

PARAMETERS AND MORPHOLOGICAL CHANGES OF ERYTHROCYTES IN COVID-19 SUBJECTS

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Abstract. *Objective:* to assess the morphological abnormalities of red blood cells (RBC) in patients with COVID-19 and cardiovascular system (CVS) pathology.

Material and methods. The study included 142 patients who were divided into 3 groups according to the following principle: combination of COVID-19 and CVS pathology (group 1, n=56); COVID-19 without CVS pathology (group 2, n=44); patients with CVS pathology without COVID-19 (group 3, n=42). Peripheral blood erythrocytes were studied. Morphological studies were carried out in the laboratories of pathological anatomy of the State Institution "RSSPMC for surgery named after V.Vakhidov" and RSH Zangiota-1.

Results. Morphological features of peripheral blood erythrocytes in COVID-19-associated cardiovascular syndrome were revealed, characterized by an increase in the frequency of elevated erythrocyte distribution width (RDW) from 45.5% to 67.9% ($p<0.05$), and the average RDW from 13.1% (11, 8-15.4%) to 14.3% (12.6-15.8%) ($p<0.0001$); an increase in the proportion of abnormal forms from 13.6% in the isolated course of COVID-19 to 32.1% in the combination of COVID-19 with cardiovascular pathology ($p<0.05$), mainly due to ridged erythrocytes from $6.4\pm 0.5\%$ to $8.2\pm 0.6\%$ ($p<0.001$) and the proportion of irreversible forms of erythrocytes from $1.6\pm 0.1\%$ to $3.1\pm 0.3\%$ ($p<0.001$) increasing the risk of transferring the patient to the ICU (RR=6.0) and death (RR=4.33). Patients with more than 20% RBC abnormality had a statistically significant higher ICU admission rate (50.0%, 9 of 18; RR=6.0) than patients with less than 10% RBC abnormality (8.33%, 1 of 12, $p=0.049$), and patients with 10-20% abnormal RBCs (7.7%, 2 of 26, $p=0.005$).

Conclusion. Patients with COVID-19-associated cardiovascular syndrome have a significant morphological heterogeneity in the shape and size of erythrocytes and their relationship with the progression and outcome of the disease.

Keywords: prospective study, COVID-19, cardiovascular system pathology, red blood cells, morphological abnormalities.

INTRODUCTION

The impact of COVID-19 on the burden of cardiovascular diseases (CVD) at the beginning of the pandemic remained unclear. It has been noted that COVID-19 has become one of the leading causes of global mortality, with a disproportionate impact on patients with CVD. Taken together, the study results suggest a heterogeneous impact of the COVID-19 pandemic on CVD prevalence, with no significant increase in CVD mortality in 2020-2021. It is known that COVID-19 poses a particular danger in terms of decompensation of existing chronic diseases and specific damage to

the cardiovascular system (CVS), especially in the case of severe COVID-19 and a high risk of adverse outcomes. As a rule, the combination of COVID-19 with cardiovascular pathology leads to additional difficulties in diagnosing, choosing management tactics, and treating patients in emergencies [2, 3].

According to R. Vasudeva, “Among CVDs registered in deaths associated with COVID-19 in all age groups, arterial hypertension (AH) is the most common (19.6%), followed by diabetes mellitus (DM) (15.9%), coronary heart disease (CHD) (10.9%), heart failure (HF) (7.7%), cardiac arrhythmias (7.5%) and cerebrovascular diseases (5%)” [4].

The authors are developing various diagnostic methods, schemes for the treatment and prevention of cardiovascular complications, among which, an important aspect is to establish the significance of genetic polymorphisms for predicting the combined course of COVID-19 with CVD [5, 6].

Direct myocardial injury due to viral damage to cardiomyocytes and the effect of systemic inflammation appear to be the most common mechanisms responsible for heart injury [7, 8]. In studies by H. Han (2020) on the analysis of the main laboratory indicators of heart damage in patients with COVID-19, and their correlation with heart damage and disease severity, it was noted that “a higher concentration in venous blood of specific enzymes of myocardial damage was associated with the severity and lethality of COVID-19” [9].

