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Results of Open-Label non-Randomized Comparative Clinical Trial: "BromhexIne and Spironolactone for CoronavirUs Infection requiring hospiTalization (BISCUIT)

Introduction	The aim of this study was to assess the efficacy and safety of a combination of bromhexine at a dose of 8 mg 4 times a day and spironolactone 50 mg per day in patients with mild and moderate COVID 19.
Material and methods	It was an open, prospective comparative non-randomized study. 103 patients were included (33 in the bromhexine and spironolactone group and 70 in the control group). All patients had a confirmed 2019 novel coronavirus infection (COVID 19) based on a positive polymerase chain reaction (PCR) for SARS-CoV-2 virus RNA and/or a typical pattern of viral pneumonia on multispiral computed tomography. The severity of lung damage was limited to stage I–II, the level of CRP should not exceed 60 mg/dL and SO_2 in the air within 92–98%. The duration of treatment is 10 days.
Results	The decrease in scores on the SHOKS-COVID scale, which, in addition to assessing the clinical status, the dynamics of CRP (a marker of inflammation), D-dimer (a marker of thrombus formation), and the degree of lung damage on CT (primary endpoint) was statistically significant in both groups and differences between them was not identified. Analysis for the group as a whole revealed a statistically significant reduction in hospitalization time from 10.4 to 9.0 days (by 1.5 days, p=0.033) and fever time from 6.5 to 3.9 days (by 2.5 days, p<0.001). Given the incomplete balance of the groups, the main analysis included 66 patients who were match with using propensity score matching. In matched patients, temperature normalization in the bromhexine/spironolactone group occurred 2 days faster than in the control group (p=0.008). Virus elimination by the 10th day was recorded in all patients in the bromhexine/spironolactone group; the control group viremia continued in 23.3% (p=0.077). The number of patients who had a positive PCR to the SARS-CoV-2 virus on the 10th day of hospitalization or longer (≥10 days) hospitalization in the control group was 20/21 (95.2%), and in the group with bromhexine/spironolactone –14/24 (58.3%), p=0.012. The odds ratio of having a positive PCR or more than ten days of hospitalization was 0.07 (95% CI: 0.008–0.61, p=0.0161) with bromhexine and spironolactone versus controls. No side effects were reported in the study group.
Conclusion	The combination of bromhexine with spironolactone appeared effective in treating a new coronavirus infection by achieving a faster normalization of the clinical condition, lowering the temperature one and a half times faster, and reducing explanatory combine endpoint the viral load or long duration of hospitalization (≥10 days).

Keywords	COVID 19; spironolactone; bromhexine; SHOKS-COVID
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The second wave of the new coronavirus pandemic in the autumn of 2020 requires finding the most effective ways to treat COVID-19 at its different stages. The top priority is to prevent the disease progression after its onset and the manifestation of viral pneumonia. Given the early viremia, there is a need

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for effective and safe treatments to reduce the viral load. Currently, there are no specific drugs for blocking replication of the SARS-CoV-2 beta-coronavirus. Attempts have been made to use drugs which reduce the replication of human immunodeficiency retrovirus (lopinavir/ritonavir), Ebola filovirus (remdesivir), and influenza virus (favipiravir) to slow the progression of COVID-19.

It is obvious now that the use of lopinavir/ritonavir is ineffective [1, 2]. Remdesivir showed discrepant results in controlled trials that varied from faster relief of symptoms by four days [3] to neutral results [4]. It also increased the risk of kidney complications [5, 6]. The full results of the favipiravir trials have not yet been published. In early July, Japanese researchers reported it to be ineffective. However, the press release following the Japanese trials reported that the time to a negative conversion of the SARS-CoV-2 RNA in PCR analysis and relief of symptom was 2.8 days shorter than in the control group [7]. The Russian clinical trial results have not been published, except for data from the Russian Direct Investment Fund's statement on the 50% reduction in the virus detection after five days of treatment [8]. However, favipiravir has been already included in the latest (9th) version of the temporary methodical guidelines of the Ministry of Health of the Russian Federation Prevention, Diagnosis, and Treatment of the New Coronavirus Disease (COVID-19) dated October 26, 2020, in which it is recommended for outpatient treatment [9]. This approach seems over-optimistic from the perspective of evidence-based medicine. Nearly every antiviral drug has adverse reactions, and its use requires careful control. Nevertheless, antiviral drugs can be beneficial only from the earliest days of the disease, and their outpatient administration seems reasonable.

Thus, the use of safe, accessible, and affordable drugs, which can slow SARS-CoV-2 entry into cells, especially alveolar epithelial cells, is of great interest. Many trials have shown that the crown-like viral spike (S) protein (corona in Latin means crown) binds to the angiotensin-converting enzyme 2 (ACE2) and then is split into S1 and S2 subunits mediated by the membrane-bound serine protease. This produces a hybrid peptide, i.e., a peptide with genetic characteristics of both the virus and the host, which helps the virus enter the cell by endocytosis. Subunit S1 mediates the attachment of a virion to receptors on the host cell's surface and is located above subunit S2 that mediates the subsequent fusion between the viral and cellular membranes of the host, thus facilitating the penetration into the host cell [10]. In view of this mechanism, inhibition of transmembrane serine protease 2 (TMPRSS2) and ACE2 can slow the progression of viremia and reduce the advance of the disease. The well-known antitussive and mucolytic drug bromhexine proved to be an effective inhibitor of TMPRSS2 [11]. In addition to its indications for use in the treatment of respiratory infectious diseases and pneumonia, it is hoped that bromhexine, which accumulates in bronchi and alveoli, has antiviral effects in COVID-19 [12].

The activity of TMPRSS2 is known to be regulated by androgens. Thus an alternative strategy, other than the selective inhibition of TMPRSS2, is to modulate the expression of TMPRSS2 by targeting androgen receptors. Therefore, the second approach was to use another well-known drug, spironolactone. Due to blockade of aldosterone, this drug has antifibrotic properties and experimentally restores respiratory function by reducing the damage to alveoli [13]. Its additional anti-androgen effects, which have always been considered to be drawbacks of spironolactone, are also important in coronavirus disease. This is particularly important in COVID-19, since activation of both the ACE2 receptor and the transmembrane serine protease is directly related to male sex hormones and hyperactivation of androgen receptors [14]. This explains the faster development and progression of the disease in male patients, especially those with severe hypergonadism [15, 16].

