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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)"

The article focuses on effective treatment of the novel coronavirus infection (COVID-19) at early stages and substantiates the requirement for antiviral therapy and for decreasing the viral load to prevent the infection progression. The absence of a specific antiviral therapy for the SARS-CoV-2 virus is stated. The authors analyzed results of early randomized studies using lopinavir/ritonavir, remdesivir, and favipiravir in COVID-19 and their potential for the treatment of novel coronavirus infection. Among the drugs blocking the virus entry into cells, the greatest attention was paid to the antimalaria drugs, chloroquine and hydroxychloroquine. The article addresses in detail ineffectiveness and potential danger of hydroxychloroquine, which demonstrated neither a decrease in the time of clinical recovery nor any improvement of prognosis for patients with COVID-19. The major objective was substantiating a possible use of bromhexine, a mucolytic and anticough drug, which can inhibit transmembrane serin protease 2 required for entry of the SARS-CoV-2 virus into cells. Spironolactone may have a similar feature. Due to its antiandrogenic effects, spironolactone can inhibit X-chromosome-related synthesis of ACE-2 receptors and activation of transmembrane serin protease 2. In addition to slowing the virus entry into cells, spironolactone decreases severity of fibrosis in different organs, including the lungs. The major part of the article addresses clinical examples of managing patients with COVID-19 at the University Clinic of the Medical Research and Educational Centre of the M. V. Lomonosov Moscow State University, including successful treatment with schemes containing bromhexine and spironolactone. In conclusion, the authors described the design of a randomized, prospective BISCUIT study performed at the University Clinic of the M. V. Lomonosov Moscow State University with an objective of evaluating the efficacy of this scheme.

Keywords COVID-19; bromhexine, spironolactone, virus load

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As the COVID-19 pandemic caused by the SARS-CoV-2 virus has emerged and developed, medicine has faced significant challenges across many countries, including the Russian Federation (RF). Currently, cases exceed 14 million worldwide with an average mortality rate of about 4.2%; the RF has seen more than 750,000 cases with an average mortality rate of about 1.6%; among patients testing positive with the polymerase chain reaction (PCR) for SARS-CoV-2 RNA, the mortality rate is about 3%. A better understanding of the disease course and an improved quality of therapy since the beginning of the pandemic has reduced mortality rates. In March 2020 (before the epidemic in the RF), the mortality rate for COVID-19 was 6.7% worldwide; it reached 7.8% in April, decreased to 4.7% in May, 3.7% in June, and 2.4% for 20 days in July, which corresponds to mortality rates in the RF (https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). There are three hypotheses for this pattern: a decrease

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in virulence, the change of seasons from winter and spring to summer (in the Northern hemisphere), and the development of more correct approaches to the treatment of COVID-19.

The beginning of the epidemic gave rise to two divergent approaches. According to one, the new disease was, in a key sense, the same as the seasonal flu – as there was no specific antiviral treatment, the goal, in this case, was to simply let the immune system work to defeat the virus. The other approach called for the early use of auxiliary ventilation, even mechanical ventilation in many patients, in the face of rapid development of severe bilateral pneumonia. This approach led swiftly to a catastrophic shortage of ventilators, an overload of intensive care units, a shortage of beds for the treatment of severe patients, and the active use of two to three different groups of antibiotics for treatment of intercurrent infection and nosocomial pneumonia.

In summary, the predominant treatment strategy in the first 2 months of the pandemic (January and February 2020) was based on three principles: assisted ventilation, proactive antibiotic treatment, and the search for antiviral therapy. Moreover, antimalarial drugs (chloroquine and hydroxychloroquine) were used as a first-line therapy along with antiretroviral treatments (lopinavir/ritonavir). This resulted in a relatively high risk of death.

In the RF, the first fatal case of COVID-19 was recorded on March 1, 2020. Social restrictions prompted by the increased incidence of the disease were introduced in the latter half of March. The most rapid increase in the incidence of COVID-19 in the RF was observed in April, 2.5 months past the outbreak in China and 6 weeks later than the outbreaks in southern Europe. This interval allowed RF physicians to critically evaluate our predecessors' experience and develop our principles for treating COVID-19 using the best of their experience.

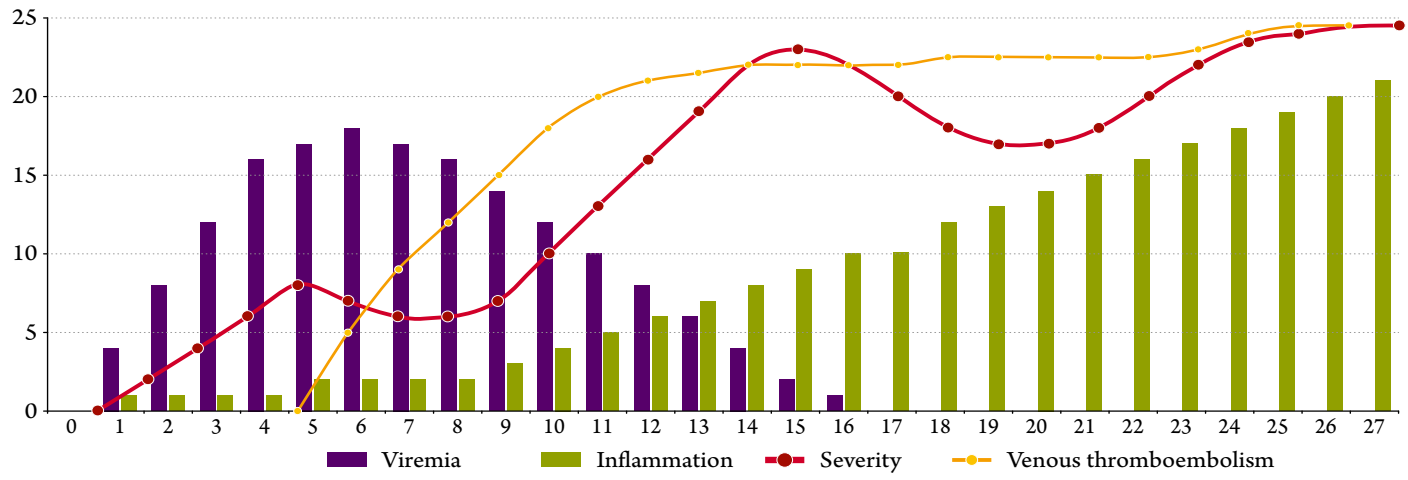
We used the experimental and clinical data and the outcomes recorded at the Moscow State University (MSU) Clinic to analyze clinicians' attempts to find the best possible combination therapy for the early treatment of COVID-19 with an increased viral load to prevent disease progression.

