Immunology and Inflammation, Medicine

Anti-inflammatory therapy with nebulised dornase alfa in patients with severe COVID-19 pneumonia A Randomised Clinical Trial

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Abstract

Background

SARS-CoV2 infection causes severe, life-threatening pneumonia. Hyper-inflammation, coagulopathy and lymphopenia are associated with pathology and poor outcomes in these patients. Cell-free (cf) DNA is prominent in COVID-19 patients, amplifies inflammation and promotes coagulopathy and immune dysfunction. We hypothesized that cf-DNA clearance by nebulised dornase alfa may reduce inflammation and improve disease outcomes. Here, we evaluated the efficacy of nebulized dornase alfa in patients hospitalised with severe COVID-19 pneumonia.

Methods

In this randomised controlled single-centre phase 2 proof-of-concept trial, we recruited adult patients admitted to hospital that exhibited stable oxygen saturation (≥94%) on supplementary oxygen and a C-reactive protein (CRP) level ≥30mg/L post dexamethasone treatment. Participants were randomized at a 3:1 ratio to receive twice-daily nebulised dornase alfa in addition to best available care (BAC) or BAC alone for seven days or until hospital discharge. A 2:1 ratio of historical controls to treated individuals (HC, 2:1) were included as the primary endpoint comparators. The primary outcome was a reduction in systemic inflammation measured by blood CRP levels over 7 days post-randomisation, or to discharge if sooner. Secondary and exploratory outcomes included time to discharge, time on oxygen, D-dimer levels, lymphocyte counts and levels of circulating cf-DNA.

Results

We screened 75 patients and enrolled 39 participants out of which 30 in dornase alfa arm, and 9 in BAC group. We also matched the recruited patients in the treated group (N=30) to historical controls in the BAC group (N=60). For the the primary outcome, 30 patients in the dornase alfa were compared to 69 patients in the BAC group. Dornase alfa treatment reduced CRP by 33% compared to the BAC group at 7-days (P=0.01). The dornase alfa group least squares mean CRP was 23.23 mg/L (95% CI 17.71 to 30.46) and the BAC group 34.82 mg/L (95% CI 28.55 to 42.47). A significant difference was also observed when only randomised participants were compared. Furthermore, compared to the BAC group, the chance of live discharge was increased by 63% in the dornase alfa group (HR 1.63, 95% CI 1.01 to 2.61, P=0.03), lymphocyte counts were improved (least-square mean: 1.08 vs 0.87, P=0.02) and markers of coagulopathy such as D-dimer were diminished (least-square mean: 570.78 vs 1656.96µg/mL, P=0.004). Moreover, the dornase alfa group exhibited lower circulating cf-DNA levels that correlated with CRP changes over the course of treatment. No differences were recorded in the rates and length of stay in the ICU or the time on oxygen between the groups. Dornase alfa was well-tolerated with no serious adverse events reported.

Conclusions

In this proof-of-concept study in patients with severe COVID-19 pneumonia, treatment with nebulised dornase alfa resulted in a significant reduction in inflammation, markers of immune pathology and time to discharge. The effectiveness of dornase alfa in patients with acute respiratory infection and inflammation should be investigated further in larger trials.

elife assessment:

This small-sized clinical trial comparing nebulized dornase-alfa to best available care in patients hospitalized with COVID-19 pneumonia is **valuable**, but in its present form the paper is **incomplete**: the number of randomized participants is small, investigators describe also a contemporary cohort of controls and the study concludes about decrease of inflammation (reflected by CRP levels) after 7 days of treatment but no other statistically significant clinical benefit.

Introduction

Severe SARS-CoV-2 infection is associated with severe pneumonia, hyperinflammation, coagulopathy, respiratory failure and death[1,2]. Hyperinflammation and a dysregulated immune response play critical roles in the pathophysiology of COVID-19 pneumonia.

In severe COVID-19 pneumonia excessive neutrophil activation drives neutrophil extracellular trap (NET) formation[3,4]. NETs are composed of DNA, histones and other components and contribute to organ damage by promoting coagulopathy and endothelial dysfunction[5]. Moreover, extracellular histones promote inflammation and lethality during sepsis[6-9]. Consistently, DNAse treatment reduces pathology in murine pulmonary viral

infections[10,11]. A treatment that enhances cf-DNA clearance is likely to reduce hyper-inflammation and coagulopathy in severe COVID-19 pneumonia and save lives. Pulmozyme®, dornase alfa, is a recombinant human DNAase approved since 1993 as a nebulised treatment for patients with cystic fibrosis (CF)[12]. Dornase alfa solubilizes NETs, reduces inflammation and improves pulmonary function in chronic and acute exacerbations of CF[13,14]. Pulmozyme is safe and well-tolerated in children and adults with CF at doses up to 10mg BD.

C-reactive protein (CRP) is a prognostic marker that correlates with clinical symptoms, hyper-inflammation and response to therapy. CRP correlates is a widely used marker of systemic inflammation. In blood the normal concentration of CRP is <0.8 mg/L but it rises rapidly peaking at 48h from disease onset. A raised CRP above 40 mg/L distinguishes patients with severe COVID-19 from those with mild illness[15]. We chose CRP as our primary endpoint as it is easily measured and is highly responsive to treatments that reduce inflammation.

Here we report the results of the COVASE study, a Phase IIa trial that evaluated the safety and the efficacy of nebulised dornase alfa in reducing hyperinflammation in hospitalised patients with severe COVID-19 pneumonia, as measured by C-reactive protein (CRP) and clinical endpoints.

Materials and Methods

Trial oversight

The trial was sponsored by University College London and carried out at University College London Hospitals (UCLH). The trial protocol and the statistical analysis plan are available in the Appendix. The COVASE trial was reviewed by the South Central - Hampshire B Research Ethics Committee Level 3 Block B Whitefriars Lewins Mead Bristol BS1 2NT, chaired by Professor Vincenzo Libri (REC reference: 20/SC/0197, Protocol number: 132333, RAS project ID:283091) and the United Kingdom Medicines and Healthcare products Regulatory Agency (MHRA). The trial was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines of the Council for International Organizations of Medical Sciences, applicable International Council for Harmonisation Good Clinical Practice guidelines, and applicable laws and regulations. All the randomized participants provided written informed consent. Consent for the historical controls was covered by the Health Service (Control of Participant Information) Regulations 2002 that allows the processing of Confidential Participant Information (CPI) for specific purposes. Regulation 3 provides for the processing of CPI in relation to communicable diseases and other threats to public health and in particular, allows the Secretary of State to require organisations to process CPI for purposes related to communicable diseases. The COVID-19 pandemic is covered by this legislation which allows a range of purposes related to diagnosing, managing, and controlling the spread of COVID-19. The sponsors designed the trial in collaboration with the investigators at the Francis Crick institute, Exploristics and Target to Treatment Consulting. The sponsors and trial investigators participated in data collection, analysis, and interpretation. The authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data presented and for the fidelity of the trial to the protocol. The COVASE study was registered on clinicaltrials.gov identifier: NCT04359654. Safety and data integrity were regularly reviewed by the Trial Monitoring Group and Data Monitoring Committee.

Participant population

Adults (≥18 years of age) admitted to UCLH with confirmed SARS-Cov2 infection by reverse-transcriptase–polymerase-chain-reaction and radiologically confirmed COVID-19 pneumonia on chest radiograph or CT-scan; an oxygen saturation below 94% requiring the use of supplemental oxygen; and evidence of hyperinflammation (C-reactive protein [CRP] ≥ 30 mg/L, after administration of dexamethasone) were eligible[16]. Participants that had unstable ventilatory requirements or needed intubation and ventilation within 24 hours of admission were excluded. Full inclusion and exclusion criteria can be found in the Protocol: Supplementary Appendix 1.

