Red Blood Cells Pathology in Patients with Coronavirus Disease 2019 (COVID-19)

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Research Article

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Abstract

Introduction

The severe acute respiratory syndrome-related coronavirus 2 (SARS-Cov-2) infection may cause hematological disorders. This pathology has been studied to a lesser extent compared to the respiratory system pathology. The main purpose of this article is to investigate the general pathology of red blood cells in coronavirus infection.

Methods

In current study 74 patients suffering from SARS-Cov-2 treated at the National Center of Infectious Diseases, Ministry of Health Armenia were included. Investigated patients were classified as: 1. Regular group (fever, respiratory symptoms and radiographic evidence of pneumonia). 2. Severe group – patients manifesting shortness of breath (over 30 breaths per minute); peripheral blood oxygen saturation below 92% in rest; pneumonia affecting over 50% of tissue; and/or respiratory failure and indicated mechanical ventilation support and/or organ failure requiring intensive care unit treatment. 3. Low saturation group with peripheral blood oxygen saturation below 85% in rest. 4. Erythroblastosis group with count of erythroblasts over 0.5% among total of nucleated blood cells. Clinical laboratory investigation included all main routine studies.

Microspectrophotometry was performed on SMP-05 Opton scanning microspectrophotometer to measure the spectra of hemoglobin (Hb) in unstained erythrocytes.

Results

Erythroblasts were common (present in about 30%) finding among SARS-Cov-2 patients but predominating mainly in the severe group. Serum ferritin and C-reactive protein (CRP) levels as well as anisocytosis highly correlate with the severity of the disease. Microspectrophotometric studies showed significant changes of hemoglobin adsorption spectra in individual erythrocytes. However, in severe form of SARS-CoV-2, increase of hemoglobin absorbance occurred in within 420 nm wavelength spectrum compared with regular group.

Conclusion

Our findings suggest that the increased levels of ferritin, CRP, anisocytosis and partially increase in Hb adsorption on 420 nm wavelength may positively correlate with adverse outcomes in SARS-Cov-2 infection.

1. Introduction

There are several subfamilies of coronaviruses. Seven coronaviruses are described to cause respiratory diseases of humans. Four of them are common human coronaviruses. And one of the sevens is the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2). SARS-CoV-2 belongs to the subfamily *Coronavirinae*. More species of coronaviruses able to induce severe human diseases are the SARS-CoV and MERS-CoV. Coronaviruses HKU1, HCoV-NL63, HCoV-OC43 and HCoV- 229E are associated with mild symptoms in humans [1, 2].

Coronavirus disease-19 (COVID-19) is an infective-inflammatory disease, which primarily affects the lungs. Severe course of disease is associated with multi-organ pathology with various pathways of injury. Though red blood cell pathology is mentioned less often, several articles suggested that SARS-Cov-2 infection may cause hematological disorders [3, 4]. Authors usually indicate differences in hematological manifestations between severe and non-

severe patients. Hemoglobinopathy, hypoxia and cell iron overload might have a possible role in SARS-CoV-2 pathology. Scientific literature has pointed out two potential pathophysiological mechanisms: a) severe acute respiratory syndrome-coronavirus-2 (SARS-CoV- 2) interaction with hemoglobin molecule, through CD147, CD26 and other receptors located on erythrocyte and/or blood cell precursors; b) hepcidin-mimetic action of a viral spike protein, inducing ferroportin blockage [3].

Serum ferritin is known as an iron storage protein and generally is measured as an indicator of iron status. Also, a prominent inflammatory marker is the significant increase of ferritin level in serum in response to inflammation and other pathologies. Serum ferritin level also correlated with the degree of disease severity in patients with SARS-CoV-2, however mechanisms for association of hyperferritinemia and disease severity in patients with SARS-CoV-2 stayed unclear [5].

Normally the erythroblasts should be totally absent in the adult blood and are usually observed in almost all forms of severe anemia (except aplastic). In patients with SARS-CoV-2 several studies observed erythroblastosis [6–8]. It has been shown, that ferritin often was located on the plasma membrane of erythroblasts [9]. Therefore, goals of this research are to investigate association of serum levels of ferritin with erythroblastosis and hemoglobin abnormalities in patients with SARS-CoV-2. The main purpose of this article is to investigate the general pathology of red blood cells in coronavirus infection.

2. Methods

2.1 Patients

This study was approved by Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of National Academy of Sciences, Yerevan, Armenia; IRB00004079, 2013

In current study 74 patients suffering from SARS-CoV-2 treated at the National Center of Infectious Diseases, Ministry of Health Armenia were included.

2.2 Clinical criteria

Investigated patients were classified as:

1. Regular group (fever, respiratory symptoms and radiographic evidence of pneumonia),

2. Severe group – patients manifesting shortness of breath (over 30 breaths per minute); peripheral blood oxygen saturation below 92% in rest; pneumonia affecting over 50% of tissue; and/or respiratory failure and indicated mechanical ventilation support and/or organ failure requiring intensive care unit treatment.