Figure 1 illustrates current views on the course of COVID-19. The red line indicates the changes in the severity of the disease. The three peaks correspond to actual changes in mortality rates, as demonstrated in the early Chinese trials [1].

The first peak is formed by elderly and old patients with increased body weight, diabetes mellitus, cardiovascular diseases, and/or chronic obstructive pulmonary disease, who debut with fever, cough, chest pain, disorientation, severe asthenia, and rapidly worsening dyspnea. Gastrointestinal disorders and diarrhea sometimes worsen the situation. The risk of death from COVID-19 is increased in this category of patients [2–5]. Our observations and analysis of the literature data allow us to provide the following pathogenetic approach to clinical management: In such cases, the best chance for survival is achieved by continued treatment of comorbidities, especially using statins and renin-angiotensin-aldosterone system inhibitors, while controlling blood pressure, cholesterol, and glucose [6, 7].

It is essential begin this treatment early because, as shown in Figure 1, this is the period of viremia (blue line), or active entry of viruses into cells, primarily the pulmonary epithelial cells. Almost half of virus carriers may have no symptoms in this period, especially young people and individuals without comorbidities. Symptoms are quite diverse among patients. For

Figure 1. Development of various presentations and the course of the new coronavirus disease by day



example, one in four patients complains of loss of smell and, more rarely, loss of taste. By days 10–12 of the disease, these symptoms disappear or significantly decrease in one-third of patients. Unfortunately, many of the COVID-19 patients admitted to hospitals present with symptoms only during this infection period. During this same period, other factors «switch on»: increasing autoimmune inflammation (green line in Figure 1) and coagulopathy with the risk of increased thrombosis (brown line in Figure 1). These factors determine the disease progression and the prognosis on days 12–14 of the disease.

In the early stages of the pandemic, the greater share of attention was focused on antiviral treatment despite the disease's novelty and the lack of specific anti-inflammatory therapy for COVID-19. The competition [8, 9] to identify an antiviral drug that would block the replication of the SARS-CoV-2 virus has received extensive coverage in the medical literature as well as the press and other media. However, four critical observations can be made:

- Testing for virus elimination using a poorly reproducible PCR test for detecting viral RNA can lead to false negative or false positive results.
- Clear, standard criteria for the progress of clinical condition remain unhelpfully basic; except for transfer to an intensive care unit (ICU) and the use of mechanical ventilation, which are considered unfavorable outcomes, and earlier discharge from the hospital, which is considered a favorable outcome, consistent criteria are lacking.
- Use of antiviral drugs indicated for other viral diseases does not warrant successful treatment of COVID-19.
- The peak viral load passes its maximum in most patients by day 10 of treatment, and it is difficult (if not possible) to rely on successful antiviral treatment on or after this point.

Various antiviral drugs have been used as «salvage therapy» to treat COVID-19, with varying degrees of success. Those included drugs used to slow the replication of retroviruses (HIV), flaviviruses (hepatitis C), filoviruses (Ebola), or influenza viruses. From the beginning of the pandemic, there has been speculation about the possibility of slowing down infection progression and preventing the development of pneumonia caused by beta-coronavirus infection with nonspecific antiviral drugs. This line of thinking has not been completely abandoned to date (!).

The first attempt, to use the combination of lopinavir/ritonavir as the main treatment, has failed. Controlled trials have not confirmed an improvement in the disease course, with a sufficiently significant number of serious adverse events [10]. Despite persistent calls to use the lopinavir/ritonavir combination as the preferred therapy for the treatment of COVID-19 in the interim guidelines of the Ministry of Health of the RF [11], we decided to restrict the extensive use of these drugs from the very beginning at the MSU Clinic. Eyes were opened worldwide only by the end of June following the publication of a press release of the large randomized RECOVERY trial that proved the lopinavir/ritonavir combination to be uselessness in the treatment of COVID-19 [12]. The World Health Organization (WHO) also announced the discontinuation of a randomized clinical trial (RCT) of this combination due to the lack of efficacy [13].

There are a few trials of ribavirin, indicated for the treatment of infection caused by the hepatitis C virus. Presently, it is impossible to reach a final conclusion about its feasibility for the successful treatment of COVID-19. In a small open comparative study, a positive effect on virus elimination, clinical manifestations, and reduction of treatment time from 15 to 8 days was observed only for the early use of the drug (during the first 7 days of illness, i.e., when viral load is increasing) [14]. No large RCTs have been registered.

