Kamalov A. A.[1,2], Mareev V. Yu.[1,2], Orlova Ya. A.[1,2], Plisyk A. G.[1,2], Akopyan Z. A.[1,2], Mareev Yu. V.[1,3,4], Mershina E. A.[1,2], Begrambekova Yu. L.[1,2], Pakhomov P. V.[2]

[1] Medical Research and Educational Center of the M. V. Lomonosov Moscow State University, Moscow, Russia

[2] Faculty of Fundamental Medicine, Lomonosov Moscow State University, Russia

[3] National Medical Research Centre for Therapy and Preventive Medicine, Moscow, Russia

[4] Robertson Centre for Biostatistics, Glasgow, Great Britain

Hydroxychloroquine in patients with novel coronavirus infection (COVID-19): a case-control study

Actuality

One of the most widely discussed treatments for patients with COVID-19, especially at the beginning of the epidemy, was the use of the antimalarial drug hydroxychloroquine (HCQ). The first small non-randomized trials showed the ability of HCQ and its combination with azithromycin to accelerate the elimination of the virus and ease the acute phase of the disease. Later, large, randomized trials did not confirm it (RECOVERY, SOLIDARITY). This study is a case-control study in which we compared patients who received and did not receive HCQ.

Material and Methods

103 patients (25 in the HCQ treatment group and 78 in the control group) with confirmed COVID-19 (SARS-CoV-2 virus RNA was detected in 26 of 73 in the control group (35.6%) and in 10 of 25 (40%) in the HCQ group) and in the rest – a typical picture of viral pneumonia on multislice computed tomography [MSCT]) were included in the analysis. The severity of lung damage was limited to stages I–II, the CRP level should not exceed 60 mg/dL, and oxygen saturation in the air within 92–98%. We planned to analysis the duration of treatment of patients in the hospital, the days until the normalization of body temperature, the number of points according to the original SHOCS-COVID integral scale, and changes in its components (C-reactive protein (CRP), D-dimer, and the percentage of lung damage according to MSCT).

Results

Analysis for the whole group revealed a statistically significant increase in the time to normalization of body temperature from 4 to 7 days (by 3 days, $p<0.001$), and the duration of hospitalization from 9.4 to 11.8 days (by 2.4 days, $p=0.002$) when using HCQ in comparison with control. Given the incomplete balance of the groups, the main analysis included 46 patients who were matched by propensity score matching. The trend towards similar dynamics continued. HCQ treatment slowed down the time to normalization of body temperature by 1.8 days ($p=0.074$) and lengthened the hospitalization time by 2.1 days ($p=0.042$). The decrease in scores on the SHOCS -COVID scale was statistically significant in both groups, and there were no differences between them (delta – 3.00 (2.90) in the HCQ group and – 2.69 (1.55) in control, $p=0.718$). At the same time, in the control group, the CRP level returned to normal (4.06 mg/dl), and with the use of GC, it decreased but remained above the norm (6.21 mg/dl, $p=0.05$). Side effects requiring discontinuation of treatment were reported in 3 patients in the HCQ group and none in the control group.

Conclusion

We have not identified any positive properties of HCQ and its ability to influence the severity of COVID-19. This antimalarial agent slows down the normalization of the body's inflammatory response and lengthens the time spent in the hospital. HCQ should not be used in the treatment of COVID-19.

Keywords Hydroxychloroquine; SHOCS COVID; COVID-19

For citation Kamalov A. A., Mareev V. Yu., Orlova Ya. A., Plisyk A. G., Akopyan Z. A., Mareev Yu. V. et al. Hydroxychloroquine in patients with novel coronavirus infection (COVID-19): a case-control study. Kardiologiia. 2021;61(2):28–39. [Russian: Камалов А. А., Мареев В. Ю., Орлова Я. А., Плисюк А. Г., Акопян Ж. А., Мареев Ю. В. и др. Гидроксихлорохин у больных с новой коронавирусной инфекцией (COVID-19): исследование по принципу случай-контроль. Кардиология. 2021;61(2):28–39]

Corresponding author Begrambekova Yu. L. E-mail: julia.begrambekova@ossn.ru

The novel coronavirus disease (COVID-19) pandemic provoked a wide variety of reactions, especially during the first outbreak in China and later in Southern Europe, when everyone, including healthcare professionals, was ready to clutch at any straw to achieve relative stabilization during the summer of 2020. However, the subsequent sharp worldwide rise in morbidity including the Russian Federation during the autumn of 2020 demonstrated a lack of effective treatments for COVID-19.

The analysis of worldwide mortality from COVID-19 shows a decline from 6.7% and 7.8% in March and April 2020, respectively, to 1.9% and 1.5% in September and October 2020, respectively. How can

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

this be explained? One possible explanation is that the disease became milder. However, since mortality in the Russian Federation rose from 1.0% and 1.2% in April and May, respectively, to 1.9% and 1.6% in September and October, respectively [1], this assumption is highly questionable. Another possible explanation is that COVID-19 treatments became more effective. Here, there are two complementary theories: one points to the use of more effective drugs, while the other also considers the abandoning of therapies that had failed to prove their efficacy and, in some cases, involved detrimental side effects.

It is undeniable that drug-based treatments with higher efficacy were developed during this period. In the Russian Federation, the use of anticoagulants became practically obligatory as early as in the spring of 2020, from the very beginning of the pandemic, and later, glucocorticoids were rehabilitated (the teaching hospital of the Moscow State University (MSU) published successful results of the WAYFARER trial in June).

However, it is also important to be able to critically evaluate ineffective treatments for COVID-19 in order to justify a timely refusal to use them. Here, particular attention should be paid to antimalarial agents such as hydroxychloroquine (HCQ), the mechanism of whose action in COVID-19 is not fully understood. While HCQ is generally defined as an anti-inflammatory agent [2, 3], more than a dozen distinct potential mechanisms of action have been described for HCQ and its metabolites in viral infection [4, 5]. The main mechanism of action consists in the ability to increase endosomal pH, provide the angiotensin-converting enzyme 2 (ACE2) receptor glycosylation and slow down the penetration of SARS-CoV-2 into cells [6, 7]. An additional potential benefit of this drug comprises its blockage of CD-145 expression and production of pro-inflammatory cytokines, which can prevent the development of cytokine storm [8].

The use of HCQ to block the virus from entering cells demonstrated in experimental in vitro studies is of interest, given that it is more effective for this purpose than remdesivir, the antiviral drug most commonly used in the US [9]. The WHO-sponsored randomized clinical trial SOLIDARITY carried out in 2020 found no evidence that remdesivir could be used to reduce mortality in COVID-19 patients [10]. Conversely, the effective use of HCQ to block the entry of SARS-CoV – a close relative of the novel coronavirus – into cells has been demonstrated experimentally [11, 12]. An initial study into the use of HCQ for providing protection from COVID-19 was carried out in Moscow. Subsequent interest in the use of antimalarial drugs to treat COVID-19 led to a small (n=42), open-label, single-arm French study published in early March 2020 demonstrated that HCQ and its combination with azithromycin accelerated the elimination of SARS-CoV-2 (PCR) [13]. This first positive news on the possibilities of COVID-19 treatment had a bombshell effect. Simultaneously, the first randomized controlled Chinese trial (n=63) also observed a statistically significant improvement in patients' clinical status during the use of HCQ [14]. Although the soft endpoint, its subjectivity, and the borderline (p=0.0476) significance of differences from the control group were widely ignored, these two studies provided hope and justified the inclusion of HCQ in most interim COVID-19 treatment guidelines. Meanwhile, unfounded comments by US President's irrational comment contributed to a more than 80-fold increase in sales of remdesivir, while global sales of HCQ increased by a mere 2-fold [15].

