Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Combined administration of inhaled DNase, baricitinib and tocilizumab as rescue treatment in COVID-19 patients with severe respiratory failure

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ABSTRACT

Aiming to reduce mortality in COVID-19 with severe respiratory failure we administered a combined rescue treatment (COMBI) on top of standard-of-care (SOC: dexamethasone/heparin) consisted of inhaled DNase to dissolve thrombogenic neutrophil extracellular traps, plus agents against cytokine-mediated hyperinflammation, namely anti-IL-6-receptor tocilizumab and JAK1/2 inhibitor baricitinib. Patients with PaO2/FiO2 < 100 mmHg were analysed. COMBI group ($n = 22$) was compared with similar groups that had received SOC alone ($n = 26$) or SOC plus monotherapy with either IL-1-receptor antagonist anakinra ($n = 19$) or tocilizumab ($n = 11$). COMBI was significantly associated with lower in-hospital mortality and intubation rate, shorter duration of hospitalization, and prolonged overall survival after a median follow-up of 110 days. *In vitro*, COVID-19 plasma induced tissue factor/thrombin pathway in primary lung fibroblasts. This effect was inhibited by the immunomodulatory agents of COMBI providing a mechanistic explanation for the clinical observations. These results support the conduct of randomized trials using combined immunomodulation in COVID-19 to target multiple interconnected pathways of immunothrombosis.

1. Introduction

In patients with COVID-19, severe respiratory failure (SRF) leading to invasive mechanical ventilation (IMV) is a leading cause of mortality [1–3]. Thus, the management of patients with SRF to prevent intubation and admission to intensive care is a challenging and crucial issue [2–4]. Numerous clinical trials have been conducted or are underway in an effort to improve the current standard-of-care (SOC) for patients with severe COVID-19, which consists of dexamethasone, heparin and supportive measures [5,6].

Deterioration of respiratory function in COVID-19 seems to be associated with a profound deregulation of immune response [7]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects predominantly alveolar epithelial cells, triggering aberrant inflammatory responses leading to acute respiratory distress syndrome (ARDS) [8,9]. Neutrophil extracellular traps (NETs) are related to the activity of various immunological mediators and the subsequent inflammatory milieu [10]. Moreover, advanced respiratory disease in COVID-19 is characterized by macrophage extravasation, while an abundance of mesenchymal cells and fibroblasts is observed and linked with the progression of respiratory failure [8,9].

Mechanistically, NET-mediated immunothrombosis leads to activation of tissue factor (TF) axis and thrombin generation. Proteases of this axis fuel a vicious cycle, in which a harmful crosstalk between

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thrombosis, inflammation and fibrosis unfolds [8,11]. Moreover, activation of complement and the NF-κB and JAK/STAT pathways have been described as partners in COVID-19 hyperinflammation [11–13]. Based on these mechanisms and in the context of randomized controlled trials (RCTs), immunomodulatory treatments including anakinra, a recombinant inteleukin-1 (IL-1) receptor antagonist [14], tocilizumab, an anti-IL-6 receptor antagonist [15], baricitinib, a selective JAK-1/JAK-2 inhibitor [16] and nebulized recombinant human DNase [17,18] an agent which dismantles NETs, have been administered separately in patients with COVID-19-related SRF.

Given the complex nature of severe COVID-19, this study describes the results of a multi-target therapeutic protocol as a rescue treatment. In particular, a combined therapeutic strategy, consisting of inhaled DNase plus baricitinib and tocilizumab on top of SOC, was found to reduce the mortality in a group of patients with severe respiratory failure due to COVID-19. Translating the clinical findings, we observed a reduced activity of the TF/thrombin axis in primary lung fibroblasts treated with the above therapeutic agents upon stimulation with COVID-19 environment.

2. Methods

2.1. Study design and patients

2.1.1. Patient selection

This is a non-randomized open-label study, conducted in the First Department of Internal Medicine, University Hospital of Alexandroupolis (NCT05279391). In total, 368 patients with COVID-19 were admitted from October 25, 2020 to August 18, 2021. Among them, we analysed 78 patients who progressed to severe respiratory failure (SRF) defined as a ratio of arterial partial pressure of oxygen to the fraction of inspired oxygen (PaO2/FiO2) less than 100 mmHg [2]. At the time of admission, they received SOC or combination of SOC with specific immunomodulatory agents, in a chronologically evolving pattern, as a compassionate/rescue treatment (Fig. 1).