3. Low saturation group with peripheral blood oxygen saturation below 85% in rest.

4. Erythroblastosis group with count of erythroblasts over 0.5% among total of nucleated blood cells.

Clinical characteristics of the 74 patients with SARS CoV-2 are presented in Table 1.

2.3. Laboratory measurements

Clinical laboratory investigation included studies of complete blood count and ferritin, C reactive protein (CRP), lactose dehydrogenase (LDH) quantitated in sera of patients.

Blood sample analyses were performed with commercially available ELISA kits normally used for clinical practice of the hospital.

2.4 Blood smears, Giemsa staining, and nucleated blood cells analysis

Fresh blood was used to prepare blood smears by routine methods. For nucleated blood cells analysis, slides were fixed in pure methanol and stained by Giemsa modified solution (azure B/azure II, eosin and methylene blue) according to the manufacturer's protocol (Sigma-Aldrich). Nucleated blood cells were examined under the light microscope at ×1250 in a random sequence. At least 300 nucleated blood cells in each sample were evaluated for cell types.

2.5 Microspectrophotometry

Microspectrophotometry was performed on SMP-05 Opton scanning microspectrophotometer to measure the spectra of hemoglobin (Hb) in unstained erythrocytes. The spectrophotometric measurement was performed only for single RBC, and extracellular area as a standard reference. Choice of microspectrophotometric method is due to the possibility to measure small spectral changes with a limited number of RBC [10]. Also, were performed cytometric investigations.

2.6 Statistical analysis

SPSS-19 software was used for data analysis. The measurement data were generally distributed in a non-normal distribution so the non-parametric Mann-Witney *u-test* was used. Initially, the 1–4 grade disease severity scale was used to record the disease severity: 1 - regular group; 2 - erythroblastosis; 3 - group with low oxygen saturation; 4 – group with severe form of disease. The following erythroblastosis scale was used to record the level of erythroblasts in blood; 1 – absence, 2–0.1%, 3–0.3%-0.5%, 4–0.6% and more. For correlation analysis Spearman correlation coefficient was used according to the data.

3. Results

3.1 Basic characteristics of the patients

Among the 74 patients, the median age was 59 years (31–83 years), and 38 (51.3%) were males. There were 35 cases in the regular group, 18 cases in the severe group with critical form of disease, 12 cases in low saturation group and 9 cases in group with a prominent erythroblastosis (at least 1% of erythroblasts from total nucleated cell count). There was no significant difference in the median age and sex ratio in each group. The main clinical manifestations of the patients were cough, fever and fatigue in all patients, and shortness of breath was more common in severe and critically ill patients.

In severe and critically severe cases ferritin level (ferritin-104.8-2929 ng/ml) was higher than in mild (ferritin-87.6-346 ng/ml) or cases with low saturation indices (ferritin-59.8-894 ng/ml) as well as in patients of erythroblastosis group. Similar data were found in the study of serum levels of CRP.

3.2 Laboratory results

Due to the small number of cases of critically ill patients, to reduce the statistical deviation, the severe and critically ill groups were combined and compared with the regular group.

As follows from Table 2, statistically significant changes in the population composition of peripheral blood cells were not revealed in the groups. Exception is the finding of erythroblasts in the group with severe cases vs. regular group.

3.3 Routine blood test and erythroblastosis

The routine blood test was collected from all the patients. The results showed that the white blood cell (WBC) in COVID-19 patients were basically in the normal reference range. Several patients (about 7–8%) showed lymphopenia. The main distinguishing feature of patients with coronavirus infection vs. the norm was the regular detection of erythroblasts in the peripheral blood. Acidophilic erythroblasts were detected in all studied groups of patients undergoing inpatient treatment. The patients with diagnosed erythroblastosis ranged from 5%-10% in regular group up to 44% in the group with severe form of coronavirus infection (Table 2). Erythroblasts were common (present in about 30%) finding among SARS-Cov-2 patients. It was predominating mainly in the severe group, and was significantly higher than regular group data ($p < 0.01$). Erythroblasts counted for 0,1%-0,5% of all nucleated cell population in the severe group patients. Additionally, polychromatophilic and basophilic erythroblasts were regularly observed in blood smears of patients with a severe form of the disease.

Considering relatively high incidence of erythroblastosis in SARS-Cov-2 infection, an individual group of patients (erythroblastosis group) was specified for more detailed study of this pathology. Patients with erythroblast count over 0,5% from all nucleated cell population were included in the group. Some of the severe group patients (16,7%) developed anisocytosis along with a erythroblastosis (Fig. 1h). As follows from Fig. 2a, the patients with severe form of SARS-Cov-2 developed erythrocytes with very large surface area as well as cells with smaller surface area. This is a manifestation of anisocytosis. Decreased hemoglobin content was revealed in the group of erythroblastosis (Fig. 2b).