Following the conversion of the MSU teaching hospital to a COVID-19 care center, HCQ was used as a routine therapy of COVID-19 from the beginning (in accordance with the Interim Guidelines of the Russian Ministry of Health "Prevention, Diagnosis and Treatment of the Novel Coronavirus Disease (COVID-19)" dated 26/10/2020) [16]. However, since no positive effect was observed, we started to withdraw this treatment. Therefore, the objective of the present study was to present the results of using hydroxychloroquine in 25 patients as compared to other treatments taking a case-control approach. We analyzed the duration of hospital stay relative to body temperature normalization, changes of the SHOCS-COVID score (original Symptomatic Hospital and Outpatient Clinical score for COVID-19) [17] and its components (C-reactive protein (CRP), D-dimer, as well as lung injury percentage on computed tomography (CT)). The background therapy was performed following the protocols of the MSU Medical Research and Educational Center.

Material and methods

The inclusion criteria were confirmed novel coronavirus disease (positive PCR for SARS-CoV-2 RNA: 26/73 (35.6%) patients in the control group and 10/25 (40%) patients in the HCQ group) or a typical picture of viral pneumonia on multispiral CT in all the others. The severity of the lung injury was limited to grade I-II, CRP not more than 60 mg/dL, and oxygen blood saturation in room air within 92–98%.

The characteristics of all 103 included patients (25 patients in the HCQ treatment group and 78 patients in the control group) are presented in Table 1a (available

in the Additional Materials section on the Journal's website). Given that 5 of the 27 baseline indicators used for the comparison were not balanced, we performed an additional analysis to exclude differences in the comparison groups (propensity score matching). The results of group comparison following this analysis are shown in Table 1.

As can be seen from Table 1, the groups were balanced. Most patients had a low-grade fever, moderately reduced oxygen blood saturation; one patient in each group required oxygen support due to having clinically significant dyspnea. CRP levels were significantly higher than normal (8-fold in the HCQ group and 6-fold in the control group). The marker of the increased risk for thrombotic complications (D-dimer) was also elevated in both patient groups. The percentage of lung injury on MSCT corresponded in the mean to grade I (according

Table 1. Baseline patient characteristics (propensity match scores)

Parameters and characteristics	Hydroxychloroquine, n=23	Control, n=23	p
General characteristics			
Age, years, mean (SD)	58.7 (13.5)	53.6 (14.4)	0.217
BMI, kg/m^2, median [Q25; Q75]	26.5 [24.5; 31.6]	28.7 [25.9; 32.0]	0.343
Male, n (%)	13 (56.5)	10 (43.5)	0.555
Arterial hypertension, n (%)	10 (43.5)	8 (34.8)	0.763
CAD, n (%)	2 (8.70)	2 (8.70)	0.999
Diabetes mellitus, n (%)	3 (13.0)	2 (8.70)	0.999
CHF, n (%)	3 (13.0)	0 (0.00)	0.243
COPD, bronchial asthma, n (%)	0 (0.00)	1 (4.35)	0.999
Clinical characteristics			
Body temperature, mean (SD)	37.0 (0.93)	36.9 (0.76)	0.594
RR, mean (SD)	19.4 (2.43)	19.4 (2.06)	0.999
HR, bpm, mean (SD)	86.5 (15.2)	90.0 (16.1)	0.449
SBP, mm Hg, median [25%; 75%]	130 [120; 140]	123 [114; 140]	0.285
SaO_2, %, median [25%; 75%]	96.0 [94.5; 98.0]	97.0 [94.0; 98.0]	0.689
Biochemical characteristics			
CRP, mg/dL, median [25%; 75%]	41.5 [12.7; 52.8]	30.5 [12.8; 72.0]	0.991
D-dimer, μg/mL, median [25%; 75%]	0.61 [0.42; 0.96]	0.59 [0.39; 0.96]	0.939
Fibrinogen, g/L, mean (SD)	5.27 (1.01)	5.54 (1.57)	0.499
Lymphocytes, 10^9/L, median [25%; 75%]	1.13 [0.98; 1.38]	1.32 [0.95; 1.62]	0.410
Neutrophils, 10^9/L, median [25%; 75%]	3.18 [2.50; 4.43]	3.69 [2.73; 4.84]	0.277
NLR, median [25%; 75%]	2.57 [1.86; 3.90]	2.65 [1.98; 3.91]	0.606
Platelets, 10^9/L, median [25%; 75%]	198 [174; 250]	221 [196; 250]	0.410
LCR, median [25%; 75%]	27.2 [20.5; 103]	39.3 [16.1; 118]	0.750
Glucose, mmol/L, median [25%; 75%]	5.78 [5.10; 6.47]	5.38 [4.99; 5.97]	0.549
Creatinine, mmol/L, median [25%; 75%]	84.0 [75.5; 104]	81.0 [71.0; 91.0]	0.222
Potassium, mean (SD)	4.10 (0.54)	4.17 (0.44)	0.682
GFR (CKD EPI), mL/min/1.73 m^2, mean (SD)	75.7 (20.7)	82.3 (15.8)	0.231
Lung lesion			
CT lesion (%), median [25%; 75%]	18.6 [5.95; 35.3]	11.5 [4.50; 24.8]	0.219
CT grade, median [25%; 75%]	1.00 [1.00; 2.00]	1.00 [1.00; 1.50]	0.245
Total severity score			
NEWS-2, score, median [25%; 75%]	3.00 [1.00; 4.00]	2.00 [1.50; 4.00]	0.770
SHOCS-COVID, score, mean (SD)	5.26 (2.18)	4.78 (1.88)	0.430
Treatment, n (%)			
Hydroxychloroquine	23 (100)	0 (0)	<0.001
Bromhexine/spironolactone	2 (8.70)	2 (8.70)	0.999
Colchicine or glucocorticoids	1 (4.35)	1 (4.35)	0.999
Paracetamol/Diclofenac	9 (39.1)	12 (52.2)	0.554
Antibiotics	23 (100)	22 (95.7)	0.999

SD – standard deviation; BMI – body mass index; CAD – coronary artery disease; CHF – chronic heart failure; COPD – chronic obstructive pulmonary disease; RR – respiratory rate; HR – heart rate; SBP – systolic blood pressure; SaO_2 – oxygen blood saturation; CRP – C-reactive protein; NLR – neutrophil-to-lymphocyte ratio; LCR – lymphocyte-to-C-reactive protein ratio; t – body temperature; GFR – glomerular filtration rate; CT – computed tomography.

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

to the guideline of the Russian Ministry of Health). The total risk of clinical manifestations (NEWS-2 score) and total risk (SHOCS-COVID score) were moderate. Concomitant therapy did not differ between the groups; almost all patients received antibiotics and preventive doses of anticoagulants.

Methods of examination

Laboratory tests including blood biochemical profile (CRP, creatinine, urea, glucose) were performed on a AU480 (Beckman Coulter, Germany) automatic biochemical analyzer; complete blood count on a XN 2000 hematological analyzer (Sysmex Corporation, Japan); hemostasis analysis (fibrinogen, D-dimer) on an SAS STA-Compact automatic hemostasis analyzer (Diagnostica Stago, France); interleukin-6 (IL-6) levels were measured on a Cobas 6000 immunochemistry analyzer (Roche Diagnostics GmbH, Germany).

Lung and chest computed tomography (CT) was performed using a 32 slice Somatom Scope scanner (Siemens, Germany). A detailed description of the CT scan procedure in COVID-19 patients in our center was published in an earlier work [18]. We used two scores to objectively determine the severity of the clinical condition and evaluate the effects of the therapy adequately: National Early Warning Score (NEWS-2) of the severity of acute respiratory distress syndrome [19] modified for patients with COVID-19 [20] and our original Symptomatic Hospital and Outpatient Clinical score for COVID-19 (SHOCS-COVID) [21].

