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Multicenter randomized trial assessing efficacy and safety of aerosolized dornase Alfa in COVID-19 ARDS

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Acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 infection is associated with high mortality rates and respiratory compromise in which excessive neutrophil extracellular trap (NET) production may amplify alveolar inflammation and injury. Dornase alfa, a recombinant DNAse 1, has been proposed to attenuate these effects by degrading extracellular DNA and enhancing alveolar clearance of NETs. In this multicenter, open-label, randomized in two parallel arms (1:1) controlled trial, intubated COVID-19 ARDS patients received either standard-of-care (SOC) alone or SOC plus aerosolized dornase alfa (2500 IU twice daily for 7 days). The primary endpoint was the proportion of patients with ARDS severity improvement at Day 7, defined by at least one-grade improvement on the Berlin criteria scale. Secondary outcomes included 28-day mortality, ventilator-free days, ICU-free days, and changes in key ventilatory parameters. Biological samples were analyzed to assess NET related markers, DNAse drug activity and indicate possible bioavailability issues associated with aerosolization of dornase alfa. Seventy-seven patients were enrolled (dornase alfa group, $n = 39$; SOC group, $n = 38$). At Day 7, ARDS severity improved in 18% of patients receiving dornase alfa compared with 29% in the SOC group (adjusted OR: 0.33; 95% CI 0.09–1.14; $p = 0.11$). Secondary endpoints, including 28-day mortality, ventilator-free days, and ICU-free days, showed no significant differences between groups. Adverse events occurred in 38.5% of patients in the dornase alfa arm versus 31.6% in the SOC arm, indicating comparable safety profiles. Despite early increases in NET plasmatic levels observed in both groups and successful ex vivo NET degradation, aerosolized dornase alfa failed to significantly enhance DNAse activity or reduce NET-related markers in patients' plasma and mucus, suggesting potential bioavailability limitations with this delivery method. In patients with COVID-19-related ARDS, dornase alfa did neither significantly reduce ARDS severity nor improve clinical outcomes over SOC. Although well tolerated, analysis of biological samples suggests that aerosol administration may have compromised drug bioavailability. Further trials are needed to determine whether specific patient subgroups could benefit more from dornase alfa or if alternative drug

delivery methods might enhance treatment efficacy. ClinicalTrials.gov, NCT04355364. Registered on 21/04/2020.

Keywords COVID-19, ARDS, Inflammation, Neutrophil extracellular traps, DNAse 1

Abbreviations

ARDS	Acute respiratory distress syndrome
CI	Confidence interval
DAMP	Damage-associated molecular patterns
ECMO	Extracorporeal membrane oxygenation
HFNO	High-flow nasal oxygenation
ICU	Intensive care unit
LOCF	Last observation carried forward
MPO	Myeloperoxidase
NET	Neutrophil extracellular trap
OR	Odd ratio
SOC	Standard of care

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Acute respiratory distress syndrome (ARDS) is a life-threatening condition characterized by acute hypoxemia, noncardiogenic pulmonary edema, and the need for mechanical ventilation. Despite advancements in supportive care, including lung-protective ventilation and fluid management strategies, ARDS remains associated with significant mortality, reaching 40–46% in severe cases[1,2]. COVID-19 has significantly increased the global burden of ARDS, with studies reporting that up to 42% of hospitalized patients with SARS-CoV-2 infection progress to ARDS[3].

The Berlin definition stratifies ARDS severity into mild, moderate, and severe categories based on oxygenation indices, reflecting the clinical and pathophysiological heterogeneity of the syndrome[1]. One of the central mechanisms underlying ARDS is an intense inflammatory response triggered by damage-associated molecular patterns, leading to the activation and recruitment of neutrophils into the pulmonary interstitium and alveolar space[4,5]. Neutrophils, key effectors of the innate immune system, exert their antimicrobial function in part through the release of neutrophil extracellular traps (NETs)—web-like structures composed of decondensed chromatin, neutrophil elastase, myeloperoxidase, and citrullinated histones[6]. While NETs play a protective role in trapping pathogens, their excessive accumulation within the alveoli can promote endothelial and epithelial injury, obstruct distal airways, and initiate thromboinflammatory cascades that contribute to lung damage[7,8]. In patients with COVID-19-related ARDS, numerous studies have reported markedly elevated levels of NET components in plasma and bronchoalveolar lavage fluid, with concentrations correlating strongly with disease severity and poor clinical outcomes[9,10]. The persistent presence of NETs is thought to hinder alveolar healing by maintaining a prothrombotic and proinflammatory microenvironment, exacerbating diffuse alveolar damage and impairing gas exchange[11]. Altogether, ARDS—particularly in its COVID-19-related form—emerges as a complex syndrome driven by both epithelial–endothelial barrier disruption and dysregulated neutrophil activity. Among the key mediators, NETs represent a pathological hallmark linking inflammation, thrombosis, and impaired alveolar repair[4,5,7,8].

Inhaled dornase alfa, a recombinant human DNase I, is approved for use in cystic fibrosis, where it enhances airway clearance by degrading extracellular DNA and reducing mucus viscosity[12]. Beyond its mucolytic properties, dornase alfa has demonstrated potential in limiting NET-related toxicity and modulating neutrophil-driven inflammation. In a murine model of viral ARDS, intravenous administration of dornase alfa led to reduced neutrophil infiltration, attenuation of diffuse alveolar damage, improved lung architecture, and restored pulmonary perfusion[13]. These findings support its potential as a therapeutic candidate in NET-driven lung injury such as ARDS[14], where excessive extracellular DNA contributes to ARDS pathophysiology[4,5,7,8].

This study evaluates the impact of aerosolized intratracheal dornase alfa on ARDS severity and clinical outcomes in mechanically ventilated COVID-19 patients. By targeting the extracellular DNA scaffold of NETs, dornase alfa may reduce hyperinflammation, improve alveolar clearance, and prevent progression to severe ARDS.

