Aprotinin is a potent multi-target drug for the combination therapy of moderate COVID-19 cases.

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Summary

We demonstrated for the first time high efficacy of Aprotinin in combination therapy - none of moderate patients hospitalized with COVID-19 were transferred to the ICU for ALV or NIV. All the patients were discharged from the hospital and no adverse events were recorded.

Abstract

Background. COVID-19 is a contagious multisystem inflammatory disease caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We have studied the efficacy of Aprotinin (nonspecific serine proteases inhibitor) and Avifavir or Hydroxychloroquine (HCQ) combinations for the therapy of COVID-19.

Methods. Three prospective single-center (cohorts 1 – 3) studies included participants with moderate COVID-19-related pneumonia, laboratory-confirmed SARS-CoV-2 and admitted to the hospitals. Patients received combinations of intravenous (IV) Aprotinin (1,000,000 KIU daily, 3 days) and HCQ (cohort 1), inhalation (inh) treatment with Aprotinin (625 KIU 4 times per day, 5 days) and HCQ (cohort 2) or IV Aprotinin (1,000,000 KIU daily for 5 days) and Avifavir (cohort 3).

Results. In the cohorts 1 – 3, the combination therapy showed 100% efficacy in preventing the transfer of patients (n = 30) to the intensive care unit (ICU). The effect of combination therapy in the cohort 3 was the most prominent and the median time to SARS-CoV-2 elimination was 3.5 days (IQR 3.0 – 4.0), normalization of CRP concentration was 3.5 days (IQR 3 – 5), of D-dimer concentration - 5 days (IQR 4 – 5); body temperature - 1 day (IQR 1 – 3), improvement in clinical status or discharge from the hospital - 5 days (IQR 5 – 5), and improvement in lung lesions of patients on 14 day - 100%.

Conclusions. The administration of Aprotinin combinations prevented the transfer of moderate COVID-19 patients to the ICU for mechanical ventilation (ALV) or non-invasive ventilation (NIV) and by shortening of their hospital stay.

Introduction

SARS-CoV-2 spread globally and for the start of November 2020 the total number of infections in the world exceeded 46 million in 190 countries, with more than 1.198 million dead [1]. Given that COVID-19 poses a serious threat to public health and the economy around the world, the urgent need exists for a new effective drugs for the treatment and prophylaxis of SARS-CoV-2 infections.

COVID-19 is a multisystem disease as SARS-CoV-2 infects cells using an angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in different tissues - lungs, kidneys, gastrointestinal tract, liver, vascular endothelial and arterial smooth muscle cells [2]. COVID-19 prognosis has largely been influenced by multiorgan involvement, largely due to the elevated concentration of fibrinogen and D-dimer in patients' blood, which activates hypercoagulation. D-dimers are not normally present in human blood plasma, their level became elevated due to the formation and degradation of fibrin clot in vivo, when the coagulation system has been activated, for instance because of the presence of thrombosis or disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT), pulmonary embolism, ect [3]. Therefore elevated D-dimer along with C-reactive protein (CRP) have a high predictive value for venous thromboembolism (VTE) in critically ill COVID-19 patients. In several studies authors have monitored an association between COVID-19 pneumonia and VTE [4]. It was suggested that the prevalence of VTE was 25%, with a sensitivity, specificity and negative predictive value of D-dimer cut-off value of 1.5 μg/mL [5]. CRP is another valuable marker to anticipate the possibility of aggravation of mild COVID-19 - the exacerbated patients have much higher levels of CRP (median 43.8 mg/L, IRQ 12.3 – 101.9) as compared to mild (median 12.1 mg/L, IRQ 0.1 – 91.4) COVID-19 patients [6]. Higher plasma CRP levels indicate severe COVID-19 pneumonia and a longer stay in hospital [7].

Currently, two broad categories of drugs for COVID-19 are in clinical use: (1) drugs that can directly target SARS-CoV-2 virus replication cycle and (2) drugs inciting innate antiviral immune responses or alleviating damage induced by the dysregulated inflammatory responses [8]. The SARS-CoV-2 replication inhibitors Favipiravir (Avifavir) [6, 9] and Remdesivir (Veklury®) [9] were among the first

drugs repurposed for COVID-19 treatment. Timely use of these antiviral drugs (up to 3-7 days after infection) have prevented the progression to more severe respiratory disease.

Due to the complex nature of SARS-CoV-2 pathogenesis and multiorgan involvement, combination of direct virus-targeted and host-targeted drugs is clinically beneficial for the therapy of COVID-19. One of the promising drug candidates for the combination therapy of COVID-19 is Aprotinin, a natural protease inhibitor with a long history of clinical use since 1960s and good safety profile [10]. Firstly, Aprotinin is a broad-spectrum antiviral drug as it is a nonspecific inhibitor of the serine proteases, especially trypsin, chymotrypsin, plasmin and kallikrein. The inactivation of kallikrein blocks factor XIIa formation and had a negative impact on the intrinsic pathway of coagulation, fibrinolysis and thrombin generation, leading to the attenuation of the pro-inflammatory response [11-13]. Secondly, Aprotinin possesses a specific mechanism of action for SARS-CoV-2 as it inhibits transmembrane serine protease 2 (TMPRSS2), a host cell protease responsible for the cleavage and activation of the spike protein of SARS-CoV-2. This mechanism of Aprotinin's action suggests that it can prevent SARS-CoV-2 penetration into susceptible cells [14]. Recently, it was shown that Aprotinin also inhibits replication of SARS-CoV-2 by downregulating cellular protease during replication cycles [15].

These data raise the hypothesis that Aprotinin is a typical multi-target drug or a "magic shotgun" [16] and can represent an efficient treatment for moderate and severe forms of COVID-19. Our first pilot clinical trials confirmed this assumption and showed that Aprotinin is effective as a drug candidate for the prevention [17] and treatment [18] of COVID-19. Therefore, we conducted a prospective clinical study and evaluated the efficacy of Aprotinin and HCQ or Avifavir combination therapy for moderate COVID-19 patients.