Also, more frequent quantitative hematological abnormalities have been reported in severe COVID-19, detected by a complete blood count (anemia, neutrophilia, neutrophilic shift to the left and lymphopenia) [10].

Red blood cells (RBC) abnormalities are known to occur in many viral infections, usually in the form of mild normomicrocytic anemia. Although some hematologic changes on automated complete blood count (including neutrophilia, lymphopenia, and increased erythrocyte distribution width (RDW)) have been associated with progression in COVID-19 severity, there is still little information on erythrocyte morphologic abnormalities, mostly in the form of case reports or small patient series that are difficult to match due to heterogeneity in sampling time and disease severity. Despite advances in automated and molecular diagnostic techniques, a simple laboratory tool that remains an invaluable tool for primary and secondary blood cell abnormalities is the peripheral blood smear.

The objective of this work was to conduct a prospective study to assess the morphological abnormalities of RBC in patients with COVID-19 and scientifically substantiate the important role of erythrocytes in the clinical course of COVID-19 in combination with cardiovascular system pathology.

MATERIAL AND METHODS.

The study included 142 patients who were divided into 3 groups according to the following principle: combination of COVID-19 and CVS pathology (group 1, n=56); COVID-19 without CVS pathology (group 2, n=44); patients with CVS pathology without COVID-19 (group 3, n=42). The main distinguishing features of the combination of COVID-19 and CVS pathology (group 1) were anemia and significantly increased RDW (erythrocyte distribution width), the morphological and morphometric significance of which is presented below.

All patients underwent studies of the morphology and morphometry of erythrocytes in a peripheral blood smear during the first 72 hours after admission to the hospital.

The mean age of patients was 64.8 (56 to 84) years in group 1, 62.9 (46 to 76) years in group 2, and 63.7 (54 to 80) years in group 3, with no intergroup statistical difference (Table 1). Also, the groups were comparable in terms of gender, a relatively equal occurrence of both men and women was noted. The presence of at least 1 comorbid condition was detected in 25.0% (14 of 56) of patients from group 1, 22.7% (10 of 44) in group 2 and 28.6% (12 of 42) in group 3. Evidence was documented based on history and laboratory findings.

Table 1.

Baseline Demographic and Clinical Characteristics

	Group 1 (n=56)	Group 2 (n=44)	Group 3 (n=42)	<i>p</i>
Age (years)	64,8±2,2	62,9±2,4	63,7±2,3	*0.382 **0.482
Gender (men)	27 (48,2%)	23 (52,3%)	20 (47,6%)	*0.754 **0.802
Presence of at least 1 comorbid pathology	14 (25,0%)	10 (22,7%)	12 (28,6%)	*0.213 **0.321
Chronic kidney disease	13 (23,2%)	6 (13,6%)	7 (16,7%)	*0.340 **0.447
Chronic liver disease	4 (7,1%)	3 (6,8%)	4 (9,5%)	*0.741 **0.958
Chronic anemia	3 (5,4%)	1 (2,3%)	1 (2,4%)	*0.234 **0.198
Chronic obstructive pulmonary disease	5 (8,9%)	4 (9,1%)	4 (9,5%)	*0.747 **0.801
Anamnesis of stroke	3 (5,4%)	0 (0,0%)	2 (4,8%)	*0.333 **0.741
Time from symptom onset to hospitalization (days)	5,5±0,6	6,2±0,8	---	*0.537
Saturation (%)	85,2±0,8	87,4±0,7	94,2±2,4	* 0.042 **< 0.01
Heart rate (bpm)	102,1±4,8	78,4±2,2	88,5±2,4	*< 0.01 ** 0.012
Mean arterial blood pressure (mm Hg)	149,3±4,2	112,7±4,6	137,6±3,8	*< 0.01 ** 0.041
Temperature (°C)	37,6±0,4	37,3±1,2	36,8±0,9	*0.813 **0.418

Note: Data are presented as n (%), mean (M±m). Statistical significance was set at $p < 0.05$ (* between groups 1 and 2, ** between groups 1 and 3).