Much more attention, especially in the United States, has been given to remdesivir, which is intended for treatment of the Ebola virus disease. The theoretical background for its use has been tested in several clinical trials [15, 16], including RCTs. In a Chinese placebo-controlled study, including 237 patients, a 10-day course of remdesivir did not accelerate the elimination of the virus, or patients' clinical improvement [17]. A much larger, international RCT (1,059 patients) showed a reduction in clinical recovery time from 15 to 11 days with a 10-day course of remdesivir (200 mg on day 1 and 100 mg/day subsequently) [18]. The odds ratio for recovery was 1.32 (95% confidence interval [CI]: 1.12–1.55; p <0.001). In addition, a downward trend was detected in the risk of death, from 11.9% to 7.1%. There was no effect of remdesivir on whether patients required mechanical ventilation, invasive ventilation, or extracorporeal membrane oxygenation. Another study in moderate COVID – 19 patients who did not require mechanical ventilation did not show differences in the effect of the 5- and 10-day courses of remdesivir on the recovery rate, which requires explanation [19]. Despite conflicting results, the U. S. Food and Drug Administration (FDA) has granted remdesivir approval a promising COVID-19

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treatment [20]. It is necessary to mention a very moderate treatment effect in nonsevere and critical patients who do not require mechanical ventilation. Most experts criticize the study with emdesivir because of the unbalanced patient groups [21]. Nevertheless, remdesivir is currently considered the «defeater» of SARS-CoV-2 in the United States.

In the RF, favipiravir, intended for treatment of severe forms of influenza, has attracted the most attention. In 2019, it was released from patent protection and reproduced in several countries, including the RF. There are only a few completed trials of favipiravir in patients with COVID-19. In an open-label comparative study, favipiravir achieved clinical effect by day 7 at a higher rate than umifenovir, but caused more serious adverse events, especially increased uric acid levels [22]. Another open comparative study demonstrated favipiravir's ability to eliminate the SARS-CoV-2 virus faster than lopinavir/ritonavir and significantly more reduce lung tissue damage on multislice computed tomography (MSCT) by day 14 of the treatment [23]. An interesting and well-planned double-blind placebo-controlled RCT is underway in 30 Russian investigational centers, including the MSU Clinic. According to the RF Ministry of Healthcare, favipiravir demonstrated a high ability to eliminate the SARS-CoV-2 virus, with a small number of adverse reactions, at the first stage (60 patients) of the trial [24]. Thus, researchers were able to proceed to the second stage, which included 270 more patients. However, official results have not yet been published. A Japanese study, which included 89 patients with COVID-19 (July 6, 2020), did not confirm the benefits of favipiravir versus control [25]. At the same time, a Bangladesh study with 50 patients reported beneficial results [26]. However, there is no official publication of these trials to date, and all information should be taken with caution. Favipiravir trials are also being conducted in the United States and India.

The concept of blocking the SARS-CoV-2 replication at the initial stages of the disease and preventing the development of severe viral pneumonia is hardly exceptional. However, it is still unclear whether it is possible to achieve success using drugs created to treat other viruses. There are still hopes for positive clinical effects of remdesivir and favipiravir, but it can only be expected at the very beginning of the disease, of mild to moderate severity. Antiviral drugs are not effective from day 7 of the disease, and or in cases in which the disease becomes more severe, since other factors are paramount in such cases (Figure 1).

An attempt can be made to block the SARS-CoV-2 infection of cells at the early stages of the disease and disrupt virus replication. Antimalarial drugs (chloroquine or hydroxychloroquine) in combination with azithromycin were used for this purpose at the beginning of the pandemic. The mechanism of action of hydroxychloroquine is not well studied, but the drug can interfere with endocytosis, when the virus is incorporated into the cell [27], and has also been shown in one study to have anti-inflammatory properties and anti-cytokine effects [28]. Hydroxychloroquine dose-dependently reduced the penetration of SARS-CoV (the causal agent of severe acute respiratory syndrome [SARS]) into cells and showed a preventive effect in this study [29]. The RNA of the human SARS-CoV-2 virus is 96% identical to that of the bat virus but, it is important to note, only 79% identical to the SARS-CoV RNA that caused SARS [30]. Moreover, SARS-CoV and SARS-CoV-2 differ in the RNA-dependent RNA polymerase responsible for virus replication [31]. Nevertheless, the possibility of using hydroxychloroquine in the treatment of COVID-19 has been suggested [32]. Enthusiasm in some quarters was fueled by the publication of an open-label, non-randomized French trial that showed rapid elimination of the virus when hydroxychloroquine was used as monotherapy and even faster elimination with a combination of hydroxychloroquine and azithromycin [33]. In the context of the absence of effective therapy, overcrowded intensive care units, and the shortage of ventilators, the search for any effective therapy led to a so-called «hydroxychloroquine storm.» Simultaneously, the first RCT of hydroxychloroquine conducted in China showed moderate but statistically significant clinical improvement [34]. In most guidelines for the treatment of COVID-19 (including the Russian guidelines), the combination of hydroxychloroquine and azithromycin was indicated as the first-line therapy despite certain doubts based on a retrospective analysis of the clinical use of the drug in four hospitals in France [35].

We used this as our main regimen from the very beginning of admitting COVID-19 patients to the MSU Clinic. The logistical working procedures involved daily online consultations, during which professors and researchers working in various fields of medicine could promptly help doctors working in the «red zone» choose the best possible therapy based on the detailed analysis of patients. Regular recording of electrocardiograms, especially in patients with COVID-19 and cardiovascular diseases, was intended to help avoid side effects, primarily associated with QT prolongation and the risk of developing ventricular arrhythmias.