Methods

Study design and patients

An open non-comparative study of the efficacy and safety of Aprotinin in patients hospitalized with COVID-19 was based on the recommendations of the WHO research and development program [19] and the Russian Ministry of Health [20]. Patients aged 18 and older, admitted to hospital with moderate COVID-19-related pneumonia (Table 1) and confirmed SARS-CoV-2 by either a positive result from a RT-PCR or CT lung scan. The study was conducted starting June 11 till August 7, 2020 at Smolensk Clinical Hospital, Russia. The COVID-19-aprotinin-01 protocol dated June 1, 2020 and the amendment to the protocol dated June 25, 2020 were approved by the Independent Ethics Committee of Smolensk Clinical Hospital (protocols no. 38 from June 2 and July 2, 2020, respectively) and registered at the U.S. National Library of Medicine [NCT04527133]. Exclusion criteria are presented in appendix.

Efficacy end points

The primary efficacy end point of the study was laboratory-confirmed time to normalization of the following parameters: elimination of SARS-CoV-2 (defined as two negative results from a RT-PCR assay with at least a 24-hour interval); CRP and D-dimer concentrations.

Key secondary clinical end points were: time to body temperature normalization (< 37°C); changes from baseline of the laboratory parameters during 14 days which included hematology: CRP values, coagulogram; changes from baseline of lung parenchyma on tomography chest CT scan on days 7 and 14; frequency of clinical status improvement by 2 scores in accordance with the WHO Ordinal scale of clinical improvement (WHO-OSCI) or discharge from the hospital before day 14; frequency of transfer to the ICU, frequency of the NIV, frequency of the invasive ventilation; mortality rate; frequency of adverse events and serious adverse events of various severity according to subjective complains, physical examination, vital signs, laboratory tests and ECG.

Procedures

Participants were divided into 3 cohorts by 10 patients: cohort 1 – received combinations of Aprotinin (IV) (Gordox® 1,000,000 KIU daily, 3 days) and HCQ (400/200 mg, twice a day, 5 – 6 days) and SOC; cohort 2 – received combinations of Aprotinin (inh) (Gordox® 625 KIU 4 times per day, 5 days) and HCQ (400/200 mg, twice a day, 5 – 6 days) and SOC; cohort 3 – received combinations of Aprotinin (IV) (Gordox® 1,000,000 KIU daily, 5 days) and Avifavir (2000 mg twice on the first day, then 800 mg twice a day for 10 days) and SOC. Patients in the cohorts 1 – 3 received thromboembolic prophylaxis with an anticoagulant enoxaparin (40 mg, once a day, 14 – 15 days). Patients with a score of 4 on the WHO-OSCI (table 1) had supportive oxygen therapy via nasal cannula or face mask. None of the patients had invasive or NIV mechanical ventilation at baseline.

The data for cohorts 1 – 3 were extracted from the clinical databases (ClinCapture, version 2.2.3) independently by investigators and uploaded into an electronic case report form. The information was collected confidentially and the computerized file used for this study was implemented according to the local cohort statements responding to all the regulatory points in accordance with the Russian regulations (law 152 FZ of July 27, 2006 N, relating to data processing, files, and freedoms) and the European General Data Protection Regulation regarding patient information and confidential treatment of all data. All data were monitored by a representative clinical team of IPHARMA LLC. The database was frozen for statistical analysis on August 10, 2020.

Clinical manifestations, including persistent fever > 38°C, respiratory rate, oxygen saturation, oxygen therapy requirement and biological parameters, including CRP, D-dimer, neutrophil, lymphocyte, and platelet counts, INR, prothrombin and fibrinogen, were recorded at baseline and at discharge from the hospital. The median time to improve the clinical state by 2 points was determined according to the WHO-OSCI. Not to be resuscitated meant that patient was not eligible for transfer to the ICU. Chest CT was done with a single inspiratory phase with patients in the supine position. Radiologists classified the CT scan as typical, equivocal, or negative for COVID-19 and described the main CT features: ground glass opacity, crazy-paving pattern and consolidation. A semi-quantitative scoring system was used to

estimate the pulmonary involvement of the observed abnormalities based on the area involved: mild (< 25%), moderate (25 – 50%), severe (51– 75%) or diffuse (> 75%) involvements [21].

Statistical analysis

The sample size was based on the exact single stage Phase II assessment at one-sided α=0·05 and 80% power [22]. Continuous variables with a normal distribution were expressed as mean (SD) and with a non-normal distribution as median with interquartile range (IQR) and compared using 2-tailed, paired *t* test for parametric data and Wilcoxon rank-sum test for nonparametric data. The categorical variables were presented as absolute and relative (in percentage) frequency and compared using chi-square test. The effect of Aprotinin for COVID-19 patients was done on a full analysis set (including all patients who signed informed consent and received at least one administration of study drug). The efficacy time points (time to viral clearance, time to CRP normalization (≤ 10 mg/L), time to D-dimer normalization (<253 ng/mL), time to temperature normalization (< 37°C), time to improvement in clinical status) were done using Kaplan-Meier curves. Groups comparison was used log-rank test (p-value < 0.05 was considered significant). We used the R (version 3.6.2) for analyses.

