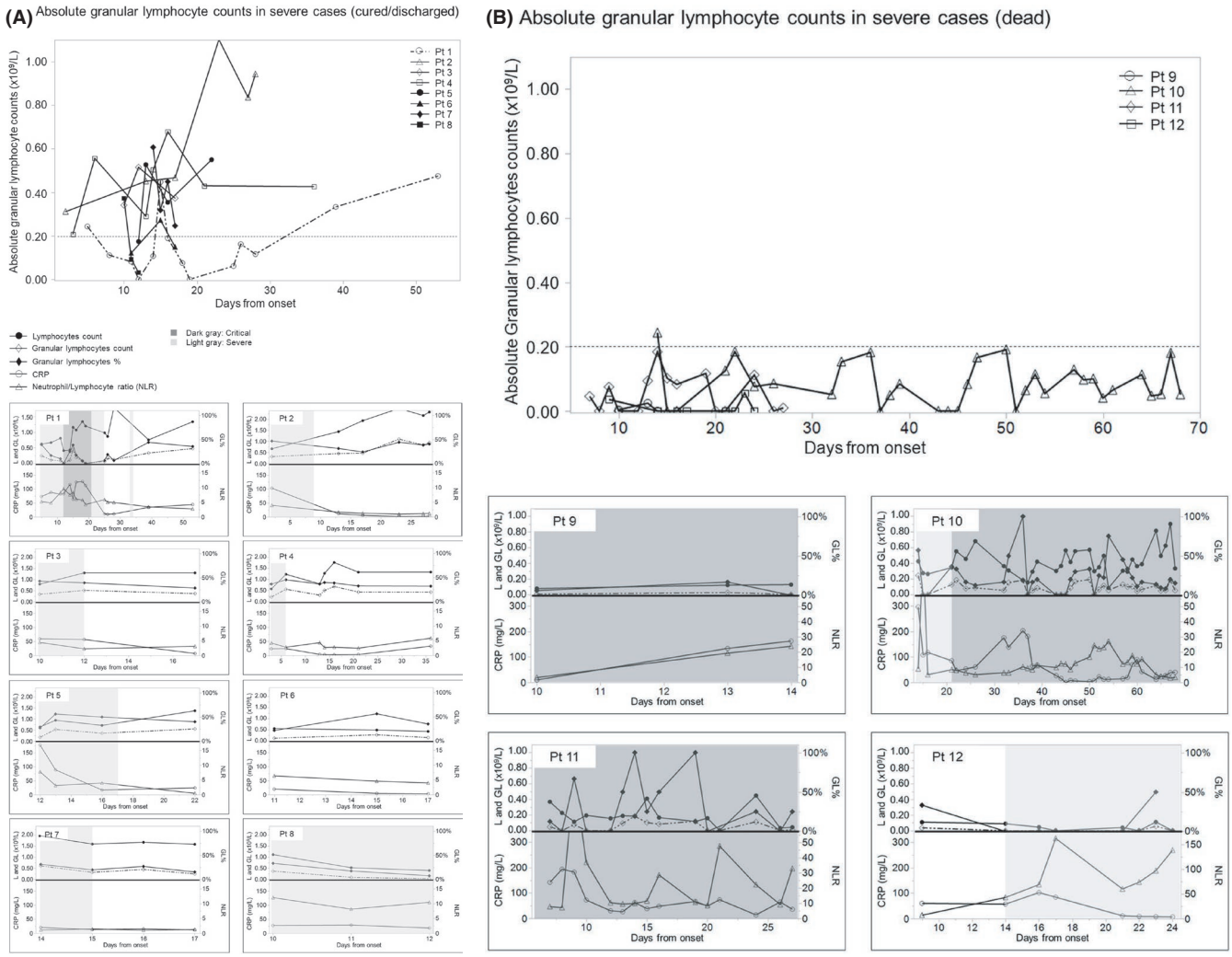

FIGURE 2 Trajectories of severe COVID-19 cases, Longitudinal changes in granular lymphocyte counts in cured or discharged severe cases of COVID-19 (A) and fatal severe cases (B) examined more than three times during the course. Progress charts of individual cases are shown with lymphocyte counts (×10^9/L;▲), granular lymphocyte counts (×10^9/L;◆), granule lymphocyte ratio (%;●), CRP (mg / L;△) and NLR (;○). Granular lymphocyte counts are indicated by dotted lines and other items are shown as solid lines. The change in severity during the course is indicated by the color of the background: white—mild, light gray—severe, and dark gray—critical. L, lymphocyte; GL, granular lymphocyte; CRP, C-reactive protein; NLR, neutrophil-lymphocyte ratio