At the same time, in the structure of comorbid pathologies, chronic kidney disease (CKD) is most often diagnosed in varying degrees (except terminal). In group 1 (patients with COVID-19 and CVS pathology), 13 (23.2%) patients had signs of CKD, in group 2 (COVID-19 without

CVS pathology) - in 6 (13.6%) and in group 3 (CVS pathology without COVID-19) – in 7 (16.7%) patients ($p=0.134$). Next in frequency in the study groups, chronic obstructive pulmonary disease (COPD) was diagnosed - in group 1 in 8.9% (5 out of 56) of patients, in group 2 in 9.1% (4 out of 44) of cases and in group 3 - 9.5% (4 out of 42). Among the groups with COVID-19, there was no difference in the timing of admission to the hospital, i.e. the time from the onset of symptoms to hospitalization was 5.5 (2-8) days in group 1 and 6.2 (3-10) days in group 2.

The groups differed from each other in terms of blood oxygen saturation, which averaged (median) 85.2% (range 82% to 90%) in group 1, 87.4% (84% to 92%) in group 2 and 94.2% (90 to 97%) in group 3. Mean arterial blood pressure (ABP), as well as heart rate, in groups with CVD pathology (groups 1 and 3) was higher than in group 2, where there were no concomitant CVDs.

Morphological studies were carried out in the laboratories of pathological anatomy of the State Institution "RSSPMC for surgery named after academician V.Vakhidov" and RSH Zangiota-1 of the Ministry of Health of the Republic of Uzbekistan.

Peripheral blood erythrocytes were studied. To assess the morphofunctional changes in peripheral blood erythrocytes, the express method of the thick drop" (EMTD) was used, which was developed to study discrete structures. This technique is patented in the Patent Office of the Republic of Uzbekistan "Method for determining the forms of erythrocytes" No. MKI 6A61V 10/00, and the program "Express diagnostics of forms of erythrocytes" No. ED-5-05 is also patented. We used the classification of the surface architectonics of erythrocytes, according to Kozinets (1987).

EMTD allows you to save the natural state of the erythrocyte and evaluate the ratio of normal and pathological forms of erythrocytes within 10-15 minutes. Based on this, it is possible to monitor the state of erythrocytes and evaluate the effectiveness of the measures taken.

Blood sampling was carried out from the finger of the patients before the start of intensive therapy. The ratio of erythrocyte shapes was calculated at a magnification of 10×40 , using a microscope Axioskop 40 - ZEISS

Light-optical micrographs were obtained using a digital camera ProGres CT3 (JENOPTIK) coupled with a microscope and a computer, with further data saving on a Pentium - IV computer using application programs. Statistical data processing was carried out on a Pentium-IV computer using the BS-STATISTICA program, Excel Office, Microsoft-Windows-Professional.

In the method of studying the forms of erythrocytes using light electron microscopy (LEM), peripheral blood erythrocytes obtained from the pad of the middle finger are fixed in 2.5% glutaraldehyde in phosphate buffer (pH-7.4). After dehydration in solutions of alcohol - acetone of increasing concentration, they were dried by passing through the critical point of nitrous oxide in the HCP-2 apparatus (Hitachi). The samples were mounted on aluminum substrates with electrically conductive glue. After ion sputtering with gold in an IB-3 apparatus (Eiko, Japan), the cells were viewed and photographed in a Hitachi S-40SA LEM.

RESULTS.

With infection, progression of immuno-inflammatory processes and the COVID-19 clinic, the proportion of discocytes and pathological forms of erythrocytes will increase towards an increase in the proportion of pathological forms, mainly due to erythrocytes with a comb (Fig. 1 and 2). Also, it was noted that the ratio of normal and pathological forms is directly proportional to the timing of the appearance of the first symptoms of the disease and the degree of respiratory failure. It is noted that the pathological forms of erythrocytes acquire an elongated shape.