Results

We identified combination therapy with Aprotinin (IV) + Avifavir in association with SOC as beneficial for CQVID-19 patients at primary and secondary efficacy points. In particular, median time to SARS-CoV-2 elimination was 3.5 (IQR 3-4) days for cohort 3, and 7.5 (IQR 6-9) and 9 (IQR 5-9) days for cohorts 1 and 2 (the difference is statistically significant p=0.019 and p=0.006 compared to patients from cohorts 3, Table 2, Figure 1A). The median time to CRP normalization (≤ 10 mg/L) was 3.5 (IQR 3-5) days for cohort 3, and 6 (IQR 6-6) days and 4 (IQR 3-5) days for cohorts 1 and 2 respectively. The difference between cohorts 1 and 3 is statistically significant (p<0.001, Table 2, Figure 1B).

The efficacy of Aprotinin combinations for the normalization of thrombosis markers (D-dimer and fibrinogen) in patients' blood are presented in Table 2 and Figure 2. Initially increased D-dimer levels (525 – 855 ng/mL, Table 1) quickly returned back to normal within median 4.5 (IQR 3 – 6), 9 (IQR 5 – 9) and 5 (IQR 4 – 5) days for cohorts 1, 2 and 3 patients, respectively (Table 2, Figure 2A). The difference between cohorts 2 and 3 is statistically significant (p=0.002). Before initiation of Aprotinin combination therapy, patients in cohorts 1 – 3 had elevated fibrinogen levels (5·0 – 9·8 g/L, Table 1), which returned back to normal on Day 4, presumably as a result of the therapy (Figure 2B). The dynamics of changes in indicators of INR (prothrombin, neutrophils, and leukocytes) defined normal both when patients were admitted and discharged from the hospital (Figure 3).

The median time to normalization of body temperature (< 37ºC) for patients in cohorts 1 – 3 was 3.0 (IQR 2 – 3), 4.5 (IQR 3 – 5), and 1.0 (IQR 1 – 3) days (Table 2), respectively. The difference between cohorts 2 and 3 is statistically significant (p<0.001). The median time to improve the clinical state by 2 points was 11.0 (IQR 6 – 11), 6.0 (IQR 6 –6) and 5.0 (IQR 5 – 5) days for cohorts 1 – 3, respectively (Table 2, Figure 4). Importantly, none of the participants in cohorts 1 – 3 administered Aprotinin combinations were transferred to the ICU for ALV or NIV. All the patients in cohorts 1 – 3 were discharged from the hospital and no adverse events were recorded.

The dynamics of INR and prothrombin changes used to monitor blood-thinning anticoagulants and to check blood clotting problem defined normal at the admission and discharge of the patients from the hospital (Figures 3A and 3B). Patients presented a normal value for neutrophils and leukocytes when admitted to the hospital and discharged from the hospital after aprotinin combination therapy (Figures 3C and 3D).

Discussion

Currently, there is no particular antiviral therapy suggested for patients admitted to the hospital due to COVID-19-associated pneumonia and requiring oxygen therapy to reduce their transfer to ICU for ALV or NIV. Remdesivir and Favipiravir are the first known drugs repurposed for the treatment of COVID-19

and registered by the US FDA [23] and Russian Ministry of Health [24], respectively. But we did not found the conclusive clinical evidence on its impact on the main markers of the COVID-19 severity - CRP and D-dimer concentration in patients' blood. At the same time, we demonstrated that Aprotinin combination therapy was significantly associated with normalization of CRP level in moderate COVID-19 patients (median 3.5 – 6 days, IRQ 3.5 – 6). The combination of IV Aprotinin and Avifavir among cohort 3 patients proved to be the most effective. The median time to normalization of CRP concentrations was 3.5 day (IRQ 3 – 5).

The patients in this clinical trial had elevated D-dimer levels (525 – 855 ng/mL), which quickly returned to normal values (median 4.5 days, IRQ 3 – 6), (9 days, IRQ 5 – 9) and (5 days, IRQ 4 – 5) for cohorts 1, 2 and 3, respectively. The similar dynamics was observed for fibrinogen.

This clinical study revealed for the first time the high potency and efficacy of Aprotinin combination therapy of the patients hospitalized with moderate COVID-19 pneumonia. Aprotinin and Avifavir combination significantly reduced the time to normalization of CRP and D-dimer concentrations in patients' blood and overall improved clinical outcome, namely:

the median time to SARS-CoV-2 elimination was 7.5 (IRQ 6-9), 9.0 (IRQ 5-9) and 3.5 (IRQ 3-4) days in cohort 1, 2 and 3 respectively; elevated fibrinogen levels returned to normal concentrations (<5.0 g/L) on day 4; the dynamics of changes in indicators of INR (prothrombin, neutrophils, and leukocytes) were normal both when patients were admitted and discharged from the hospital; the median time to normalization of body temperature was 3.0 (IRQ 2-3), 4.5 (IRQ 3-5), and 1.0 (IRQ 1-3) days in cohort 1, 2 and 3 respectively; the median time to improve the clinical state by 2 points was 11.0 (IRQ 6-11), 6.0 (IRQ 6-6) and 5.0 (IRQ 5-5) days in cohorts 1 – 3, respectively. Significantly, none of the participants in cohorts 1 – 3 treated with aprotinin combinations were transferred to the ICU for ALV or NIV. All the patients in cohorts 1 – 3 were discharged from the hospital and no adverse events were recorded.

The results obtained in this pilot clinical study are promising and open the possibility for the initiation of the multicenter, placebo-controlled, randomized trial of the combination Aprotinin therapy in patients with moderate and severe COVID-19.

Authors' contributors

OSR and ENS were the Principal Investigators responsible for the recruitment of patients, study treatment, and data collection in compliance with the Protocol. AAI, VGL, EVY and AVI conceived this project, suggested a variants of its organization and controlled the implementation. VNA, NPS, SVP and ANE developed the clinical trial protocol, worked on the statistical aspects of the study and the analysis of the results. EAM and NVK organized the clinical trial and collection of the data. RNK developed the preclinical study design, organized its implementation, did literature search and edited the manuscript. DVK developed the technology and coordinated the production of the substance. AVI carried out scientific management of the project, wrote and edited the manuscript. All the authors read and approved the final manuscript.