Inclusion criteria were as follows: 1) adult patients ≥18 years old, 2) positive polymerase-chain-reaction (PCR) test for SARS-CoV-2 RNA in nasopharyngeal swab, 3) pulmonary infiltrates suggestive of COVID-19, 4) progression to SRF as defined by PaO2/FiO2 < 100 mmHg, 4) written informed consent from the patients or their legal representatives for the current compassionate therapeutic protocol.

Patients were excluded from the analysis according to the following criteria: 1) need for intubation/IMV during the first 24 h after the initiation of treatment, 2) multi-organ failure, 3) systemic co-infection, 4) SRF due to cardiac failure or fluid overload, 5) glomerular filtration rate (GFR) <30 ml/min/1.73 m^2), 6) any stage IV solid tumor or immunosuppression due to hematological disorders, 7) any immunosuppressive therapy and/or chemotherapy during the last 30 days, 8) low patient's functional performance status as defined by a Palliative Performance Scale (PPS) score ≤ 30% [19], 9) pregnancy.

2.1.2. Study groups and endpoints

The selection of the different rescue treatments that have been used throughout the study period, was initially decided, and periodically revised, through considering any new RCT evidence, as well as emerging research findings by our group and others on the role of different pathways in the inflammatory milieu of severe COVID-19, including the critical role of NETs, the triggering of NFκB pathway and the induction of IL-6 and Janus kinases (JAKs). In total, 4 SRF groups were compared and analyzed, including the group of patients treated with SOC only and 3 additional chronologically consecutive groups in which apart from SOC treatment, different rescue therapeutic immunomodulatory regimens had been added (Fig. 1).

SOC group comprised patients who received only standard of care in accordance to our department protocol including dexamethasone 6–8 mg once daily, low molecular weight heparin at therapeutic doses, antibiotic prophylaxis and supportive care.

TOCI group included patients who received SOC plus the IL-6 receptor inhibitor tocilizumab. Tocilizumab was administered as a single intravenous (iv) dose of 8 mg/kg actual body weight up to 800 mg.

ANA group included patients who received SOC plus the IL-1 receptor antagonist anakinra. Anakinra was administered iv 200 mg/twice daily for 3–6 days, then 100 mg/twice daily, for up to 10 days in total.

COMBI group included patients who were treated with SOC and the following combination regimen: a) tocilizumab (as described above), b) selective JAK-1/JAK-2 inhibitor baricitinib, 4 mg *per os* once daily, for up to 14 days (2 mg once daily, if GFR was 30–60 ml/min/1.73 m^2) and c) nebulized dornase alfa, a recombinant human DNase I (inhaled DNase), 2500 U/twice daily for up to 14 days, in order to dismantle the NETs accumulated in the lung. Inhaled DNase was administered simultaneously with inhaled budesonide (800 μg/twice daily) and bronchodilators (salbutamol or/and ipratropium at standard doses). During the course of the study, the same general care protocols were used.

The primary endpoint was the reduction of the in-hospital mortality rate, whereas secondary endpoints included intubation/IMV rate, days of hospitalization and overall survival as derived from the last follow-up visit, either at the office or remotely.

The study was in accordance with the Declaration of Helsinki.

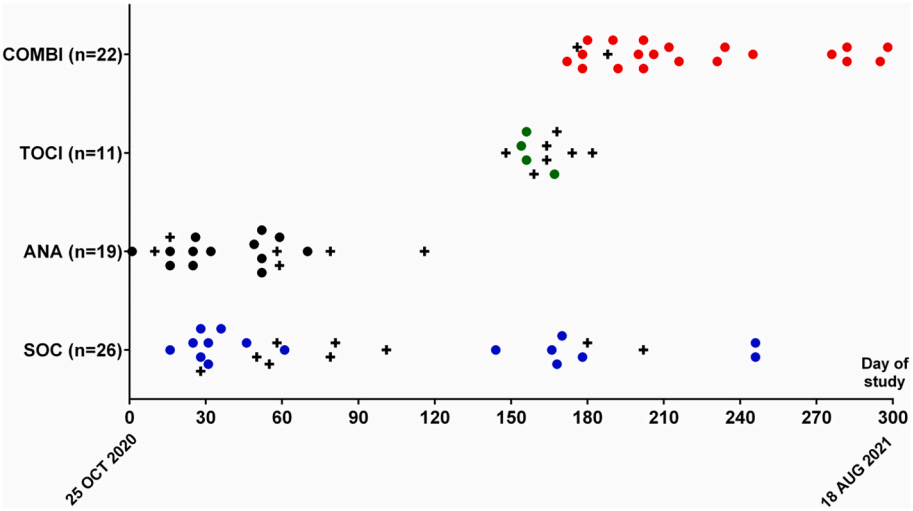

Fig. 1. Consecutive groups of therapies and number (n) of patients enrolled during the study period; the symbol + denotes in-hospital deaths.