These two ideas prompted us to plan an open-label, prospective controlled trial of bromhexine and spironolactone in patients with moderate COVID-19: BromhexIne And Spironolactone For CoronaVirUs Infection Requiring HospiTalization (BISCUIT) [17]. Results were compared to those of standard recommended therapy.

Organization and general characteristics of the trial

When the University Clinic of Moscow State University began to admit patients with the new coronavirus disease, we used standard temporary treatment protocols recommended by the Ministry of Health of the Russian Federation and the Moscow Health Department. However, not fully satisfied with the outcomes, we have developed the BISCUIT program. A detailed discussion of the concept and the trial protocol has been published earlier [17]. Both drugs are approved in the Russian Federation and indicated for treatment of respiratory infectious diseases (bromhexine) and as an antifibrotic agent (spironolactone). The protocol was approved on May 12, 2020 by the Ethics Committee of the Medical Research and Educational Center of

Moscow State University (MSU). Even if we were wrong that these drugs slow the progression of COVID-19, these drugs could not worsen the course of the disease. This is the difference between our treatment regimen and, e.g., hydroxychloroquine, azithromycin, and lopinavir/ritonavir, which have a broad range of serious adverse effects, including cardiac effects.

We had initially planned to conduct an open-ended, prospective randomized, controlled clinical trial of the combination of bromhexine (8 mg x 4 times a day) and spironolactone (25–50 mg/day) for 10 days in patients with moderate COVID-19 vs. standard recommended therapy [18]. However, the pandemic settings, our desire to improve the condition of potentially vulnerable patients as much as possible, and the rapidly emerging confidence of clinical physicians in the effectiveness of the regimen resulted in only 23 patients randomized in the study. Moreover, bromhexine was ordered openly in some patients, even before their hospital admission. Patients were then included without randomization. When the hospital of the MSU Medical Research and Educational Center was transferred from COVID-19 to regular operation, 33 patients were included who received the combination of bromhexine and spironolactone. Seventy patients have been included in the control group by that time. Thus, a total of 103 patients were treated under the program.

The inclusion criterion was confirmed new coronavirus disease. PCR data were avaible for 66 patients in control group and 32 in treatment group. 23 of 66 (34.8%) patients in the control group and 13 of 32 (40.6%) patients in the treatment group were SARS-CoV-2 RNA positive by PCR. The remainder had a typical MSCT pattern of viral pneumonia. The severity of lung damage according to the X-ray computed tomography (CT) was grade I–II (CT1 and CT2 following the temporary methodical guidelines of the Ministry of Health of the Russian Federation, versions 6–9). The C-reactive protein (CRP) levels should not exceed 60 mg/dl, and oxygen blood saturation was within 92–98%.

It was planned in the initial study to evaluate changes of the original SHOKS-COVID score first used in the WAYFARER trial as the primary endpoint [10]. This score, which includes clinical severity, inflammation (CRP), thrombosis risk (D-dimer), and lung damage severity (CT), showed the efficacy of hormone pulse therapy. We used it in this trial as well.

The number of days before normal temperature (<37°C), the number of days in hospital, and the three components of the SCOKS-COVID score (changes in CRP, D-dimer, and the percentage of lung damage on CT) were planned to be analyzed in the final analysis, as well as the SHOKS-COVID score. The elimination of the virus by the end of the treatment course (negative PCR for SARS-CoV-2 RNA) was evaluated as an additional exploratory endpoint.

Material and Methods

The characteristics of all 103 patients included in the trial (33 patients in the treatment group and 70 patients in the control group) are presented in Table 1a. (Additional materials for this article available on the journal's website). Given that the original groups were not balanced by several indicators, we performed an additional analysis to exclude differences in the groups, i.e., a propensity match score. The results of group comparison after this analysis are shown in Table 1.

As can be seen from Table 1, the groups were balanced. The median age was 53 (25–89) years, with an equal number of male and female patients. Most patients had a fever and reduced oxygen blood saturation and had clinically significant dyspnea. One patient in each group required oxygen support. The mean CRP was three times higher than normal, with a normal baseline coagulation profile (D-dimer). The percentage of lung damage in CT mainly corresponded to grade 1 (mild) severity of COVID-19-related pneumonia (CT1). The total risk of clinical manifestations (NEWS-2 score) and the total risk (SHOKS-COVID score) were moderate.

Treatment analysis showed that bromhexine was openly ordered as symptomatic therapy in about 36% of patients in the control group, which could slightly affect the intergroup differences. One in five patients in the control group received hydroxychloroquine. There were no significant differences in the administration of antibacterial therapy, anticoagulants, and anti-inflammatory drugs (colchicin, glucocorticosteroids) between the groups.

Laboratory biochemical testing of CRP, creatinine, urea, and glucose was performed with an automatic biochemical analyzer AU480 Beckman Coulter, Germany. Complete blood count (5 diff) was measured by a hematological analyzer XN 2000 Sysmex Corporation, Japan, and hemostasis analysis (fibrinogen, D-dimer) was performed in an automatic hemostasis analyzer STA-Compact Diagnostics Stago SAS, France.