Declaration of interests

The Sponsor of the study – Aviron LLC, Skolkovo Innovative Centre, Moscow, Russian Federation. AAI, AVI, NPS, and DVK are reporting patent RU 2731932 (09.09.2020). Other authors declare no competing interests.

Appendix.

Exclusion criteria were the following: refusal of the patient to participate, patients with respiratory rate > 35 per min that does not decrease after the body temperature drops to normal or sub-febrile values; blood oxygen saturation ≤ 93% at rest; partial pressure of oxygen in arterial blood (SpO2) < 60 mm Hg; oxygenation index, SpO2 per fraction of inspired oxygen (SpO2/FiO2) ≤ 200 mm Hg; septic shock; chronic liver and kidneys diseases in terminal stage; refusal of other organs requiring control and treatment in the ICU; patients with HIV; using aprotinin within 6 months prior to screening; hypersensitivity to any of the components of the study therapy; patients participating in other clinical trials or taking other investigational drugs within 28 days of screening; pregnant or lactating women or women planning a pregnancy during a clinical study; women capable of childbirth who do not use adequate methods of contraception; patients unable to read or write; unwilling to understand and follow research protocol procedures; non-compliance with the regimen of taking medications or performing procedures, which, in the opinion of the investigator, may affect the results of the study or the safety of the patient and prevent the patient's further participation in the study; patients with any other comorbid medical or serious mental health conditions that render them ineligible for participation in clinical research, limit their ability to obtain informed consent, or may affect their ability to participate in the research.

References

1. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. November 1, 2020. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6/.

2. Zheng KI, Feng G, Liu W-Y, et al. Extrapulmonary complications of COVID-19: A multisystem disease? *J Med Virol* 2020 Jul 22: 10.1002/jmv.26294, doi: 10.1002/jmv.26294.

3. Hayıroğlu MI, Çınar T, Tekkeşin AI. Fibrinogen and D-dimer variances and anticoagulation recommendations in Covid-19: current literature review. *Rev Assoc Med Bras* 2020, 66(6). https://doi.org/10.1590/1806-9282.66.6.842.

4. Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 2020, 191, 145-147. doi: 0.1016/j.thromres.2020.04.13

5. Cui S, Chen S, Li X, et al. Wang. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J Thromb Haemost* 2020, 18(6), 1421-1424. doi: 10.1111/jth.14830.

6. Ivashchenko AA, Dmitriev KA, Vostokova NV et al. AVIFAVIR for Treatment of Patients with Moderate COVID-19: Interim Results of a Phase II/III Multicenter Randomized Clinical Trial. *Clin Infect Dis* 2020 Aug 9: ciaa1176, https://doi.org/10.1093/cid/ciaa1176.

7. Wang G, Wu C, Zhang O, et al. C-Reactive Protein Level May Predict the Risk of COVID19 Aggravation. *OFID* 2020, 7(5), ofaa153, https://doi.org/10.1093/ofid/ofaa153.

8. Tu Y-F, Chien C-H, Yarmishun AA, et al. A Review of SARS-CoV-2 and the Ongoing Clinical Trials. *Int J Mol Sci* 2020, *21*(7), 2657. https://doi.org/10.3390/ijms21072657.

9. Scavone C, Brusco S, Bertini M, et al. Current pharmacological treatments for COVID-19: What's next? *Br J Pharmacol* 2020 Apr 24; 10.1111/bph.15072. doi: 10.1111/bph.15072.

10. Scheule AM, Beierlein W, Wendel HP, et al. Aprotinin in fibrin tissue adhesives induces specific antibody response and increases antibody response of high-dose intravenous application. *J Thorac Cardiovasc Surg* 1999, 118(2), 348-353.

11. Ascenzi P, Bocedi A, Bolognesi M. The bovine basic pancreatic trypsin inhibitor (Kunitz inhibitor): a milestone protein. *Curr Protein Pept Sci* 2003 Jun; 4(3), 231–251.

12. Engles L. Review and application of serine protease inhibition in coronary artery bypass graft surgery *Am J Health Syst Pharm* 2005 Sep 15; 62(18 Suppl 4), S9–14.

13. Solun B, Shoenfeld Y. Inhibition of metalloproteinases in therapy for severe lung injury due to COVID-19. *Med Drug Discov* 2020 Sep; 7, 100052.

14. Hoffmann M, Kleine-Weber H, Schroeder 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.

15. Bojkova D, Bechtel M, McLaughlin K-M, et al. Aprotinin Inhibitors SARS-CoV-2 Replication. *Cells* 2020, *9*(11), 2377; https://doi.org/10.3390/cells9112377.

16. Science News. Cancer's next magic bullet may be magic shotgun. University of California - San Francisco, June 15, 2012. https://www.sciencedaily.com/releases/2012/06/120615141716.htm.

17. Ivashchenko AA, Svistunov AA, Khorobryh TV, et al. Aprotinin - a New Drug Candidate for The Prevention of SARS-CoV-2 (COVID-19). *COVID19-PREPRINTS* 2020-09-13. https://covid19-preprints.microbe.ru/files/140.

18. Ivashchenko AA, Svistunov AA, Khorobryh TV, et al. Aprotinin - a new multi-target drug candidate or "magic shotgun" for the therapy of COVID-19. *COVID19-PREPRINTS* 2020-10-03. https://covid19-preprints.microbe.ru/files/148.

19. WHO R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis. February 18, 2020, Geneva, Switzerland.

20. Minister of Health of the Russian Federation. Interim guidelines prevention, diagnostics and treatment of new coronavirus infection (2019-nCoV) Version 7 (2020.06.03) (*Russian language*). https://static-0.rosminzdrav.ru/system/attachments/attaches/000/050/584/original/03062020_%D0%9CR_COVID-19_v7.pdf.