3.4 Influence of coronavirus infection on erythrocyte parameters, determined by microspectrophotometry

Mean values of hemoglobin content, concentration of a single red blood cell and cell size were determined in 200 erythrocytes. It was found that coronavirus infection caused variation of Hb adsorption spectra in erythrocytes.

Figure 2 show data of changes of adsorption spectra in a single erythrocyte. Main changes occurred in spectra between 414 nm and 420 nm (Fig. 3A, B). Differences in adsorption spectra in main type of erythrocytes and variable absorbance induced by SARS-CoV-2 are shown in Fig. 3c. As shown in Fig. 3d, the absorption maximum at the wavelength 418–422 band changed markedly. Absorption maximum increased in single erythrocytes in patients from groups with severe form of disease and in patients with erythroblastosis at the wavelength 418–422. Normally, the erythrocyte population is quite homogeneous (Fig. 3d), and hemoglobin spectrophotometry reveals a significant decrease in hemoglobin absorption with an increase in wavelength from 414 to 420 nm. However, certain erythrocytes in SARS-CoV-2 (20%-35% patients from severe form of disease and from group with erythroblastosis; see below) demonstrated less decrease in absorption compared to control (Fig. 3d).

Microspectrophotometry recorded increased amounts of erythrocytes with elevated Hb absorption at wavelength 420 nm in patients with erythroblastosis and severe form of SARS-Cov-2.

Microspectrophotometric studies showed significant changes of hemoglobin absorption spectra in individual erythrocytes. However, in severe form of SARS-CoV-2, increase of hemoglobin absorbance occurred in within 420 nm wavelength spectrum (Fig. 4); this increase was statistically significant (*u*-criterion) compared with regular group. Hemoglobin absorbance index in erythroblastosis group varied between values of control and the severe form of SARS-CoV-2.

It follows from the Table 3 that serum ferritin and CRP levels as well as anisocytosis highly correlate with the severity of the disease.

4. Discussion

The changes we identified in the peripheral blood after pathology induced by coronavirus infection mimicked the process of restoring red blood cells. These major changes are expressed in release of erythroblasts from the bone marrow, anisocytosis and deviations in Hb absorption spectra. Coronavirus infection develops in mild or asymptomatic form in majority of population who are not subject to hospitalization. Erythroblastosis was found in hospitalized patients with initially more severe course of the disease. However, erythroblastosis was not a predominant finding of only patients with a poor prognosis as described by some others [7]. Erythroblasts were detected in all investigated groups of patients, and higher levels of erythroblastosis did not show a significant correlation (only tendency $p < 0.1$) with a more severe course or worse prognosis of the disease. Additionally, higher erythroblastosis was comparable between regular and severe groups, and did not correlate with the level of oxygen saturation. Erythroblastosis is frequently associated with hypoxia [11]. However, our data found no correlation between the levels of erythroblastosis and reduced level of saturation (a tendency towards correlation is shown only between the level of erythroblastosis and the severe course of the disease), even though hemoglobinopathy can be an essential factor in general pathology of SARS-CoV-2 [12, 13]. So, such abnormalities of erythropoiesis as erythroblastosis may be an indirect effect of the systemic hyperinflammation that occurs in patients with severe form of disease, but also by direct targeting of erythroid progenitors by SARS-CoV-2 [14]. This data observe presence of the virus after 14 days in the erythroid cells without impairing their viability suggest that the erythroblastosis detected in severe patients may be due to direct infection.

Virus effect on hemoglobin could be mediated through ACE2, CD147, CD26 and other receptors on erythrocytes and/or blood cell precursors [3]. In this aspect, [4] suggestions were made about possible interactions between COVID-19 and hemoglobin that may reduce both oxygen affinity and total hemoglobin content.

A similar mechanism of entry into erythrocytes through CD147 is also practiced by *Plasmodium*, which is targeted by anti-malarial drugs also that may express anti-SARS-CoV-2 effect as well. They were applied for prevention of non-structural SARS-CoV-2 proteins from attacking heme and forming porphyrin complex [15].