Trial design

The COVASE trial was a single-site, randomised, controlled, parallel, open-label investigation of the effect of dornase alfa on hyperinflammation in hospitalised participants with COVID-19. Screening was performed within 24 hours prior to the administration of dornase alfa (**Figure 1A**). Eligible, consented participants were randomly assigned at a 3:1 ratio with the use of a closed envelope system to receive either best available care (BAC) plus nebulised dornase alfa or BAC alone. On Day 1 the first baseline sample was collected prior to dornase alfa administration. Thereafter, from Day 1 to Day 7 of the trial participants randomised to the active arm received 2.5mg BD of nebulised dornase alfa in addition to BAC. In all cases BAC included dexamethasone (6 mg/day) for 10 days or until hospital discharge, whichever was shorter as per the RECOVERY trial[17]. Participants received additional treatments at the discretion of their physicians. The primary analysis was performed on samples up to Day 7. The final trial visit occurred at day 35. In addition to participants randomised to receive BAC alone, for every COVASE participant randomised to active treatment, 2 matched historical controls were included. Historical controls (HCs) had been admitted to UCLH and treated with the same BAC treatment strategy, including dexamethasone. HCs were identified from a database of >600 patients, and were matched for age, gender, BMI, comorbidities and CRP (either pre-or post-dexamethasone to provide 2 HC populations). In most cases the patients in the historical controls were admitted concomitantly with the participants recruited to the trial. Additional details regarding the trial design are provided in the protocol (SA1).

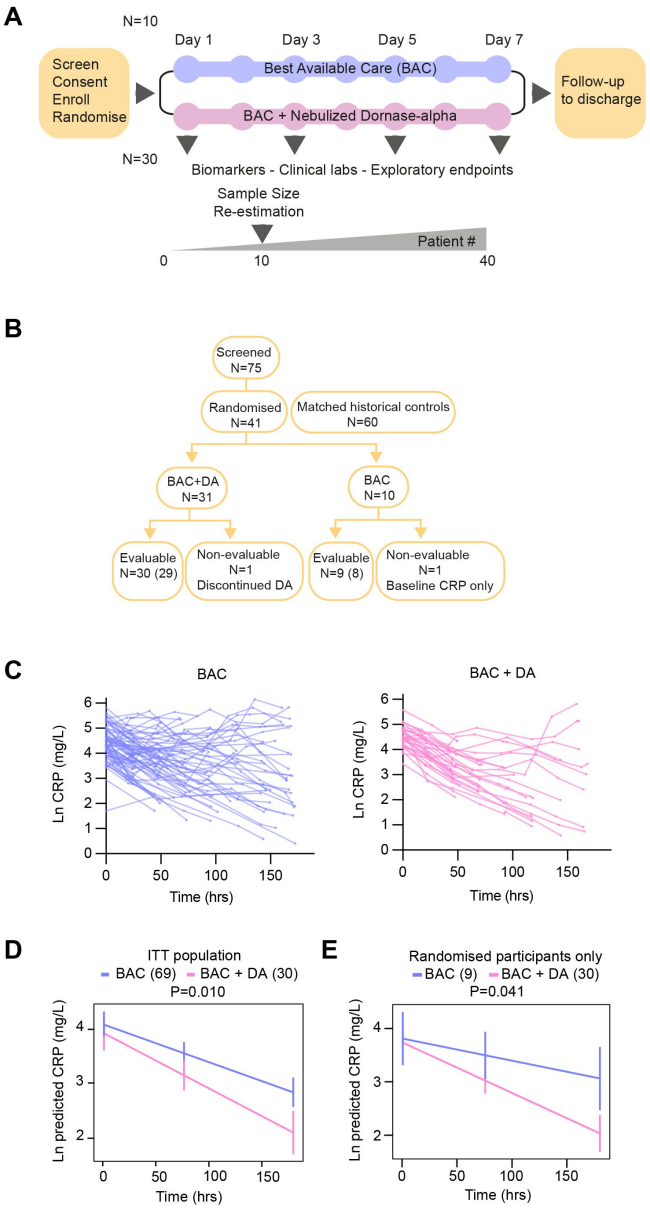

Figure 1.

Prespecified Primary and Secondary endpoints.

A. COVASE Trial Design.

B. Consort diagram. Numbers not in parentheses indicate those in the intention-to-treat population, numbers in parentheses indicate the numbers in the per-protocol population.

C. (left panel) Natural log CRP in BAC (HC and randomised participants; blue). (Right panel) Natural log CRP in participants randomised to BAC+DA (pink).

D. Fitted mean (95% confidence interval) from mixed model, with natural log (CRP) over 7 days follow-up as the outcome, adjusting for natural log baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease or hypertension), time and a treatment × time interaction. P-value generated by comparing least-square means between arms. Intention to treat (ITT) population (Blue: HC and participants randomised to BAC, N=69; Pink: participants randomised to BAC+DA, N=30).

E. Same output as in (**D**) but examining randomised participants only: (Blue: participants randomised to BAC, N=9; Pink: participants randomised to BAC+DA, N=30).

Evaluations

The baseline was defined as the last observation before the administration of dornase alfa on Day 1 for participants randomized to BAC+ dornase alfa, or the first observation after the first dose of dexamethasone for participants randomized to BAC and for HC. The participants' clinical status was assessed each day for requirement for supplementary oxygen, admission to the intensive care unit (ICU), ventilation or high flow oxygen, and standard clinical measurements: respiration rate, oxygen saturation, systolic blood pressure, pulse rate, level of consciousness, and temperature. On day 1, and alternate days thereafter until day 7 or discharge, whichever was sooner, blood was collected for routine analysis of CRP, clinical laboratory parameters and exploratory endpoints. Participants were followed until discharge or death and/or at a follow up of at least 28 days after the last treatment day (Day 35).

Outcomes

The primary efficacy outcome was the least square mean CRP up to 7 days or at hospital discharge whichever was sooner. Pre-specified secondary efficacy outcomes included days on oxygen; time to hospital discharge; clinical status at day 7 on the ordinal scale; mortality by day 35; changes in clinically relevant biomarkers including blood lymphocyte count and D-dimer levels. Other secondary outcomes were the time until discharge alive, initiation of mechanical ventilation, or ICU transfer; and the duration of ICU stay. Adverse events were recorded according to the system organ class and preferred terms in the Medical Dictionary for Regulatory Activities, version 23.0.

Statistical analysis

As pre-specified in the statistical analysis plan, efficacy assessments of the primary and secondary outcomes in the modified intention-to-treat population were performed on all randomised participants and received at least one dose of dornase alfa if randomized to treatment. A sample size of 90 participants was calculated to provide a power of 80% to determine a between-group difference of >40% in the primary outcome (CRP at day 7) which we considered both achievable and clinically relevant, yielding 30 participants in the active treatment group and 60 in the control group. An additional 10 participants were randomised to BAC alone as a controls for exploratory endpoints and to compare the characteristics of enrolled participants with historical controls. In summary, 40 participants enrolled in the study (30 on BAC plus dornase alfa and 10 on BAC alone, of which 30 and 9 were evaluable) and 60 historical controls which were selected using propensity score matching, with age, sex, BMI, baseline CRP, and key comorbidities defined as one or more of hypertension, diabetes or cardiovascular disease included as covariates to ensure these characteristics were as balanced as possible between those randomised to dornase alfa and the historical controls.

All baseline data, demographics, endpoints, safety, and tolerability were summarised overall and by treatment group and by day. In general, continuous data were summarised using the mean (standard deviation), median (1st and 3rd quartiles), minimum and maximum, and categorical data were represented as frequency counts (percentages).

For analyses relating to the primary objective, group comparisons were performed using a repeated measures mixed model, adjusted for baseline factors and with treatment as the main effect. Prior to analysis, primary and secondary endpoints were assessed for conformance to normality assumptions and the appropriate transformation was conducted if necessary.

A re-estimation of the sample size was carried out following an interim analysis when 12 participants had been randomised.

Safety was assessed in the randomised population. Full details of the planned statistical analysis are presented in the Statistical Analysis Plan (SAP; Supplementary Appendix 2).