21. Huet T, Beaussier H, Voisin O, et al. Anakinra for severe forms of COVID-19: a cohort study. *Lancet Rheuvatology* 2020, 2(7), e393-e400. www.thelancet.com/rheumatology.

22. Thorlund K, Dron L, Park J, et al. A real-time dashboard of clinical trials for COVID-19. *Lancet Digital Health* 2020, 2(6), e286-e287. https://doi.org/10.1016/S2589-7500(20)30086-8.

23. FDA approves first treatment for COVID-19. *FDA news release* October 22, 2020. https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19.

24. Khrennikov I. Russian Health Ministry Approves Anti-Coronavirus Drug Avifavir. *Bloomberg.com* 2020. https://www.bloomberg.com/news/articles/2020-05-31/russian-health-ministry-approves-anti-coronavirus-drug-avifavir.

Table 1. Demographic and baseline characteristics of participants

Characteristic	Cohort 1	Cohort 2	Cohort 3
Age			
Years, mean (SD)	44.9 (11.2)	48.2 (10.4)	46.7 (10.6)
Age category, no. (%)			
18 – 44	4 (40)	4 (40)	4 (40)
45 – 59	6 (60)	3 (30)	5 (50)
≥ 60	0	3 (30)	1 (10)
Male, no. (%)	3 (30)	1 (10)	8 (80)
Female, no. (%)	7 (70)	9 (90)	2 (20)
Body mass, kg (SD)	74.3 (4.7)	76.7 (6.4)	80.7 (6)
Body-mass index, kg/m^2 (SD)	25.9 (1.7)	26.9 (2.1)	26.4 (1.5)
Positive swab by RT- PCR, %	100	100	100
Duration of illness, days (SD)	3.4 (1.1)	2.7 (0.7)	3.4 (0.8)
≤ 7 days, no. (%)	10 (100)	10 (100)	10 (100)
> 7 days, no. (%)	0	0	0
WHO Ordinal scale of clinical improvement (WHO-OSCI, score 0 to 8)			
Score 3, no. (%)	4 (40)	0	0
Score 4, no. (%)	6 (60)	10 (100)	10 (100)
Oxygen saturation, % (SD)	96.7 (1.1)	94.3 (0.7)	95.0 (0.9)
$SpO_2 \geq 95\%$, no. (%)	10 (100)	6 (60)	6 (60)
$SpO_2 < 95\%$, no. (%)	0	4 (40)	4 (40)
Fever, ºC (SD)	38.3 (0.1)	38.3 (0.3)	38.5 (0.4)
< 37 ºC, no. (%)	0	0	0
37 – 38 ºC, no. (%)	0	1 (10)	1 (10)

Characteristic	Cohort 1	Cohort 2	Cohort 3
> 38 ºC, no. (%)	10 (100)	9 (90)	9 (90)
Respiratory rate, min (SD) (normal range 16 – 20 min)	21.4 (1.6)	22.6 (0.7)	21.8 (1)
≤ 22 min, no. (%)	8 (80)	5 (50)	7 (70)
> 22 min, no (%)	2 (20)	5 (50)	3 (30)
CRP, mg/L (SD) (normal < 5 mg/L)	21.5 (8.2)	38.9 (8.1)	37.8 (6.7)
D-dimer, ng/mL (SD) (normal < 243 ng/mL)	525.4 (175.7)	820.1 (133.1)	855.5 (142.5)
Neutrophil count x 10^9 cells per L, no. (SD) (normal range 1.8 – 6.5)	3.0 (0.5)	2.2 (0.4)	2.7 (1.0)
Leukocyte count x 10^9 cells per L, no. (SD) (normal range 3.2 – 10.6)	4.4 (0.7)	3.4 (0.4)	3.9 (1.5)
INR*, no. (SD) (normal range 0.85 –1.15)	1.0 (0.2)	1.0 (0.1)	1.1 (0.1)
Prothrombin, % (SD) (quick test, normal range 95 – 105%)	103.3 (8.1)	78.5 (5.7)	78.2 (8.7)
Fibrinogen, g/L (SD) (normal range 2 – 4)	9.8 (2.6)	5.0 (1.2)	5.1 (2.2)
Involvement of the lung parenchyma, % (SD)	28.3 (7.6)	20.6 (6.8)	21.8 (6.1)
Chest CT 1 (< 25% abnormality), no. (%)	4 (40)	3 (30)	6 (60)
Chest CT 2 (25% –50% abnormality), no. (%)	6 (60)	7 (70)	4 (40)
* International Normalized Ratio or prothrombin time.			

Table 2. Efficacy of Aprotinin and HCQ or Avifavir combination therapy in COVID-19 patients

Cohort 1	Cohort 2	Cohort 3
Median time to elimination of SARS-CoV-2 virus confirmed by RT-PCR, days (IQR), *p		
7.5 (6 – 9), p = 0.019	9.0 (5 – 9), p = 0.006	3.5 (3 – 4)
Median time to normalization of CRP concentration (≤ 10 mg/L) in patient's blood, days (IQR)		
6.0 (6 – 6), $p < 0.001$	4.0 (3 – 5), p =0.821	3.5 (3 – 5)
Median time to normalization of D-dimer concentration (< 253 ng/mL) in patient's blood, days (IQR)		
4.5 (3 – 6), p = 0.675	9.0 (5 – 9), p = 0.002	5.0 (4 – 5)
Median time to normalization of body temperature (< 37ºC), days (IQR)		
3.0 (2 – 3), p = 0.090	4.5 (3 – 5), p <0.001	1.0 (1 – 3)
Median time to improvement in clinical status by 2 points on the WHO-OSCI, days (IQR)		
11.0 (6 – 11), p = 0.004	6.0 (6 – 6), p = 0.036	5.0 (5 – 5)
Changes in lung lesions according to chest CT data on day 14 after hospitalization		
Improvement, no. (%)		
6 (60)	10 (100)	10 (100)
Without changes, no. (%)		
4 (40)	0	0
*p < 0.05, compared between characteristics in patients from cohorts 1 and 2 and those in patients from cohort 3 (Aprotinin (IV) + Avifavir + SOC) using log-rank test.		