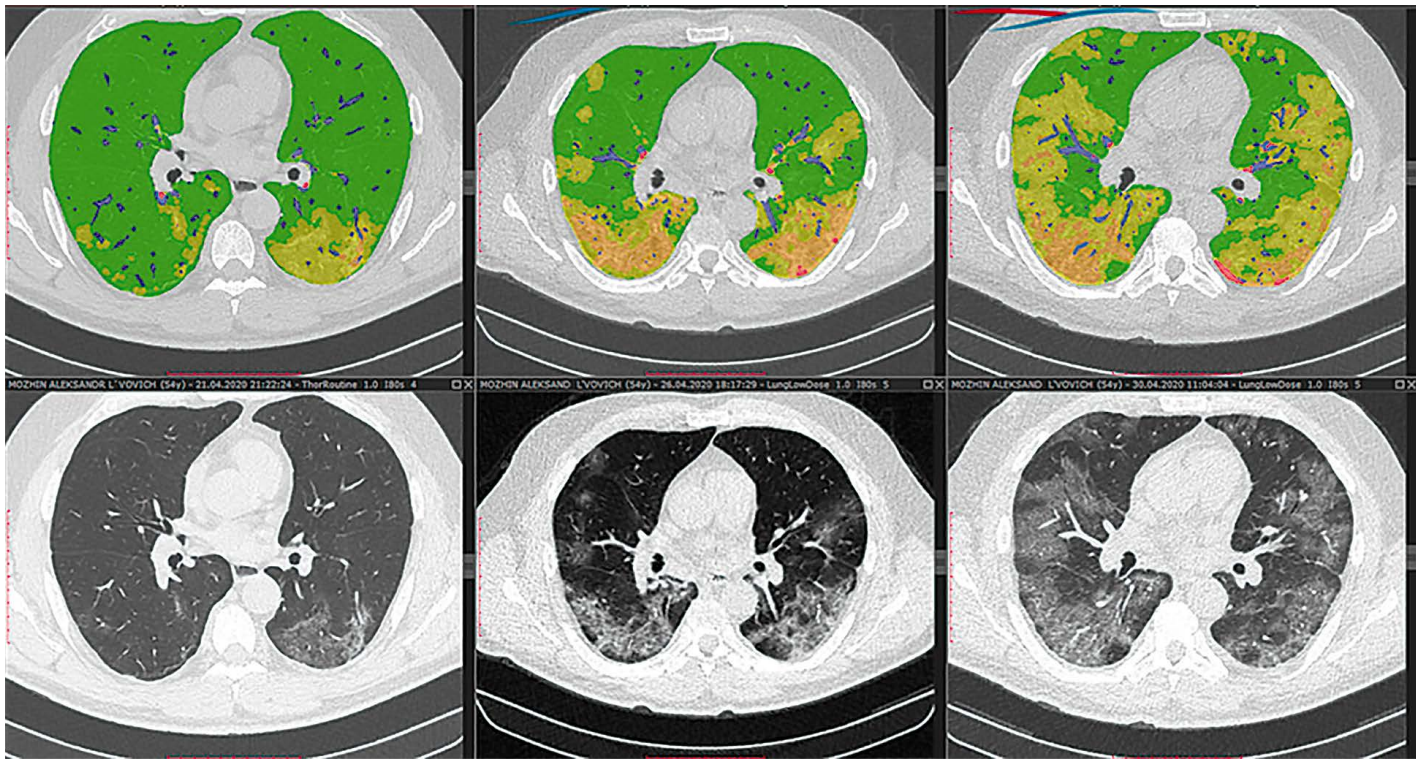
The daily discussions and quick reaction to the changing situation and the outcomes allowed us to doubt the objective usefulness and efficacy of such treatment by days 10–11 of the clinic's work. We present here a case study of patient M., 54 years old, admitted on April 21, 2020 (the first day of the clinic's work), on day 5 after the onset of symptoms with complaints of shortness of breath, worsening on exertion, severe weakness, headache, dry cough, sore throat, diarrhea, bile vomiting, pain in the anterior chest, worsening with cough, calf heaviness, fever above 38°C for 5 days. He received hydroxychloroquine 200 mg bid, azithromycin 500 mg once a day, and paracetamol to treat fever. At the time of admission he had a body temperature of 36.7°C, blood pressure 100/70 mmHg, heart rate 100 bpm, and oxygen saturation 97%; oxygen support was not required. No severe comorbidities requiring additional treatment were identified.

MSCT (Figure 2) at admission (April 21, 2020, day 5 of the disease) showed minimal damage in the lung tissue, more on the left side. The total area of the lesion was less than 10% (stage 1 under the RF Ministry of Healthcare Guidelines) [11].

Laboratory findings were the following: lymphocytes 0.95 × 109/L; erythrocyte sedimentation rate (ESR) 9 mm/h; potassium 4.0 mmol/L; C-reactive protein (CRP) 65 mg/dL; fibrinogen 5.37 g/L; D-dimer 0.4 μg/mL. Based on our knowledge at the time, specific anti-inflammatory treatment was not initiated, despite the increased CRP level, due to the minimal lung damage, good oxygen saturation, and normal D-dimer values. A second antibiotic was added to the treatment: amoxicillin/clavulanic acid 1.0 g tid. Acetylcysteine 600 mg od and enoxaparin sodium 40 mg od were given as an anticoagulant treatment.

After 5 days (day 10 of the disease), the patient's condition worsened, although the body temperature remained subfebrile, and the cough cleared up a bit. Shortness of breath worsened, and oxygen saturation decreased to 94%. It was difficult for the patient to lie on his stomach due to obesity (body mass index 32.5 kg/m^2). Laboratory tests showed that the lymphocyte count had decreased to 0.66 × 109/L, ESR increased to 24 mm/h; potassium was 3.8 mmol/L; CRP 69 mg/dL, D-dimer 0.84 μg/mL, fibrinogen 5.51 g/L. MSCT found an increase in lung tissue damage on both sides, mainly presenting as ground-glass opacity, and the total area of the lesion had expanded to up to 31% (stage 2 under the RF Ministry of Healthcare Guidelines) [11]. Azithromycin was replaced with moxifloxacin. The dose of enoxaparin sodium was increased to 40 mg bid, and spironolactone 25 mg/day was added.