Exploratory endpoints were only available in the randomised participants and not in the historical controls. In this case, a *post hoc* within group analysis was conducted to compare baseline and post-baseline measurements.

Exploratory endpoint analysis

Peripheral venous blood was collected into EDTA or Heparin tubes, depending on clinic availability, and layered on Histopaque 1119 (Sigma-Aldrich) and centrifuged for 20 min at

800x g. The plasma, PBMC and neutrophil layers were collected. Plasma was centrifuged and frozen in liquid nitrogen, thawed and DNA levels were measured in a fluorescence plate reader using the Quant-iT™ PicoGreen dsDNA Assay Kit (P7589,Thermofisher). Data were analysed using Microsoft Excel and Graph Pad Prism software.

Informed consent and ethics for healthy control samples

For the cf-DNA measurements in the blood of healthy control donors, peripheral blood was isolated from consenting healthy adult volunteers, according to approved protocols of the ethics board of the Francis Crick Institute and the Human Tissue act.

Results

Patient characteristics

From May 2020-October 2021, 41 participants were randomised but 1 participant in the BAC group was discharged from hospital before a second CRP measurement (**Figure 1B**). This participant was excluded from all analyses, except for the safety analyses. One participant withdrew consent prior to receiving any dose of dornase alfa and was replaced and excluded from all analyses. 39 participants were included in the intention-to-treat analysis set, 30 in the BAC + dornase alfa group and 9 in the BAC group. There was one treatment discontinuation, after one dose of dornase alfa, which was the participant's decision due to a "tingling of the mouth, cough, shortness of breath" reported after receiving dornase alfa. This participant was removed from the per protocol population. All 39 participants were followed up for 35 days or until death whichever was sooner.

Two participants were excluded from the per-protocol population. One from the BAC as randomisation occurred prior to dexamethasone being widely used in the treatment of COVID-19 and they were the only participant in the analysis set not to be on dexamethasone at the start of follow-up; and a second participant who withdrew after one dose of dornase alfa due to side-effects from the medication (**Figure 1B**).

Baseline characteristics were generally well balanced across groups (BAC + dornase alfa, BAC, and historical controls/BAC; Table 1). Selection of historical controls via propensity score matching was successful in ensuring the means of the characteristics included in the propensity score matching were similar to those observed in the BAC + dornase alfa arm (**Table 1**), as well as having similar overall distributions (**Supplementary Figure 1**).

Table 1.

Patient baseline characteristics

	Randomised to BAC + dornase-alfa (N=30)	Randomised to BAC (N=9)	Historical controls (N=60)	All BAC (N=69)	Total (N=99)
Age (years)					
N	30	9	60	69	99
Mean	56.8	53.3	57.3	56.8	56.8
SD	12.5	13.7	14.5	14.3	13.7
Median	58.0	53.0	57.0	57.0	57.0
Min	32.0	31.0	23.0	23.0	23.0
Max	77.0	76.0	86.0	86.0	86.0
Gender					
Female N (%)	7 (23.3)	2 (22.2)	15 (25.0)	17 (24.6)	24 (24.2)
Male N (%)	23 (76.7)	7 (77.8)	45 (75.0)	52 (75.4)	75 (75.8)
BMI (kg/m²)					
N	30	9	60	69	99
Mean	27.8	30.8	27.8	28.2	28.0
SD	4.7	7.8	5.6	6.0	5.6
Median	26.5	28.9	27.9	28.2	27.7
Min	20.7	22.6	16.3	16.3	16.3
Max	41.7	48.4	43.8	48.4	48.4
Baseline CRP (mg/L)					
N	30	9	60	69	99
Mean	101.9	91.9	100.7	99.5	100.2
SD	52.2	68.1	68.3	67.8	63.3

Median	86.3	74.6	75.8	75.3	79.6
Min	25.2	18.9	30.8	18.9	18.9
Max	261.5	221.6	336.4	336.4	336.4
Key Comorbidity					
No N (%)	16 (53.3)	3 (33.3)	28 (46.7)	31 (44.9)	47 (47.5)
Yes N (%)	14 (46.7)	6 (66.7)	32 (53.3)	38 (55.1)	52 (52.5)

The overall mean age was 56.8 years (mean in BAC + dornase alfa group=56.8 years, mean in BAC group=56.8 years). The percentage of males was 75.8% overall (76.7% BAC + dornase alfa group, 75.4% BAC group). The most prevalent ethnicity was “White British”, with 30.3% of participants identifying in that category overall (33.3% BAC + dornase alfa group, 29.0% BAC group). The overall mean BMI was 28.0kg/m2 (mean in BAC + dornase alfa group=27.8kg/m2, mean in BAC group=28.2kg/m2). The mean baseline CRP (post dexamethasone) as defined in the primary analysis was 100.2mg/L (mean in BAC + dornase alfa group=101.9mg/L, mean in BAC group=99.5mg/L). The overall proportion of participants with a key comorbidity, defined as one or more of hypertension, diabetes, or cardiovascular disease, was 52.5% (46.7% BAC + dornase alfa group, 55.1% BAC group).

All but one (38/39) of the randomised participants received dexamethasone prior to randomisation, and 48 of the total 99 participants also received remdesivir or tocilizumab in addition to dexamethasone within the first 7 days.

The last pre-dexamethasone CRP was also similar between groups, with an overall mean of 125.0mg/L (mean in BAC + dornase alfa group=128.1mg/L, mean in BAC group=122.7mg/L). The number of days between dexamethasone initiation and baseline was 1.2 days overall (mean in BAC + dornase alfa group=0.7 days, mean in BAC group=1.3 days).

There were imbalances noted at baseline between the groups in white blood cell count, neutrophil count, procalcitonin count and D-dimer **(Supplementary Table S1)**.

Clinical outcomes

Primary outcome

Individual CRP traces over time for each patient are shown in **Figure 1C**. Blood collection for both BAC and BAC + DA groups occurred at similar times and frequencies over the course of treatment (**Supplementary Figure 2A**). For the ITT group, the LS mean log (CRP) over 7 days follow-up was 3.15 (95% confidence interval [CI] 2.87 to 3.42) in the BAC + dornase alfa group (n=30), and 3.55 (95% CI, 3.35 to 3.75) in the BAC group (n=69; Table 2; **Figure 1D**), p=0.01. This indicates a reduction in mean CRP of approximately 33% in the BAC + dornase alfa group (23.23 mg/mL) compared to the BAC group (34.82 mg/mL) at the mean follow-up over 7 days.

Table 2.

Primary endpoint and sensitivity analysis:

CRP (mg/L)	Randomised to BAC + dornase-alfa	BAC	Difference between BAC + dornase-alfa and BAC	p-value*
Primary analysis of Primary Endpoint: ITT population including all individuals: (BAC + dornase-alfa, BAC & historical controls).				
N	30	69		
Least-squares mean log(CRP) * (95% CI)	3.15 (2.87 to 3.42)	3.55 (3.35 to 3.75)	-0.4 (-0.71 to –0.10)	0.010
Least-square mean CRP ** (95% CI)	23.23 (17.71 to 30.46)	34.82 (28.55 to 42.47)	0.67 (0.49 to 0.91)	
Sensitivity Analyses				
PP population including all individuals: (BAC + dornase-alfa, BAC & historical controls)				
N	29	68		
Least-squares mean log(CRP)* (95% CI)	3.12 (2.85 to 3.39)	3.55 (3.36 to 3.74)	-0.43 (-0.73 to –0.13)	0.006
Least-square mean CRP** (95% CI)	22.64 (17.35 to 29.54)	34.82 (27.7 to 42.21)	0.65 (0.48 to 0.88)	
ITT population including randomised individuals only				
N	30	9		
Least-squares mean log(CRP)* (95% CI)	3.1 (2.84 to 3.35)	3.59 (3.13 to 4.06)	-0.5 (-0.97 to –0.02)	0.041
Least-square mean CRP** (95% CI)	22.12 (17.16 to 28.5)	36.34 (22.79 to 57.94)	0.61 (0.38 to 0.98)	
ITT population including randomised individuals to BAC + dornase-alfa and historical controls only.				
N	30	60		
Least-squares mean log(CRP)* (95% CI)	3.18 (2.91 to 3.45)	3.56 (3.35 to 3.76)	-0.37 (-0.68 to –0.06)	0.019