Figure 1. Effect of Aprotinin and HCQ or Avifavir (cohorts 1 – 3) combination therapy on time to elimination of SARS-CoV-2 (A) and dynamics of normalization of CRP level (B) in COVID-19 patients.

Normal concentration of CRP is ≤ 10 mg/L. The plot A shows time since initiation of treatment (days). The plot B shows time from inclusion (days).

Figure 2. Effect of Aprotinin and HCQ or Avifavir (cohorts 1 – 3) combination therapy on dynamics of normalization of D-dimer (A) and fibrinogen (B) concentrations in COVID-19 patients.

Normal concentration of D-dimer is ≤ 253 ng/mL. Normal range of fibrinogen is 2 - 4 g/L. The plot shows time from inclusion (days).

Figure 3. Effect of Aprotinin and HCQ or Avifavir (cohorts 1 – 3) combination therapy on dynamics of INR (A) prothrombin (B), neutrophils (C), and leukocytes (D) concentrations in COVID-19 patients.

INR is a ratio of the patient's prothrombin time (PT) to a control PT standardized for the potency of the thromboplastin reagent developed by the World Health Organization (WHO) using the following formula: INR = Patient PT ÷ Control PT. Normal range of INR is 0.85 – 1.15. The Quick's Prothrombin (PC) test is used in decoding the coagulogram and compares the patient's coagulogram with the reference value of normal plasma. Normal range of prothrombin according to Quick Prothrombin test is 75 – 140 %. Normal range of neutrophils counts is 1·8 –6·5 x 10^9 cells per L. Normal range of leukocytes counts is 3·2 – 10·6 x 10^9 cells per L . The plot shows time from inclusion (days).

Figure 4. Effect of Aprotinin and HCQ or Avifavir (cohorts 1 – 3) combination therapy on the median time to normal body temperature (<37ºC) (A) and the average time to improve the clinical state by 2 points (B) in COVID-19 patients.

Clinical state was estimated according to the WHO-OSCI. The plot shows time since the initiation of treatment (days).