Various articles show that ferritin levels increase in patients with progressive form of SARS-Cov-2 and poor outcomes [16, 17, 18]. Serum ferritin is an acute-phase reactant, which levels correlate with the degree of acute and chronic inflammation in infections [19]. Our data are consistent with the conclusions of the cited authors stating that highest levels of serum ferritin were found in the group of severe cases of SARS-Cov-2 and in the group of patients with low oxygen saturation, a significant part of the studied ferritin levels were increased compared to the patients from regular group. An increased ferritin level observed in some SARS-Cov-2 patients probably documents an inflammatory reaction or is related to viral entry into a bloodstream and to its impact on iron metabolism [20]. We also detected anisocytosis which is a predictor of anemia. Anisocytosis is also associated with a higher all-

cause mortality rate in the general population [21]. Our data revealed that high ferritin values have a positive correlation with the severity of the disease, anisocytosis and changes in the microspectrophotometric characteristics of hemoglobin in erythrocytes, but have no correlation with erythroblastosis. Several studies demonstrated an association between disturbances in erythrocyte size distribution and inflammation [21, 22]. However, despite a clear association between increased anisocytosis levels and CRP, this phenomenon is only a part of a complex pathology of erythrocytes developed in SARS-Cov-2 infection.

5. Conclusion

Increased levels of ferritin, CRP, anisocytosis and partially increase in Hb absorption on 420 nm wavelength may positively correlate with adverse outcomes in SARS-Cov-2 infection.

6. Abbreviations

SARS-COV-2: severe acute respiratory syndrome-related coronavirus 2

RBC: red blood cells

Hb: hemoglobin

WBC: white blood cells

CRP: C-reactive protein (CRP)

LDH: lactose dehydrogenase

7. Declarations

Ethics approval This study was approved by Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of National Academy of Sciences, Yerevan, Armenia; IRB00004079, 2013.

Conflict of interest The authors declare no competing interests.

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Author's contribution ZK created the concept and wrote the paper. LH, LA, AA, EK, SH, HA, NB, HV carried out cells analysis,microspectrophotometry analyses, carried out the preparation of blood smears, LN, MD, KS, TG supervised and carried out the production of blood samples, clinical and laboratory analysis, AS, EKassisted with interpretation of the data and critical revision of the manuscript.

Availability of data and material Not applicable.

Code availability Not applicable.

Consent to participate Informed consent was obtained from all individual participants in the study.

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9. Tables

Table 1

Clinical characteristics of the 74 patients with SARS CoV-2

Characteristic	Regular group (n = 35)	Severe group (n = 18)	Low saturation group (n = 12)	Erythroblastosis group (n=9)
Age—year	61±4.5	58±3.3	56±4.8	57±5.5
Male sex—%. (N)	51.4 (18)	50.0 (9)	50.0 (6)	55.5 (5)
Symptoms—%. (N)				
Fever	80% (28)	94.5% (17)	16.7 (2)	66.7% (6)
Cough	77.1% (27)	22.2% (4)	75% (8)	22.2% (2)
Loss of smell/taste	57.1 (20)	16.7% (3)	16.7% (2)	-
Shortness of breath	22.8% (8)	16.7% (3)	50% (6)	22.2%(2)
O_2 saturation	92.2±2.8	88.5±4.9	75.7±5.3	91.2±5.1
Anorexia	8.7% (3)	5.5% (1)	-	-
Diarrhea	5.7% (2)	5.5%(1)	-	-
Fatigue	82.9% (29)	22.2% (4)	83.3% (10)	77.8% (7)
Myalgia or arthralgia	28.6% (10)	16.7% (3)	25% (3)	44.4% (4)
Coexisting disorder—%. (N)				
Hypertension	42.9% (15)	38.8% (7)	33.3% (4)	44.4% (4)
Diabetes	31.4% (11)	27.8% (5)	25% (3)	22.2% (2)
Coronary heart disease	8.7% (3)	5.5% (1)	8.3% (1)	22.2% (2)
Cerebrovascular disease	-	5.5% (1)	-	-
Chronic renal disease	2.9% (1)	5.5% (1)	-	-
Malignant tumor	5.7% (2)	5.5% (1)	-	-
other coexisting chronic disorder	20% (7)	11.1% (2)	8.3% (1)	-
Ferritin level (ng/ml)	87.6-346	104.8-2929*	59.8-894	87-285
CRP (mg/l)	46.5 (8-81)	89.3 (21-231)	47.9 (14-95)	19.1 (7-72)

Table 2

Blood cell populations.

Blood cells	Regular group (n = 35)*	Severe group (n = 18)**	Low saturation group (n = 12)***	Erythroblastosis group (n = 9)
Basophilic erythroblast	-	0.1	-	0.7
Polychromatophilic erythroblast	-	0.1	-	0.7
Acidophilic erythroblast	0.1	0.3	0.1	0.1
Lymphoblast	1.8±1.4	2.5	1.4	2.1
Lymphocyte	22.0±6.4	25.3	28.8	28.5
Lymphocyte aberrant	1.1±2.1	0.4	1.4	1.8
Monoblast	0.4±0.7	0.3	0.4	0.7
Monocyte	2.1±1.4	2.6	2.6	1.6
Myeloid cell	2.8±2.5	1.6	3.7	1.3
Metamyelocyte	14.7±7.4	16.3	17.7	12.2
Band neutrophil	39.9±9.6	36.8	34.4	39.1
Segmented neutrophil	12.3±1.1	10.9	7.7	8.2
Pathological neutrophil	0.8±0.9	0.7	0.7	1.6
Eosinophil	0.4±0.2	0.9	0.2	0.5
Basophil	0.1±0.2	0.1	0.0	0.1
Destructed cells	1.3±1.2	1.1	1.0	0.9
*Erythroblastosis present in 5.7% cases (2 patients from 35). **Erythroblastosis present in 44.4% cases (8 patients from 18). ***Saturation below 85 percent.				