Methods

Study design

COVIDornase was an investigator-initiated, multicenter, randomized (1:1) study in two parallel arms, and open-label clinical trial conducted in seven intensive care units (ICUs) in France. The study was approved by the Comité de Protection des Personnes Ouest IV Nantes (EUDRACT: 2020-001492-33). All procedures were performed in compliance with relevant laws and institutional guidelines and regulations. Written informed consent was obtained from patients or their legal representatives prior to inclusion whenever possible. In cases requiring emergency inclusion, consent was obtained retrospectively from the patient or their relatives. The trial was registered in ClinicalTrials.gov on 21/04/2020, before the enrollment of the first patient and was overseen by an independent data and safety monitoring committee (DSMC). The trial registration number is NCT04355364. A full trial protocol was published previously[15]. No design changes occurred after trial initiation. Interim safety analyses based on a Bayesian analysis of the delta between the two groups on the primary endpoint were conducted after the inclusion of 20, 50, and 75 patients. In a Bayesian context, the trial continuation decisions were based on the following posterior probabilities: $P3 = P(\Delta < 0 \mid \text{data})$ inefficacy or harmful effect; $P4 = P(\Delta < -0.2 \mid \text{data})$ clinically significant harmful effect. The following decision rules were proposed: Stop with evidence of inefficacy if $P3 > 0.8$; Stop with evidence of a harmful effect if $P4 > 0.67$. The trial was not stopped based on these criteria, but due to a lack of recruitment.

Patients

Patients aged ≥ 18 years with confirmed severe COVID-19 pneumonia and ARDS, as defined by the Berlin criteria ($PaO_2/FiO_2 < 300$ mmHg and PEEP ≥ 5 cmH_2O), were eligible for enrollment. Additional inclusion criteria included intubation for < 8 days and an anticipated need for mechanical ventilation > 48 h. Patients with known hypersensitivity to dornase alfa or any of the excipients, pregnant or breastfeeding woman and patient under legal protection measures were excluded. Baseline demographics, clinical characteristics (e.g., comorbidities, concomitant medications), and laboratory data were collected at enrollment. Patients were recruited between November 2020 and August 2021 (2nd, 3rd and 4th epidemic waves in France). One third of the patients (26/77) were included in Parisian hospitals (Hôpital Fondation Adolphe de Rothschild and Hôpital Pitié-Salpêtrière), others were included in roughly equivalent proportions in the hospitals of Valenciennes (15/77), Metz (15/77), Chartres (12/77), and Strasbourg (9/77).

Randomization and blinding

Patients were randomized in a 1:1 ratio to receive either standard-of-care (SOC) alone according to current international guidelines[16] or SOC plus nebulized dornase alfa. Randomization was stratified by center and according to the average PaO_2/FiO_2 ratio over the 24 h preceding inclusion (< 100, 100–200, or 200–300 mmHg). The allocation sequence was based on blocks of variable sizes (2 or 4) and generated centrally by a statistician to ensure balance between groups. Randomization was accessible for the physician enrolling the patient via a web-based module and secured and concealed until assignment. For budgetary reasons, this study being conducted on an exploratory basis, physicians, patients, and outcome assessors were not blinded to treatment assignment due to the open-label design.

Intervention

In the intervention group, Dornase alfa (Pulmozyme®, Roche, Switzerland) was administered by aerosol, at a dose of 2500 IU twice daily, 12 h apart, for 7 consecutive days, using a vibrating mesh nebulizer (Aerogen Solo®, Aerogen, Ireland), in addition to SOC. This regimen was derived from its established clinical use in cystic fibrosis, where 2500 IU once or twice daily by nebulization has been validated and shown to be safe[17]. The first dose was administered within 1 h after randomization and the whole content of the Pulmozyme® vial was poured directly in the Aerogen chamber by the nurse in charge without dilution. Duration of aerosolization was not specifically recorded but varied between 3 and 5 min for each dose. In every study site, Pulmozyme® was stored at 4 °C in temperature-controlled refrigerators and was prepared extemporaneously before administration. The Aerogen device was shown to optimize dornase alfa deposition in the distal lung airways[18]. The nebulizer was placed upstream in the inspiratory limb of the ventilator. Active humidification was authorized according to the preference of the attending physician, in which case, the aerogen device was placed upstream the humidification chamber, on the "dry limb" of the inspiratory ventilator circuit, as recommended by the manufacturer. In any circumstances, guidelines published by the International Society of Aerosols in Medicine were followed. Ventilator settings were not altered during aerosol administration. Administration of dornase alfa twice daily was also allowed using NIV and high-flow nasal oxygenation (HFNO) in patients randomized to the intervention group when they were extubated within 7 days after inclusion. The vibrating mesh nebulizer was placed on the same position on the inspiratory circuit.

SOC for ARDS during the study period included the use of corticosteroids (dexamethasone 6 mg intravenously per day for 7 to 10 days), antithrombotic agents (enoxaparin 4000 IU twice per day, increased to 6000 IU twice per day in patients weighing more than 120 kg), and protective mechanical ventilation strategies (tidal volume set to 6 mL/kg ideal body weight, plateau pressure and driving pressure kept under 30 and 15 cm H_2O, respectively) in accordance with institutional protocols and international recommendations[16]. The use of adjunctive therapies, such as neuromuscular blocking agents (usually when PaO_2/FiO_2 ratio was under 150) prone positioning (usually when PaO_2/FiO_2 ratio was under 100 or when neuromuscular blockade failed to improve PaO_2/FiO_2 ratio above 150), or extracorporeal membrane oxygenation (ECMO, as a third line rescue therapy), was at the discretion of the treating ICU team for both groups. The use of tocilizumab was not universally approved when patients were included, and its prescription was marginal among participating centers. Patients in the control group received SOC alone.