Figure 1

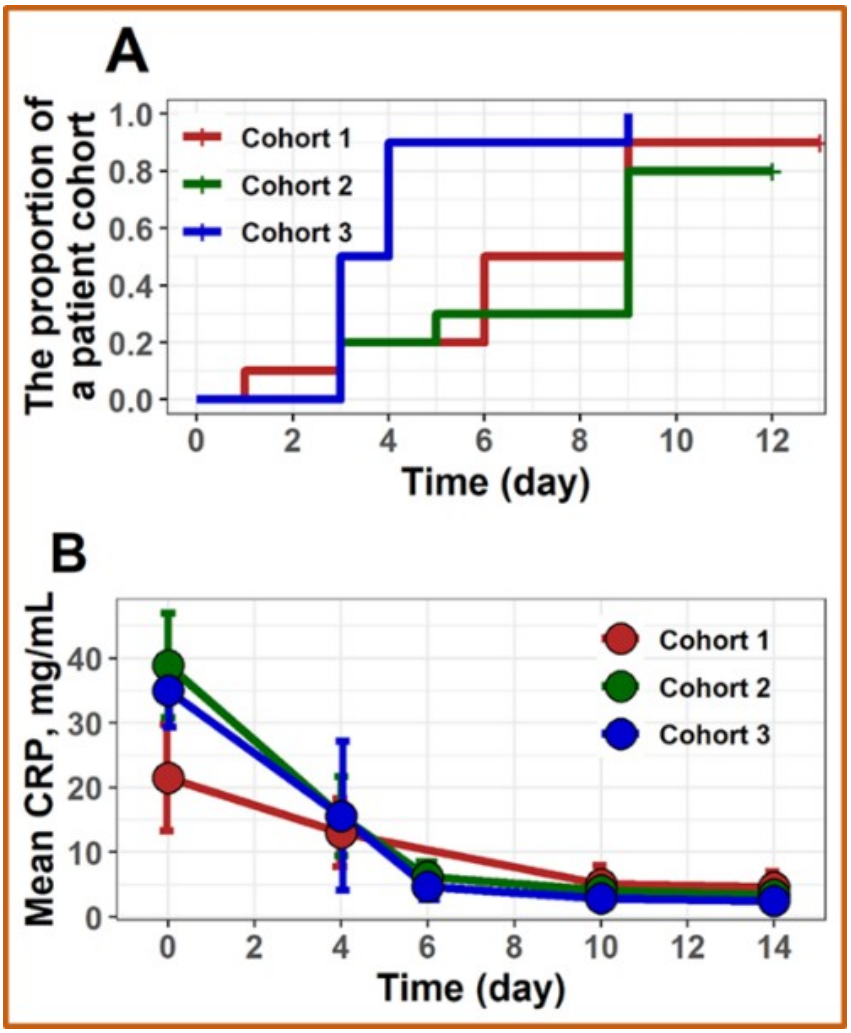


Figure 2

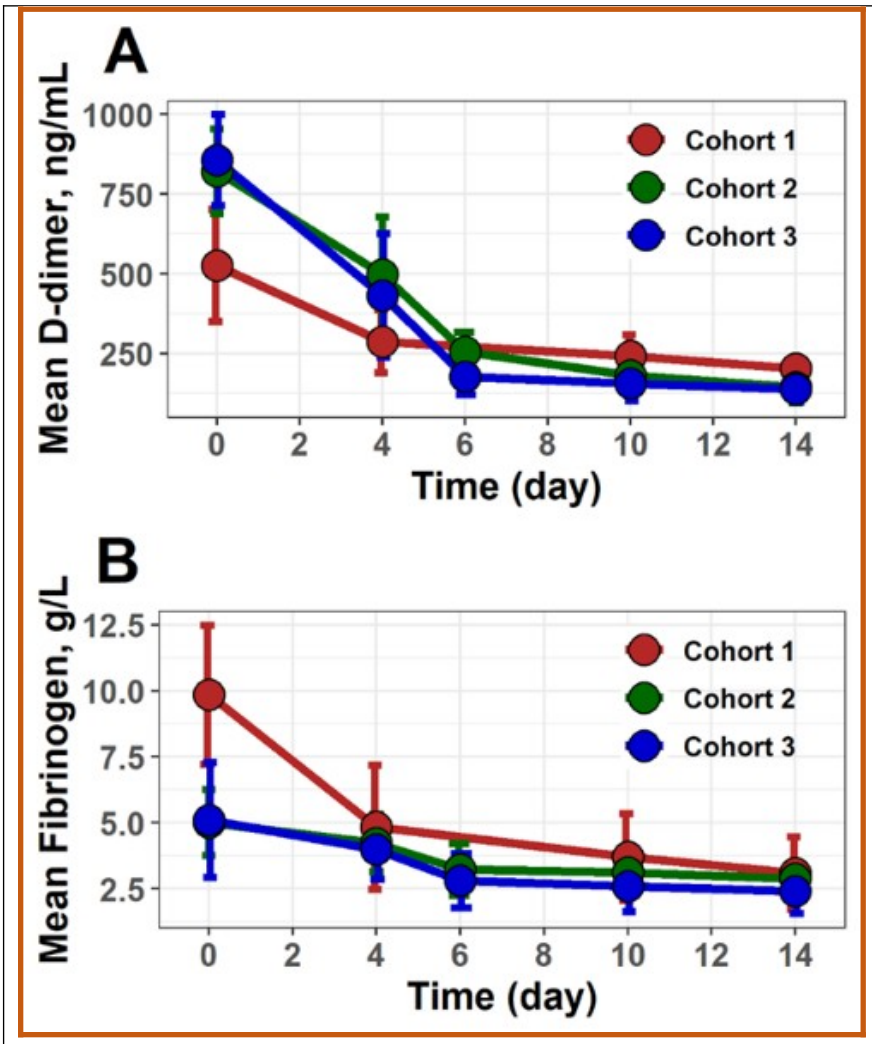

A
Mean D-dimer, ng/mL
1000
750
500
250
0
2
4
6
8
10
12
14
Time (day)
Cohort 1
Cohort 2
Cohort 3
B
Mean Fibrinogen, g/L
12.5
10.0
7.5
5.0
2.5
0
2
4
6
8
10
12
14
Time (day)
Cohort 1
Cohort 2
Cohort 3