Least-square mean CRP** (95% CI)	24.09 (18.36 to 31.6)	35.03 (28.44 to 43.15)	0.69 (0.5 to 0.94)	
ITT population including randomised individuals to BAC and historical controls only, i.e. the comparator population				
N	9	60		
Least-squares mean log(CRP)* (95% CI)	3.78 (3.23 to 4.33)	3.53 (3.3 to 3.77)	0.24 (-0.32 to 0.8)	0.386
Least-square mean CRP** (95% CI)	43.69 (25.18 to 75.8)	34.23 (27.11 to 43.24)	1.28 (0.73 to 2.23)	
Area under the log(CRP), standardised by days followed up, over 7 days follow-up: ITT population including all individuals (BAC + dornase-alfa, BAC & historical controls).				
N	30	69		
Least-squares mean area [a] (95% CI)	3.45 (3.22 to 3.68)	3.72 (3.55 to 3.88)	-0.27 (-0.53 to –0.01	0.043
ITT population including all individuals: (BAC + dornase-alfa, BAC & historical controls matched including last pre-Dexamethasone CRP value).				
N	30	69		
Least-squares mean log(CRP)* (95% CI)	3.16 (2.83 to 3.5)	3.69 (3.44 to 3.93)	-0.53 (-0.91 to –0.14)	0.007
Least-square mean CRP** (95% CI)	23.57 (16.85 to 32.970	39.92 (31.32 to 50.89)	0.59 (0.4 to 087)	
ITT population including all individuals: (BAC + dornase-alfa, BAC & historical controls). Stratified by BAC treatment				
No Remdesivir or Tocilizumab				
N	12	39		
Least-squares mean log(CRP)* (95% CI)	3.29 (2.83 to 3.76)	3.75 (3.45 to 4.04)	-0.45 (-0.96 to 0.05)	0.079
Least-square mean CRP** (95% CI)	26.97 (16.87 to 43.11)	42.35 (31.44 to 57.04)	0.64 (0.38 to 1.06)	
Remdesivir no Tocilizumab				

N	16	23		
Least-squares mean log(CRP)* (95% CI)	3.16 (2.79 to 3.53)	3.5 (3.18 to 3.83)	-0.35 (-0.79 to 0.1)	0.123
Least-square mean CRP** (95% CI)	23.53 (16.29 to 33.99)	33.26 (23.97 to 46.15)	0.71 (0.45 to 1.1)	
Tocilizumab no Remdesivir				
N	1	5		
Remdesivir and Tocilizumab				
N	1	2		

* From linear repeated measures model, adjusted for natural log(baseline CRP, age, sex, BMI, serious condition, time, treatment, a treatment*time interaction, and subject as a random effect. Least squares means compared at mean follow-up time.

** Antilog of estimates from *. Ratio of BAC + dorna-alfa: BAC shown in the difference column.

[a] From linear model, adjusted for natural log(baseline CRP, age, sex, BMI, serious condition, and treatment.

This effect of dornase alfa on CRP was confirmed in various other subgroup analyses and are shown in Table 2: the per-protocol population only; participants who were randomised into the COVASE trial, excluding the historical controls (**Figure 1E**); participants who were randomised to BAC + dornase alfa in the COVASE trial, and HC,. excluding those randomised to BAC only (**Table 2**).

In addition, to ensure that the HCs did not have a significantly different CRP trajectory to those randomised to BAC, we compared participants who were randomised to BAC with HC by excluding those randomised to BAC + dornase alfa and found no significant differences (**Table 2**).

Sensitivity analyses were also conducted and continued to support the observed effect on CRP. These are also shown in Table 2 and included: log(CRP) as an area under the curve; historical controls matched for their last pre-dexamethasone CRP measurement as opposed to their first CRP after starting dexamethasone; and the effect of remdesivir or tocilizumab.

Secondary outcomes

Length of hospitalisation was analysed as a time-to-event outcome of alive discharge from hospital censored at 35 days. The hazard ratio observed in the Cox proportional hazards model was 1.63 (95% CI, 1.01 to 2.61), p=0.03 (**Table 3 and Figure 2A**). Showing that throughout 35 days follow-up, there was a 63% higher chance of discharge alive at any given time-point in the BAC + dornase alfa group compared to the BAC group. Although the rate of discharge was similar in 50% of patients, 80% discharge occurred by 8 days in the dornase alfa group whereas, whereas the same proportion was reached at 30 days in the BAC group, suggesting that dornase alfa may be beneficial to patients that do not respond efficiently to dexamethasone alone. This trend was also seen when only the COVASE participants were considered, although not powered to reach significance and with a smaller HR of 1.18 (95% CI, 0.52-2.69), p=0.62, (**Supplementary Table 2 and Supplementary Figure 2B**).

Table 3.

Secondary Endpoints

Secondary Endpoints	Randomised to BAC + dornase-alfa	BAC	Difference between BAC + dornase-alfa and BAC	p-value*
Time to discharge from hospital (days)				
Number discharged	27	51		
Median time to discharge [a]	6	7	-1	
(95% CI)	(4 to 7)	(6 to 12)		
Hazard ratio [b]			1.63	0.030
(95% CI)			(1.01 to 2.61)	
D-dimer (ug/L) FEU [d]				
N	28	11		
Least-square mean [c]	570.78	1656.96	0.34	0.004
(95% CI)	(384.51 to 847.3)	(876.93 to 3130.81)	(0.17 to 0.69)	
Lymphocyte count (×10⁹/L)**				
N	30	61		
Least-square mean [c]	1.08	0.87	1.25	0.021
(95% CI)	(0.92 to 1.27)	(0.76 to 0.98)	(1.03 to 1.51)	
Procalcitonin levels (ng/mL)				
N	26	7		
Least-square mean [c]	0.18	1.31	-1.13	0.005
(95% CI)	(-0.2 to 0.56)	0.56 to 2.05)	(-1.88 to –0.37)	

a Estimated from Kaplan-Meier curve.

b From Cox proportional hazard model, adjusting for age, baseline CRP and treatment

c From linear repeated measures model, adjusted for baseline endpoint, age, sex, BMI, serious condition, time, treatment, a treatment*time interaction, and subject as a random effect. Least squares means compared at mean follow-up time.

d Modelled by log transforming the outcome. Estimates shown are the antilog of the estimates from the fitted model. Ratio of BAC + dornase-alfa: BAC shown in the difference column.

* From linear repeated measures model, adjusted for natural log(baseline CRP), age, sex, BMI, serious condition, time, treatment, a treatment*time interaction, and subject as a random effect. Least squares means compared at mean follow-up time.