Data collection

Demographic and baseline clinical data, including comorbidities, corticosteroid use, timing from ARDS diagnosis to randomization, and laboratory values, were recorded at enrollment. Daily data were collected for the first 14 days on the use of mechanical ventilation, oxygen support modalities (high-flow nasal cannula, noninvasive ventilation), prone positioning, neuromuscular blockade, and ECMO. Diagnosis of new infections, changes in clinical status, and other outcomes were recorded through day 28 or until hospital discharge, whichever occurred first. The data were collected every day until day 28 or hospital discharge from the patient's medical record by clinical research technicians in an open-label manner.

Plasma and mucus collection

Plasma and mucus samples were collected from patients at Day 0, Day 2, Day 7, and Day 28. DNAse I activity in plasma and mucus from COVIDornase patients was quantified using the DNAse I Assay Kit Fluorometric (Ref. ab234056, Abcam, Cambridge, UK). Neutrophil extracellular traps (NETs) were evaluated in plasma from COVIDornase patients using the protocol developed by Sun et al.[19] Evaluation of NETs in mucus was not possible due to technical constraints. To address this, myeloperoxidase (MPO) in mucus from COVIDornase patients was measured using the MPO Human ELISA kit (Ref. HK324, Hycult Biotech, PB UDEN, The Netherlands), and extracellular DNA in mucus was measured using the Quant-iT PicoGreen (Ref. P7589, Invitrogen, Carlsbad, USA). To further evaluate the ex vivo lytic efficacy of dornase alfa, fresh mucus samples obtained at Day 0 from ten patients were subjected to fluorescence imaging. Each sample was first incubated with Hoechst 33,342 (Ref. H3570, Invitrogen, Carlsbad, USA) at a final concentration of 10 µg/mL for 30 min at 37 °C. Then, 25 µL of mucus was treated for 10 min at 37 °C with either PBS ($n=5$) or Pulmozyme at 1 mg/mL ($n=5$). A small volume of each treated sample was spread onto a microscope slide, and images were acquired using a Zeiss Axio Observer microscope (Carl Zeiss, Oberkochen, Germany). Total DNA fluorescence per field was quantified using ImageJ software to determine the extent of DNA degradation. Excitation/emission wavelengths used: 375–407/420–450 nm (DAPI) and 542–566/578–610 nm (MPO).

Outcomes

Outcomes were pre-specified. The primary outcome was improvement in ARDS severity, defined as at least one-grade improvement on the Berlin criteria scale (e.g., from "severe" to "moderate" or "moderate" to "mild") between baseline (D0) and Day 7 (D7). Secondary outcomes included all-cause mortality at 28 days, the number of ventilator-free days between Day 0 and Day 28 (defined as days alive and free of mechanical ventilation for ≥ 48 consecutive hours) and the number of ICU-free days between Day 0 and Day 28. Changes in physiological parameters (PaO_2/FiO_2 mean ratio, FiO_2 and PEEP) between Day 0 and D 7 were also compared between groups (post-hoc analysis). We had planned to analyze the quality of life at Day 28 for patients surviving to Day 28; however, this information was not collected and could not be analyzed.

Statistical analysis

The COVIDornase study was exploratory, and sample size was not based on power calculations but on feasibility in terms of recruitment. However, the inclusion of 36 patients per group allowed for the detection of a 60% improvement in experimental group compared to a 28% improvement in the control group (bilateral alpha risk of 5% and power of 80%). Analyses were conducted on an intention-to-treat basis, with all randomized patients included in their assigned group regardless of protocol adherence and imputation of primary outcome using last observation carried forward (LOCF) method if missing. Confidence intervals (CI) were calculated using Wald interval method. A sensitivity analysis on available data was carried out. Descriptive statistics were used to summarize baseline characteristics. Differences between groups regarding concomitant medication over the first 7 days were assessed using chi-square tests or Fisher exact tests. Mixed models adjusted for center (random effect), prone position and administration of anti-infective (antibiotics and lopinavir-ritonavir) and anti-inflammatory treatments (tocilizumab and corticosteroids) within the first 7 days were used for outcomes comparisons between groups (logistic regressions for primary outcome and mortality, Poisson regressions for numbers of ventilator-free days and ICU-free days and linear regressions for PaO_2/FiO_2 mean ratio, FiO_2 and PEEP variations). Odds ratios (ORs) are displayed with 95% confidence intervals (CIs). Regarding the small sample size, no subgroup analyses were performed.

Concomitant medications

Details of concomitant therapies, such as corticosteroids, are provided in Table 2. These medications were considered SOC for COVID-19 ARDS during the study period. Full protocol was previously published[15].

Results

Patients

Between December 2020 and August 2021, a total of 77 patients were enrolled and randomized to either the dornase alfa group ($n=39$) or the standard-of-care (SOC) group ($n=38$). Patients were followed for 28 days or discharge. Details regarding randomized patients, treatment administration and reason for missing data regarding primary outcome in each group are provided in Fig. 1. Baseline demographic and clinical characteristics were well balanced between the groups (Table 1). The median age was 67 years, and 32.5% of participants were female. The prevalence of comorbidities, including hypertension, diabetes, and chronic kidney disease, was similar across groups, and most patients had severe COVID-19 pneumonia requiring high levels of oxygen support upon inclusion (median PaO_2/FiO_2 ratio: 143 [123, 169]). Most patients (89% in SOC and 85% in dornase alfa) received systemic corticosteroids dexamethasone 6 mg/day for 10 days) as part of the SOC for COVID-19 ARDS during the study period.