Lung and chest computed tomography (CT) scans were produced using a 32 slice SOMATOM Scope CT scanner (Siemens, Germany). The scans were obtained with 1-mm slices. During the first examination, the standard CT protocol (tube voltage 120 kV, automatic tube current modulation 200–400 mA) was used. The later examinations were carried out using a low-

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Table 1. Baseline patient characteristics (propensity match scores)

Characteristics	Control, n=33	Bromhexine Spironolactone, n=33	p
General characteristics			
Age, years, mean (SD)	53.0 (13.4)	52.4 (17.0)	0.873
BMI, kg/m^2, Me [Q25; Q75]	26.9 [25.6; 30.6]	27.5 [25.0; 32.0]	0.840
Male, n (%)	15 (45.5%)	16 (48.5%)	1.000
Hypertension, n (%)	13 (39.4%)	9 (27.3%)	0.433
CAD, n (%)	1 (3.03%)	2 (6.06%)	1.000
Diabetes mellitus, n (%)	4 (12.5%)	1 (3.03%)	0.200
CHF, n (%)	0 (0.00%)	2 (6.06%)	0.470
COPD, asthma, n (%)	2 (6.06%)	1 (3.03%)	1.000
Clinical characteristics			
Temperature, median [25%; 75%]	37.7 [36.8; 37.9]	37.7 [36.6; 38.0]	0.852
RR, median [25%; 75%]	20.0 [17.0; 21.0]	20.0 [18.0; 20.0]	0.964
HR, bpm, mean (SD)	89.9 (16.1)	91.9 (12.1)	0.569
SBP, mmHg, median [25%; 75%]	120 [120; 132]	124 [119; 132]	0.902
SO_2, median [25%; 75%]	97.0 [95.0; 99.0]	96.0 [95.0; 99.0]	0.655
Biochemical characteristics			
CRP, mg/dL, median [25%; 75%]	15.2 [7.20; 23.5]	15.3 [6.07; 31.2]	0.974
D-dimer, μg/ml, median [25%; 75%]	0.35 [0.28; 0.68]	0.31 [0.18; 0.50]	0.273
Fibrinogen g/L, median [25%; 75%]	4.87 (1.15)	4.51 (1.10)	0.212
Lymphocytes 10^9/L, median [25%; 75%]	1.53 (0.41)	1.38 (0.52)	0.180
Neutrophils 10^9/L, median [25%; 75%]	3.39 [2.72; 4.31]	4.07 [2.31; 4.56]	0.509
Neutrophils/Lymphocytes, median [25%; 75%]	2.10 [1.64; 3.04]	2.52 [1.78; 4.32]	0.102
Platelets 10^9/L, median [25%; 75%]	208 [172; 250]	209 [169; 250]	0.748
Lymphocytes/CRP, median [25%; 75%]	110 [56.6; 232]	93.9 [44.1; 184]	0.705
Glucose, mmole/l, median [25%; 75%]	5.68 [5.10; 6.14]	5.80 [5.30; 6.93]	0.210
Creatinine, mmole/l, median [25%; 75%]	84.8 (15.2)	87.0 (19.0)	0.612
Potassium, median [25%; 75%]	4.10 [3.90; 4.40]	4.00 [3.90; 4.40]	0.890
GFR, ml/min/1.73 m^2 (CKDEpi), median [25%; 75%]	79.1 (12.9)	78.8 (15.8)	0.932
Lung lesion			
CT lesion (%), median [25%; 75%]	5.80 [2.50; 11.5]	7.60 [3.60; 13.4]	0.320
CT grade (%), median [25%; 75%]	1.00 [1.00; 1.00]	1.00 [1.00; 1.00]	1.000
Total severity score			
NEWS-2, median [25%; 75%]	2.00 [1.00; 4.00]	3.00 [1.00; 4.00]	0.222
NEWS-2, score, mean (SD)	2.84 (1.82)	2.75 (1.83)	0.838
SHOKS-COVID, score, median [25%; 75%]	4.00 [2.00; 5.00]	4.00 [3.00; 4.00]	0.922
SHOKS-COVID, score, mean	4.00 (1.59)	3.69 (1.64)	0.441
Therapy			
Bromhexine, n (%)	12 (36.4%)	33 (100%)	< 0.001
Spironolactone, n (%)	2 (6.06%)	33 (100%)	< 0.001
Antibiotics, n (%)	33 (100%)	29 (93.5%)	0.233
Colchicin or hormones, n (%)	6 (18.2%)	3 (9.09%)	0.489
Hydroxychloroquine, n (%)	7 (21.2%)	2 (6.06%)	0.155
Anticoagulants, n (%)	33 (100%)	33 (100%)	1.000

SD, standard deviation; BMI, body mass index; CAD, coronary heart disease; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; RR, respiratory rate; HR, heart rate; SO_2, oxygen saturation; CRP, C-reactive protein. Both mean and median values are provided for the NEWS2 and SHOKS-COVID scores.

dose CT protocol with reduced parameters of tube voltage (100 or 110 kV) and automatic tube current modulation (40–120 mA). The mean radiation exposure was 3.9±0.4 mSv under the standard protocol and 0.9±0.2 mSv under the low-dose protocol. CT scans were performed at the admission and discharge and repeated during the hospitalization period as deemed clinically necessary, but at least once every five days. All the scans were stored in DICOM format in the radiological information network (PACS/RIS) of the

MSU Medical Research and Educational Center. The CT scans were processed and analyzed in the Syngo.via workstations (Siemens). A semi-quantitative score for assessing the amount of infiltration and consolidation areas of the lung tissue was used to process and interpret CT findings, as was recommended by the Interim Guidelines of the Russian Ministry of Health «Prevention, Diagnosis and Treatment of New Coronavirus Disease (COVID-19) versions 7–9 (CT1 – CT4),» and by software for quantitative analysis of the COVID-19-related lung infiltrations, Multivox (Gammamed, Moscow) and Botkin.AI (Intelogic, Moscow).

We used two scores to objectify the severity of the clinical condition and to adequately assess effects of the therapy. The first one was the NEWS-2 distress syndrome severity score [19] updated for patients with COVID-19 [20]. The other one was our original clinical assessment score for patients with coronavirus disease (SHOKS-COVID) published earlier and referred to above [10].

Statistical Analysis

Statistical analysis was perfomed with R-studio software and programming language R. The normality of distributions was evaluated using the Shapiro-Wilk test.

Quantitative data are described as the median and interquartile range if non-normaly distributed or as the average and standard deviation if normally distributed. Qualitative indicators were compared between groups with the Mann-Whitney test for the non-normal distributions and the Student t-test for the normal distributions.