** Antilog of estimates from *. Ratio of BAC + dorna-alfa: BAC shown in the difference column.

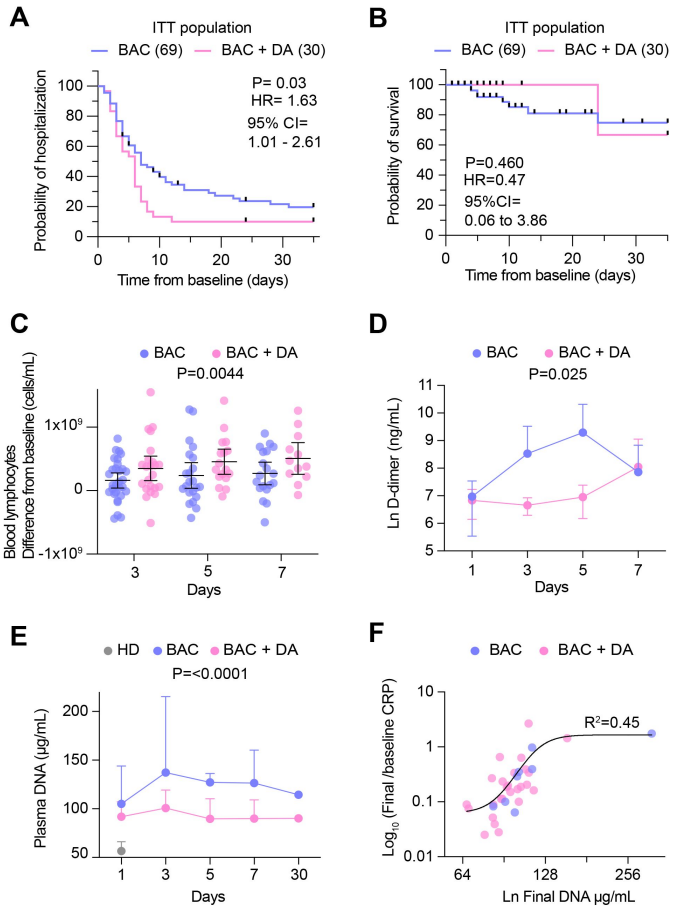

Figure 2.

Analysis of secondary endpoints and exploratory endpoints.

A. Kaplan-Meier plot showing time to discharge from hospital from baseline. ITT population. Hazard ratio from Cox proportional hazards model adjusted for baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease of hypertension). P-value from log-rank test. (Blue: HC and participants randomised to BAC, N=69. Pink: participants randomised to BAC+DA, N=30).

B. Kaplan-Meier plot showing time to death over 35 days follow up. ITT population. Hazard ratio from Cox proportional hazards model adjusted for baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease of hypertension). P-value from log-rank test. (Blue: HC and participants randomised to BAC, N=69. Pink: participants randomised to BAC+DA, N=30). Abbreviations: BAC-best available care, CRP-C-reactive protein, DA-dornase alfa, ITT-intention-to-treat.

C. Difference between the lymphocyte count for each day of the treatment period and the baseline in each patient who exhibited lymphopenia at baseline (<1×10^9 lymphocytes/mL). Mean and 95%CI interval is shown with statistical analysis by two-way Anova.

D. Mean D-dimer levels per day in randomised BAC (blue) and BAC+DA (pink) patients with error bars depicting 95% CI in randomised BAC (blue) and BAC+DA (DA) patients (pink). Statistical difference by by mixed effects Anova analysis.

E. Mean cf-DNA levels per day in randomised BAC (blue) and BAC+DA (pink) patients, with error bars depicting standard deviation. Statistical analysis by mixed effects Anova.

F. Correlation between the final cf-DNA levels and ratio of CRP at day-7 normalized to the baseline CRP ($CRP_{final}/CRP_{baseline}$) per patient. Fitting by non-linear regression.

Over 7 days of follow up there was no significant difference between BAC + dornase alfa versus BAC alone in either the fraction of participants admitted to ICU (23.3% versus 21.74%), p=0.866, or the length of ICU stay, least squares mean 21.25 (95% CI, 4.65 to 37.84) hours versus 19.85 (95% CI, 8.00 to 31.70) hours, p=0.883. The same was seen over 35-day follow-up, with least squares mean 55.21 95% CI, -23.59 to 134.00) hours versus 60.60 (95% CI, 4.34 to 116.86) hours, p=0.905. At any point during the 35 days follow-up, 23% of the BAC + dornase alfa group were admitted to ICU compared to 23.19% in the BAC group, p= 0.983 (**Supplementary Table 3**).

There was no significant difference in time requiring oxygen between the two groups, at either 7 days, least squares mean 94.32 (95% CI, 72.8 to 115.79) hours, versus 88.96 (95% CI, 73.64 to 104.29) hours, p=0.662, or 35 days, least squares mean 133.22 (95% CI, 52.01 to 214.43) hours versus 156.35 (95% CI, 98.36, 214.33) hours, p=0.618. At 35 day follow up if we

look at COVASE participants, there are only 9 participants to evaluate, but mean oxygen use tends to a reduction of 123 hours with BAC + dornase alfa, versus 241 hours for BAC, p=0.187 (**Supplementary Table 3**)

Over 35 days follow up, 1 person amongst the 30 patients in the BAC + dornase alfa group died, compared to 8 of the 69 participants in the BAC group. The hazard ratio observed in the Cox proportional hazards model was 0.47 (95% CI, 0.06 to 3.86), indicating a trend towards a reduced chance of death at any given time-point in the BAC + dornase alfa group compared to the BAC group, but this did not reach significance p= 0.460 (**Figure 2B**).

There was no significant difference at either 7- or 35-days follow-up, in the number of participants that required mechanical ventilation in the BAC + dornase alfa group compared with the BAC group (16.67% vs 13.04%), p=0.628. Amongst participants that were ventilated, the mean length of mechanical ventilation at 7 days follow-up in the BAC + dornase alfa group was 76.8 hours, compared to 88.78 in the BAC group. At 35 days follow-up, the mean length of mechanical ventilation in the BAC + dornase alfa group was 76.8, compared to 411.17 in the BAC group (**Supplementary Table 3**).

There was no significant difference in superadded bacterial pneumonia at either 7- or 35-days follow-up: 7 days, 1 (3.33%) participant in the BAC + dornase alfa group compared to 3 (4.35%) participants in the BAC group, p= 0.934; 35 days, 2 (6.67%) participants in the BAC + dornase alfa group had bacterial pneumonia, compared to 3 (4.35%) participants in the BAC group, p=0.548 (**Supplementary Table 3**).

Blood analysis with no adjustment for multiple testing showed a significant treatment effect in BAC + dornase alfa group vs. BAC group for three parameters: lymphocyte counts, D-dimer, a marker of coagulation and procalcitonin (PCT).

First, the dornase alfa treated group exhibited higher lymphocyte counts with a least-squares mean of 0.87 (95% CI, 0.76-0.98) in the BAC group vs. 1.08 (95% CI, 0.92-1.27) in the BAC + dornase alfa group, p=0.02 (**Table 3 and Supplementary Table 2**). In particular, patients with lymphopenia at baseline ($<1\times10^9$ lymphocytes/L) exhibited a greater increase in blood lymphocyte numbers in the BAC + dornase alfa group than in the BAC group during the entire length of treatment (**Figure 2C**).

Furthermore, D-dimer levels were lower in the BAC + dornase alfa group compared to the BAC group, with a least-squares mean D-dimer difference of 1657 (95% CI, 3131-877) (**Table 3**, **Supplementary Table 2**, **Figure 2D and Supplementary Figure 2C**).

Procalcitonin (PCT) is marker of bacterial infection that is also elevated in many patients with severe COVID-19 infection. Our analysis indicated lower levels of PCT in patients that received dornase alfa, with a mean value of 0.18 ng/mL (95% CI, -0.2-0.56) compared to those treated with BAC alone with a mean of 1.31 ng/mL (95% CI, 0.56-2.05), p=0.005 (**Table 3**). On repeat analysis excluding the historical control population the results were replicated and changes in these 3 parameters reached statistical significance (**Supplementary table 2**).

Exploratory outcomes

Given the role of circulating cf-DNA in pathology, we examined whether the pulmonary administration of dornase alfa influenced systemic cf-DNA levels in plasma. There was no difference in baseline plasma cf-DNA levels on Day 1 between the two groups. However, during the treatment period cf-DNA was reduced in participants randomised to BAC+ dornase alfa compared to BAC alone (**Figure 2E and Supplementary Figure 2D**). We also examined whether cf-DNA levels correlated with D-dimer and CRP levels. Samples that contained cf-DNA above 100µg/mL exhibited significant higher D-dimer levels compared to

samples containing cf-DNA levels below 100µg/mL (**Supplementary Figure 2E**). Moreover, there was a positive correlation between the levels of cf-DNA in the final sample collected during the treatment period and the ratio of final to baseline CRP ($CRP_{final}/CRP_{baseline}$) indicating that changes in CRP were inversely proportional to the final cf-DNA levels in all patients independently of treatment (**Figure 2F**).