Figure 2. Lung MSCT scan, patient M., 54 years old (see details in the text); results of quantitative tomography of the lung parenchyma, MultiVox (in collaboration with Faculty of Fundamental Medicine, Moscow State University)



4/21/2020	Ground glass 6% Consolidation 2%	26/04/2020	Ground glass 25% Consolidation 6%	30/04/2020	Ground glass 39% Consolidation 6%

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Despite the treatment, the patient's condition continued to worsen. In 4 days (day 14 of the disease), shortness of breath worsened further, oxygen saturation decreased to 92%, and asthenia progressed, making the lack of therapeutic efficacy certain. Laboratory tests showed an even more significant decrease in lymphocyte count up to 0.53 × 109/L, increased ESR up to 32 mm/h, potassium 3.5 mmol/L, a twofold increase in CRP to 134 mg/dL, and D-dimer to 1.73 μg/mL, compared to results from 3 days ago; fibrinogen 5.81 g/L. MSCT data showed a significant increase in the zone of destruction of lung tissue up to 48%, plus moderate hydrothorax and hydroperiod, which corresponded to the 3rd stage of lung disease (according to guidelines by the RF Ministry of Healthcare). The condition was considered progressing to inflammation (developing a cytokine storm). It was decided to conduct pulse therapy with high doses of glucocorticosteroids: methylprednisolone 1,000 mg for 3 days, followed by dexamethasone 8 mg bid [36]. Hydroxychloroquine was canceled due to the lack of efficacy. The spironolactone dose was increased to 50 mg/day. Bromhexine 8 mg qid was added. The patient's condition gradually improved after pulse therapy, CRP decreased to 78.8 mg/dL, but the D-dimer level increased to 10.12 μg/mL. With a decrease in shortness of breath and a gradual increase in oxygen saturation, the area of lung damage was about 40% on MSCT (stage 2 under the RF Ministry of Healthcare Guidelines). However, pain appeared in the right arm. Using Doppler ultrasonography, occlusive thrombosis was detected in the saphenous vein (v. intermedia cubiti) at the ulnar fossa level, which required further increase in the dose of enoxaparin sodium to 80 mg bid and the local application of heparin ointment. The patient's condition slowly improved. As of May 18, 2020 (day 32 of the disease), there was no shortness of breath, oxygen saturation was 98%, lymphocyte count 1.51 × 109/L; ESR 12 mm/h; potassium 4.3 mmol/L; CRP 2.85 mg/dL, D-dimer 0.61 μg/mL, fibrinogen 4.21 g/L. The area of lung tissue damage, according to MSCT, was still 35% but presented as mild ground-glass opacity indicative of the resolution of the process.

The patient was discharged from the hospital on May 19, 2020 (day 28 of the hospital stay). The outpatient use of spironolactone for 2 weeks and rivaroxaban 20 mg/day for 3 months, followed by 10 mg/day, was recommended. A control visit and MSCT was scheduled 45 days after discharge.

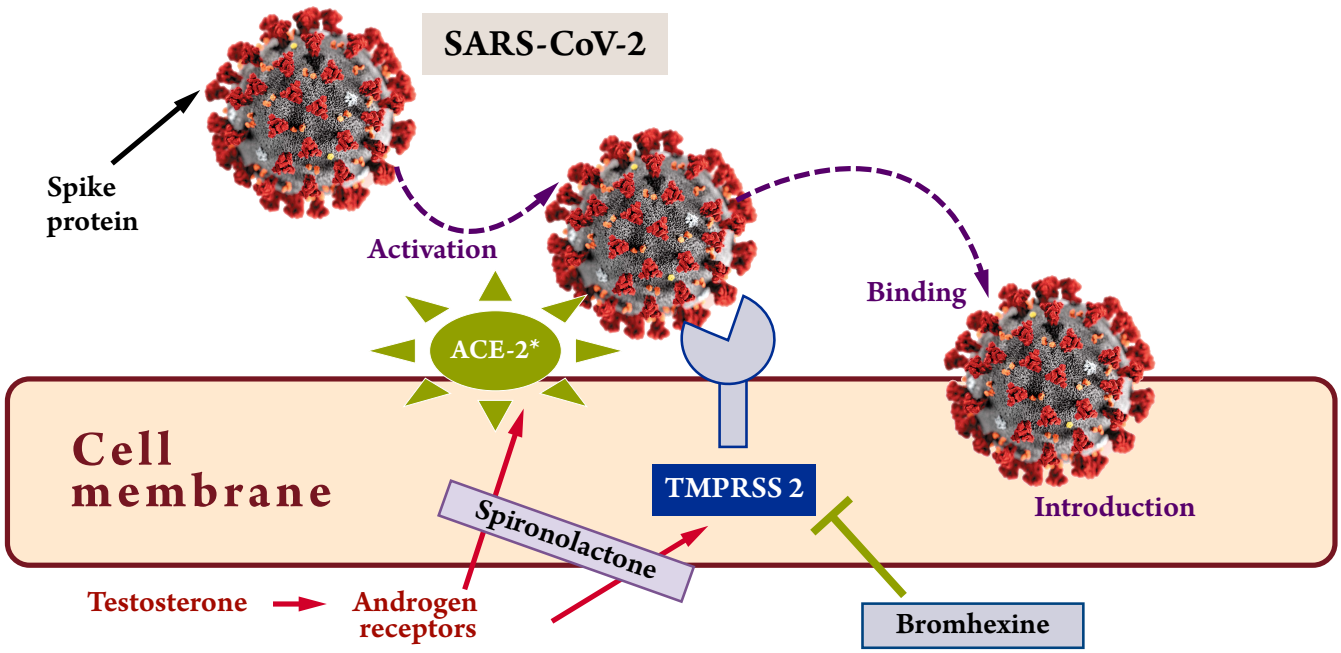
This case study taught us many lessons. First, it demonstrated the low efficacy of hydroxychloroquine. Since then, most retrospective cohort analyses involving a large number of patients have not confirmed this drug's safety and efficacy in the treatment of COVID-19 [37, 38]. A retrospective analysis of the U. S. Department of Veterans Affairs database showed a significant increase in mortality with hydroxychloroquine, but not a combination of hydroxychloroquine and azithromycin [39]. Azithromycin cannot be associated with prognosis deterioration in patients with COVID-19 [38]. The meta-analysis «showed feeble and unreliable evidence for both the benefit and risk of hydroxychloroquine in COVID-19» [40]. Moreover, clinical trials have shown that this drug is associated with an increase in the corrected QT interval and an increased risk of sudden cardiac death in patients with COVID-19 [38, 41]. A U. S. prospective study on the protection of healthcare professionals also showed no beneficial effects of hydroxychloroquine [42]. A U. S. RCT showed no benefits of hydroxychloroquine in treating patients with COVID-19 [43].