Table 3

Correlation analysis of red blood alterations and disease severity by Spearman coefficient.

	disease severity	erythroblastosis	ferritin	CRP	Increased Hb absorption on 420 nm (%)	anisocytosis (%)	decreased Hb in erythrocyte (%)
disease severity	1.000	.316	1.000*	1.000*	.800	1.000*	-.105
erythroblastosis	.316	1.000	.316	.316	.632	.316	.833
ferritin levels in serum	1.000*	.316	1.000	1.000*	.800**	1.000*	-.105
CRP levels in serum	1.000*	.316	1.000*	1.000	.800**	1.000*	-.105
increase in Hb absorption on 420 nm (%)	.800**	.632	.800**	.800**	1.000	.800**	.105
anisocytosis (%)	1.000*	.316	1.000*	1.000*	.800**	1.000	-.105
decreased Hb amount in erythrocyte (%)	-.105	.833	-.105	-.105	.105	-.105	1.000

* Correlation is significant at the 0.01 level (2-tailed).

** Tendency at 0.1 level (2-tailed).

Figures

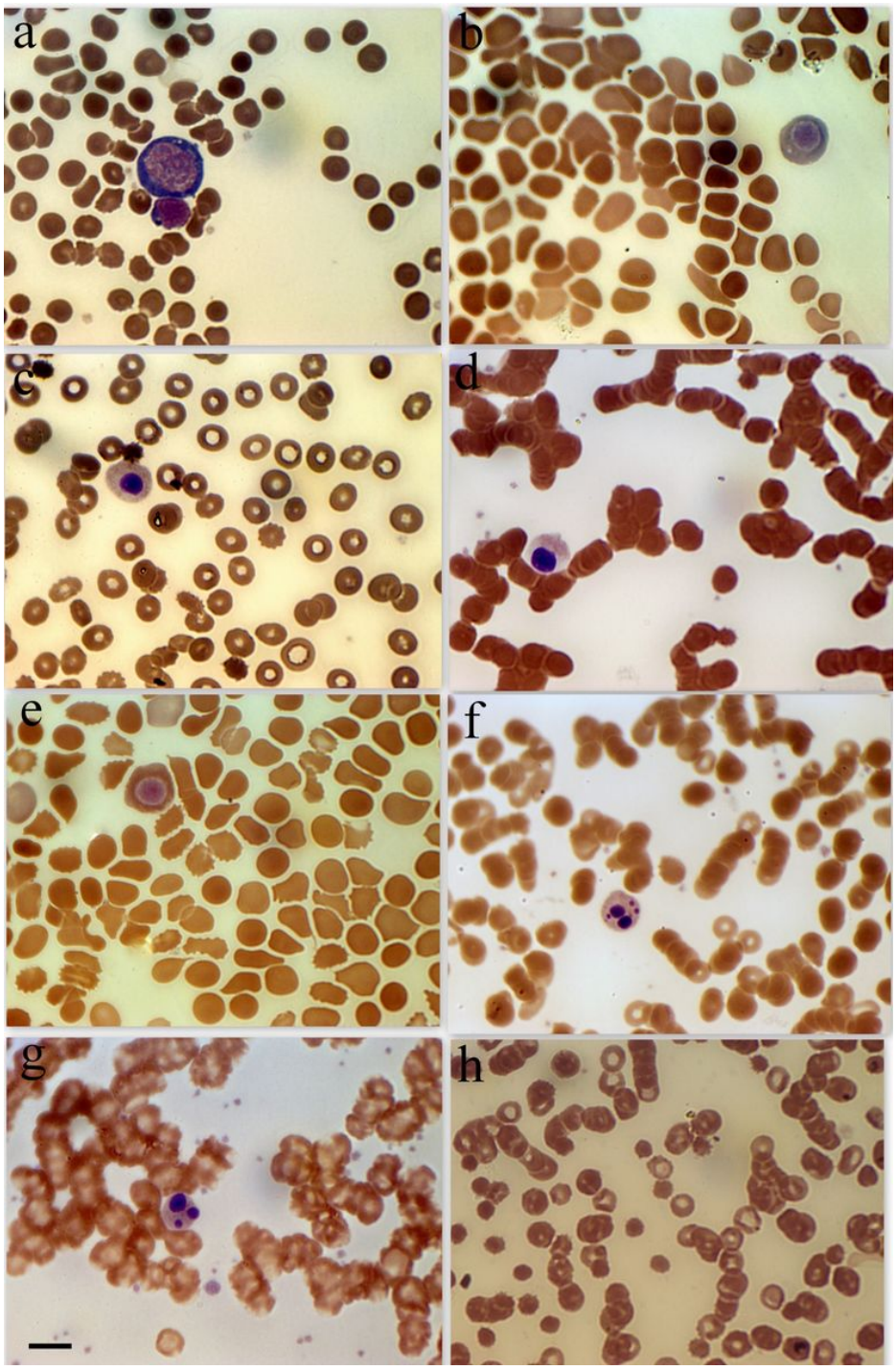

Figure 1

Erythroid cell morphology in peripheral blood in patients with SARS-CoV-2 a. Basophil erythroblast in female patient with severe form of coronavirus. b. Polychromatophilic erythroblast female patient with moderate form of coronavirus. c. Polychromatophilic erythroblast, female 48 year old patient with diabetes and hypertension. d. Acidophilic erythroblast, female patient with severe form of coronavirus. e. Acidophilic erythroblast, female patient with moderate form of coronavirus. f. Acidophilic erythroblast with cleaved nucleus, male patient with moderate form of coronavirus. g. Acidophilic erythroblast with cleaved nucleus, female patient with moderate form of coronavirus. h. Anisocytosis in female patient with severe form of coronavirus. Scale bar 10 μm