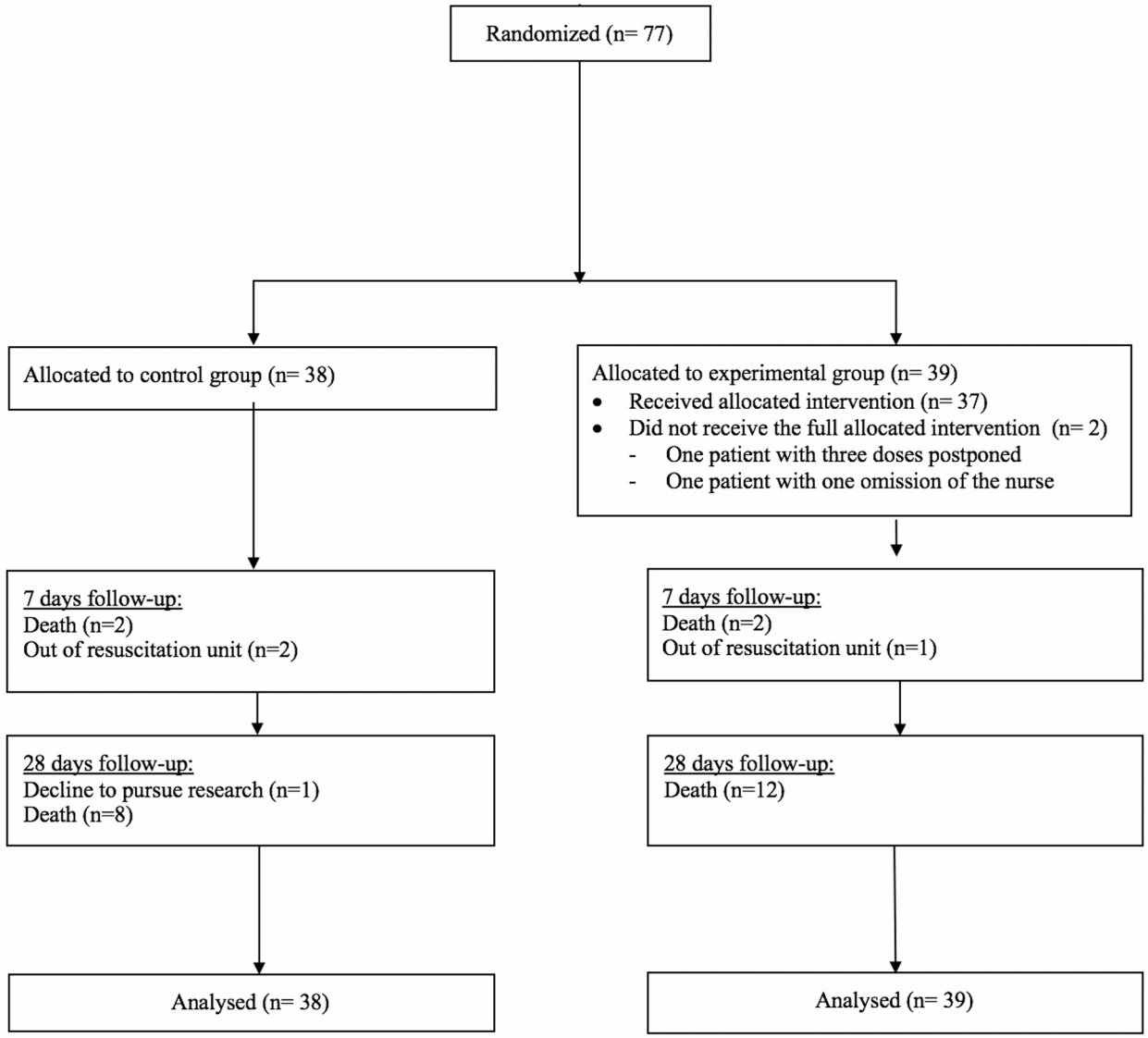

Fig. 1. Flow-chart.

Interventions and treatments during the first 7 days

Treatments administered during the first 7 days are summarized in Table 2. The use of prone positioning (81% in SOC vs. 68% in dornase alfa, $p = 0.2$) and antibiotics (95% in both groups) was consistent across groups. The use of tocilizumab and other anti-inflammatory agents was infrequent and similar between groups. These findings indicate comparable management practices between treatment arms during the initial intervention period.

Primary outcome

The primary outcome, an improvement of at least one grade on the ARDS severity scale (Berlin criteria) between Day 0 and Day 7, was observed in 18% of the dornase alfa group and 29% of the SOC group (adjusted odds ratio [OR]: 0.33; 95% CI 0.09–1.14, $p = 0.11$) (Table 3). Primary outcome was imputed with LOCF method for 7 patients. Sensitivity analyses using available case data showed similar results, with no significant association between treatment group and ARDS improvement (adjusted OR: 0.34; 95% CI 0.10–1.17).

Secondary outcomes

Key secondary outcomes are detailed in Table 3. All-cause mortality at Day 28: mortality was 36% in the dornase alfa group and 26% in the SOC group (adjusted OR: 2.66; 95% CI 0.88–8.03). Ventilator-free days: the median number of days alive and free from mechanical ventilation at day 28 was 1 day in the dornase alfa group versus 2 days in the SOC group (adjusted Incidence Rate Ratios [IRR]: 0.71; 95% CI 0.39–1.28). ICU-free days: patients in both groups had a median of 0 days outside the ICU within the 28-day follow-up period (adjusted IRR: 0.69; 95%

Median (Q1, Q3) or *n*/*N* (%)	CONTROL (*N* = 38)	EXPERIMENTAL (*N* = 39)	OVERALL (*N* = 77)
Female gender	13/38 (34%)	12/39 (31%)	25/77 (32.5%)
Age at inclusion (years)	67 (61, 71)	66 (57, 73)	67 (60, 73)
BMI (kg/m^2)*	27.3 (24.5, 33.7)	31.1 (27.4, 33.7)	29.9 (25.7, 33.9)
Medical history cancer	3/38 (8%)	3/39 (8%)	6/77 (8%)
High blood pressure	16/38 (42%)	16/39 (41%)	32/77 (42%)
Diabetes	13/38 (34%)	16/39 (41%)	29/77 (38%)
Immunodeficiency	3/38 (8%)	0/39 (0%)	3/77 (4%)
Chronic kidney disease	3/38 (8%)	3/39 (8%)	6/77 (8%)
Heart failure	1/38 (3%)	4/39 (10%)	5/77 (6.5%)
SAPS II score	38 (30, 51)	39 (32, 49)	39 (31, 50)
SOFA score*	8 (6, 11)	7.5 (5, 10)	8 (6, 10)
PaO_2/FiO_2 mean ratio at D0	141 (119, 165)	148 (125, 171)	143 (123, 169)
FiO_2 (%)*	60 (50, 74)	54 (44, 67)	55 (50, 70)
PEEP (cmH_2O)	10 (8, 12)	12 (8, 14)	10 (8, 12)
Time since symptoms onset (days)*	13 (9, 15)	12 (8, 18)	12 (8, 16)
Time since intubation (days)	2 (1, 4)	2 (1, 3)	2 (1, 4)