infection can induce multi-lineage changes in blood cell counts and morphological anomalies: (1) dysplastic anomaly of neutrophils, such as APHA and monolobated neutrophils, degranulation/hypogranulation, and chromatin abnormalities, in all COVID-19 cases irrespective of severity; (2) systemic inflammatory response-induced changes in neutrophils, such as toxic changes, Döhle bodies, vacuoles, and giant neutrophils mainly in severe cases; (3) increases in granular lymphocytes in mild cases, but persistent granular lymphocyte decrease in fatal cases; and (4) largely elevated RDW in severe cases.

Because the morphological analysis of blood cells can be subjective, we utilized a digital platform equipped with the CNN-based automatic image-recognition system.[19] The digital CNN analyzing system revealed difference in morphological anomalies of neutrophils, including APHA, and toxic changes, and numerical change in granular lymphocytes and reactive lymphocytes between patients with mild and severe COVID-19, whereas a previous study using a CNN-based analyzing system had identified only an increase in reactive lymphocytes in COVID-19[18]; these differences may be partially due to the fact that the system in the previous study focused on lymphocyte morphology. As SARS-CoV-2 can cause various anomalies of all lineage blood cells,[9] our digital CNN analyzing system can be suitable to detect blood cell morphological anomalies in COVID-19.

4.1 | Granulocyte change

We observed neutrophil dysplasia, including APHA and other monolobated neutrophils, in mild-to-severe COVID-19 and increased levels of toxic granulation/Döhle bodies in severe cases, similar to the previous reports.[9,19] Several infections, including tuberculosis and viral infections by HIV, EBV, and parvovirus, are known to cause bone marrow stress, resulting in hematopoietic dysfunction and the appearance of APHA.[20] Neutrophil anomalies, including toxic granule and Döhle bodies, appear in secondary bacterial or fungal infections, including sepsis.[21]

4.2 | Lymphocyte change

Lymphopenia is one of the most predominant hematologic changes caused by SARS-CoV-2 infection,[5] and lymphocyte subset alterations are associated with disease activity in patients with COVID-19.[19] Our data indicate that absolute counts of granular lymphocytes and reactive lymphocytes were significantly decreased in the severe cases, while normal or higher levels of granular lymphocyte ratios were present in the mild cases despite decrease in total lymphocytes. Liu et al reported that survived severe COVID-19 cases showed a decrease in $CD3^+/CD8^+$ T cells compared with mild cases.[22] However, our novel finding is that patients who showed an increase in granular lymphocytes, even transiently, tended to recover. By detailed kinetic case study including fatal cases, we revealed that the granular lymphocyte counts, consisting of natural killer (NK) cells and activated T cells that are the major mediators of cellular cytotoxicity,[23,24] remained at extremely low levels throughout the clinical course in the deceased cases, but increased temporarily during the clinical course of patients with severe disease who recovered (Figure 2A). $CD8^+$ killer T cells have been shown to play a critical role in mediating viral clearance after acute respiratory infections of respiratory syncytial virus (RSV), influenza A virus (IAV), and human metapneumovirus.[25,26] A study using single-cell RNA sequencing suggested that impaired granular lymphocytes in patients with severe COVID-19 may consist of active state T cells and cytotoxic natural killer cells.[27] Our results and these previously reported findings indicate that the increase in granular lymphocytes and reactive lymphocytes is associated with an effective immune response. Therefore, detection of numerous changes in these cells in the PB smear may be a promising marker in the early screening stage for critical illness and recovery from severe COVID-19.