The dominant type of erythrocytes in group 1 (patients with COVID-19 and cardiovascular pathology) were discocytes, which averaged $77.3 \pm 1.7\%$ (from 54 to 93%), which was statistically significantly less ($p=0.003$) than in group 2 (patients with COVID-19 without cardiovascular pathology) – $84.1 \pm 1.54\%$ (from 72% to 93%) and less ($p<0.001$) than in group 3 (patients with cardiovascular pathology without COVID-19) – $88.4 \pm 1.4\%$ (from 81% to 96%). Pathological forms of erythrocytes in patients of group 3 averaged 8-12%, echinocytes were $2.7 \pm 0.2\%$, stomatocytes $3.3 \pm 0.2\%$, erythrocytes with a comb - $4.4 \pm 0.3\%$, irreversible forms - $1.2 \pm 0.1\%$.

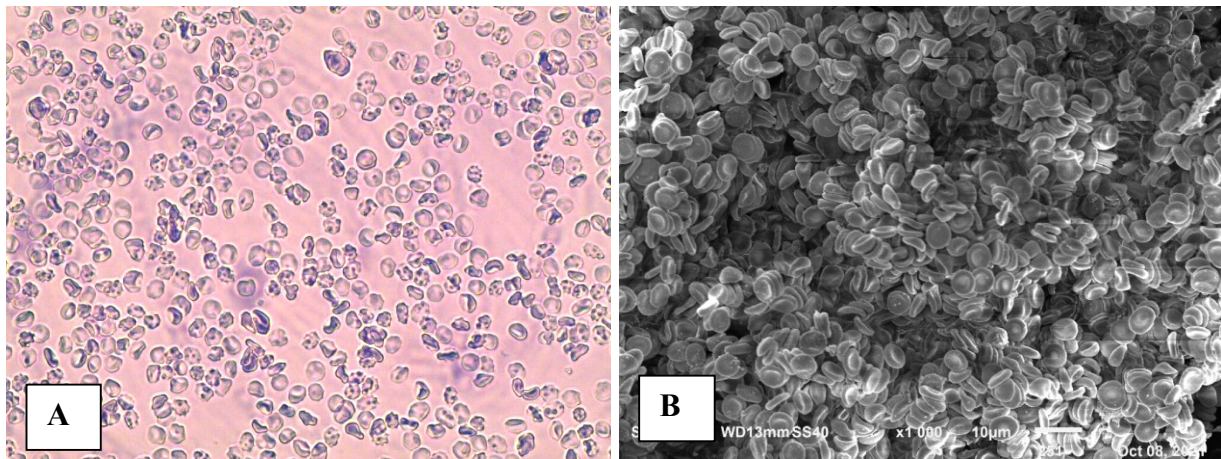


Figure 1. A significant shift in the ratio of normal and pathological forms of erythrocytes. Sludge of normal and pathological erythrocytes. A 64-year-old man with severe COVID-19. Hemoglobin 97 g/l, the width of the distribution of erythrocytes - 15.9%. EMTD, 10×40 and LEM×1000