Table 1. Description of baseline characteristics. *3 missing data in control group and 7 in experimental group for BMI. 1 missing data in each group for SOFA score. 1 missing data for FiO_2 in experimental group. 1 missing data for time since symptoms onset in control group.

n/*N* (%)	CONTROL (*N* = 38)	EXPERIMENTAL (*N* = 39)	*p* value
Any prone position	30/37 (81%)	26/38 (68%)	0.2[a]
Any tocilizumab	0/33 (0%)	1/36 (3%)	> 0.9[b]
Any steroids	32/36 (89%)	33/39 (85%)	0.7[b]
Any antibiotics	36/38 (95%)	35/37 (95%)	> 0.9[b]

Table 2. Treatments during the first 7 days. [a] Chi^2 test. [b] Fisher exact test.

Median (Q1, Q3) or *n*/*N* (%)	CONTROL (*N* = 38)	EXPERIMENTAL (*N* = 39)	Adjusted** OR [95CI] or IRR [95CI] or β [95CI]
Improvement of at least one grade in ARDS scale between D0 and D7 (imputed)	11/38 (29%)	7/39 (18%)	0.33 [0.09–1.14]
All causes mortality at D28	10/38 (26%)	14/39 (36%)	2.66 [0.88–8.03]
Number of days alive without invasive mechanical ventilation between D0 and D28	2 (0, 17)	1 (0, 14)	0.71 [0.39–1.28]
Number of days alive out of resuscitation unit between D0 and D28	0 (0, 8)	0 (0, 10)	0.69 [0.32–1.48]
Variation of PaO_2/FiO_2 mean ratio between D0 et D7*	−13 (−54, 38)	−3 (−36, 22)	10.8 [-19.24–40.85]
Variation of FiO_2 between D0 et D7*	0 (−9, 10)	−5 (−10, 10)	−1.91 [−12.61–8.78]
Variation of PEEP between D0 et D7*	1 (−1, 5)	1 (0, 3)	−1.11 [−3.32–1.10]

Table 3. Outcomes assessment. * 4 missing data for Variation of PaO_2/FiO_2 mean ratio between D0 et D7 in control group and 3 in experimental. 4 missing data for Variation of FiO_2 between D0 et D7 in each group. 5 missing data for Variation of PEEP between D0 et D7 in control group and 6 in experimental. Q1: first quartile, Q3: third quartile, OR: odds ratio, IRR: Incidence Rate Ratio. ** All models are adjusted for PaO_2/FiO_2 mean ratio at inclusion (except Variation of PaO_2/FiO_2 mean ratio between D0 et D7), prone position, administration of anti-inflammatory drugs and administration of anti-infectious drugs during the first 7 days.

CI 0.32–1.48). Changes in physiological variables, including PaO_2/FiO_2 mean ratio, FiO_2, and PEEP, between Day 0 and Day 7 were not significantly different between groups (Table 3). For example, the variation in PaO_2/FiO_2 ratio was −3 (−36, 22) in the dornase alfa group and −13 (−54, 38) in the SOC group (adjusted β: 10.8; 95% CI −19.24–40.85).

Safety and adverse events

The safety profile of dornase alfa was consistent with expectations. Adverse events were reported in 38.5% of dornase alfa patients compared to 31.6% in the SOC group. Serious adverse events, including secondary infections and worsening organ failure, occurred in 41% of the dornase alfa group and 34% of the SOC group.

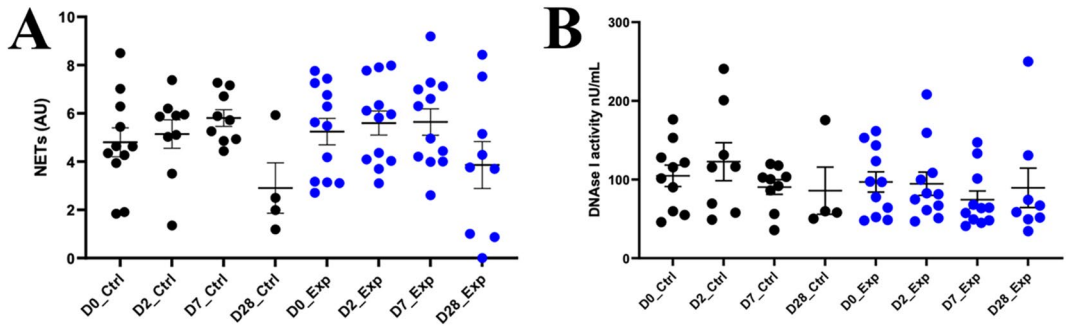

Fig. 2. Impact of in vivo DNAse administration on NET levels (**A**) and DNAse activity (**B**) in Plasma. Comparison of DNAse-treated (blue) and control (black) patients at Day 0, Day 2, Day 7, and Day 28. Both treated patients and controls showed a trend of increasing NET levels in plasma between J0 and J7 ($p = 0.71$ and $p = 0.11$ respectively), which subsequently dropped at J28 ($p = 0.38$ and $p = 0.22$, respectively). However, DNAse-treated patients did not exhibit lower plasma NET formation compared to controls. DNAse activity was not significantly augmented in plasma of treated patients compared to controls.