Statistical analysis

The normality of distribution was evaluated using the Shapiro-Wilk test. The quantitative data were expressed as the median and interquartile range (25%; 75%) in the case of the non-parametric distribution and as the mean and standard deviation if the distribution was normal. The qualitative indicators were compared between the groups using the Mann-Whitney test in the non-parametric distribution and Student's t-test in the normal distribution. The qualitative data are presented as absolute and relative values. The significance of intergroup differences in qualitative characteristics was assessed using the $\chi 2$ test and two-tailed Fisher's exact test.

Changes of the parameters within each group were compared with the Wilcoxon signed-rank test in the non-parametric distribution and with the Student's t-test for dependent samples in the normal distribution.

Nearest neighbor matching was used for the propensity match score. The selection was based on the following baseline parameters: levels of CRP,

Figure 1. Group-wide number of days in hospital and days with fever in the hydroxychloroquine group compared to the control group

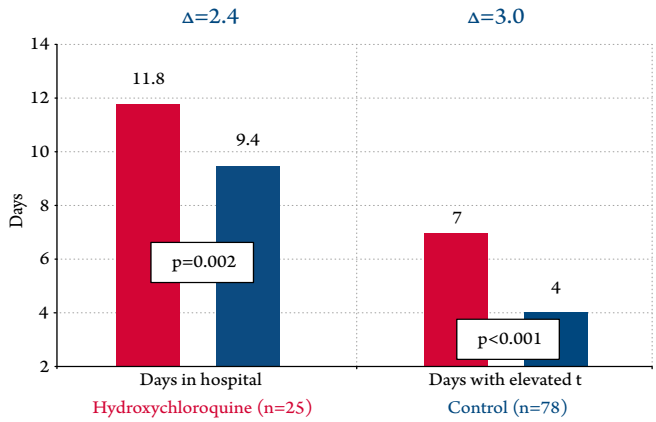
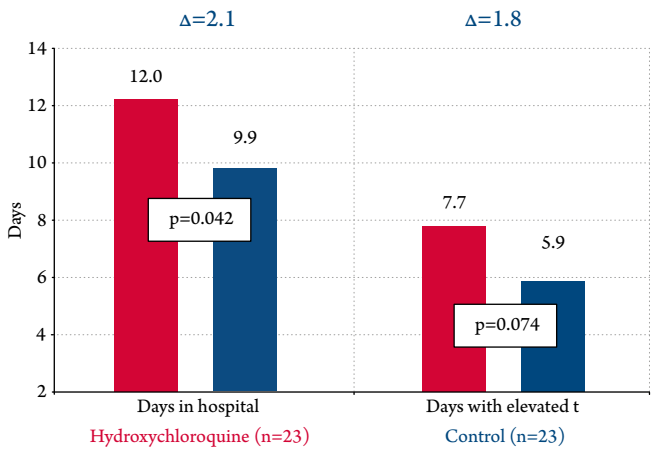


Figure 2. Number of days in hospital and days with fever in the hydroxychloroquine group compared to the control group the propensity score-matched groups (n = 46)



D-dimer, CT injury %, the use of bromhexine and/or spironolactone, SHOCS-COVID score, age and heart rate.

The significance threshold for the statistical hypotheses was 0.05. Statistical analysis was performed using the R programming language in R Studio.

Results

The group-wide analysis showed that statistically significant increases in time to body temperature normalization from 4 to 7 days (by 3 days, p<0.001), and the duration of hospital stays from 9.4 to 11.8 days (by 2.4 days, p=0.002), when HCQ was used to treat COVID-19, as shown in Figure 1.

A similar analysis was performed in the matched patient groups (propensity score matching), as shown in Figure 2. As can be seen, the trend of similar changes continued. The HCQ therapy increased time to body temperature normalization by 1.8 days (p=0.074) and

duration of the hospital stay by 2.1 days (p=0.042). Other indicators were analyzed later during the treatment (Table 2).

Although there were no differences in the cumulative SHOCS-COVID score decreases during treatment, the trend for these indicators to remain higher continued in the HCQ group by the end of the follow-up period (p=0.082) continued (Table 2 and Figure 3). Most of the indicators, mainly of the clinical condition severity and included in this scale, showed significant improvement in both groups. Dyspnea (RR) decreased, while oxygen blood saturation (SaO2) increased by equal amounts. The heart rate (HR) trend was unexpected. Given the mechanism of action,

Table 2. Changes in the main parameters during treatment (propensity scores matching)

Parameters	Hydroxychloroquine, n = 23		Control, n = 23		p (intergroup differences)
	Baseline	Treatment	Baseline	Treatment	Before/after
SHOCS-COVID, score, mean (SD)	5.26 (2.18)	2.75 (1.69)	4.78 (1.88)	2.00 (1.35)	0.430/0.196
Δ treatment – baseline	-3.00 (2.90); p<0.001		-2.69 (1.55); p<0.001		0.718
SHOCS-COVID, score, median [25%; 75%]	5.00 [4.00; 7.00]	2.50 [2.00; 3.00]	5.00 [3.50; 6.00]	2.00 [1.00; 2.00]	0.436/0.082
Δ treatment – baseline	-3.00 [-3.00; -2.00]		-3.50 [-5.25; -1.75]		0.506
RR, brpm, median [25%; 75%]	19.0 [18.0; 20.0]	17.0 [16.0; 17.0]	20.0 [18.0; 20.0]	17.0 [16.0; 17.0]	0.999/0.863
Δ treatment – baseline	-2.00 [-4.00;0.00]; p<0.001		-2.00 [-4.00;-1.00]; p<0.001		0.698
SaO_2, %, median [25%; 75%]	96.0 [94.5; 98.0]	98.0 [97.0; 99.0]	97.0 [94.0; 98.0]	98.0 [97.0; 99.0]	0.689/0.614
Δ treatment – baseline	2.00 [0.00;3.00]; p<0.001		2.00 [0.00;3.00]; 0.012		0.520
HR, bpm, mean (SD)	86.5 (15.2)	75.3 (13.7)	90.0 (16.1)	72.0 (7.83)	0.383 /0.449
Δ treatment – baseline	-11.26 (16.0)		-20.91 (14.4)		0.037
CRP, mg/dL, median [25%; 75%]	41.5 [12.7; 52.8]	6.21 [4.06; 12.5]	30.5. [12.8; 72.0]	4.06 [2.33; 7.03]	0.991/0.050
Δ treatment – baseline	-25.40 [-48.09; -2.80]; p=0.004		-27.06 [-66.27; -10.34]; p<0.001		0.264
D-dimer, μg/mL, median [25%; 75%]	0.61. [0.42; 0.96]	0.54. [0.36; 0.72]	0.59. [0.39; 0.96]	0.34. [0.22; 0.71]	0.939/0.333
Δ treatment – baseline	-0.12 [-0.66;0.18]; p=0.211		-0.04 [-0.26;0.05]; p=0.252		0.937
CT lung injury (%), median [25%; 75%]	18.6 [5.95; 35.3]	14.7 [5.65; 29.8]	11.5 [4.50; 24.8]	9.75 [5.75; 18.7]	0.219/0.386
Δ treatment – baseline	-3.70 [-14.95;0.70]; p=0.101		-4.50 [-10.68;1.48]; p=0.112		0.694
Lymphocytes, 10^9/L, mean (SD)	1.24 (0.45)	1.80 (0.62)	1.33 (0.47)	1.91 (0.70)	0.410/0.603
Δ treatment – baseline	0.56 (0.53); p<0.001		0.64 (0.58); p<0.001		0.634
NLR, median [25%; 75%]	2.10 [1.64; 3.04]	1.31 [0.96; 1.55]	2.52 [1.78; 4.32]	1.43 [1.13; 1.84]	0.606/0.121
Δ treatment – baseline	-0.63 [-1.89; -0.18]; p<0.001		-1.31 [-2.52; -0.89]; p<0.001		0.124
LCR, median [25%; 75%]	27.2 [20.5; 103]	289 [152; 477]	39.3 [16.1; 118]	364 [200; 1150]	0.750/0.084
Δ treatment – baseline	276 [70.0; 369]; p>0.001		341 [186; 963]; p>0.001		0.053
Creatinine, μmol/L, median [25%; 75%]	84.0 [75.5; 104]	83.0 [71.0; 89.0]	81.0 [71.0; 91.0]	70.0 [65.0; 81.0]	0.108/0.104
Δ treatment – baseline	-7.20 (16.9); p=0.103		-12.42 (24.5); p=0.05		0.444
GFR, mL/min/1.73 m^2, median [25%; 75%]	76.0 [64.0; 93.0]	82.0 [79.5; 92.5]	83.0 [74.0; 92.5]	84.0 [79.0; 97.0]	0.231/0.448
Δ treatment – baseline	8.00 [-6.00; 18.0]; p=0.073		6.00 [-5.00; 15.0]; p=0.03		0.957
Glucose, mmol/L, median [25%; 75%]	5.78 [5.10; 6.47]	4.93 [4.76; 5.24]	5.38 [4.99; 5.97]	5.02 [4.45; 5.36]	0.549/0.862
Δ treatment – baseline	-0.70 [-0.99;0.01]; p=0.455		0.58 [-0.96;0.06]; p=0.064		0.817
Potassium, mmol/L, medium (SD)	4.10 (0.54)	4.64 (0.62)	4.17 (0.44)	4.59 (0.53)	0.682/0.760
Δ treatment – baseline	0.67 (0.69); p<0.001		0.42 (0.56); p=0.002		0.229
NEWS-2, score, median [25%; 75%]	3.00 [1.00; 4.00]	1.00 [0.25; 3.00]	2.00 [1.50; 4.00]	1.00 [000; 2.00]	0.652/0.443
Δ treatment – baseline	-1.00 [-3.00;0.00]; p=0.010		-1.00 [-2.00; -0.50]; p=0.003		0.635