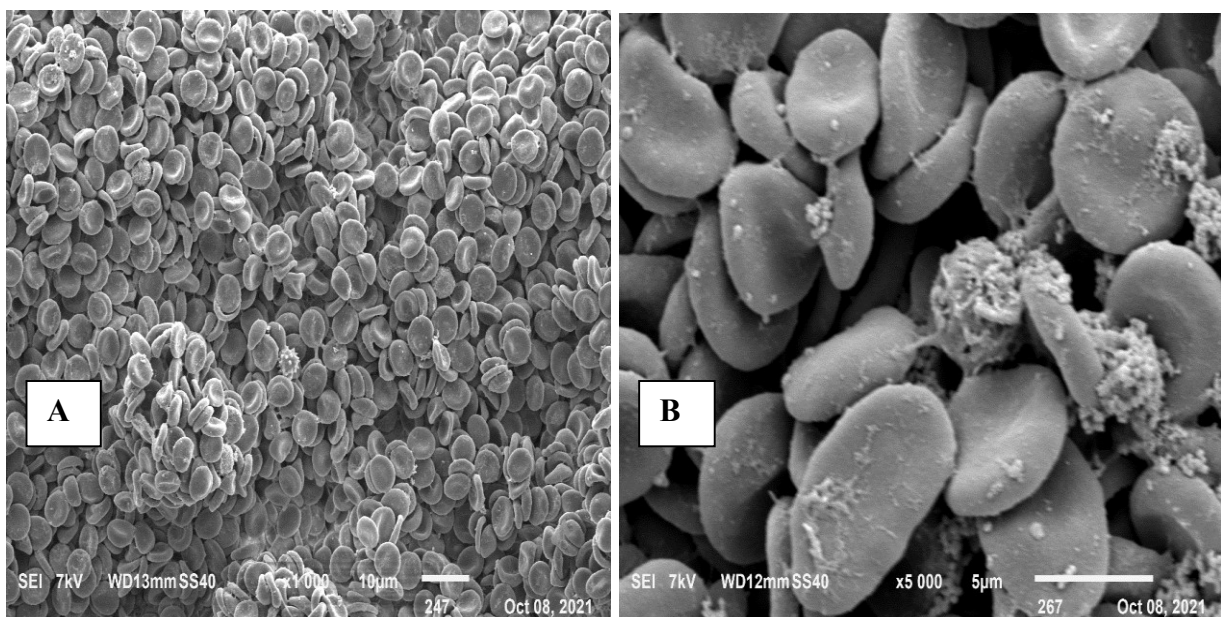


Figure 2. Blood erythrocytes of a 48-year-old patient infected with COVID-19, moderate course, accompanied by coronary heart disease and hypertension. Sludge is noted. Dominance of pathological forms of erythrocytes in peripheral blood. The distribution width of erythrocytes is 17.2%. A) LEM ×1000; B) Various superficial protein formations are visible. LEM ×5000

Patients of the COVID-19 group without any pathology from the cardiovascular system have pronounced shifts in the proportion of discocytes and pathological forms of erythrocytes. There are more echinocytes, increasing up to $3.4 \pm 0.3\%$, stomatocytes up to $4.5 \pm 0.4\%$, erythrocytes

with a crest $6.4 \pm 0.5\%$, as well as irreversible forms up to $1.6 \pm 0.1\%$. The proportion of normal erythrocytes decreases by almost 20% (Table 2).

Table 2.

The ratio of discocytes and pathological forms of erythrocytes

	Group 1 (n=56)	Group 2 (n=44)	Group 3 (n=42)	<i>p</i>
Discocytes	77,3±1,6%	84,1±1,5%	88,4±1,4%	*0.003 **<0.001
Echinocytes	5,2±0,4%	3,4±0,3%	2,7±0,2%	*<0.001 **<0.001
Stomatocytes	6,2±0,5%	4,5±0,4%	3,3±0,2%	*<0.001 **<0.001
RBCs with a comb	8,2±0,6%	6,4±0,5%	4,4±0,3%	*<0.001 **<0.001
Irreversible forms	3,1±0,3%	1,6±0,1%	1,2±0,1%	*<0.001 **<0.001

Note: Data are presented as mean ($M \pm m$, %). Statistical significance was set at $p < 0.05$ (* between groups 1 and 2, ** between groups 1 and 3).

Another feature of the combination of COVID-19 with CVD is the increase in cases with a higher proportion of abnormal RBC (Fig. 3).