Subjects provided written informed consent before participating in the study. Patients' records were anonymized and deidentified prior to analysis, to ensure confidentiality and anonymity. The study protocol was approved by the local scientific and ethics committees and institutional review board of the University Hospital of Alexandroupolis (Ref. No. 87/08-04-2020).

2.2. Statistical analysis

2.2.1. Analysis of clinical data

Chi square test along with adjusted residuals was used to compare outcomes among the four treatment groups. Similarly, chi square and ANOVA were used to compare binary and discrete/continuous variables, respectively, that are considered to be potential confounders. To consolidate the independent correlation of each treatment group with outcome, a Generalized Linear Model using outcome as dependent variable, treatment groups as independent variables and potential confounders as factors was further utilized. For that purpose, all scale variables were turned into binary ones with the use of Optimal Scaling along with ridge regression, random initial configuration and bootstrapping. Secondary outcomes were evaluated with ANOVA; *ad-hoc* analysis was performed using Tuckey's HSD test. The repeated measures General Linear Model was used for analysis of within-subjects and between-subjects variance of the same variable measured several times on each patient. Kaplan-Meier curves were used to depict survival data; comparisons were performed by the Logrank test. A Cox proportional-hazards regression model was introduced to examine simultaneously the effects of multiple covariates on overall survival. Median follow-up was approached by the reverse Kaplan-Meier estimator.

2.2.2. Analysis of experimental data

Comparisons between two groups were performed using Student's *t*-test (2-tailed). For comparisons among more than two groups, Kruskal-Wallis test, followed by Dunn's test for multiple comparisons, was performed.

For both clinical and experimental results, the level of statistical significance was set at 0.05. Bonferroni correction was applied in case of multiple testing. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 26.0 software.

Detailed information for all methods can be found in the Supplementary Materials section.

3. Results

3.1. Clinical outcomes

The baseline demographics and clinical characteristics of the patients included in the study are shown in Table 1. There were no significant differences among groups.

In the SOC group, 9 deaths were recorded among 26 patients (34.6%). Ten out of 26 patients were intubated (38.5%); eight of them did not survive. The mean (SD) duration of hospitalization was 19.4 (7.2) days. In the ANA group, 6 deaths and 6 intubations were recorded among 19 hospitalized patients (31.6%). Five intubated patients did not survive. The mean (SD) duration of hospitalization was 23.9 (10.8) days. In the TOCI group, 7 deaths were recorded out of 11 patients, all of who had been intubated (63.6%). The mean (SD) duration of hospitalization was 19.4 (7.8) days. In the COMBI group, there were 2 deaths out of 22 patients (9.1%), both in intubated patients. There was a reduction in the mean (SD) duration of hospitalization, which was 15.6 (5.3) days.

Taken together, COMBI treatment was associated with a significantly lower in-hospital mortality ($p = 0.014$), as well as intubation rate ($p = 0.013$) and shorter duration of hospitalization ($p = 0.019$) compared to other treatment groups of patients with COVID-related SRF (Fig. 2A, B). Moreover, COMBI treatment was correlated with a prolonged survival ($p = 0.003$) after a median follow-up of 110 days; loss to follow-up was 1.3% (1 patient in the SOC group) (Fig. 2C).

Table 1
Baseline demographical and clinical characteristics of the patients studied ($n = 78$).