Safety

Dornase alfa was very well tolerated, with no systemic effects and this was consistent with its short half-life and lack of systemic exposure. There were 10 reported AEs by 9 participants in the randomised BAC arm versus 30 AEs reported by 30 participants in the BAC + dornase alfa arm. Of these, one was reported by the clinical team as definitely related to the study drug and one as unlikely to be related to the study drug (Supplementary Table S4). 'Tingling of the mouth' after using the nebuliser was attributable to the drug whilst 'headache' was unlikely to be related to the study drug. The AE data reflect the clinical trial and post-marketing experience of using Pulmozyme at the recommended dose regimen. Adverse reactions attributed to Pulmozyme are reported as rare (< 1/1000). No treatment-related serious adverse events (SAEs) occurred in any participants.

Discussion

In this Phase IIa trial involving hospitalised participants with severe COVID-19 pneumonia and systemic inflammation, we found that nebulised human recombinant DNA-ase (dornase alfa) significantly reduced CRP over 7 days, versus BAC, which included dexamethasone, alone. The finding was robust after several sensitivity analyses and no safety concerns resulted from the use of dornase alfa in this patient group. No significant mortality benefit was associated with this reduction, although this phase IIa trial was not powered for this outcome. Moreover dornase alfa reduced time to discharge over 35 days follow up. Adverse events were balanced between the two groups with no treatment related SAEs.

The RECOVERY trial resulted in dexamethasone becoming standard care in patients with COVID-19 pneumonitis requiring supplemental oxygen. We recruited participants dependent on a CRP ≥ 30 mg/L, on the day after receiving their first dexamethasone dose to minimise steroid-dependent effects on CRP. The finding that dornase alfa can significantly reduce CRP in participants receiving dexamethasone suggests a complementary mode of action to deliver sustained reduction in inflammation. Dornase alfa may also prove suitable for the treatment of patients with mild viral pneumonia, in contrast to dexamethasone which is only effective in patients with an oxygen requirement, and potentially harmful in milder cases[17].

Given the lack of support for COVID-19 studies outside approved UK platform trials, we employed historical controls to allow us to complete the study more quickly and match patients more closely. Still these historical controls include patients that had been admitted on days when competing studies were unable to recruit because of clinical trials pharmacy closures, or patients that had been recruited to the BAC arm of other clinical trials. The historical controls all met the inclusion criteria for COVASE and did not meet any exclusion criteria. They were matched as closely as possible for key parameters and comorbidities. Despite this potential heterogeneity we were still able to show a reduction in CRP and a tendency to reduce hospital stay in dornase alfa treated participants. Our trial was not powered to overcome confounders, such as the use of antivirals and tocilizumab, an IL-6 inhibitor, recognised to reduce CRP[18]. Although underpowered, we demonstrate a trend to a

reduction in CRP with dornase alfa in participants that had received tocilizumab and/or remdesivir, and those that had not.

By stripping the DNA from chromatin, dornase alfa suppresses the proinflammatory properties of histones and potentiating their degradation by serum and NET proteases[14,19]. Suprisingly, dornase alfa reduced cf-DNA levels in the circulation athough the treatment is only localized to the lung due to the short half-life of the enzyme in the bloodstream. Hence, decreases in blood cf-DNA levels likely reflect the enhanced local clearance of pulmonary NETs and DNA released by other cell types. Consistently, we noted an inverse correlation between the final readings of circulating cf-DNA and the magnitude of decrease in CRP within the 7-day treatment period, further supporting a functional link between chromatin levels and systemic inflammation in COVID-19 pneumonitis. The reduction in D-dimer is also consistent with the pro-thrombotic role of NETs in the alveoli of these patients[3]. Consistently, CRP and D-dimer correlated with cf-DNA in all patients. Moreover, the increased recovery of lymphopenia is consistent with our recent finding that cf-chromatin promotes lymphocyte death in sepsis[8]. By countering these pathogenic properties of cf-chromatin, dornase alfa counters coagulopathy and lymphopenia in these patients. Recombinant DNAse treatment may also be beneficial due to a significant number of SARS-CoV-2 and microbial sepsis patients exhibiting defects in plasma cf-DNA degradation[19] which is a critical factor in patient survival.

Whilst immunisation has greatly reduced the numbers of patients admitted to hospitals with COVID-19 pneumonia there is still a need for virally agnostic therapies that retain efficacy even as viruses mutate. Moreover, nebulised dornase alfa can be safely administered outside the health-care setting. Three other trials of dornase alfa in patients with COVID-19 have data published in peer reviewed manuscripts reporting improvements in oxygen requirements[20-22] (Holliday et al., 2021; Okur et al., 2020; Weber et al., 2020). One small study indicated improvements in the plasma and sputum proteomic profile (Fisher et al., 2021). However, these studies examined a small number of patients and the study designs, patient populations and endpoints differ from those used here.

In conclusion, we have demonstrated that nebulised dornase alfa significantly reduces inflammation in hospitalised patients with severe COVID-19 pneumonia with a trend towards clinical benefit with reduced oxygen requirements and earlier discharge from hospital in patients that received this treatment. These very encouraging preliminary findings warrant further investigation in larger studies.

Data Availability

All data produced in the present work are contained in the manuscript

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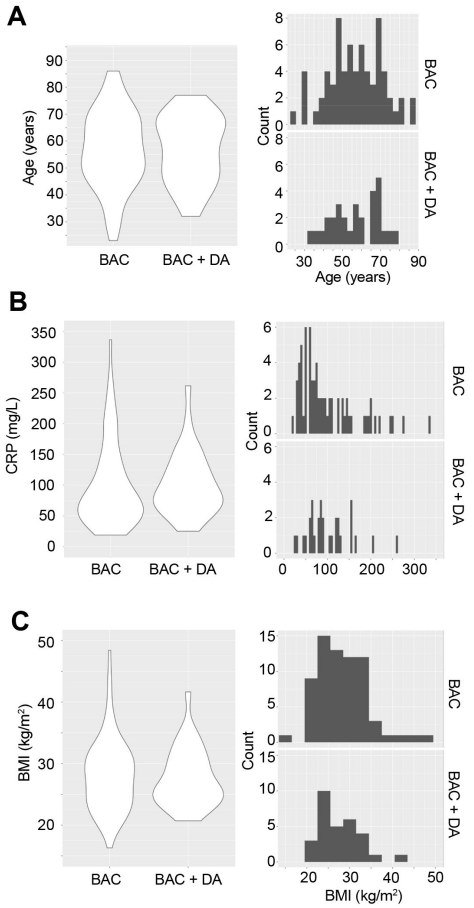
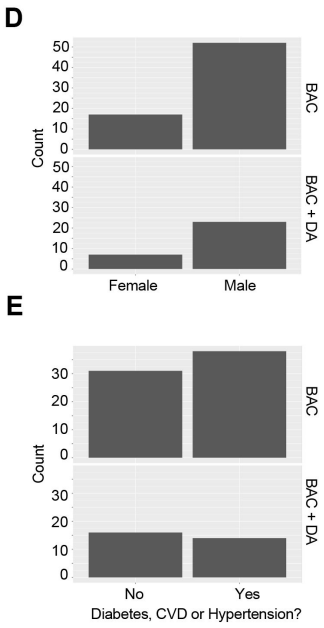
Disclosure

forms provided by the authors are available with the full text of the published article.