Qualitative data are presented as absolute and relative values. The significance of intergroup differences in qualitative characteristics was assessed using the χ^2 test and the two-way Fischer's exact test.

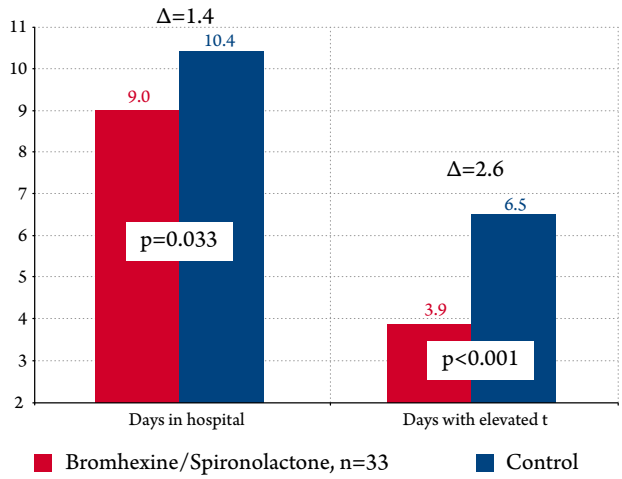
Changes of the parameters within each group were compared with the Wilcoxon signed-rank test for non-normal distributions and with the Student t-test for dependent samples with normal distributions. Nearest neighbor matching was used for the propensity match score.

The matching was carried out using the following baseline parameters: CRP and D-dimer levels, CT stage, temperature, SHOKS-COVID scores. The significance threshold for statistical hypotheses was p=0.05.

Results

Group-wide analysis showed statistically significant reductions in the duration of hospital stay from 10.4 to 9.0 days (by 1.4 days, p=0.033) and the duration of elevated temperature from 6.5 to 3.9 days (by 2.6 days, p<0.001), see Figure 1. Patients of the treatment group noted subjectively a significant decrease in cough, dyspnea, and chest pain, as well as faster normalization of temperature.

Figure 1. The number of days in hospital and days with elevated temperatures during the use of the combination of bromhexine and spironolactone vs. the control. Data for all patients



The main efficacy and safety analysis was performed in patient groups after the propensity matching. Figure 2 shows the changes in the SHOKS-COVID scores in both groups by day 10 of treatment, the primary endpoint of the trial. Statistically significant improvements were observed in both patient groups, with no differences between the groups.

Changes of the main indicators that characterize the severity of patients with COVID-19, including those included in the SHOKS score, are provided in Table 2. For both groups, the clinical condition of patients (RR, oxygen blood saturation, and NEWS-2 scores) significantly improved, the CRP levels normalized, and lymphocyte count increased by day 10.

There was no increase in the D-dimer levels during anticoagulant therapy, which can be associated with anticoagulant therapy in all patients. According to CT, disease progression and extension of lung damage was completely blocked. The two main indicators of autoimmune inflammation, neutrophil-to-lymphocytes ratio (NLR) and lymphocyte-to-CRP ratio (LCR), improved significantly in both groups. The latter increased more in the treatment group than in the control group (+367 vs. +252), but the differences were not significant.

Figure 3 shows the changes in the main secondary endpoint: days before temperature normalization and days in hospital. As can be seen, the normalization of

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Table 2. Changes in the main clinical, biochemical, and instrumental indicators during the use of bromhexine and spironolactone vs. the control. Propensity match score data

Indicator	Control, n=33		p	Bromhexine/Spironolactone, n=33		p
	Pretreatment	Treatment		Pretreatment	Treatment	
SHOKS-COVID, mean (SD)	4.56 (1.98)	2.39 (1.59)	0.007	3.67 (1.61)	2.12 (1.39)	<0.001
SHOKS-COVID, median [25%; 75%]	4.00 [2.00; 5.00]	2.00 [1.00; 3.00]	0.013	4.00 [3.00; 4.00]	2.00 [1.00; 3.00]	<0.001
RR per minute, median [25%; 75%]	20.0 [17.0; 21.0]	17.0 [16.0; 18.0]	0.001	20.0 [18.0; 20.0]	17.0 [16.0; 18.0]	0.001
SaO_2, median [25%; 75%]	97.0 [95.0; 99.0]	99.0 [98.0; 99.0]	0.001	96.0 [95.0; 99.0]	98.0 [97.0; 99.0]	0.077
CRP, mg/dL, median [25%; 75%]	15.2 [7.20; 23.5]	4.32 [2.42; 9.91]	<0.001	15.3 [6.07; 31.2]	4.09 [2.36; 8.19]	<0.001
D-dimer, μg/ml, median [25%; 75%]	0.35 [0.28; 0.68]	0.36 [0.22; 0.72]	0.900	0.31 [0.18; 0.50]	0.28 [0.19; 0.58]	0.850
CT lesion (%), median [25%; 75%]	5.80 [2.50; 11.5]	6.60 [2.60; 13.7]	0.577	7.60 [3.60; 13.4]	7.35 [3.15; 14.2]	0.951
Lymphocytes 109/l, median [25%; 75%]	1.45 [1.22; 1.67]	1.84 [1.47; 2.41]	<0.001	1.34 [0.98; 1.67]	2.04 [1.43; 2.33]	<0.001
Neutrophils/Lymphocytes, median [25%; 75%]	2.10 [1.64; 3.04]	1.31 [0.96; 1.55]	<0.001	2.52 [1.78; 4.32]	1.43 [1.13; 1.84]	<0.001
Lymphocytes/CRP, median [25%; 75%]	110 [56.6; 232]	362 [192; 954]	<0.001	93.9 [44.1; 184]	461 [205; 1189]	<0.001
News-2, medium (CO)	2.33 (1.85)	1.00 (1.37)	<0.001	2.85 (1.89)	1.48 (1.70)	<0.001
News-2, median [25%; 75%]	2.00 [1.00; 4.00]	0.00 [0.00; 2.00]	<0.001	3.00 [1.00; 4.00]	1.00 [0.00; 3.00]	0.001

SD, standard deviation; RR, respiratory rate; SO2, oxygen saturation; CRP, C-reactive protein.