Parameter	SOC n = 26	ANA n = 19	TOCI n = 11	COMBI n = 22	*p*
Age (years)					
Mean ± SD	62.8 ± 10.6	62.9 ± 10.9	63.6 ± 8.7	56.9 ± 11.1	0.154
Sex					
Male (%)	18 (69.2)	15 (78.9)	8 (72.7)	16 (72.7)	0.912
Female (%)	8 (30.8)	4 (21.1)	3 (27.3)	6 (27.3)	
Body Mass Index (BMI)					
Mean ± SD	30.3 ± 4.0	29.7 ± 5.3	29.8 ± 4.7	30.9 ± 5.1	0.853
Number of comorbidities					
Mean ± SD	2.2 ± 1.4	2.0 ± 1.4	2.0 ± 1.1	1.8 ± 1.1	0.722
0 (%)	3 (11.5)	4 (21.1)	1 (9.1)	2 (9.1)	
1 (%)	6 (23.1)	3 (15.8)	3 (27.3)	8 (36.4)	
2 (%)	6 (23.1)	5 (26.3)	2 (18.2)	6 (27.3)	
3 (%)	6 (23.1)	4 (21.1)	5 (45.4)	5 (22.7)	
4 (%)	4 (15.4)	3 (15.8)	0 (0)	1 (4.5)	
5 (%)	1 (3.8)	0 (0)	0 (0)	0 (0)	
Disease day at admission*					
Mean ± SD	9.5 ± 2.9	9.3 ± 3.9	7.7 ± 3.1	9.5 ± 1.8	0.361
Disease day at enrollment					
Mean ± SD	11.3 ± 2.6	11.6 ± 3.1	10.0 ± 2.4	10.6 ± 2.2	0.358
PaO2/FiO2 at enrollment (mmHg)					
Mean ± SD	80.9 ± 12.5	89.8 ± 29.2	87.9 ± 24.9	96.0 ± 21.6	0.129
SOC treatment					
Dexamethasone	26	19	11	22	
Low molecular weight heparin	26	19	11	22	1.000
Antibiotics	26	19	11	22	

* The onset of the disease was defined as the date of the first symptoms consistent with COVID-19 or, whenever this was not feasible, the date of the first positive PCR.

A Generalized Linear Model was used to assess the effect of potential confounders on mortality rate; similarly, a Cox regression proportional hazards model, incorporating the same potential confounders as covariates, was introduced to estimate survival probability in a stochastic manner. Taken together, these data further supported the initial findings (Supplementary Table 1, Supplementary Fig. 1).

Overall immunomodulatory treatments were well tolerated. All the recorded serious adverse events were related to severe COVID-19 disease (Supplementary Table 2). There were no serious infections associated with the immunomodulatory therapies.

3.2. Disease-dependent biomarkers

There is sufficient evidence showing that inflammatory and hematological markers such as the absolute neutrophil and lymphocyte count (ANC, ALC), LDH, C-reactive protein (CRP) and D-dimers, are associated with the progression and severity of COVID-19 [20–22]. As a result, we recorded these markers on the first day of progression to SRF, as well as on day 7 after the initiation of each treatment regimen. Comparison between groups, showed statistically significant results in favor of the COMBI group as regards the increase of ALC count ($p = 0.021$) and the reduction of CRP levels ($p = 0.002$) on day 7 (Fig. 3A, B). There was no statistically significant difference between groups, regarding ANC, LDH