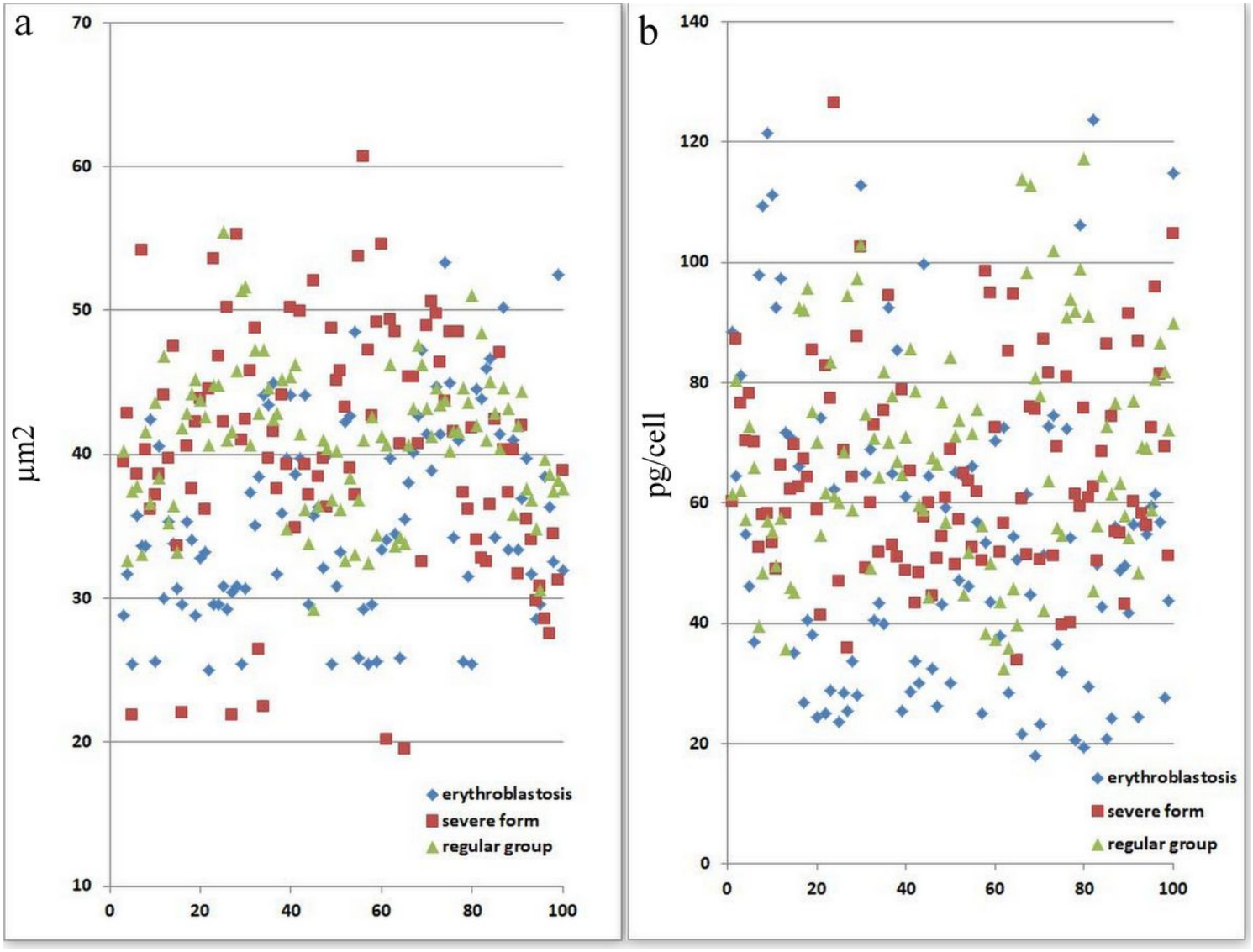

Figure 2

Morphological abnormalities of erythrocytes in patients with SARS-Cov-2 infection. Each point is the average of erythrocytes from three patients, systematized by size. a. Size of erythrocytes b. Hb amount in erythrocytes

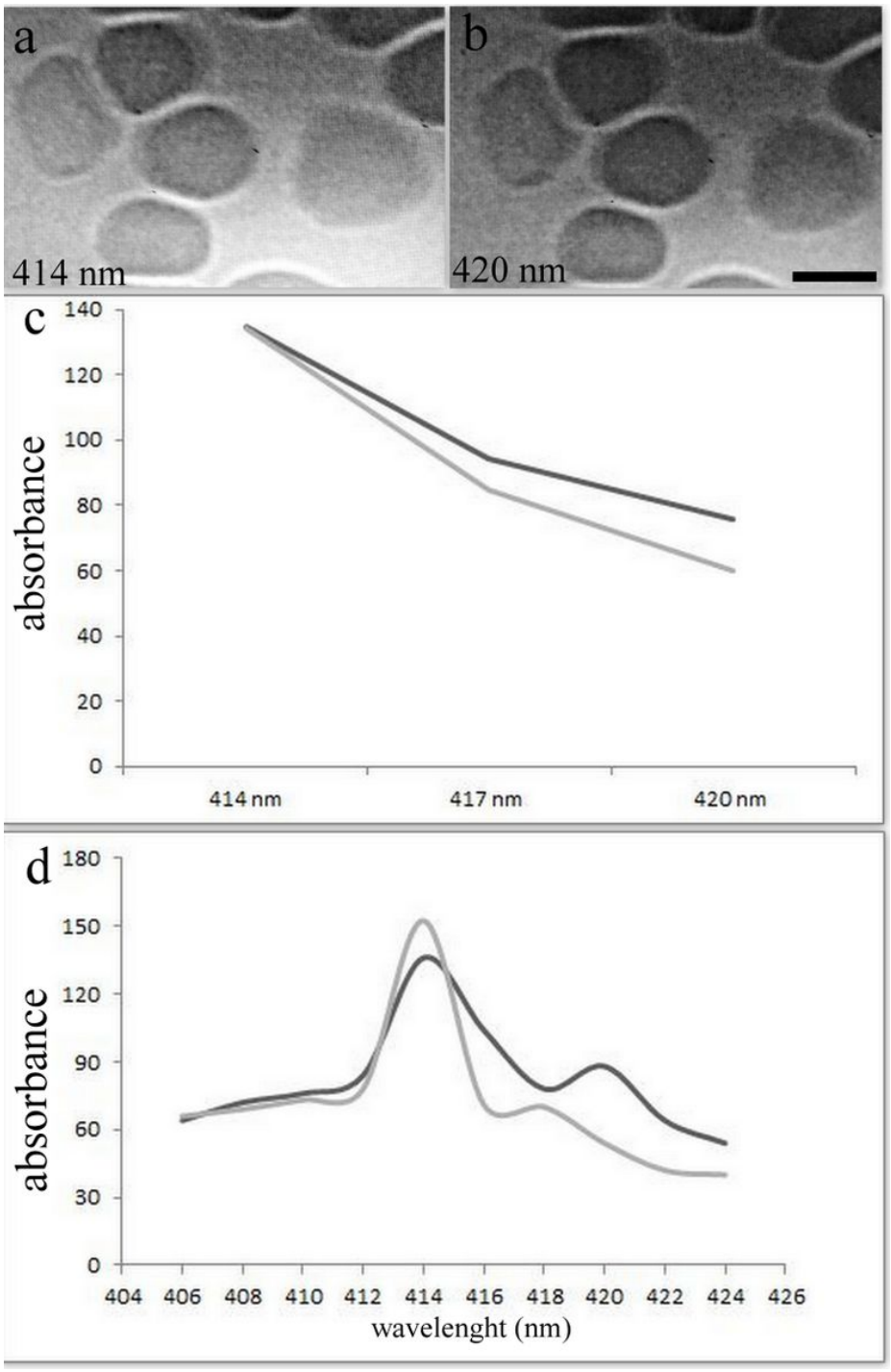

Figure 3

Soret absorption spectroscopy of hemoglobin The graph shows the data of microspectrophotometry of erythrocytes with an identical area (differences less than 1%) on different wavelengths. Black line: main type of erythrocytes Gray line: erythrocytes with variable adsorbance in SARS-CoV-2 infection. a. Erythrocytes in 414 nm wavelengths. b. Erythrocytes in 420 nm wavelengths. Scale bar 5 μm c. Spectral changes of erythrocytes in patients with SARS-CoV-2 (black curve), increase in hemoglobin adsorbance occurs about the 420 nm wavelength spectrum. d. Adsorption spectra of single erythrocytes (with same surface area) studied by microspectrophotometry on different wavelengths.