Table 3. Changes of the main clinical, biochemical, and instrumental indicators during the use of bromhexine and spironolactone starting from the first day or later

Indicator	Therapy from day 1, n=23		Therapy initiated after day 1, n=10		p between the groups, before and after the treatment
	Pretreatment	Treatment	Pretreatment	Treatment	
SHOKS-COVID score, median [25%; 75%]	3.00 [2.00; 4.00]	2.00 [1.00; 2.75]	4.50 [4.00; 5.75]	2.00 [1.00; 2.50]	0.006/0.898
SHOKS-COVID, mean (SD)	3.13 (1.25)	2.06 (1.26)	4.90 (1.73)	2.29 (1.80)	0.002/0.729
Δ baseline – treatment	–1.07 (1.45) [0.008]		–2.61 (1.50) [0.009]		0.012
HR, bpm, mean (SD)	18.7 (1.91)	17.5 (1.27)	20.4 (2.12)	18.0 (2.40)	0.047/0.558
Δ baseline – treatment	–1.26 (2.22) [0.019]		–2.40 (3.31) [0.037]		0.332
SaO_2, %, mean (SD)	97.1 (1.81)	97.6 (1.20)	95.6 (1.96)	96.9 (3.93)	0.032/0.601
Δ baseline – treatment	0.52 (2.27)		1.30 (3.86)		0.564
CRP, mg/dL, median [25%; 75%]	9.75 [4.89; 17.5]	3.71 [1.52; 5.38]	39.8 [20.6; 65.2]	6.87 [3.92; 9.22]	0.001/0.117
Δ baseline – treatment	–6.04 [–11.75; –0.54]		–32.90 [–56.31; –12.50]		0.003
D-dimer, μg/ml, median [25%; 75%]	0.25 [0.14; 0.43]	0.24 [0.18; 0.38]	0.44 [0.32; 0.52]	0.37 [0.24; 0.76]	0.048/0.160
Δ baseline – treatment	0.01 [–0.01; 0.10]		–0.07 [–0.27; –0.04]		0.058
CT lesion (%), median [25%; 75%]	5.90 [2.80; 12.2]	7.35 [3.15; 13.7]	10.8 [6.85; 15.1]	9.75 [5.47; 21.2]	0.170/0.573
Δ baseline – treatment	1.45 (9.42)		–1.05 (12.4)		0.967
Lymphocytes 109/l, median [25%; 75%]	1.47 (0.53)	1.97 (0.56)	1.16 (0.47)	1.78 (0.58)	0.111/0.397
Δ baseline – treatment	0.50 (0.56)		0.62 (0.49)		0.545
Neutrophils/Lymphocytes, median [25%; 75%]	2.29 [1.55; 4.11]	1.43 [1.03; 1.71]	3.51 [2.53; 6.38]	1.43 [1.31; 2.71]	0.078/0.196
Δ baseline – treatment	–0.86 (1.98)		–2.08 (2.59)		0.211
Lymphocytes/CRB, median [25%; 75%]	148 [77.3; 360]	551 [379; 1309]	35.8 [14.3; 55.9]	230 [184; 460]	0.001/0.002
Δ baseline – treatment	397 [54.6; 594]		205 [148; 366]		0.457
Potassium, mmole/l, medium (SD)	4.21 (0.34)	4.70 (0.37)	3.50 (1.17)	4.61 (0.50)	0.069/0.635
Δ baseline – treatment	0.49 (0.43) [0.000036]		1.11 (1.33) [0.018]		0.181

SD, standard deviation; RR, respiratory rate; SO_2, oxygen saturation; CRP, C-reactive protein.

temperature during the treatment with bromhexine and spironolactone occurred by day 4, which is significantly faster by 2 days than in the control group. The results questioned the correctness of choosing a single time endpoint, i.e., 10 days of treatment, since significant differences between the groups were noted earlier and could disappear by day 10. This is also known from other trials [21, 14].

Thus, we performed additional tests, including the virus detection using PCR. Baseline data were available for 62 of 66 matched patients at the enrolment and in 30 patients (13 control and 17 treatment) by the end of the trial since some were discharged before day 10. As seen in Figure 4, the elimination of the virus by day 10 in the treatment group was reported in all patients, and 3 of 13 (23.1%) control patients still had viremia. Differences were not statistically significant, but there was an obvious trend in favor of the combination of bromhexine and spironolactone (p=0.077).

The number of patients who reached the combined end point of a positive PCR to the SARS-CoV-2 virus on the 10th day of hospitalization or had longer (≥10 days) hospitalization was lower in the treatment group (treatment vs control: 14/24 (58.3%) vs. 20/21 (95.2%), p=0.012). Limitation of this analysis was that data for this analysis were avaible only for 45 patients (24 treatment and 21 control) due to absent of PCR data in some patients. As we had lack data of PCR at 10th day we did the analysis of last PCR test before discharge. This analysis include 41 patients. The PCR tests for SARS- CoV-2 before discharge were positive in 3 (15.8%) control patients and positive in 0 (0%) treated patients (p=0.097).

The analysis of time to the initiation of treatment with the combination of bromhexine and spironolactone was also of interest (Table 3). In the treatment group, treatment started on day 1 of hospital stay in 23 patients and, on average, on day 3.5 [2,0; 5,0] or 2.5 days later in the remaining 10 patients.