Figure 3

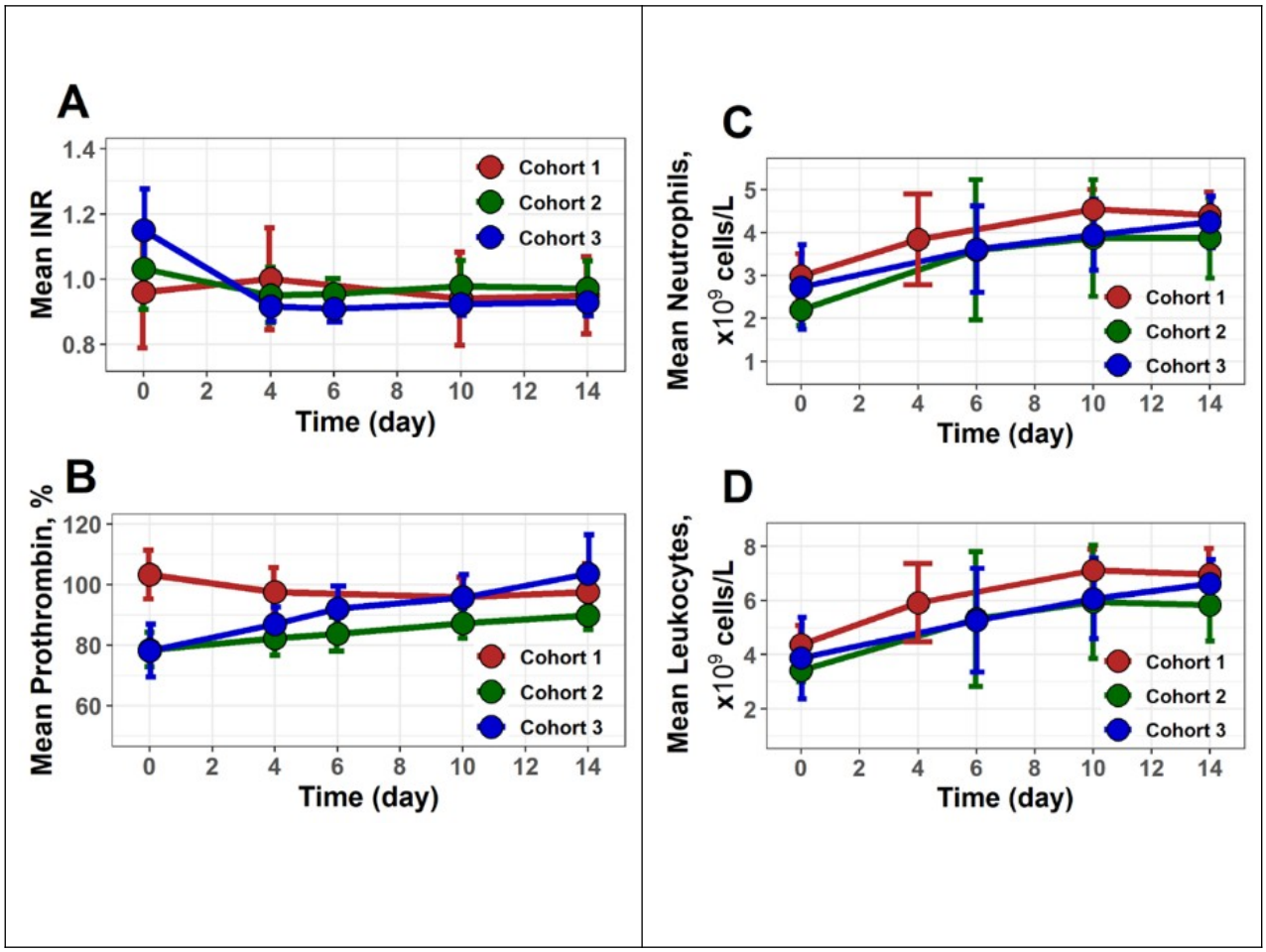
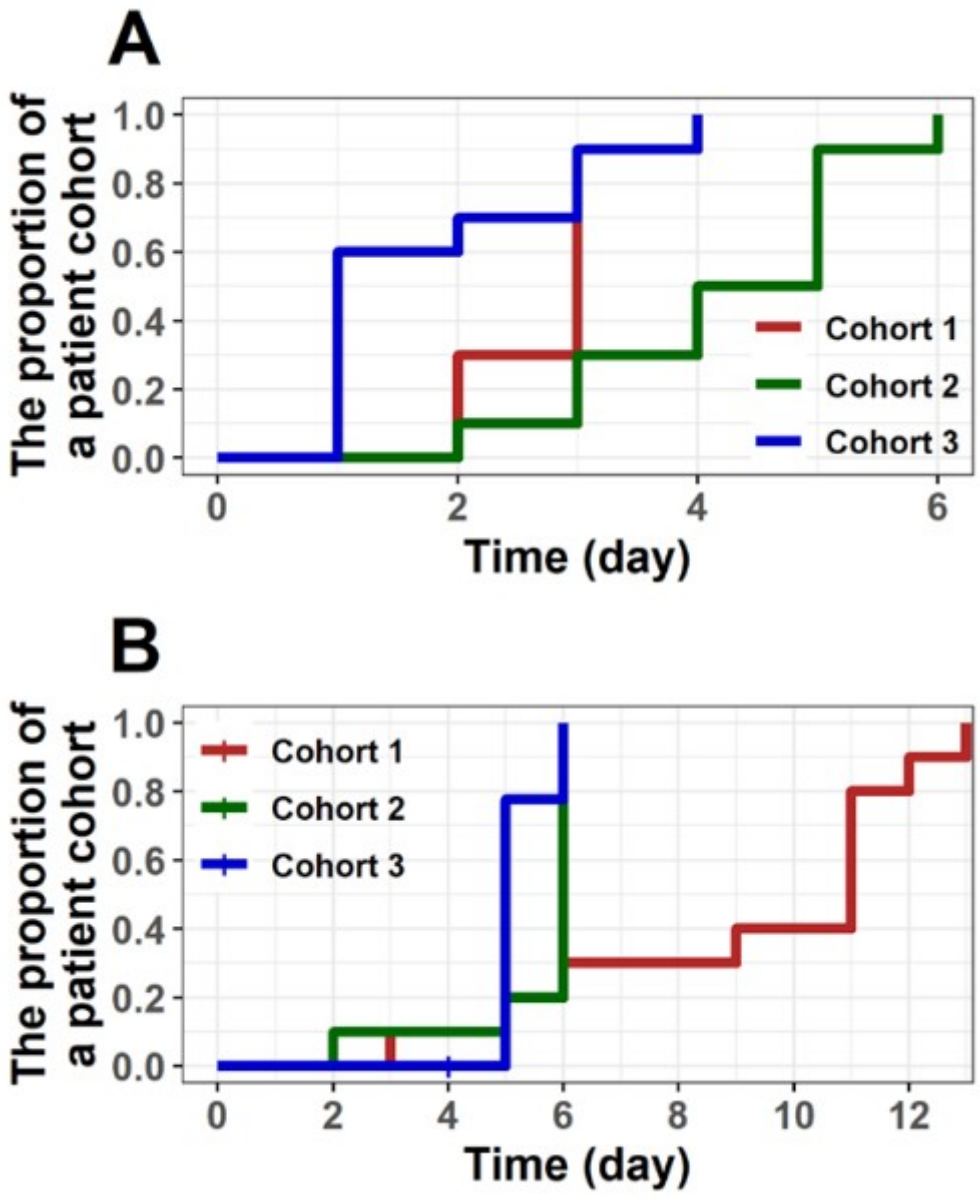

A
Mean INR
1.4
1.2
1.0
0.8
0 2 4 6 8 10 12 14
Time (day)
Cohort 1
Cohort 2
Cohort 3
B
Mean Prothrombin, %
120
100
80
60
0 2 4 6 8 10 12 14
Time (day)
Cohort 1
Cohort 2
Cohort 3
C
Mean Neutrophils, x10^9 cells/L
5
4
3
2
1
0 2 4 6 8 10 12 14
Time (day)
Cohort 1
Cohort 2
Cohort 3
D
Mean Leukocytes, x10^9 cells/L
8
6
4
2
0 2 4 6 8 10 12 14
Time (day)
Cohort 1
Cohort 2
Cohort 3

Figure 4


A
The proportion of a patient cohort
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
Time (day)
Cohort 1
Cohort 2
Cohort 3
B
The proportion of a patient cohort
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Time (day)
Cohort 1
Cohort 2
Cohort 3