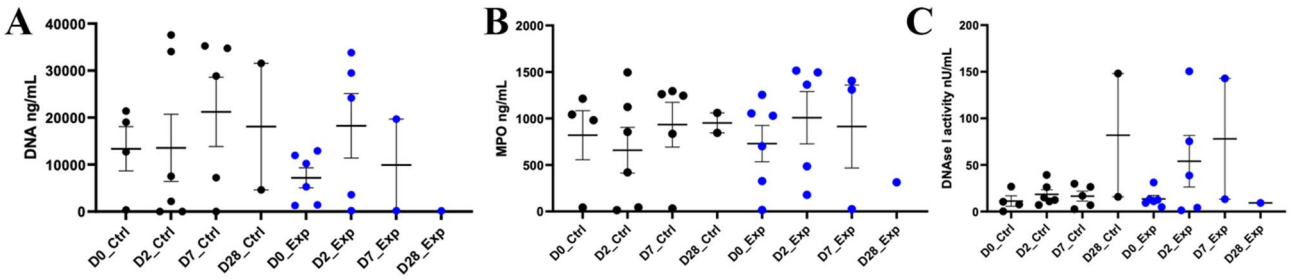

Fig. 3. Effect of in vivo DNAse administration on extracellular DNA (**A**), MPO (**B**), DNAse Activity (**C**) in Mucus. Comparison of DNAse-treated and control patients at Day 0, Day 2, Day 7, and Day 28. DNAse activity was not significantly augmented in mucus of treated patients compared to controls. Similarly, MPO levels and extracellular DNA were not diminished in mucus of DNAse-treated patients.

Impact of dornase Alfa on biomarkers of NETs and inflammatory markers in plasma and mucus

In both the dornase alfa and control groups, plasma NET levels non-significantly increased between Day 0 and Day 7 and then declined by Day 28 (Fig. 2), indicating early NET accumulation in ARDS. Dornase alfa treatment did not significantly reduce plasma NET formation relative to controls, and DNAse activity in plasma or mucus was not markedly elevated in treated patients (Figs. 2 and 3). Correspondingly, MPO levels and extracellular DNA in mucus were unaffected by dornase alfa administration (Fig. 3), suggesting a potential bioavailability issue with aerosolized delivery. Notably, preincubation of fresh mucus with DNAse effectively degraded NETs ex vivo (Fig. 4), highlighting the therapeutic potential of dornase alfa under optimal delivery conditions.

Discussion

In this multicenter randomized controlled trial, aerosolized dornase alfa did neither significantly reduce ARDS severity nor improve clinical outcomes in mechanically ventilated patients with COVID-19–related ARDS compared to standard-of-care (SOC). Although dornase alfa's capacity to degrade extracellular DNA theoretically positions it to mitigate neutrophil extracellular trap (NET)–mediated lung injury, our findings underscore the practical limitations of aerosolized administration and the challenges of achieving meaningful clinical impact in this population with a single target therapy.

Key findings and interpretation of primary outcome

The primary endpoint -an improvement in ARDS severity by at least one Berlin criteria grade at Day 7- was achieved by 18% of patients receiving dornase alfa versus 29% of those on SOC (adjusted OR: 0.33; 95% CI 0.09–1.14; $p = 0.11$). Secondary endpoints, including 28-day mortality, ventilator-free days, and ICU-free days, likewise did not favor dornase alfa. Although mortality was slightly higher in the dornase alfa arm (36 vs. 26%), wide confidence intervals precluded definitive conclusions. This absence of clear benefit, consistent with other negative trials targeting specific pathophysiological processes in ARDS[20–22]. Even if a lack of power may account for the negative results the most likely explanations either include wrong target (NETs), insufficient alveolar concentrations of dornase alfa or inactivation of dornase alfa by vibrating mesh nebulizer. Recent bench studies clearly demonstrated that vibrating mesh technology does not alter dornase alfa activity, concentration and

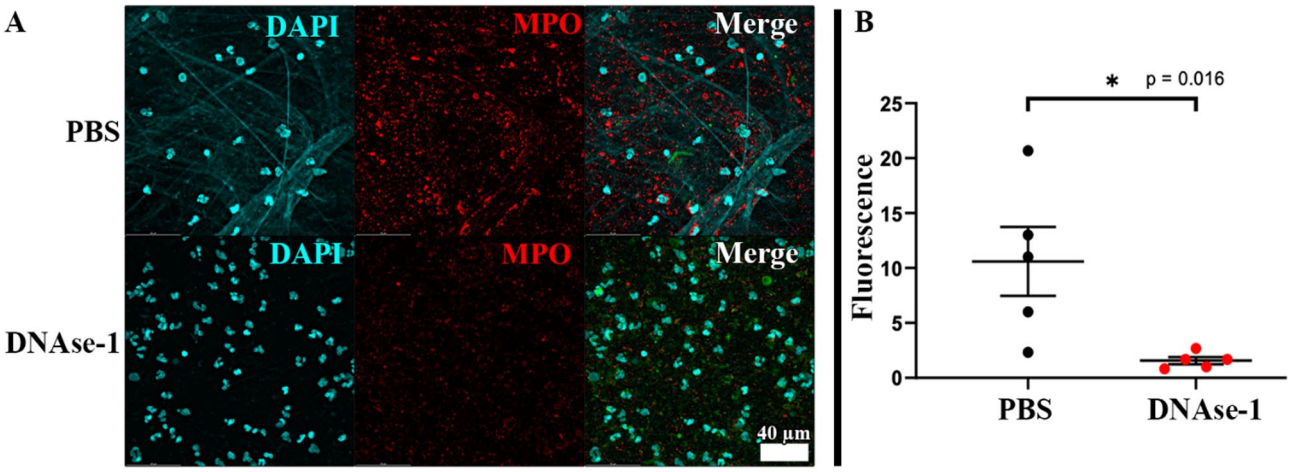

Fig. 4. Effect of ex vivo DNase-1 Pre-incubation on patient mucus. (**A**) Representative fluorescence images of patient mucus stained for DNA (DAPI, blue) and MPO (red), illustrating extensive extracellular DNA coated with MPO. Pre-incubation with DNase-1 leads to marked degradation of these DNA structures. Scale bar = 40 µm. Excitation/emission wavelengths used: 375–407/420–450; 542–566/578–610. (**B**) Quantification of extracellular DNA degradation following DNase-1 pre-incubation, measured by fluorescence intensity ($n = 5$ per group).

structural integrity after nebulization[23]. Our biomarker analysis provides additional insights worth discussing further.