Finally, the largest prospective randomized RECOVERY study (which ended June 5, 2020) showed no positive effects of antimalarial drugs on COVID-19 patients' prognosis [44]. Based on the findings for more than 4,700 patients with COVID-19, hydroxychloroquine did not affect the risk of death but increased the risk of mechanical ventilation and prolongation of hospital stay [45]. In early July, the WHO announced the termination of the study of hydroxychloroquine for the treatment of COVID-19 due to the lack of efficacy [13].

To safely inhibit virus penetration into the cell, we tried to use medications generally indicated for the treatment of pneumonia and its consequences (pulmonary fibrosis).

As shown in Figure 3, beta-coronavirus uses the S1 spike protein and connects to the angiotensin-converting enzyme type 2 (ACE2) receptor and can interact with transmembrane serine protease 2 (TMPRSS2) after activation. Only then can SARS-CoV-2 pass through the membrane and enter the cells [45]. In theory, drugs that block the ACE2 receptor and/or disrupt the activity of transmembrane serine protease can have antiviral activity. We have already written about the benefits of renin-angiotensin-aldosterone system blockers in treating cardiovascular diseases in patients with COVID-19 and their neutral effect on ACE2 receptors [47]. Bromhexine and spironolactone deserve special attention among the available drugs indicated for the treatment of viral pneumonia and pulmonary fibrosis that can affect not only the ACE2 receptor but also TMPRSS2, and reduce the ability of SARS-CoV-2 viruses to enter cells.

Figure 3. Possibility of pharmacological blockade of SARS-CoV-2 in a cell



*** ACE, angiotensin-converting enzyme type 2**

Bromhexine is a well-known antitussive and mucolytic drug that reduces cough, congestion, and chest pain, facilitates breathing, and is indicated in acute respiratory tract infections [48]. Therefore, its use as an additional symptomatic agent that improves the clinical condition of COVID-19 patients is unquestioned and unexceptionable. Bromhexine has been shown to additionally block the activity of TMPRSS2 in the trials with influenza viruses [49]. Both the bromhexine's attachment points to TMPRSS2 and the ability to block its active centers in three-dimensional modeling were studied [50]. Thus, bromhexine can be considered a promising antiviral agent that can reduce the viral load in the treatment of COVID-19 [50, 51].

Spironolactone is an effective antifibrotic agent that can reduce the severity of fibrosis of various tissues and organs, including the myocardium and interstitial pulmonary fibrosis, by blocking aldosterone's effects on receptors [52]. The ability to increase the aeration of the lung by affecting exudative and infiltrative alveolar lesions has been proven both experimentally and in the real-world treatment of acute respiratory distress syndrome and chronic heart failure [52, 53]. Our clinical case study shows how persistent lung damage is and how difficult it is to get rid of pulmonary fibrosis (at day 34 of the disease, the area of lung tissue damage was still about 35%). Therefore, it seems reasonable to use spironolactone to speed up the restoration of aeration of the lung in patients who had COVID-19. The efficacy of spironolactone can be related mainly to its anti-androgenic activity. One reason for the higher morbidity and mortality in men with COVID-19 is the difference in the synthesis of the ACE2 and TMPRSS2 membrane receptors associated with the X chromosome and testosterone levels [54, 55]. Therefore, anti-androgenic spironolactone decreases the testosterone level, blocks androgen receptors, and can reduce ACE2 expression levels and the activity of TMPRSS2 at the same time (Figure 3). Thus, spironolactone, with antifibrotic and antihypertensive properties, can also be used as an antiviral drug in the treatment of COVID-19 [56]. Another essential property of spironolactone in treating chronic heart failure and renal dysfunction is increasing potassium levels [57, 58]. Hypokalemia is detected in 40–55% of patients with COVID-19 and up to 85% in severe cases. Reduced potassium levels are inversely correlated with CRP levels – that is, systemic inflammation [59]. Our patient also had hypokalemia and did not use diuretics. Spironolactone helped to correct potassium levels and stabilize the situation.

In search of possible ways to reduce the viral load in the first 10 days of the disease, we decided to use a combination of bromhexine hydrochloride 8 mg qid and spironolactone 50 mg/day (25 mg/day if potassium levels increase above 5.2 mmol/L). At the same time, we focused on the positive clinical (symptomatic) effects of both drugs in pneumonia of any etiology. A prospective, randomized, comparative clinical trial