Figure Legends





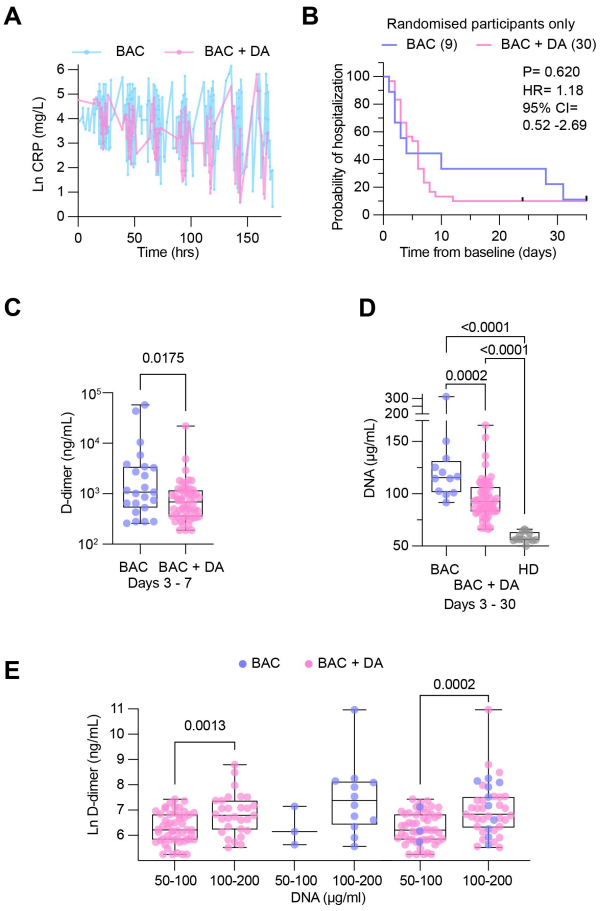
Supplemental Figure 1.

Baseline characteristics of patients analysed in the trial.

(**A-C**). Violin plots (left) and frequency distribution (right) of baseline clinical parameters between patients in the historical control and randomised BAC group and the randomised BAC+Dornase alfa (BAC+DA) group. **A**. Age, **B**. Baseline CRP and **C**. Body mass index (BMI).

D. Number of male and female patients in the two groups.

E. Incidence of cardiovascular comorbidities in the two groups.



Supplemental Figure 2.

A. Graph depicting the periodicity and frequency of blood sample collection for all post-baseline CRP values from historical control and randomised BAC (blue) or BAC+Dornase alfa (BAC+DA, pink) patients pooled into a single timeline.

B. Kaplan-Meier plot showing time to discharge from hospital from baseline. Randomised participants only. Hazard ratio from Cox proportional hazards model adjusted for baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease of hypertension). P-value from log-rank test (Blue: participants randomised to BAC, N=9. Pink: participants randomised to BAC+DA, N=30).

C. D-dimer concentration in randomised patient post-baseline blood samples pooled into BAC and BAC + DA groups. Statistical analysis by two-tailed unpaired parametric t-test.

D. DNA concentration in randomised patient post-baseline blood samples pooled into BAC and BAC + DA groups. Statistical analysis by one-way Anova.

E. Correlation between D-dimer and cell-free (cf) DNA levels in the blood of patients randomised to BAC (blue) or to BAC+DA (DA) (pink), where samples have been segregated depending on whether the corresponding levels of cf-DNA were below or above 100 µg/mL. Statistical analysis by unpaired parametric t-test.

Supplementary Tables

Supplementary Table S1.

Randomised individuals and historical control, additional baseline characteristics. characteristics: white blood cell count, neutrophil count, procalcitonin count and D-dimer

	Randomised to BAC + dornase-alfa (N=30)	Randomised to BAC (N=9)	Historical controls (N=60)	All BAC (N=69)	Total (N=99)
White blood cell count ($\times 10^9$/L)					
N	30	9	60	69	99
Mean	6.7	7.0	10.6	10.2	9.1
SD	2.5	2.7	9.2	8.7	7.6
Median	6.5	7.0	9.5	8.9	7.9
Min	3.1	1.8	1.8	1.8	1.8
Max	12.9	10.3	72.6	72.6	72.6
Neutrophil count ($\times 10^9$/L)					
N	30	9	60	69	99
Mean	5.7	5.6	9.1	8.7	7.8
SD	2.3	2.6	8.8	8.4	7.2
Median	5.3	5.8	7.9	7.9	6.7
Min	2.4	1.2	1.2	1.2	1.2
Max	10.9	8.6	69.5	69.5	69.5
Lymphocyte count ($\times 10^9$/L)					
N	30	9	60	69	99

Mean	0.7	0.9	0.9	0.9	0.9
SD	0.3	0.4	0.5	0.5	0.5
Median	0.5	0.9	0.8	0.8	0.7
Min	0.2	0.4	0.1	0.1	0.1
Max	1.5	1.5	3.7	3.7	3.7
Monocyte count ($\times 10^9$/L)					
N	30	9	60	69	99
Mean	0.4	0.4	0.5	0.5	0.4
SD	0.2	0.3	0.3	0.3	0.3
Median	0.3	0.3	0.4	0.4	0.4
Min	0.1	0.1	0.1	0.1	0.1
Max	0.9	0.8	1.7	1.7	1.7
Eosinophil count ($\times 10^9$/L)					
N	30	9	60	69	99
Mean	0.0	0.0	0.0	0.0	0.0
SD	0.1	0.0	0.1	0.1	0.1
Median	0.0	0.0	0.0	0.0	0.0
Min	0.0	0.0	0.0	0.0	0.0
Max	0.2	0.1	0.6	0.6	0.6
Basophil count ($\times 10^9$/L)					
N	30	9	60	69	99

Mean	0.0	0.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0
Median	0.0	0.0	0.0	0.0	0.0
Min	0.0	0.0	0.0	0.0	0.0
Max	0.2	0.1	0.1	0.1	0.2
Procalcitonin count (ng/ml)					
N	27	8	1	9	36
Mean	0.3	0.3	19.3	2.4	0.8
SD	0.4	0.3	-	6.3	3.2
Median	0.1	0.2	19.3	0.2	0.2
Min	0.1	0.1	19.3	0.1	0.1
Max	1.8	0.8	19.3	19.3	19.3
D-dimer (ug/L) FEU					
N	30	9	15	24	54
Mean	885.0	909.1	1059.3	1003.0	937.4
SD	1154.5	1054.1	1115.0	1071.8	1109.6
Median	545.0	570.0	600.0	585.0	570.0
Min	190.0	1.9	280.0	1.9	1.9
Max	6580.0	3570.0	4460.0	4460.0	6580.0
WHO ordinal COVID-19 severity score*					
N	30	9	0	9	39
Mean	5.0	5.0	-	5.0	5.0
SD	0.0	0.5	-	0.5	0.2
Median	5.0	5.0	-	5.0	5.0
Min	5.0	4.0	-	4.0	4.0
Max	5.0	6.0	-	6.0	6.0

Supplementary Table S2.

Randomised participants only

	Randomised to BAC + dornase-alfa	Randomised to BAC only	Difference between BAC + dornase-alfa and BAC	p-value*
Time to discharge from hospital (days)				
Number discharged	27	8	19	
Median time to discharge**	6 (4 to 7)	4 (2 to-n.a.)	2	
Hazard ratio***			1.18 (0.53 to 2.69)	0.62
D-dimer (ug/L) FEU				
N	28	6		
Least-squares mean (log)* (95% CI)	6.37 (6.01 to 6.74)	7.55 (6.71 to 8.39)	-1.18 (-2.02 to -0.33)	0.008
Least-square mean** (95% CI)	586.87 (407.44 to 845.31)	1903.82 (821.57 to 4411.69)	0.31 (0.13 to 0.72)	
Lymphocyte count ($\times 10^9$/L)				
N	30	9		
Least-squares mean (log)* (95% CI)	-0.06 (-0.25 to 0.12)	-0.46 (-0.82 to -0.1)	0.4 (0.03 to 0.76)	0.033
Least-square mean** (95% CI)	0.94 (0.78 to 1.13)	0.63 (0.44 to 0.9)	1.49 (1.03 to 2.13)	
Procalcitonin count (ng/ml)				
N	26	7		
Least-square mean* (95% CI)	0.18 (-0.2 to 0.56)	1.31 (0.56 to 2.05)	-1.13 (-1.88 to -0.37)	0.005

*From log-rank test with treatment as a stratification variable.
**Estimated from Kaplan-Meier curve.
***From Cox proportional hazard model, adjusting for age, baseline CRP and treatment.