4.3 | RBC Change

Our data showed that hypochromic anemia and increased RDW[14] were associated with severe infection. It has been postulated that SARS-CoV-2 interacts with Hb molecules via ACE2, CD147, and CD26 receptors, leading to hemolysis.[28] Appearance of hypochromic RBCs could be further associated with inflammation and iron deficiency although it is difficult to determine which is the dominant factor.

RDW increases in many diseases due to impaired turnover of RBCs.[29] Although COVID-19 is known to be associated with altered turnover in all blood cell lineages,[30] specific mechanisms for RDW alterations in COVID-19 remain unclear. In this study, we observed several morphological changes in RBCs that cause elevated RDW. For example, polychromatic RBC %, which reflects the presence of relatively large immature reticulocytes,[31] was increased in patients with COVID-19, with no significant difference between mild and severe cases. This suggests that SARS-CoV-2 infection stimulates the release of reticulocytes from the bone marrow to the peripheral circulation.

We also observed a significant increase in RBC fragmentation and schistocytes in the severe group compared with the mild group. This may be due to DIC and/or invasive therapies, such as hemodiafiltration and plasmapheresis. Interestingly, schistocytes were observed more frequently in the mild cases of infection than in HCs, suggesting that the appearance of schistocytes was not only associated with DIC and mechanical intervention. Fragmented red blood cell (FRC) by XN-3000 may be useful for quantitative estimation of schistocytes.

A recent study reported the frequent occurrence of bone marrow hemophagocytosis in patients with fatal COVID-19.[32] Excess bone marrow stress in severe cases may cause catastrophic changes, such as leukoerythroblastosis,[10] which was observed in the fatal case in our study. These findings further indicate that the hematopoietic progenitor cells may be involved in COVID-19 pathogenesis.

4.4 | Limitations

This study has some limitations. This was a single-center study involving a small number of patients. As the numbers of cases of infection and death by COVID-19 are low in Japan,[33] it was difficult to collect a large number of severe cases. However, our data showed important information regarding clinical and laboratory data that were distinct from other regions. We only examined CBCs and PB morphology, while lymphocyte surface antigens and cytokines were not analyzed. Bone marrow involvement was not studied. Further investigations, including bone marrow smear morphology, are required to elucidate the underlying mechanisms of increased APHA and other neutrophil dysplasia. In addition, the sole use of the Sysmex DI-60 in combination with CNN-based analysis is another potential limitation.

Steroid treatment may have affected the blood test results. Furthermore, long-term follow-up is necessary to confirm the recovery of hematopoietic systems.

5 | CONCLUSION

Our data demonstrated that basic PB examination can detect decreased granular lymphocytes and increased RDW as well as blood cell morphological changes in cases of severe COVID-19. These changes were dynamically correlated with disease severity and may be associated with disrupted hematopoiesis and immunological systems due to bone marrow stress in severe infection. To the best of our knowledge, this is the first work to describe the importance of the detection of a temporary increase in granular lymphocytes in patients with severe COVID-19 who survived. We propose that PB examination can be useful to predict the prognosis of COVID-19.