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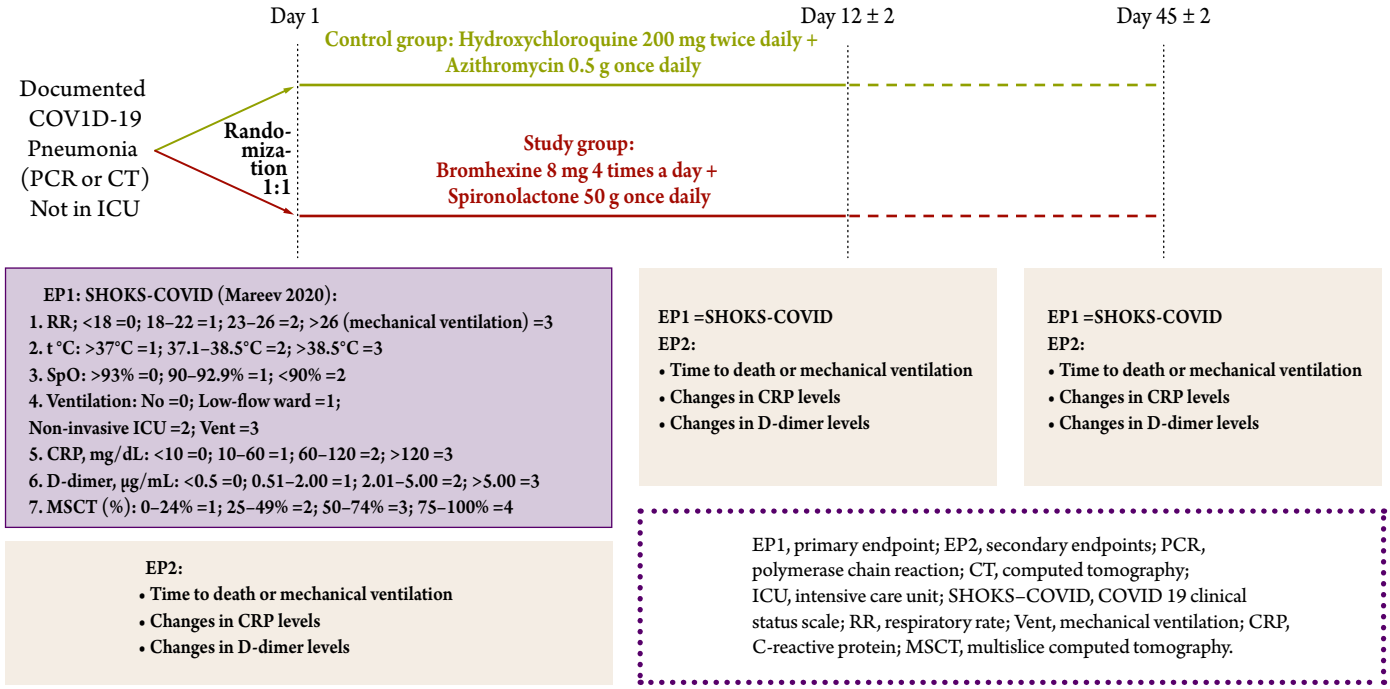
Figure 4. Study design and endpoints: BromhexIne and Spironolactone for Coronavirus Infection Requiring Hospitalization (BISCUIT)



conducted in our clinic to test this hypothesis was titled «BromhexIne and Spironolactone for Coronavirus Infection Requiring Hospitalization» (BISCUIT)[60]. Figure 4 shows the study design.

Patients received bromhexine and spironolactone in the treatment group and hydroxychloroquine and azithromycin in the control group, as recommended by the RF Ministry of Healthcare [11]. We used changes in the original SHOKS-COVID score as a primary endpoint (Figure 4) [36]. Secondary endpoints were:

- Combined endpoint: time to death or a need for mechanical ventilation.
- Changes in CRP levels.
- Changes in D-dimer levels.

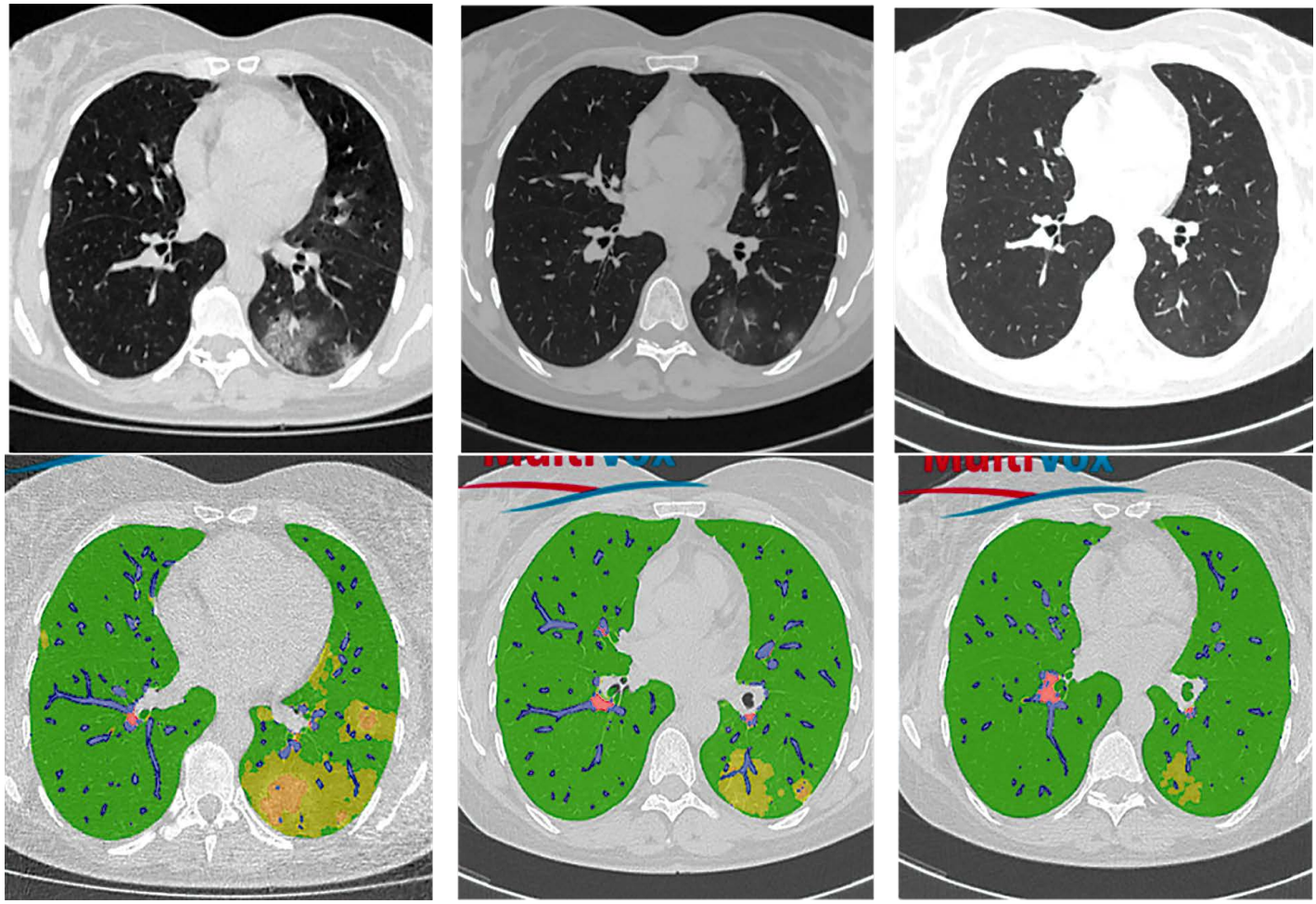
All endpoints were evaluated from the inclusion in the study until days 12 and 45.