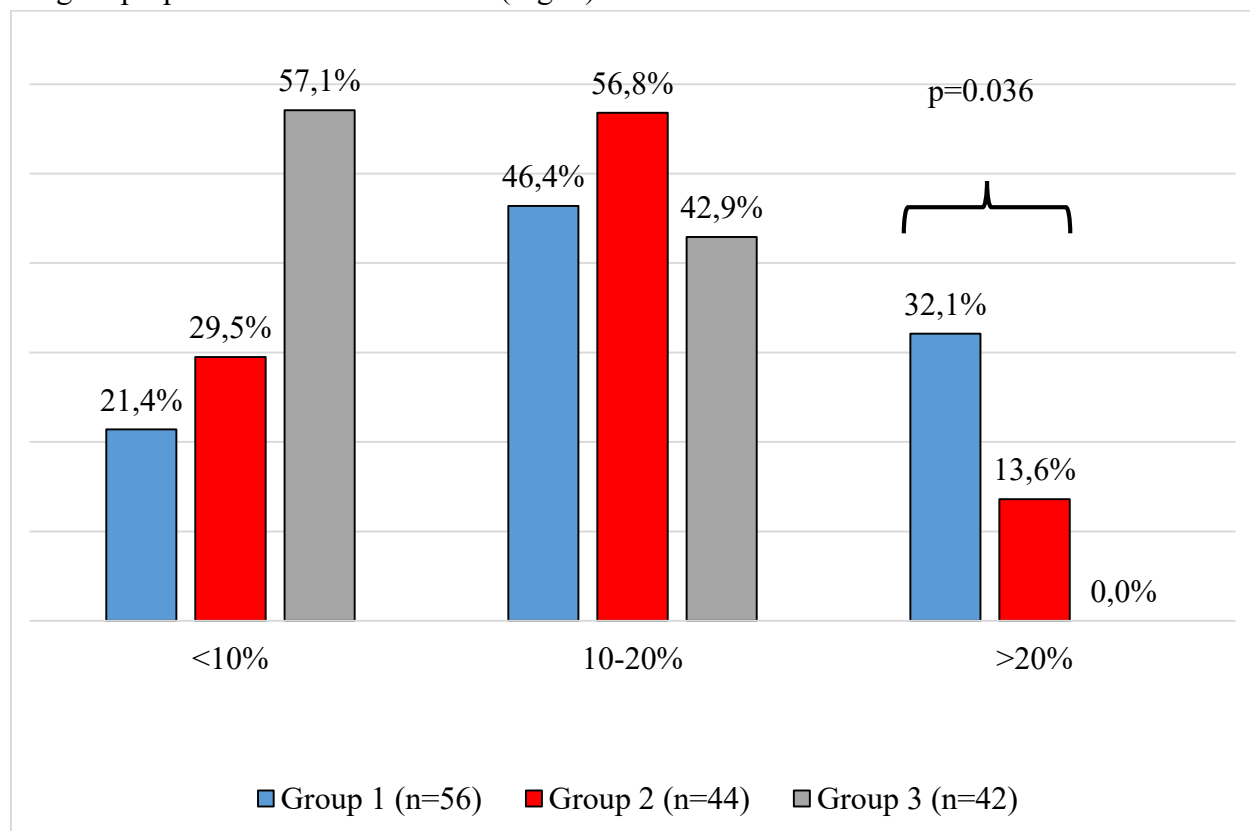


Figure 3. Distribution of patients in study groups depending on the percentage of pathological forms (anomalies) of erythrocytes in peripheral blood detected

Moreover, changes are noted both in the blood of patients with a mild course of the disease, and to an even greater extent in patients with a serious condition requiring respiratory support. This leads to the fact that the proportion of pathological forms of erythrocytes reaches 40-45% of

all RBC. The proportion of stomatocytes and erythrocytes with a comb increases especially significantly and exceeds the normal values by more than 3 times.

Table 3.
Description of comorbidities and the presence of RBC abnormalities on a peripheral blood smear

Considered factor	Pathological morphology of erythrocytes			<i>p</i>
	<10% (n=12)	10-20% (n=26)	> 20% (n=18)	
Chronic kidney disease	3 (25,0%)	4 (11,5%)	6 (33,3%)	*0.626 **0.303
Chronic liver disease	1 (8,33%)	1 (3,8%)	2 (11,1%)	*0.710 **0.741
Anemia	0 (0,0%)	1 (3,8%)	2 (11,1%)	*0.655 **0.741
Arterial hypertension 2-3 degree	4 (33,3%)	11 (42,3%)	13 (72,2%)	* 0.036 ** 0.048
Atrial fibrillation	1 (8,33%)	4 (15,9%)	8 (44,4%)	* 0.025 ** 0.034
NYHA class IV	3 (25,0%)	8 (30,8%)	13 (72,2%)	* 0.031 ** 0.017
Diabetes mellitus	3 (25,0%)	4 (11,5%)	6 (33,3%)	*0.626 **0.303
Chronic obstructive pulmonary disease	4 (33,3%)	4 (11,5%)	6 (33,3%)	*0.626 **0.303
Anamnesis of stroke	1 (8,33%)	1 (3,8%)	2 (11,1%)	*0.710 **0.741
1 or more comorbid conditions	4 (33,3%)	4 (11,5%)	6 (33,3%)	*0.693 **0.303