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CONFLICT OF INTEREST

The Department of Next Generation of Hematology Laboratory Medicine at Juntendo University Graduate School of Medicine has been endowed by Sysmex (Kobe, Japan) to develop and validate new diagnostic technology and to conduct academic research in hematology through collaborations. FH, YI, and KU are employees of Sysmex. The study was performed by scientifically proper methods without any bias.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol*. 2020;20:363-374.
2. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. 2020;20:269-270.
3. Yamada T, Wakabayashi M, Yamaji T, et al. Value of leukocytosis and elevated C-reactive protein in predicting severe coronavirus 2019 (COVID-19): a systematic review and meta-analysis. *Clin Chim Acta*. 2020;509:235-243.
4. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. 2020;130:2620-2629.
5. Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther*. 2020;5:33.
6. Cheng A, Hu L, Wang Y, et al. Diagnostic performance of initial blood urea nitrogen combined with D-dimer levels for predicting in-hospital mortality in COVID-19 patients. *Int J Antimicrob Agents*. 2020;56:106110.
7. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis*. 2020;34:101623.
8. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8:420-422.
9. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. *Am J Hematol*. 2020;95:870-872.
10. Mitra A, Dwyre DM, Schivo M, et al. Leukoerythroblastic reaction in a patient with COVID-19 infection. *Am J Hematol*. 2020;95:999-1000.
11. Kimura K, Tabe Y, Ai T, et al. A novel automated image analysis system using deep convolutional neural networks can assist to differentiate MDS and AA. *Sci Rep*. 2019;9:13385.
12. Palmer L, Briggs C, McFadden S, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol*. 2015;37:287-303.
13. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054-1062.
14. Foy BH, Carlson JCT, Reinertsen E, et al. Association of red blood cell distribution width with mortality risk in hospitalized adults with SARS-CoV-2 infection. *JAMA Netw Open*. 2020;3:e2022058.
15. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical utility of reticulocyte parameters. *Clin Lab Med*. 2015;35:133-163.
16. Loughran TP Jr, Kadin ME, Starkebaum G, et al. Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. *Ann Intern Med*. 1985;102:169-175.
17. Chan WC, Link S, Mawle A, Check I, Brynes RK, Winton EF. Heterogeneity of large granular lymphocyte proliferations: delineation of two major subtypes. *Blood*. 1986;68:1142-1153.
18. Merino A, Vlagea A, Molina A, et al. Atypical lymphoid cells circulating in blood in COVID-19 infection: morphology, immunophenotype and prognosis value. *J Clin Pathol*. 2020:jclinpath-2020-207087.
19. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis*. 2020;221:1762-1769.

20. Ayan MS, Abdelrahman AA, Khanal N, Elsallabi OS, Birch NC. Case of acquired or pseudo-pelger-huët anomaly. *Oxf Med Case Reports*. 2015;4:248-250.
21. Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal co-infection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis*. 2020;71(9):2459-2468.
22. Liu J, Li S, Liu J, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine*. 2020;55:102763.
23. Prager I, Watzl C. Mechanisms of natural killer cell-mediated cellular cytotoxicity. *J Leukoc Biol*. 2019;105:1319-1329.
24. Zhang R, Shah MV, Loughran TP Jr. The root of many evils: indolent large granular lymphocyte leukaemia and associated disorders. *Hematol Oncol*. 2010;28:105-117.
25. Wells MA, Ennis FA, Albrecht P. Recovery from a viral respiratory infection. II. Passive transfer of immune spleen cells to mice with influenza pneumonia. *J Immunol*. 1981;126:1042-1046.
26. Cannon MJ, Stott EJ, Taylor G, Askonas BA. Clearance of persistent respiratory syncytial virus infections in immunodeficient mice following transfer of primed T cells. *Immunology*. 1987;62:133-138.
27. Zhang JY, Wang XM, Xing X, et al. Single-cell landscape of immunological responses in patients with COVID-19. *Nat Immunol*. 2020;21:1107-1118.
28. Cavezzi A, Troiani E, Corrao S. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. a narrative review. *Clin Pract*. 2020;10:1271.
29. Goyal H, Lippi G, Gjymishka A, John B, Chhabra R, May E. Prognostic significance of red blood cell distribution width in gastrointestinal disorders. *World J Gastroenterol*. 2017;23:4879-4891.
30. Spiezia L, Boscolo A, Poletto F, et al. COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. *Thromb Haemost*. 2020;120:998-1000.
31. An X, Mohandas N. Erythroblastic islands, terminal erythroid differentiation and reticulocyte maturation. *Int J Hematol*. 2011;93:139-143.
32. Harris CK, Hung YP, Nielsen GP, Stone JR, Ferry JA. Bone marrow and peripheral blood findings in patients infected by SARS-CoV-2. *Am J Clin Pathol*. 2021;155(5):627-637.
33. Iwasaki A, Grubaugh ND. Why does Japan have so few cases of COVID-19? *EMBO Mol Med*. 2020;12:e12481.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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