Mechanistic considerations and aerosol delivery limitations

Importantly, our biomarker analyses corroborated the clinical findings: in vivo, neither DNAse activity nor levels of MPO and extracellular DNA in plasma and mucus changed significantly with dornase alfa treatment compared to SOC. In contrast, preincubation of fresh mucus with dornase ex vivo effectively degraded NETs, confirming that although the enzyme can dissolve NET structures under ideal conditions, it likely did not reach or remain active in distal airspaces in sufficient concentrations in vivo. Although we used vibrating mesh nebulizers, recognized for their efficiency[24], all patients were managed with heated humidifiers—an approach that can reduce effective drug deposition and potentially limit any therapeutic impact of aerosolized dornase alfa[25]. Indeed, when the heated humidifier is switched on, aerosol may undergo hygroscopic growth while traversing the humidifier chamber, thereby reducing its deposition to the distal airways[26]. A recent in vitro and in vivo study using a radiolabeled aerosol suggest that placing the nebulizer upstream of a heated humidifier reduces lung delivery by two- to three-fold compared to specialized circuits for aerosol therapy, largely due to increased extrapulmonary deposition[27]. When our trial took place (December 2020-August 2021), these specific bioavailability data were not yet published, and we followed then current recommendations from both the manufacturer and international societies[28]. Moreover, heated humidifiers were routinely employed to minimize endotracheal tube obstruction by tracheobronchial slough[29] and have since been formally shown to outperform heat and moisture exchangers in preventing such obstructions[30].

Comparison with prior studies and NET-Targeted therapies

Consistent with previous studies reporting a significant presence of NETs in severe COVID-19 patients, our findings show elevated NET markers in both plasma and mucus. This observation aligns with the work of Zuo et al. [31], who demonstrated that neutrophils in SARS-CoV-2–infected individuals release higher levels of NETs, and Middleton et al. [11], who detected abundant NET components in the bronchoalveolar lavage fluid of COVID-19 patients. The detection of these NET structures in our cohort's plasma and airway secretions bolsters the evidence that excessive NET formation is a key contributor to the inflammatory and thrombotic processes underlying COVID-19–related ARDS. Early description of clinical improvement after aerosolized dornase alfa in COVID-19 patients was reported in September 2020[32]. Favorable outcomes were also reported in 5 ventilated COVID-19 patients in whom dornase alfa was co-administered with albuterol[33] which may improve dornase alfa delivery to the alveoli. A separate randomized trial by Porter et al.[34] evaluated nebulized dornase alfa in hospitalized, non-intubated patients with COVID-19 pneumonia, using reduction in C-reactive protein (CRP) as the primary endpoint. They showed anti-inflammatory effects in this less severe population, supporting the biological relevance of targeting extracellular chromatin across different stages of COVID-19. In contrast, our study focused on invasively ventilated patients with moderate-to-severe ARDS and was powered for clinical endpoints rather than systemic inflammatory markers. At the time our protocol was designed, the study by Porter et al. had not yet been published; otherwise, we would have incorporated CRP or similar biomarkers in our analysis. We recognize the value of such markers and will include them in future trials. While both studies confirm the safety of nebulized dornase alfa, differences in disease severity, treatment setting, and outcome measures limit direct comparison but together reinforce the potential value of this therapeutic strategy. In a

non-randomized case-controlled clinical trial, using similar administration scheme (2.5 mL dornase alfa twice daily for 3 days) Holliday et al. observed a transient improvement of PaO_2/FiO_2 ratio together with an improved static lung compliance[35]. The location of the vibrating mesh aerosol was not described, but the authors found a significant reduction in bronchoalveolar lavage fluid myeloperoxidase-DNA complexes. Dornase alfa was one of the seven agents tested in the phase 2, open label, adaptive platform randomized controlled I-SPY COVID trial[36]. It did not meet the prespecified criteria for a large efficacy signal.

Study strengths and limitations

Strengths of our trial include its robust multicenter (7 sites), randomized design and extensive biomarker profiling. Nevertheless, several limitations merit consideration. The open-label approach and lack of outcome assessor blinding may have introduced performance bias, observer bias and treatment bias, despite standardized protocols and explicit, objective outcome criteria. Future studies should be assessor-blinded and placebo-controlled. A modest sample size provided results with large confidence intervals, potential imprecision and limited power to detect moderate but clinically meaningful effects. The confidence intervals presented for OR/IRR rely on approximate methods assuming asymptotic normality and are therefore not exact in the strict statistical sense. These approximations are generally reliable for moderate to large sample sizes but should be interpreted with caution in small or highly unbalanced datasets. Rapidly changing viral variants and SOC for COVID-19 ARDS during the study period could have influenced clinical outcomes. Finally, the broad inclusion criteria likely encompassed a heterogeneous spectrum of ARDS severity and etiologies, potentially diluting any benefit specific to specific phenotypes[37]. These findings should be interpreted in the context of high-resource ICU settings; differences in healthcare infrastructure, patient management practices, and drug availability may limit the applicability of results to other regions, particularly low-resource environments.