Note: Data are presented as n (%). Statistical significance was set at $p < 0.05$ (* - between groups >20% and <10%, ** - between groups >20% and 10-20%).

Also, it should be noted that patients with more than 20% erythrocyte abnormalities had more persistent lymphopenia and thrombocytopenia compared to patients without anomalies or less than 20% erythrocyte abnormalities ($p=0.012$ and $p=0.02$, respectively), which confirms a possible association with an overall greater resistant immuno-inflammatory "stress" hematopoiesis.

Subsequent analysis showed different levels of COVID-19 progression in patients with CVS comorbidities, with the highest rate in cases with more frequent erythrocyte morphological changes compared to patients with less than 20% or no abnormalities. Thus, our results point to the importance of research into changes in erythropoiesis in the pathophysiology of COVID-19.

A more thorough analysis of the results of the morphology and morphometry of erythrocytes in patients with COVID-19 associated with cardiovascular syndrome (group 1) with the study of anamnestic and clinical characteristics, it was found that in cases with more than 20% erythrocyte abnormalities (18 out of 56) it was statistically significant 2-3 degree AH, atrial fibrillation, and NYHA class IV were more common, and in terms of the incidence of CKD,

chronic liver disease, kidney disease, and anemia, as well as diabetes mellitus, COPD, and anamnesis of stroke, patients with varying degrees of erythrocyte anomalies were comparable, indicating a significant effect violations of the activity of the CVS in COVID-19 on the morphofunctional state of erythrocytes.

Table 4.

Rate of admission to the ICU or death during hospital stay. Data are shown for the entire cohort and grouped according to the presence of RBC abnormalities

	Total (n=56)	Pathological morphology of erythrocytes			p
		<10% (n=12)	10-20% (n=26)	> 20% (n=18)	
Transferred to ICU	12 (21,4%)	1 (8,33%)	2 (7,7%)	9 (50,0%)	* 0.049 ** 0.005
Transferred to the ICU with at least one risk factor	6 (10,7%)	0 (0,0%)	1 (3,8%)	5 (27,8%)	*NaN **0.068
Lethal outcomes	4 (7,1%)	0 (0,0%)	1 (3,8%)	3 (16,7%)	*NaN **0.357
Deaths with at least one risk factor	4 (7,1%)	0 (0,0%)	1 (3,8%)	3 (16,7%)	*NaN **0.357

Note: Data are presented as n (%). Were considered risk factors from the table. 1, which can potentially affect erythrocyte morphology. Statistical significance was set at $p < 0.05$ (* - between groups >20% and <10%, ** - between groups >20% and 10-20%).

Patients with more than 20% RBC abnormality had a statistically significant higher ICU admission rate (50.0%, 9 of 18; RR=6.0) than patients with less than 10% RBC abnormality (8.33%, 1 of 12, $p = 0.049$), and patients with 10-20% abnormal RBCs (7.7%, 2 of 26, $p = 0.005$). The proportion of patients with at least one risk factor did not statistically differ between the studied patients both in terms of the frequency of transfers to the ICU and in terms of hospital mortality (Table 4).

CONCLUSION

In cases of COVID-19 and cardiovascular pathology, there is a significant morphological heterogeneity in the shape and size of erythrocytes and their relationship with the progression and outcome of the disease. Also, a close relationship is determined between the ratio of normal and pathological forms of erythrocytes and the timing of the onset of the first symptoms of the disease, the degree of respiratory failure, the progression of immuno-inflammatory processes and the COVID-19 clinic.

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