RR – respiratory rate; HR – heart rate; CT – computed tomography; NLR – neutrophil-to-lymphocyte ratio; LCR – lymphocyte-to-C-reactive protein ratio; GFL – glomerular filtration rate; SD – standard deviation; SaO_2 – oxygen blood saturation; CRP – C-reactive protein.

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

bradycardia should have been expected during the use of HCQ, which would require regular QT interval monitoring. However, relatively more significant decrease in HR in the control group may be attributed to a more significant overall improvement in the clinical condition. However, the cumulative improvement in clinical status (NEWS-2 score, which includes HR) was statistically significant in both patient groups.

The trend of higher SHOCS-COVID scores in the HCQ group may have been associated to a certain extent with less significant decreases in the indicators of systemic inflammation in COVID-19 patients (Table 2 and Figure 4).

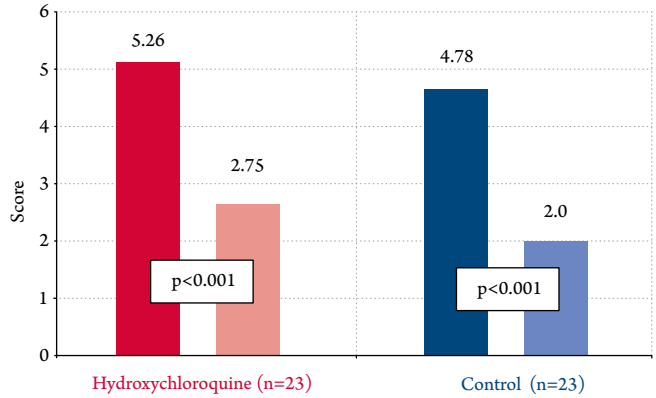
The decrease in CRP and increase in the lymphocyte to CRP ratio (LCR) were statistically significant in both groups. However, CRP normalized (4.06 mg/dL) in the control group, however, decreased in the HCQ group but remained normal (6.21 mg/dL, p=0.05). There was also a clear trend to a more significant increase in another marker that characterizes inflammatory status, the lymphocytes to CRP ratio in the control group (p=0.053), lower values were achieved in the HCQ group. Nevertheless, there were no statistically significant differences (p=0.084).

There were no significant differences in the D-dimer changes, which normalized in the control group (0.34 μg/mL) and remained above normal in the HCQ group (0.54 μg/mL). Although a decrease in CT lung injury was observed in both groups, these changes were not statistically significant, probably due to the short monitoring period.

Also of interest were the differences in creatinine changes, which decreased statistically significantly in the control group although remaining within the normal values. At the same time, the calculated glomerular filtration rate changed significantly only in the control group. However, it is worth noting that these indicators did not deteriorate in the HCQ group.

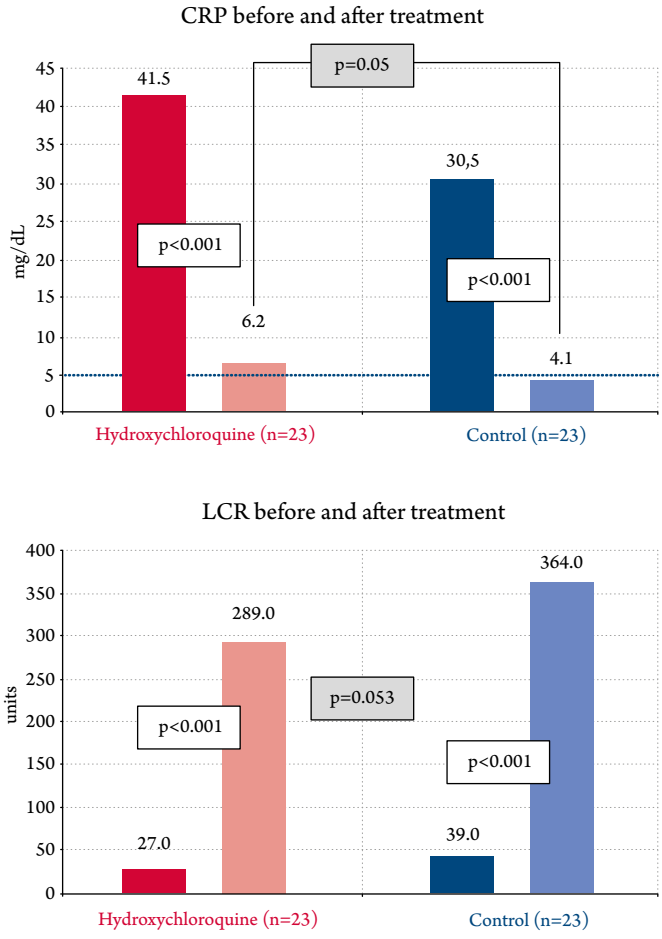
Three (13%) patients who received HCQ experienced serious side effects that required the treatment to be discontinued. In two cases, a prolongation of the corrected QT interval and development of heart rhythm disorders were observed. In another case, gastrointestinal disorders, such as nausea, vomiting and persistent diarrhea were experienced. These phenomena disappeared when HCQ was withdrawn. In the control group, there were no serious adverse events and treatment was not discontinued.

Figure 3. Changes in the SHOCS-COVID (modification by V.Yu. Mareev) in the hydroxychloroquine and control groups (means and SD)*



* The medians are provided in Table 2.