Implications for clinical practice and future research

Optimizing drug delivery

Given the limitations of aerosol delivery highlighted by our results, future trials should explore optimized strategies to ensure effective alveolar drug deposition[38]. Direct intra-tracheal administration[39], next-generation vibrating mesh nebulizers, optimized for heated-humidified circuits or closed-loop systems, could improve bioavailability[40]. Another lead could come from a more stable DNase-1. One drawback of native dornase alfa is its short half-life (at least in plasma)[41]. "Long-acting DNase-1", namely recombinant DNase-1-coated polydopamine-poly(ethylene glycol) nanoparticulate provides longer enzymatic activity and was shown to reduce NETosis in a septic mouse model and provided longer survival compared to native DNase-1[42]. This is even more promising as PEGylated DNase was recently shown to remain stable enough to withstand aerosolization with vibrating mesh technology even at higher concentrations than conventional DNase formulation. Protein integrity and enzymatic activity were also preserved[43]. Intravenous dornase alfa administration may reduce both blood NET burden and blood extracellular mitochondrial DNA[44] which binds Toll Like Receptor 9 and induces NETs formation. Intravenous dornase alfa was shown efficient in a mouse model of viral ARDS[13], but the currently commercially available products have short plasma half-lives and are neither EMA nor FDA-approved for intravenous administration in man. Yet, intravenous administration of recombinant dornase alfa was performed 25 years ago in 14 patients with lupus nephritis and was reported to be safe, well tolerated and not associated with the development of neutralizing antibodies to DNase[45]. Pending EMA and FDA approval and marketing authorization, intravenous dornase alfa may represent an alternative approach to nebulization.

Identifying subgroups of patient

Although our trial did not demonstrate a statistically significant clinical benefit in an unselected COVID-19 ARDS population, the intervention was safe and well tolerated, supporting its potential use in more targeted contexts. Given the established involvement of NETs and mucus plugging in the pathogenesis of COVID-19-related ARDS[11,31,46], future research should aim to identify subgroups of patients most likely to benefit from dornase alfa. Patients with radiological signs of mucus impaction—such as lobar atelectasis or tree-in-bud opacities—may reflect phenotypes where impaired mucus clearance plays a central role in respiratory failure[47]. Similarly, individuals with elevated systemic NET markers (e.g., circulating cell-free DNA, MPO-DNA complexes, or citrullinated histones) could represent a biologically enriched population in which DNase-based therapies are more likely to have mechanistic relevance[11] and clinical efficacy. Other possible markers of potential dornase alfa responsiveness include low baseline endogenous DNAse activity or high concentrations of extracellular DNA in blood, bronchoalveolar lavage fluid or endotracheal secretions[48,49]. Incorporating such criteria into eligibility or stratification in future trials—either through biomarker screening, imaging assessment, or composite clinical scores—may improve the time window, precision and efficacy of interventional strategies targeting NETs and mucus-related pathology in ARDS.

Combination therapies

In the setting of COVID-19-related ARDS, systemic anti-inflammatory agents such as corticosteroids[50] and IL-6 receptor antagonists[51] have demonstrated clear mortality benefits and are now part of standard of care. These agents primarily act by attenuating the systemic hyperinflammatory response and dampening cytokine-mediated lung injury. However, COVID-19 ARDS is increasingly recognized as a multifactorial syndrome involving not only inflammation but also immunothrombosis, mucus plugging, dysregulated alveolar repair, and altered neutrophil responses[37]. Dornase alfa represents a mechanistically distinct intervention. Acting locally within the airways, it degrades extracellular DNA, a key component and scaffold of NETs, and reduces mucus viscosity, thereby potentially improving alveolar ventilation and reducing distal airway obstruction. Given these distinct but complementary mechanisms, combining dornase alfa with systemic immunomodulators[52] or

antithrombotics[53] may provide a broader therapeutic impact. For example, corticosteroids may limit upstream neutrophil activation[54], while dornase alfa could eliminate NET-related debris that accumulate downstream in the alveolar spaces. Similarly, targeting NETs through DNase activity may enhance the efficacy of anticoagulants and antiplatelet agents by dismantling the pro-thrombotic scaffold that contributes to immunothrombosis[11], a hallmark of severe COVID-19 lung pathology[55,56]. Similarly, dornase alfa may be associated with α-1 antitrypsin, a serine protease inhibitor which limits epithelial injury by inhibiting neutrophil elastase and was shown to be safe and effective in reducing systemic inflammation in a small randomized controlled trial[57]. The failure of many monotherapies in ARDS clinical trials has highlighted the limitations of targeting single pathways in a heterogeneous and dynamic syndrome[37]. Future research should prioritize multimodal therapeutic strategies tailored to specific biological phenotypes, supported by preclinical models and biomarker-driven designs to guide combination regimens. Defining optimal timing, dosing, and sequencing will be critical to maximizing potential synergy while minimizing adverse effects such as immunosuppression or bleeding risk. For instance, associating synthetic nuclear peptidylarginine deiminase 4[58] (which is needed for chromatin decondensation and NETs formation) and dornase alfa (which degrades already formed NETs) may prove synergistic, but this has to be proven.

Conclusion

Despite its favorable in vitro profile, aerosolized dornase alfa did not confer significant clinical or biological benefits in this trial. These results underscore the complexity of effectively targeting NETs in COVID-19–related ARDS and emphasize the need to refine delivery methods, optimize patient selection, and consider multifaceted therapeutic strategies in future trials.

Data availability

The database can be obtained upon reasonable request from the corresponding author.

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Author contributions

BHTN, MSN, JG, MH, MRL, FL, SLT, MC, NE and AY participated in conceptualization, investigation, and data curation. CLC and PT participated in conceptualization, investigation, data curation, supervision, and project administration. LDM participated in writing— original draft, writing—review & editing, and supervision. CG, JPD and JP participated in conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing—original draft, writing—review & editing, supervision and project administration. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval and consent to participate
The study was approved by the Comité de Protection des Personnes Ouest IV Nantes (EUDRACT: 2020-001492-33). Written informed consent was obtained from patients or their legal representatives prior to inclusion whenever possible. In cases requiring emergency inclusion, consent was obtained retrospectively from the patient or their relatives. The trial was registered on ClinicalTrials.gov (NCT04355364) before the enrollment of the first patient and was overseen by an independent data and safety monitoring committee (DSMC).

Consent for publication
Not applicable.

Additional information
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