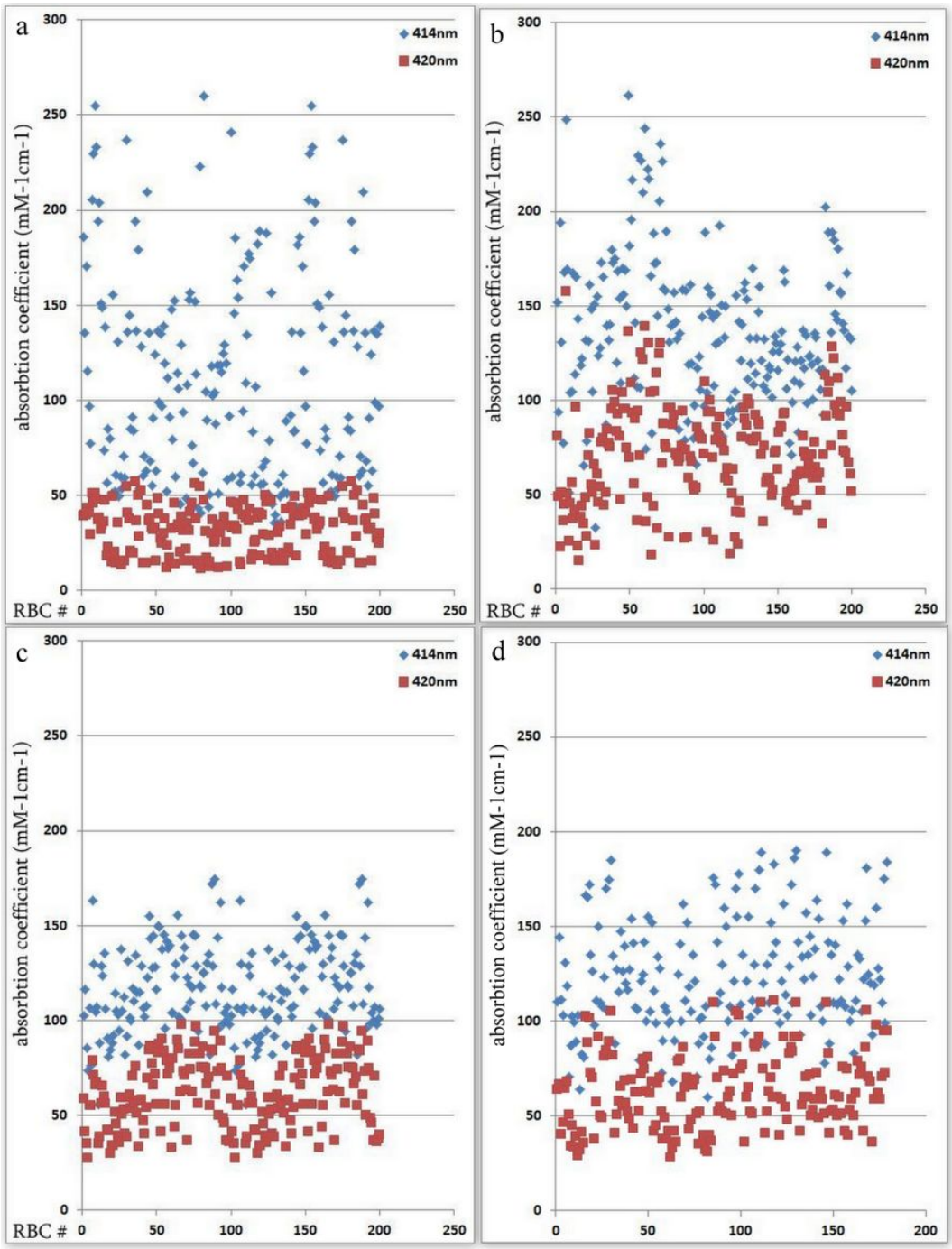

Figure 4

Distribution of single erythrocytes by absorption spectroscopy of hemoglobin on different wavelengths in norm and in patients with SARS-CoV-2. Each point is the average of erythrocytes from three patients, systematized by size. a. Norm. b. Regular group. c. Severe group d. Group with erythroblastosis