Figure 4. Changes in the inflammation markers: CRP and LCR in the hydroxychloroquine and control groups



Discussion

HCQ was commonly used to treat COVID-19 in the first months of the pandemic. Early uncontrolled studies, such as the one carried out in France, showed that the drug is able to accelerate virus elimination [13]. While the almost simultaneous published results of an observational study conducted in 4 hospitals did not show prognosis improvement for COVID-19

patients receiving HCQ, there was a trend of decreasing mortality in moderate-risk patients along with an increased risk of acute respiratory distress syndrome [22]. The first small but randomized trial conducted in China showed a marginal improvement in the clinical condition during the use of HCQ [14]. In contrast to a powerful wave of optimistic assessments, the works of those who called for caution about HCQ remained in a minority were very little cited in non-medical periodicals. However, a risk associated with increased doses of the drug [23] was reported, as well as the risk of QT interval prolongation in almost every fifth patient, especially in the combined use of HCQ and azithromycin [24]. At the time of its conversion to a COVID-19 care center, HCQ and its combination with azithromycin was the standard treatment of COVID-19 in the MSU teaching hospital. Our work was arranged to include daily multidisciplinary case conferences involving the MSU Medical Research and Educational Center experts, which allowed a rapid response to the treatment results. Therefore, in the absence of apparent clinical benefits of HCQ, the need for too many tests to avoid QT interval prolongation and cardiac complications, as well as doubts about the presence of positive mechanisms of action of the drug, we abandoned this treatment relatively quickly (by early May) [25]. At approximately the same time, the first results of the relatively large (n=1 438) observational study carried out in New York were published [26]. No positive effects of HCQ on the prognosis were found (relative risk (RR)=1.08; 95% confidence interval (CI): 0.63–1.85). Moreover, there was a clear trend to cardiac arrest, which became statistically significant for the combination of HCQ and azithromycin. Here, it should be noted that the mean hospital stay duration was three days more for COVID-19 patients treated with HCQ [26]. We cannot say that these findings were a wake-up call, although other serious studies showed no effects of HCQ on the risk of death and admission to intensive care units and putting on ventilation during the treatment of COVID-19 [27]. It was only when the results of a large observational multinational study that demonstrated a double increase in mortality associated with the use of chloroquine were published in the Lancet that serious doubts were raised about HCQ and its combinations with macrolides [28, 29]. The WHO even stopped the enrollment of patients receiving HCQ in the controlled SOLIDARITY trial [30]. However, following the withdrawal of "mischief-making" article eight days later due to the unreliability of evidence, there was a calm period before the results of the randomized trials were obtained.

The first of these was the RECOVERY protocol, which included more than 4,600 patients (more than 1,500 receiving HCQ). HCQ was used at the dose of 1600 mg on day 1 and 400 mg twice daily on the remaining 10 days [28]. No positive effects of HCQ on the prognosis for patients or adverse effects including death or the need for ventilation by day 28 of treatment were demonstrated in patients who did not require ventilation (RR=1.14; 95% CI 1.03–1.27). In this case, patients treated with HCQ stayed in hospital for three days longer than the control patients.

Two more randomized controlled trials (RCTs) showed virtually the same results. In the Brazilian trial, the therapeutic dose of HCQ was 800 mg/day for 7 days. There were no statistically significant changes in mortality; the duration of hospital stays increased by one day though insignificantly in the HCQ group [31].

In the Spanish trial, the use of HCQ in patients with mild to moderate COVID-19 was neither associated with faster elimination of the virus nor a significant decrease in days in hospital and deaths [32].

Finally, the WHO-sponsored SOLIDARITY study confirmed that there were no significant effects of HCQ (1600 mg on day one and 800 mg/day for 10 days) on the prognosis for COVID-19 patients, those admitted to intensive care units and given ventilation. There was also a trend of an increasing rate of adverse outcomes (RR=1.26, 95% CI: 0.76–2.10). A more significant percentage of patients treated with HCQ stayed in hospital by 7, 14, and even 21 days, while the mean number of days in hospital was higher by 2.2 days [10].

The results of these studies have a clear focus. No actual decrease in mortality and severity of infection is achieved when HCQ is used; moreover, there is a trend of a moderate deterioration of the prognosis. In all studies, HCQ-group patients stayed longer in hospital than the control group. The results of our study are almost precisely the same as those of the large RCTs. The analysis of all included patients showed that they stayed in hospital for 2.4 (p=0.002) days longer or 2.1 (p=0.042) days more following group matching. Moreover, it took longer to achieve body temperature normalization in the HCQ group, which indicates a longer acute phase of the disease. The odds ratio of hospital stay for less than 10 days during the use HCQ was 0.40 [95% CI 0.11–1.46], i.e., it was 2.5 times less compared to the control, although the differences were statistically insignificant (p=0.17). At the same time, the odds ratio for virus elimination and negative PCR for SARS-CoV-2 RNA in the HCQ group was 0.65 [95% CI 0.25–1.68, p=0.37], i.e., it was 1.6 times lower compared to the control. Of course, while this cannot

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

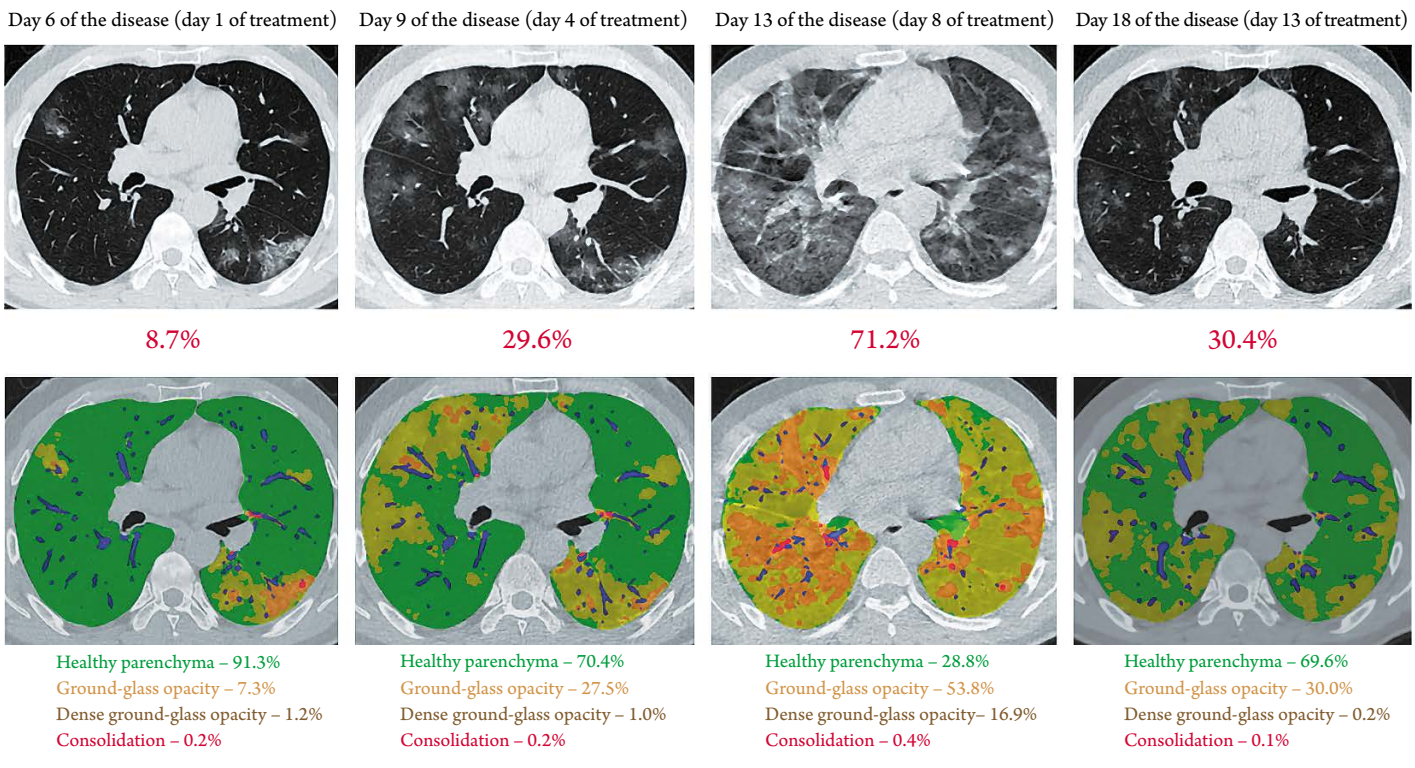
indicate a evident negative effect of HCQ on the course of COVID-19, it definitely excludes the positive effects of this drug in patients with COVID-19.