Supplementary Table S3.

Secondary clinical endpoints

- Admission to ICU:
    - Over 7 days of follow up BAC + dornase-alfa versus BAC alone % of participants admitted to ICU (23.3% versus 21.74%), p=0.866,
    - length of ICU stay, LSM 21.25 (95% CI, 4.65 to 37.84) hours versus 19.85 (95% CI, 8.00 to 31.70) hours, p=0.883.
    - over 35-day follow-up, LSM 55.21 95% CI, -23.59 to 134.00) hours versus 60.60 (95% CI, 4.34 to 116.86) hours, p=0.905.
    - At any point during 35 days follow-up, 23% of the BAC + dornase-alfa group were admitted to ICU compared to 23.19% in the BAC group, p= 0.983.
- time requiring oxygen at
    - 7 days, LSM 94.32 (95% CI, 72.8 to 115.79) hours, versus 88.96 (95% CI, 73.64 to 104.29) hours, p=0.662, or
    - 35 days, LSM 133.22 (95% CI, 52.01 to 214.43) hours versus 156.35 (95% CI, 98.36, 214.33) hours, p=0.618.
    - At 35 day follow up mean oxygen use tends to a reduction of 123 hours with BAC + dornase-alfa, versus 241 hours for BAC, p=0.187
- participants that required mechanical ventilation and mean length of ventilation at either
    - 7- or
    - 35-days follow-up,
- Superadded bacterial pneumonia at either
    - 7- or
    - 35-days follow-up:

Time on Oxygen over 7 days follow-up (hours)				
N	30	69		
Least-square mean* (95% CI)	94.32 (72.86 to 115.79)	88.96 (73.64 to 104.29)	5.36 (-18.92 to 29.65)	0.662
Time on Oxygen over 35 days follow-up (hours)				
N	30	69		
Least-square mean* (95% CI)	133.22 (52.01 to 214.43)	156.35 (98.36 to 214.33)	-23.12 (-115.02 to 67.77)	0.618

Proportion of individuals on mechanical ventilation over 7 days follow-up				
N (%)	5 (16.67)	9 (13.04)	-4 (3.62)	
Odds ratio* (95% CI)			1.36 (0.39 to 4.66)	0.628
Proportion of individuals on mechanical ventilation over 35 days follow-up				
N (%)	5 (16.67)	9 (13.04)	-4 (3.62)	
Odds ratio* (95% CI)			1.36 (0.39 to 4.66)	0.628
*From Logistic regression model, adjusted for age, sex, BMI, baseline CRP, serious condition and treatment.				
Proportion of individuals with Superadded Bacterial Pneumonia over 7 days follow-up				
N (%)	1 (3.33)	3 (4.35)		
Odds ratio* (95% CI)	0.9 (0.08 to 10.21)			0.934
Proportion of individuals with Superadded Bacterial Pneumonia over 35 days follow-up				
N (%)	2 (6.67)	3 (4.35)		
Odds ratio* (95% CI)	1.81 (0.26 to 12.61)			0.548
*From Logistic regression model, adjusted for age, sex, BMI, baseline CRP, serious condition, and treatment.				

Supplementary Table S4.

Safety

Adverse Events in the BAC + dornase alfa group

Subject	BAC + dornase-alfa, or BAC only?	Adverse event	Serious?	Relationship to study drug
COV002	Dornase-alfa + BAC	Cough & SOB	No	Not related
COV003	Dornase-alfa + BAC	Mild depression	No	Not related
COV003	Dornase-alfa + BAC	Mild cognitive impairment	No	Not related
COV005	Dornase-alfa + BAC	Struggle to sleep	No	Not related
COV005	Dornase-alfa + BAC	Transaminitis (ALT 91 - NR 10-35 iu/L)	No	Not related
COV005	Dornase-alfa + BAC	Constipation	No	Not related
COV007	Dornase-alfa + BAC	Blood stain in sputum	No	Not related
COV012	Dornase-alfa + BAC	Small Pericardial Effusion	No	Not related
COV012	Dornase-alfa + BAC	Dysphonia	No	Not related
COV012	Dornase-alfa + BAC	Hypercapnia	No	Not related
COV013	Dornase-alfa + BAC	Ulcerative Colitis flare	No	Not related
COV013	Dornase-alfa + BAC	Bradycardia	No	Not related
COV015	Dornase-alfa + BAC	Mechanical Fall	No	Not related
COV015	Dornase-alfa + BAC	Dizziness	No	Not related
COV018	Dornase-alfa + BAC	Dehydration	No	Not related

COV018	Dornase-alfa + BAC	Lower Respiratory Tract Infection	No	Not related
COV018	Dornase-alfa + BAC	Haemoptysis	No	Not related
COV020	Dornase-alfa + BAC	Chest pain	No	Not related
COV022	Dornase-alfa + BAC	Microcytic anaemia	No	Not related
COV022	Dornase-alfa + BAC	Elevated Blood glucose	No	Not related
COV023	Dornase-alfa + BAC	Tachypnoea (PR 32BPM)	No	Not related
COV023	Dornase-alfa + BAC	Hyperglycaemia (BM 14.9)	No	Not related
COV031	Dornase-alfa + BAC	Chest Pain	No	Not related
COV035	Dornase-alfa + BAC	Left leg spasm	No	Not related
COV035	Dornase-alfa + BAC	Rectal bleed due to haemorrhoids	No	Not related
COV037	Dornase-alfa + BAC	Chest Pain	No	Not related
COV002	Dornase-alfa + BAC	Tingling of the mouth	No	Definitely
COV035	Dornase-alfa + BAC	Headache	No	Unlikely

Appendices

Supplementary appendix 1:Protocol

Supplementary appendix 2: Statistical Analysis Plan (SAP)

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Joint Public Review:

In this study, Porter et al report on outcomes from a small, open-label, pilot randomized clinical trial comparing dornase-alfa to the best available care in patients hospitalized with COVID-19 pneumonia. As the number of randomized participants is small, investigators describe also a contemporary cohort of controls and the study concludes about a decrease of inflammation (reflected by CRP levels) after 7 days of treatment but no other statistically significant clinical benefit.

Suggestions to the authors:
• The RCT does not follow CONSORT statement and reporting guidelines
• The authors have chosen a primary outcome that cannot be at least considered as clinically relevant or interesting. After 3 years of the pandemic with so much research, why investigate if a drug reduces CRP levels as we already have marketed drugs that provide beneficial clinical outcomes such as dexamethasone, anakinra, tocilizumab and baricitinib.
• Please provide in Methods the timeframe for the investigation of the primary endpoint
• Why day 35 was chosen for the read-out of the endpoint?
• The authors performed an RCT but in parallel chose to compare also controls. They should explain their rationale as this is not usual. I am not very enthusiastic to see mixed results like Figures 2c and 2d.
• Analysis is performed in mITT; this is a major limitation. The authors should provide at least ITT results. And they should describe in the main manuscript why they chose mITT analysis.
• It is also not usual to exclude patients from analysis because investigators just do not have serial measurements. This is lost to follow up and investigators should have pre-decided what to do with lost-to-follow-up.
• In Table 1 I would like to see all randomized patients (n=39), which is missing. There are also baseline characteristics that are missing, like which other treatments as BAT received by those patients except for dexamethasone.
• In the first paragraph of clinical outcomes, the authors refer to a cohort that is not previously introduced in the manuscript. This is confusing. And I do not understand why this analysis is performed in the context of this RCT although I understand its pilot nature.
• Propensity-score selected contemporary controls may introduce bias in favor of the primary study analysis, since controls are already adjusted for age, sex and comorbidities.
• The authors do not clearly present numerically survivors and non-survivors at day 34, even

though this is one of the main secondary outcomes.
• It is unclear why another cohort (Berlin) was used to associate CRP with mortality. CRP association with mortality should (also) be performed within the current study.