The mean SHOKS-COVID score was 3.13 when treatment started on day 1 of hospital stay vs. 4.90 (p=0.012)

Figure 2. Changes of SHOKS-COVID scores (primary endpoint) during the use of the combination of bromhexine and spironolactone vs. the control. Propensity match score data

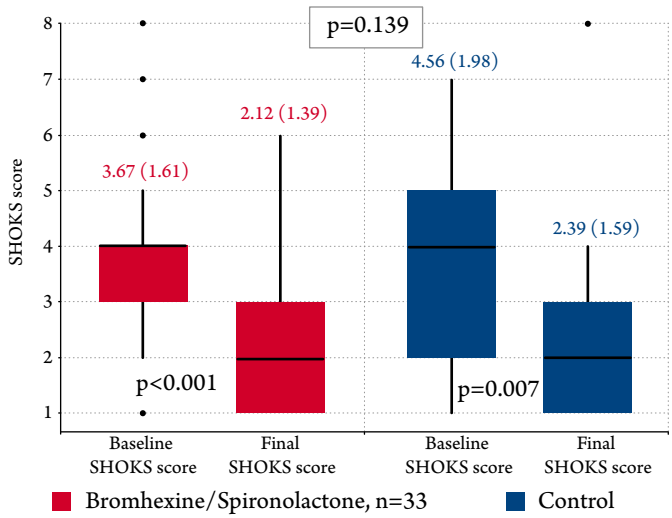


Figure 3. The number of days with elevated temperatures and days in hospital during the use of the combination of bromhexine and spironolactone vs. the control. Propensity match score data

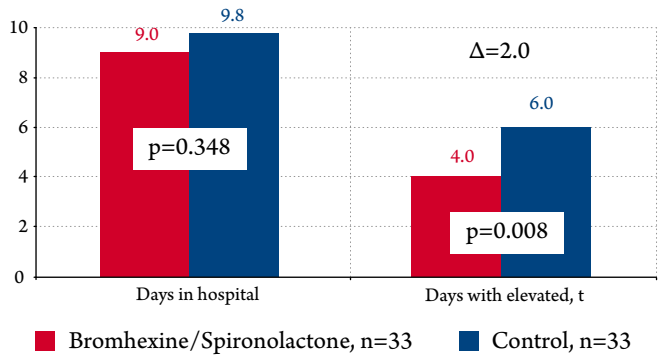
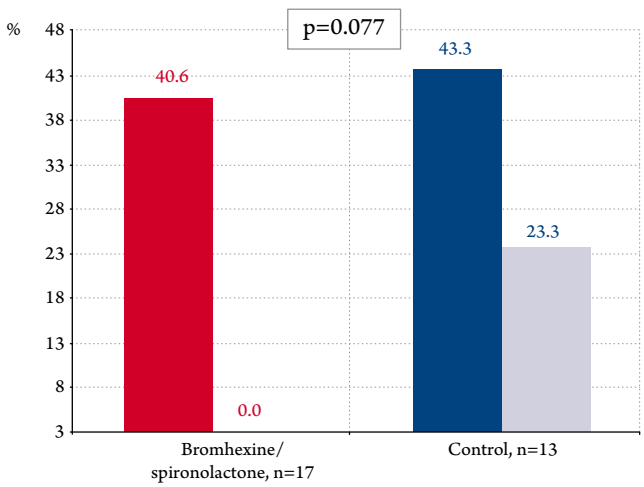
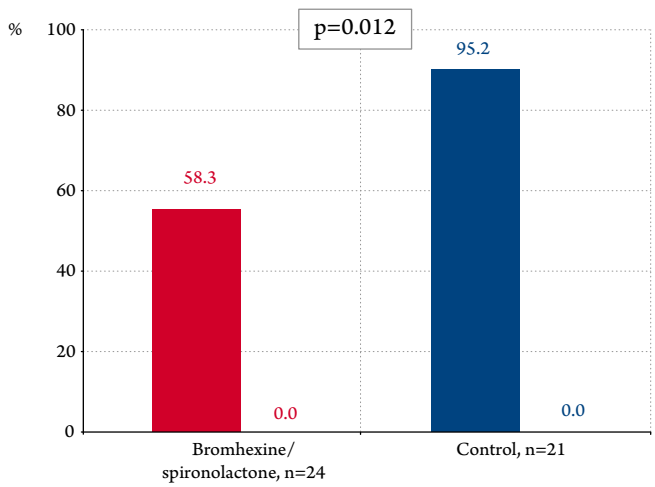


Figure 4. Changes in the virus detection (PCR test) and the combination of positive PCR on day 10 and longer (>10 days) hospital stay during the use of the combination of bromhexine and spironolactone vs. the control. Propensity match score data



(+) PCR reaction for SARS-Cov-2 RNA



(+) PCR reaction and hospitalization > 10 days

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with later initiation of therapy, also oxygen saturation was 98% and 95% (p=0.032), and shortness of breath was 18.7 and 20.4 breaths per minute (p=0.047), respectively. The CRP levels increased 408% from 9.75 to 39.8 mg/dl (p<0.001) in patients with later treatment initiation, and LCR decreased from 148 to 35.8 (p<0.001), which was indicative of a severe exacerbation of systemic inflammation. D-dimer was within the normal range in the group with delayed start of therapy with bromhexine and spironolactone, but significantly higher than in the comparison group (p=0.048). The mean severity of lung damage in CT was also higher with later initiation of bromhexine and spironolactone, 10.7% vs. 5.9%, but the difference was not statistically significant. Moreover, there were noticeable, although not statistically significant, differences in plasma potassium, 4.20 mmole/l when treatment started on day 1 of hospital stay and 3.51 mmole/l (p=0.069) when treatment was delayed to after day 1. Treatment was effective in both groups, with significantly lower SHOKS-COVID scores (p=0.012) in case of the delayed treatment and no differences by day 10. The same applied to clinical manifestations. There were no differences in RR and oxygen saturation between the groups by day 10. The time to temperature normalization was 4 [3; 4] days at the early initiation of treatment and 3.5 [2.25; 4.75] days when the treatment was delayed (p=0.786). However, non-steroidal anti-inflammatory drugs were used in 77.8% of patients whose treatment started later vs. 22.7% in the group of treatment initiation from day 1 (p=0.007). There were also noticeable differences in the number of days in the hospital. In the group who started treatment on day 1, patients were discharged at 8.26 days, and in the delayed treatment group at 10.6 days (p=0.088).