The natural desire was to analyze why HCQ did not live up to expectations in the treatment of COVID-19. As shown in our study, the cumulative SHOCS-COVID score scale, which reflects the severity of disease, tended to be higher in the HCQ group after treatment compared to the control group (p=0.082). In the control group, the PCR results for SARS-CoV-2 RNA were negative in all patients after 10 days of treatment but remained positive in 21.2% of patients in the HCQ group (p=0.053). The level of CRP, being a cumulative indicator of systemic inflammation, was 6.21 [95% CI 4.06-12.5] mg/dL, i.e., above the upper limit of normal in the HCQ group. However, CRP normalized in the control group – 4.06 [95% CI 2.33;7.03] mg/dL; the intergroup differences were statistically significant (p=0.05). An increase in lymphocytes-to-CRP ratio most accurately reflects the positive changes during the treatment of COVID-19, i.e., a reduction in systemic inflammation. The increase of this indicator was much more significant in the control group (+341 [95% CI: 186–963], p > 0.001) versus HCQ (+276 [95% CI: 70;369], p > 0.001), while the intergroup differences between were on the verge of statistical significance (p=0.053). Once again, this at least demonstrates the absence of a distinct anti-inflammatory effect of HCQ. The better clinical condition of the control patients is indirectly confirmed by a statistically more significant decrease in heart rate (–20.91 (14.4) bpm vs. –11.26 (16.0) bpm (p=0.037). This is important since HCQ, given its mechanism of action, can increase the QT interval. While changes in creatinine levels and calculated glomerular filtration rate were slightly better (and statistically more significant) in the control group, all the indicators remained normal in both groups.

Let us provide a case study to highlight our findings. Patient S., 36 years old, male, without comorbidities, body mass index 25.8 kg/m2, was admitted on day 6 of the disease with complaints of general weakness, fever up to 39.3°C, a hardly productive cough with blood-streaked sputum, headache, one-off vomiting and diarrhea up to 3 times a day. Azithromycin, levofloxacin, and lopinavir/ritonavir had been administered for 2 days prior to hospital admission on the orders of a primary care physician. PCR test for SARS-CoV-2 RNA was negative at admission. There were signs of bilateral multisegmental pneumonia on lung CT (Figure 5), probably of viral origin, mean injury volume 8.7% (grade 1).

At admission, body temperature was 37.8°C, respi ratory rate (RR) – 22 brpm, oxygen blood saturation – 95% in room air. The patient's chest was evenly involved in the act of breathing. Accessory muscles were not

Figure 5. Trends of changes in lung injury on CT in patient S., 36 years old, who received hydroxychloroquine, azithromycin, and low-molecular-weight heparin



involved in breathing. Blood pressure – 126/80 mm Hg; HR – 105 bpm.

The dissonance between the very small volume of pulmonary tissue injury and the pronounced dyspnea is of interest. Some researchers associate this phenomenon with the possible penetration of the virus into the central nervous system and the activation of the central mechanisms of difficulty breathing [33]. At the same time, many symptoms, such as weakness, apathy, headaches, depressive anxiety, are associated with the central mechanisms, i.e., the effects of SARS-CoV-2 virus on various brain regions [34].

Laboratory findings: WBCs – 1.5×10^9/L; neutrophils – 4.53×10^9/L, neutrophil-to-lymphocyte ration (NLR) – 3.0; erythrocyte sedimentation rate (ESR) – 24 mm/h; D-dimer – 0.29 μg/mL; CRP – 11.9 mg/dL; LCP – 126; ferritin – 642 μg/L.

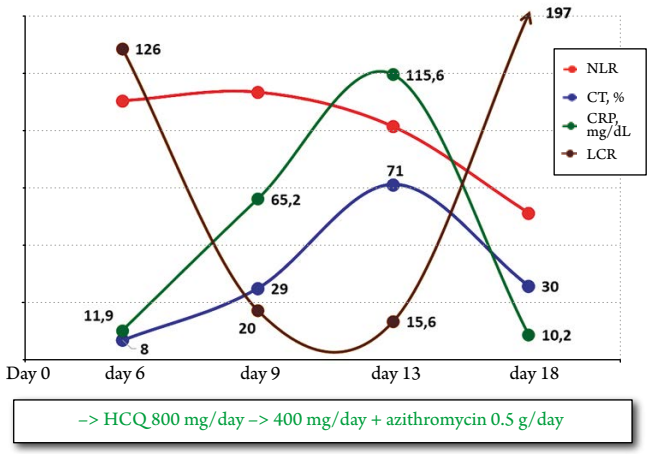
HCQ 400 mg bid was started on day 1 followed be 200 mg bid for up to 2 weeks, azithromycin 0.5 g/day, and enoxaparin 0.4 mg od, subcutaneously.

Three days later (on day 4 of treatment), his body temperature normalized, and dyspnea improved (RR 18 brpm), oxygen saturation was 96%, but weakness and headache remained, and the cough did utterly resolve. The levels of neutrophils, lymphocytes, and ESR were almost unchanged. CRP significantly increased to 65.2 mg/dL. Ferritin remained elevated (655 μg/L), although did not increased compared to the baseline values, which indicated an increase in the body's inflammatory response, yet without excessive activation of the cytokine mechanism. The neutrophil-to-lymphocyte ratio (3.1), which is a marker of the thrombosis risk, did not change significantly. The volume of pulmonary tissue injury increased significantly to 29.6% (grade 2). PCR test for SARS-CoV-2 RNA was positive.

By day 8 of treatment with HCQ, the patient's clinical condition had not significantly improved. CRP was increased to 115 mg/dL, with persistently elevated ferritin of 652 μg/l; ESR was increased to 50 mm/h. That is, despite the use of HCQ, the degree of inflammatory response increased. However, stable ferritin levels provided grounds for hope that no cytokine storm was oncoming. The volume of pulmonary tissue injury on CT significantly increased to 71.2%. RR was 18 brpm, i.e., there was no increase in shortness of breath. Oxygen saturation was 97%, which generated dissonance with constantly increasing lung injury (53.8% ground glass and 16.9% dense ground-glass opacities).

As treatment continued, there was a clear improvement after another 5 days (day 13 of treatment):

Figure 6. Trends of changes in the inflammation markers



CRP (green line), marker of thrombosis risk LCR (brown line), and CT lung injury volume (blue line) in patient S., 36 years old, who received hydroxychloroquine, azithromycin, and low-molecular-weight heparin.

RR – 16 brpm; oxygen saturation – 99%; normal body temperature; HR – 76 bpm; corrected QT interval (Bazett) – 448 ms (normal up to 430 ms); no serious heart rhythm disturbances. CRP decreased to 10.8 mg/dL, NLR to 1.7, injured pulmonary tissue volume decreased to 30.2% (grade 2), although it was still larger than at admission. PCR test for SARS-CoV-2 RNA was again negative.

Two days later (day 15), the patient was discharged from hospital in good condition to be followed up for the volume of pulmonary tissue injury.