Statistical analysis of the results is being completed. Here we present a case study of patient C., 40 years old, with confirmed COVID-19 (by MSCT), concomitant stage 3 (!) hypertension, and antiphospholipid syndrome (!), who became acutely ill on April 28, 2020. First, she experienced severe weakness and muscle pain; fever 38.8°C, dry cough, shortness of breath, panic on the next day, and runny nose and loss of smell a day later. PCR test for SARS-CoV-2 RNA was negative. Hydroxychloroquine, azithromycin, and paracetamol were recommended as outpatient therapy to reduce the temperature. On April 30, 2020 (day 3 of the disease), the patient received online consultation at the MSU Clinic and was given the following recommendations: discontinue hydroxychloroquine, start bromhexine 8 mg qid, spironolactone 50 mg/day, colchicine 1 mg on the first day, then 0.5 mg. Antihypertensive therapy (azilsartan with chlorthalidone 40+25 mg/day, amlodipine 10 mg/day, moxonidine 0.4 mg bid) and anticoagulant therapy (apixaban 5 mg bid) were continued.

Outpatient MSCT (Figure 5) on May 2, 2020 (day 5 of the disease) revealed signs of left-sided coronavirus pneumonia (stage 2 under to the RF Ministry of Healthcare Guidelines). Coughing and shortness of breath persisted, as well as anosmia, asthenia, and panic attacks. The body temperature was 37.5°C. Laboratory tests: lymphocyte count 0.97 × 109/L; ESR 31 mm/h; CRP 38 mg/dL; D-dimer 1.03 μg/mL.

Patient C. was admitted to the MSU Clinic on May 5, 2020 (day 8 of the disease), with complaints of weakness, breathing difficulties, cough with moderate sputum production, body temperature 37.0°C, and a feeling of fear. Oxygen saturation was 98%, BP 135/80 mmHg, heart rate 90 bpm. MSCT showed the lung lesion area (on the left) was about 10% versus 18% on the previous MSCT scan (May 2, 2020). Laboratory tests: lymphocyte count 1.23 × 109/L; ESR 18 mm/h; CRP 21 mg/dL; D-dimer 0.25 μg/mL. Treatment with bromhexine, spironolactone, and colchicine was continued. Ceftriaxone 1000 mg bid was prescribed intravenously instead of azithromycin, and hydroxyzine 25 mg/day was added. Antihypertensive and anticoagulant therapy was continued.

Figure 5. Lung MSCT scan, patient C., 40 years old (see details in the text); results of quantitative tomography of the lung parenchyma, MultiVox (in collaboration with Faculty of Fundamental Medicine, Moscow State University)



05/02/2020	Lesion area is ≈18%	06/05/2020	Lesion area is ≈10%	12/05/2020	Lesion area is ≈2%

As a result, on May 12, 2020 (day 15 of the disease), the patient's condition improved. She had no complaints. Cough and shortness of breath regressed, the sense of smell normalized, and body temperature was 36.5°C, oxygen saturation 99%. The fear and panic attacks were gone. Laboratory tests: lymphocyte 1.91 × 109/L; ESR 12 mm/h; CRP 0.48 mg/dL; D-dimer 0.19 μg/mL. There were minimal residual changes in the lung tissue (about 2%) on MSCT. She was discharged with a recommendation to take colchicine for a further 2 weeks and continue antihypertensive and anticoagulant therapy.

As follows from the data above, the patient with new coronavirus pneumonia had a more extensive initial lung tissue damage than the first patient. There was an increase in CRP, just as in the first case. Serious deterioration factors were comorbidities presented by stage 3 – hypertension controlled by only four (!) antihypertensive agents and antiphospholipid syndrome – due to which the patient took anticoagulant therapy to prevent thrombotic complications. However, a too-early (day 3 of the disease) start of the treatment with the combination of bromhexine and spironolactone plus preemptive anti-inflammatory therapy with colchicine and adequate anticoagulation therapy made it possible to slow down the development and progression of COVID-19 quickly.

In contrast, antiviral therapy (hydroxychloroquine) was not very effective in the first case. Anti-inflammatory therapy with glucocorticosteroids was prescribed very late, in the ICU, when invasive mechanical ventilation had become necessary. The efficacy of steroid therapy for COVID-19 has been demonstrated by the RECOVERY trial [61], but it was an experiment when we used it instead of interleukin-6 blockers (due to their absence). This case study demonstrates both the advantages (decrease in the CRP levels, decrease in inflammation, and improvement of the clinical condition) and the disadvantages (prothrombotic effects) of glucocorticosteroid therapy, which was studied in detail in the PUTNIK trial [36].

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After days 7–10 days of the disease, other factors (other than viral load) played a more critical role in the progression of the disease and poor prognosis: systemic inflammation (green line in Figure 1) and coagulopathy with high-risk thrombosis and thromboembolism (brown line in Figure 1). Therefore, preemptive anti-inflammatory therapy and aggressive anticoagulation treatment should be prescribed after 10–12 days of the novel coronavirus disease. The specifics of the treatment of this phase of the COVID-19 course require analysis and detailed discussion in a separate article.

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