The most distinct difference was observed for the baseline CRP levels. However, these striking differences almost disappeared during the treatment, as the decrease was much more noticeable in the delayed treatment group (p=0.003). The CRP levels reduded by 83% of baseline level and almost normalized with the later start of treatment. Moreover, LCR, another indicator of inflammation, increased significantly in both groups, but the intergroup differences remained statistically significant.

There were differences in the other indicators between the groups at the end of the observation on day 10. Most remarkably, plasma potassium significantly increased and normalized in both subgroups, from 4.21 to 4.79 mmole/l when the treatment started on day 1 of the hospital stay and from 3.50 to 4.61 mmole/l when the bromhexine and spironolactone were initiated later.

No serious adverse events were reported in the bromhexine/spironolactone group during treatment, compared to 4.3% in the control group.

Discussion

The only treatment for patients with COVID-19 and bilateral pneumonia that can have a beneficial effect on the course of the disease and its prognosis is presently anti-inflammatory therapy with glucocorticosteroids [10, 13, 15, 22]. However, attempts continue to prevent the progression of the new coronavirus disease at relatively early stages. Unfortunately, the hopes for hydroxychloroquine and the non-specific antiviral drug, lopinavir/ritonavir, came to nothing in the RECOVERY prospective randomized clinical trial (RCT) [2, 16, 23]. Not only did these drugs not shorten the course of the disease or prevent mortality, they also had serious adverse side effects. Another promising antiviral drug, remdesivir, also disappointingly lacks strong evidence of beneficial effect in COVID-19. The largest (405 hospitals in 30 countries, 11,266 patients, 253 lethal outcomes) WHO-supported RCT SOLIDARITY study reported remdesivir to be ineffective: odds ratio of death was 0.95 (0.8–11.11, p=0.50; 301/2743 in the remdesivir group vs. 303/2708 in the control group); the same was found for hydroxychloroquine: odds ratio of death was 1.19 (0.89–1.59, p=0.23; 104/947 in the hydroxychloroquine group vs. 84/906 in the control group). For lopinavir/ritonavir the odds ratio of death was 1.00 (0.79–1.25, p=0.97; 148/1399 vs. 146/1,372 in the control group). For interferon beta-1a the odds ratio of death was 1.16 (0.96–1.39, p=0.11; 243/2050 vs. 216/2050 in the control group) [24, 17]. There are no convincingly positive findings of controlled trials of favipiravir. In the first Chinese study, favipiravir improved clinical condition more significantly than arbidol by day 7 of treatment (p=0.02). The duration of hyperthermia decreased only by 1.7 days and cough by 1.75 days, without statistically significant differences in the number of patients who required oxygen support, transfer to the intensive care unit (ICU), and mechanical ventilation [25, 18]. Moreover, 13.8% of patients who received favipiravir had serious adverse reactions, including elevated uric acid levels. The second study of favipiravir (35 patients) showed faster elimination of the virus compared to lopinavir/ritonavir, from a mean of 11 to 4 days, and adverse reactions in 11.4% of patients [26, 19].

However, the press statement of the Japanese controlled trial of 156 patients treated with favipiravir suggested that the acute stage of the disease might be reduced by 2.8 days [7, 32, www.pharmaceutical-technology.com/news/fujifilm-

avigan-covid-data/#:~:text=Japan's%20Fujifilm%20Toyama%20Chemical%20plans,in%20Japan%20to%20treat%20influenza].

In any case, these agents, like any antiviral drug, are the more effective, the earlier they are used [27, 20]. Unlike seasonal flu, viroemia in COVID-19 usually precedes the onset of symptoms, which is why the start of therapy is delayed.

Thus, the present trial focused on the use of drugs that can block or slow the entry of SARS-CoV-2 virus into cells and reduce the rate and speed of viral replication. It should be noted now that almost all patients in both groups were treated with low-molecular-weigh heparins. The trial was based on the search of available and safe drugs that are expressly recommended for the treatment of respiratory infectious diseases, pneumonia, and pulmonary fibrosis. The symptomatic use of the well-known antitussive and mucolytic drug bromhexine in pneumonia is beyond doubt reasonable. Its additional property of blocking TMPSS-2, which is responsible for the entry of SARS-CoV-2 virus into alveolar epithelial cells, was detected several years ago [28, 21]. Recent experimental studies have confirmed that bromhexine can block the activity of the main cellular receptor ACE2 [29, 22]. Moreover, the most recent controlled trial of bromhexine in 78 patients demonstrated a statistically significant decrease in the rate of transfer to the ICU (2/39 vs. 11/39, p=0.006), mechanical ventilation (1/39 vs. 9/39, p=0.007), and decrease in mortality (0 vs. 5, p=0.027) [30, 23].

Spironolactone, a well-known available antifibrotic drug with minimal side effects when used in low doses, has similarly shown ability to diminish the process of pulmonary damage in experimental and drug-induced alveolitis [13, 31, 9, 39, http://ijrr.com/article-1–2461-en.html].

Moreover, 37% of patients with COVID-19 have hypokalemia (3–3.5 mmole/l), and another 18% have severe hypokalemia (<3.0 mmole/l), which is associated with the severity of the inflammatory process and the prognosis [32, 24]. The overactivation of the renin-angiotensin-aldosterone system is supposed to be a counter-regulatory response to ACE2 receptor blockade by the virus through hypokalemia associated with inflammatory factors. Spironolactone has a pronounced antialdosterone and potassium-sparing effects. In some cases, the effect is even excessive [33, 25].