Figure 6 shows the patient's disease course during the used of HCQ in combination with azithromycin. As shown in the figure, not all the changes were associated with the treatment. The levels of immune response and inflammatory reaction as evaluated by CRP and LCR, were consistent with those previously assumed for young patients without overweight and comorbidities [35]. The dome-shaped curve (CRP, green line) has a maximum on day 13 of the disease and a return to the baseline values by day 18. LCR (brown line) also decreased most by day 11 and then recovered. We previously discussed in detail the types of inflammatory immune responses in COVID-19 and the need for proper and timely administration of adequate treatment in each particular case [35]. Following the current standards, this patient needed pro-active anti-inflammatory therapy; nevertheless, at that time, such therapeutic interventions were not a standard procedure in the MSU Medical Research and Educational Center and had not yet been considered in the official guidelines of the Russian Ministry of Health.

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

If there is no intervention at the peak activation of inflammation, there is a possibility of an adverse course of the disease, development of cytokine storm and an acute progression of the disease. Although the stable ferritin level constituted reasons for calm, but this indicator had not normalized by the end of the monitoring period. We conclude that HCQ has not shown significant protective and anti-inflammatory properties. As shown previously, changes in NLR corresponding to the risk of thrombotic complications were also registered [21]. Changes were linear (red line) and tended to decrease gradually. The figure indirectly confirms that the choice of anticoagulant treatment was correct in our patient. Changes in CT lung injury (blue line) were similar to changes in the inflammatory immune response, although lagging by activation and recovery rate. Therefore, the complete normalization of the CT lung pattern should be expected with a delay compared to the normalization of clinical condition, body temperature, oxygen blood saturation, recovery of lymphocyte counts and CRP levels.

In this case, the absence of any anti-inflammatory effect of HCQ did not contribute to the prognosis in the young patient with a normal body weight and no serious comorbidities. However, in more serious clinical cases, leaning on such therapy may be accompanied by a failure of compensatory adaptation reactions, development of cytokine storm and potentially life-threatening complications.

When this article was being prepared, results of an RCT conducted by the National Heart, Lung, and Blood Institute were published, in which the 14-day clinical efficacy of HCQ with/without azithromycin in patients with moderate-to-severe COVID-19 was investigated [36]. The results of the ORCHID study, which did not confirm any positive effect of HCQ in COVID-19, are entirely consistent with our findings. Interestingly, recent experimental studies both in vitro and in primates also found no evidence to support the claimed anti-inflammatory properties of HCQ or its ability to slow down the entry of SARS-CoV-2 into cells [37]. Thus, both the latest experimental data and the trial results demonstrate the absence of efficacy of HCQ in COVID-19 [38]. It should be noted that hydroxychloroquine is not currently included in the major international COVID 19 treatment guidelines.

Conclusion

It is concluded that HCQ has no favorable properties or positive effects on the severity of COVID-19. The use of this antimalarial agent slows down the normalization of the inflammatory response and prolongs hospital stay. HCQ should not be used to treat COVID-19.

Funding

The work has been performed within the framework of the state order to the MSU Medical Research and Educational Center.

No conflict of interest is reported.

The article was received on 01/02/2021

REFERENCES

1. Our World in Data. Mortality Risk of COVID-19 - Statistics and Research. [Internet] Available at: https://ourworldindata.org/mortality-risk-covid
2. Fujita Y, Matsuoka N, Temmoku J, Furuya MY, Asano T, Sato S et al. Hydroxychloroquine inhibits IL-1β production from amyloid-stimulated human neutrophils. Arthritis Research & Therapy. 2019;21(1):250. DOI: 10.1186/s13075-019-2040-6
3. Li X, Wang Y, Agostinis P, Rabson A, Melino G, Carafoli E et al. Is hydroxychloroquine beneficial for COVID-19 patients? Cell Death & Disease. 2020;11(7):512. DOI: 10.1038/s41419-020-2721-8
4. Hashem AM, Alghamdi BS, Algaissi AA, Alshehri FS, Bukhari A, Alfaleh MA et al. Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: A narrative review. Travel Medicine and Infectious Disease. 2020; 35: 101735. DOI: 10.1016/j.tmaid.2020.101735
5. Rajaiah R, Abhilasha KV, Shekar MA, Vogel SN, Vishwanath BS. Evaluation of mechanisms of action of re-purposed drugs for treatment of COVID-19. Cellular Immunology. 2020; 358: 104240. DOI: 10.1016/j.cellimm.2020.104240
6. Diaz-Griffero F, Hoschander SA, Brojatsch J. Endocytosis Is a Critical Step in Entry of Subgroup B Avian Leukosis Viruses. Journal of Virology. 2002;76(24):12866–76. DOI: 10.1128/JVI.76.24.12866-12876.2002
7. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271-280.e8. DOI: 10.1016/j.cell.2020.02.052
8. Wu S-F, Chang C-B, Hsu J-M, Lu M-C, Lai N-S, Li C et al. Hydroxychloroquine inhibits CD154 expression in CD4+ T lymphocytes of systemic lupus erythematosus through NFAT, but not STAT5, signaling. Arthritis Research & Therapy. 2017;19(1):183. DOI: 10.1186/s13075-017-1393-y
9. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Research. 2020;30(3):269–71. DOI: 10.1038/s41422-020-0282-0
10. WHO Solidarity trial consortium, Pan H, Peto R, Karim QA, Alejandria M, Henao-Restrepo AM et al. Repurposed antiviral drugs for COVID-19 – interim WHO SOLIDARITY trial results. Infectious Diseases (except HIV/AIDS). [Av. at: http://medrxiv.org/lookup/doi/10.1101/2020.10.15.20209817]. 2020.
11. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virology Journal. 2005;2(1):69. DOI: 10.1186/1743-422X-2-69
12. Zhou D, Dai S-M, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. Journal of Antimicrobial Chemotherapy. 2020;75(7):1667–70. DOI: 10.1093/jac/dkaa114

13. Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents. 2020;56(1):105949. DOI: 10.1016/j.ijantimicag.2020.105949
14. Chen Z, Hu J, Zhang Z, Jiang S, Han S, Yan D et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. 2020. [Av. at: http://medrxiv.org/lookup/doi/10.1101/2020.03.22.20040758].
15. Bull-Otterson L, Gray EB, Budnitz DS, Strosnider HM, Schieber LZ, Courtney J et al. Hydroxychloroquine and Chloroquine Prescribing Patterns by Provider Specialty Following Initial Reports of Potential Benefit for COVID-19 Treatment – United States, January–June 2020. MMWR. Morbidity and Mortality Weekly Report. 2020;69(35):1210–5. DOI: 10.15585/mmwr.mm6935a4
16. Ministry of Health of Russian Federation. Temporary methodical recommendations. Prevention, diagnosis and treatment of new coronavirus infection (COVID-2019). Version 9 (26.10.2020). Av. at: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/052/548/original/%D0%9C%D0%A0_COVID-19_%28v.9%29.pdf?1603730062. 2020. [Russian: Министерство здравоохранения РФ. Временные методические рекомендации «Профилактика, диагностика и лечение новой коронавирусной инфекции (COVID-19)». Версия 9 (26.10.2020). Доступно на: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/052/548/original/%D0%9C%D0%A0_COVID-19_%28v.9%29.pdf?1603730062]
17. Mareev V.Yu., Begrambekova Yu.L., Mareev Yu.V. How evaluate results of treatment in patients with COVID-19? Symptomatic Hospital and Outpatient Clinical Scale for COVID-19 (SHOCS-COVID). Kardiologiia. 2020;60(11):35–41. [Russian: Мареев В.Ю., Беграмбекова Ю.Л., Мареев Ю.В. Как оценивать результаты лечения больных с новой коронавирусной инфекцией (COVID-19)? Шкала Оценки Клинического Состояния (ШОКС–КОВИД). Кардиология. 2020;60(11):35-41]. DOI: 10.18087/cardio.2020.11.n1439
18. Mareev V.Yu., Orlova Ya.A., Plisyk A.G., Pavlikova E.P., Matskeplishvili S.T., Akopyan Zh.A. et al. Results of an open prospective controlled comparative study on the treatment of new coronavirus infection (COVID-19): Bromhexine and spironolactone for the treatment of coronavirus Infection requiring hospitalization (BISCUIT). Kardiologiia. 2020;60(11):4–15. [Russian: Мареев В.Ю., Орлова Я.А., Плисюк А.Г., Павликова Е.П., Мацкеплишвили С.Т., Акопян Ж.А. и др. Результаты открытого проспективного контролируемого сравнительного исследования по лечению новой коронавирусной инфекции (COVID-19): Бромгексин И Спиронолактон для лечения КоронаВирусной Инфекции, Требующей госпитализации (БИСКВИТ). Кардиология. 2020;60(11):4-15]. DOI: 10.18087/cardio.2020.11.1440
19. Royal College of Physicians. National Early Warning Score (NEWS) 2. [Av. at: https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2]. 2017.
20. Liao X, Wang B, Kang Y. Novel coronavirus infection during the 2019–2020 epidemic: preparing intensive care units – the experience in Sichuan Province, China. Intensive Care Medicine. 2020;46(2):357–60. DOI: 10.1007/s00134-020-05954-2
21. Mareev V.Yu., Orlova Ya.A., Pavlikova E.P., Matskeplishvili S.T., Krasnova T.N., Malahov P.S. et al. Steroid pulse -therapy in patients with coronAvirus Pneumonia (COVID-19), sYstemic inFlammation And Risk of vEnous thRombosis and thromboembolism (WAYFARER Study). Kardiologiia. 2020;60(6):15–29. [Russian: Мареев В.Ю., Орлова Я.А., Павликова Е.П., Мацкеплишвили С.Т., Краснова Т.Н., Малахов П.С. и др. Пульс-терапия стероидными гормонами больных с коронавирусной пневмонией (COVID-19), системным воспалением и риском венозных тромбозов и тромбоэмболий (исследование ПУТНИК). Кардиология. 2020;60(6):15-29]. DOI: 10.18087/cardio.2020.6.n1226
22. Mahévas M, Tran V-T, Roumier M, Chabrol A, Paule R, Guillaud C et al. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data. BMJ. 2020;369:m1844. DOI: 10.1136/bmj.m1844
23. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M et al. Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients Hospitalized with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection: A Randomized Clinical Trial. JAMA Network Open. 2020;3(4):e208857. DOI: 10.1001/jamanetworkopen.2020.8857
24. Mercuro NJ, Yen CF, Shim DJ, Maher TR, McCoy CM, Zimetbaum PJ et al. Risk of QT Interval Prolongation Associated with Use of Hydroxychloroquine With or Without Concomitant Azithromycin Among Hospitalized Patients Testing Positive for Coronavirus Disease 2019 (COVID-19). JAMA Cardiology. 2020;5(9):1036–41. DOI: 10.1001/jamacardio.2020.1834
25. Mareev V.Yu., Orlova Ya.A., Pavlikova E.P., Matskeplishvili S.T., Akopyan Zh.A., Plisyk A.G. et al. Combination therapy at an early stage of the novel coronavirus infection (COVID-19). Case series and design of the clinical trial "BromhexIne and Spironolactone for CoronavirUs Infection requiring hospiTalization (BISCUIT)". Kardiologiia. 2020;60(8):4–15. [Russian: Мареев В.Ю., Орлова Я.А., Павликова Е.П., Мацкеплишвили С.Т., Акопян Ж.А., Плисюк А.Г. и др. Возможности комбинированной терапии на раннем этапе течения новой коронавирусной инфекции (COVID-19). Разбор клинических случаев и дизайн исследования: Бромгексин И Спиронолактон для лечения КоронаВирусной Инфекции, Требующей госпитализации (БИСКВИТ). Кардиология. 2020;60(8):4–15]. DOI: 10.18087/cardio.2020.8.n1307
26. Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J et al. Association of Treatment with Hydroxychloroquine or Azithromycin with In-Hospital Mortality in Patients With COVID-19 in New York State. JAMA. 2020;323(24):2493. DOI: 10.1001/jama.2020.8630
27. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. New England Journal of Medicine. 2020;382(25):2411–8. DOI: 10.1056/NEJMoa2012410
28. The RECOVERY Collaborative Group. Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. New England Journal of Medicine. 2020;383(21):2030–40. DOI: 10.1056/NEJMoa2022926
29. Mehra MR, Desai SS, Ruschitzka F, Patel AN. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. The Lancet. 2020;S0140673620311806. DOI: 10.1016/S0140-6736(20)31180-6
30. World Health Organisation. Coronavirus disease (COVID-19): Hydroxychloroquine. [Internet] Available at: https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-hydroxychloroquine
31. Cavalcanti AB, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A et al. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. New England Journal of Medicine. 2020;383(21):2041–52. DOI: 10.1056/NEJMoa2019014
32. González R, García-Otero L, Pons-Duran C, Marbán-Castro E, Goncé A, Llurba E et al. Hydroxychloroquine efficacy and safety in preventing SARS-CoV-2 infection and COVID-19 disease severity during pregnancy (COVID-Preg): a structured summary of a study protocol for a randomised placebo controlled trial. Trials. 2020;21(1):607. DOI: 10.1186/s13063-020-04557-y
33. Li Y, Bai W, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. Journal of Medical Virology. 2020;92(6):552–5. DOI: 10.1002/jmv.25728
34. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. ACS Chemical Neuroscience. 2020;11(7):995–8. DOI: 10.1021/acschemneuro.0c00122
35. Mareev V.Yu., Orlova Ya.A., Pavlikova E.P., Akopyan Zh.A., Matskeplishvili S.T., Plisyk A.G. et al. Proactive anti-inflammatory and anticoagulant therapy in the treatment of advanced stages of novel coronavirus infection (COVID-19). Case Series and Study Design:

ISSN 0022-9040. Kardiologiia. 2021;61(2). DOI: 10.18087/cardio.2021.2.n1548

COLchicine versus ruxolitinib and secukinumab in open prospective randomIzed trial (COLORIT). Kardiologiia. 2020;60(9):4–21. [Russian: Мареев В.Ю., Орлова Я.А., Павликова Е.П., Акопян Ж.А., Мацкеплишвили С.Т., Плисюк А.Г. и др. Упреждающая противовоспалительная и антикоагулянтная терапия в лечении продвинутых стадий новой коронавирусной инфекции (COVID-19). Разбор клинических случаев и дизайн исследования: колхицин против руксолитиниба и секукинумаба в открытом проспективном рандомизируемом исследовании у пациентов с COVID-19 (КОЛОРИТ). Кардиология. 2020;60(9):4-21]. DOI: 10.18087/cardio.2020.9.n1338

36. Self WH, Semler MW, Leither LM, Casey JD, Angus DC, Brower RG et al. Effect of Hydroxychloroquine on Clinical Status at 14 Days in Hospitalized Patients With COVID-19: A Randomized Clinical Trial. JAMA. 2020;324(21):2165. DOI: 10.1001/jama.2020.22240
37. Hoffmann M, Mösbauer K, Hofmann-Winkler H, Kaul A, Kleine-Weber H, Krüger N et al. Chloroquine does not inhibit infection of human lung cells with SARS-CoV-2. Nature. 2020;585(7826):588–90. DOI: 10.1038/s41586-020-2575-3
38. Saag MS. Misguided Use of Hydroxychloroquine for COVID-19: The Infusion of Politics Into Science. JAMA. 2020;324(21):2161. DOI: 10.1001/jama.2020.22389