The activation of ACE2 receptors and TMPRSS2 is more pronounced in men, explaining the more severe course of the new coronavirus disease in male patients [16, 11]. The high levels of ADAM17 receptors responsible for the ACE2 clearance are observed in the testicles and prostate and decrease with age, which is associated with a decrease in testosterone synthesis [34, 40, https://www.proteinatlas.org/ENSG00000151694-ADAM17/tissue]. As well as stimulating the expression of ACE2, male sex hormones, testosterone and dihydrotestosterone, activate androgen receptors responsible for the increased expression of TMPRSS2 necessary for SARS-CoV-2 endocytosis and entry into cells [35, 26]. The Spanish trials suggested a faster and more aggressive course of the new coronavirus disease in male patients with high testosterone levels as reflected by baldness, excessive face and body hair, acne. There was an adverse prognosis in this category of patients [15, 10]. Androgen receptors are known to be present in the pulmonary tissue, partly explaining the high prevalence of male lung cancer [36, 27]. No wonder that cancer significantly increased the risk of positive PCR for SARS-CoV-2 by 79% (p<0.0001) in male patients, and prostate cancer increased mortality of COVID-19 more than 100% [37, 28]. The risk of COVID-19, including a severe form of the disease, decreased 99.8% (p<0.0001) in patients receiving androgen deprivation therapy, which suppresses synthesis of sex hormones.

As well as blocking the mineralocorticoid receptors, spironolactone influences androgen receptors [38, 29]. Due to the additional antiandrogenic effect of reducing testosterone levels, this drug causes the development of gynecomastia, which is a side effect in the treatment of cardiac patients [39, 30]. However, while reducing the effects of testosterone and dihydrotestosterone, spironolactone in COVID-19 can simultaneously block two key components of the SARS-CoV-2 entry into cells: ACE2 receptors and auxiliary protease TMPRSS2 [40, 41, 31, 41, Cadegiani F et al., www.researchgate.net/publication/341103985].

The BISCUIT findings confirmed our hypothesis. Therapy with the combination of bromhexine and spironolactone reduced significantly the time to temperature normalization by 2.6 days in all subjects and by 2 days or by half in patients after group matching (p<0.001). This was accompanied by a rapid (by day 4) improvement in the clinical condition, such as reduction of cough, the feeling of chest tightness and congestion without any adverse reactions.

Simple trial protocols, which may still be highly burdensome for health care professionals, are needed due to the difficulties of managing patients with COVID-19, which has an unpredictable and rapidly progressive course. This is why we planned only two points in the BISCUIT protocol: start and end of the 10-day treatment. Retrospectively, it is obvious that an additional intermediate test on days 4–5 could haved revealed earlier and more significant benefits of

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bromhexine and spironolactone therapy compared to the standard management of patients with COVID-19. Other researchers have reported similar observations [21, 14]. We observed no side effects in the bromhexine/spironolactone group under our protocol compared to 4.33% in the control group. This therapy also unloaded the staffs' burden by reducing the number of electrocardiograms with QT-interval calculations, as is relevant for hydroxychloroquine, azithromycin, and lopinavir/ritonavir. In the analysis of all 103 patients included in the study, those patients treated with bromhexine and spironolactone were discharged significantly sooner (by 1.5 days, p=0.033). There was a trend to more rapid virus elimination in the treatment group compared to the control group day (treatment vs control: 0% vs 23%; p=0.077). However, follow-up PCR data were avaible only in 30 patients (13 control and 17 treatment). The number of patients who reached the combined end point of a positive PCR to the SARS-CoV-2 virus on the 10th day of hospitalization or longer (≥10 days) hospitalization was lower in the treatment group (treatment vs control: 14/24 (58.3%) vs. 20/21 (95.2%), p=0.01, Odds ratio 0.07 (95% CI: 0.008–0.61, p=0.016). However, this analysis included 45 (24 treatment and 21 control) of 66 matched patients due to anavalibity of follow-up PCR data in some patients. We were also encouraged because another bromhexine trial produced noticeable positive results, as mentioned earlier [30, 23].

Days to temperature normalization, days to the recovery from the acute stage of the disease, and the rate of virus elimination are used as the endpoints in most trials designed to treat the new coronavirus disease, especially in the early stages of the disease. We tried to use the composite indicator, SHOKS-COVID score used in the WAYFARER trial to treat severe patients with COVID-19 with glucocorticosteroids, as the primary endpoint [10, 13]. This score, which includes clinical severity, inflammation (CRP), thrombosis risk (D-dimer), and lung damage severity (CT), showed the efficacy of hormone pulse therapy. In this trial, the SHOKS-COVID scores were significantly correlated with the number of days in hospital and the number of days with elevated temperature, which allowed us to positively assess the significance and adequacy of this tool.

In this study, changes in the SHOKS-COVID score also was indicative of a significant improvement in both patient groups. However, this indicator proved insufficiently sensitive to identify differences in the changing condition in two groups of patients with COVID-19 at early stages, especially only with two prefixed trial points. Nevertheless, even in the absence of statistically significant differences in all the studied indicators, we can state that the therapy with bromhexine and spironolactone carries no risk of adverse events and is effective in patients with the new coronavirus disease.

The second perspective of this trial was a comparative analysis of this combination's efficacy from day 1 of hospital stay or if treatment was delayed up to 2.5 days. Most importantly, however, therapy with bromhexine and spironolactone not only preserved efficacy but also proved more effective in patients with more severe symptoms whose treatment started later. By the end of the observation on day 10, those patients whose treatment started from day 1 and those not from day 1 were similar in most parameters (Table 3). They had a statistically more significant decrease in CRP, SHOKS-COVID score, and normalized plasma potassium. However, the most sensitive indicator, LCR, remained higher in patients with a delayed start of the treatment. This highlights the usefulness of early treatment, but it does not offset the positive effects of bromhexine/spironolactone even with delayed start of the treatment, unlike that of antiviral drugs.

Conclusion

There were similar significant improvement in the SHOKS-COVID scores in the bromhexine and spironolactone and the control groups day 10 of treatment which was the primary endpoint of the trial. In the same time the combination of bromhexine with spironolactone appeared effective in treating a new mild to moderate coronavirus infection by achieving a faster normalization of the clinical condition, lowering the temperature one and a half times faster, and reducing explanatory combine endpoint the viral load or long duration of hospitalization (≥ 10 days).

Limitations

The study was limited by the absence of appropriate randomization, incomplete PCR data by day 10 of the treatment, and open-label ordering of bromhexine to some patients in the control group.

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