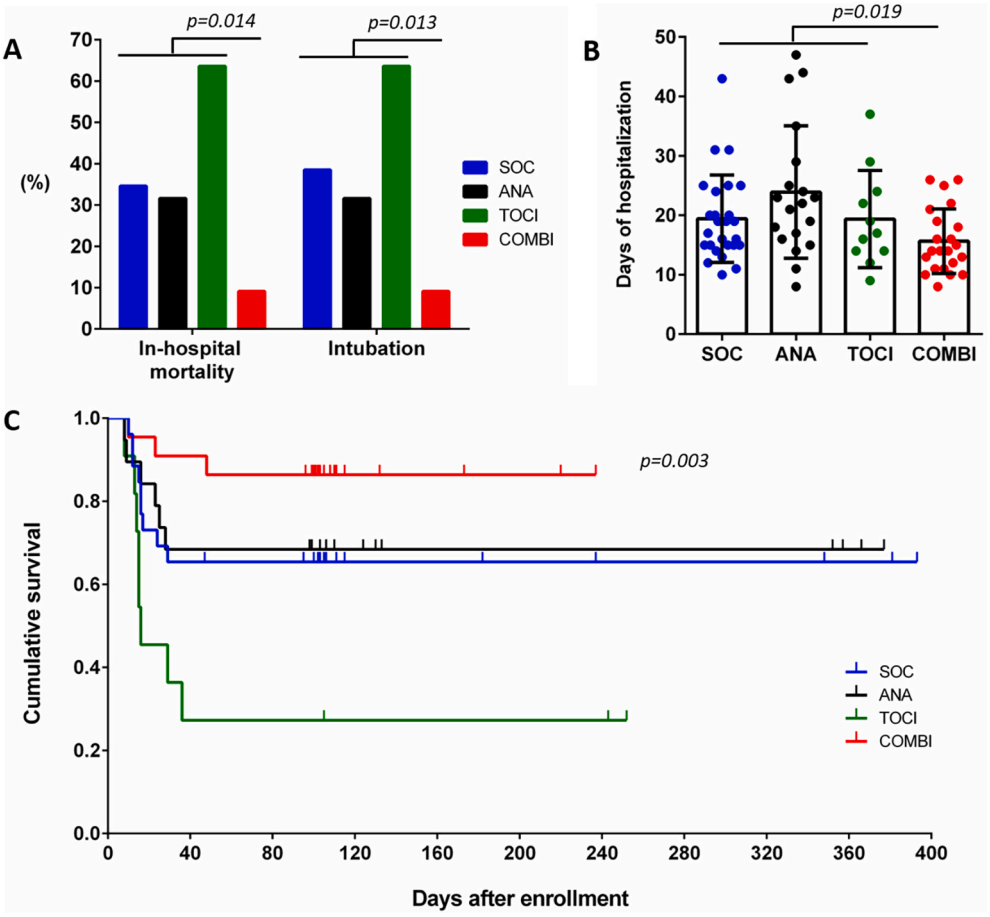

Fig. 2. COMBI treatment reduces (A) mortality and intubation rate, and (B) days of hospitalization, whereas (C) prolongs overall survival when compared with other treatments.

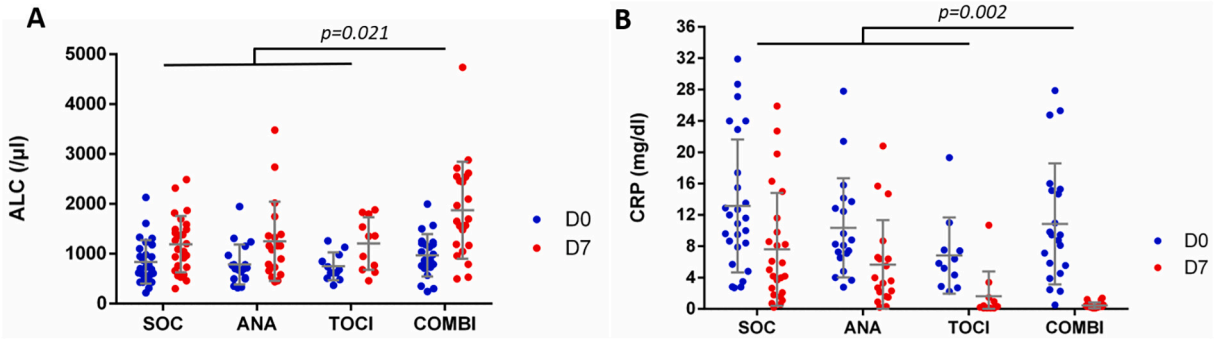

Fig. 3. COMBI treatment (A) increased Absolute Lymphocyte Count (ALC) and (B) diminished CRP when compared with other treatments; comparisons were made between the first day of progression to SRF (D0) and the day 7 (D7) after the initiation of each treatment.

and D-dimers values.

3.3. Primary lung fibroblasts, in vitro treated in COVID-19 environment, are involved in TF expression

Since immunothrombosis is crucially involved in the pathophysiology of COVID-19-related ARDS [23], mesenchymal cell/fibroblast accumulation in the lung is linked with the progression of COVID-19 severe respiratory failure [8] and fibroblasts under certain inflammatory conditions express TF [24,25], we examined whether COVID-19 environment could activate the TF/thrombin pathway in lung fibroblasts (LFs). We observed that plasma samples from treatment-naive COVID-19 patients markedly induced TF expression in LFs, compared to untreated cells, as indicated by TF real-time quantitative PCR (qPCR), in-cell ELISA and immunofluorescence microscopy (Fig. 4A, B and D). TF released by plasma-stimulated LFs was bioactive, as assessed by a TF activity quantitative assay (Fig. 4C). Together, our findings suggest that COVID-19 inflammatory microenvironment is a potent activator of the thrombotic potential of LFs.

3.4. Agents of the COMBI protocol have inhibitory effect on TF/thrombin axis in fibroblasts treated with COVID-19 plasma

According to previous findings, and considering that the proteases of

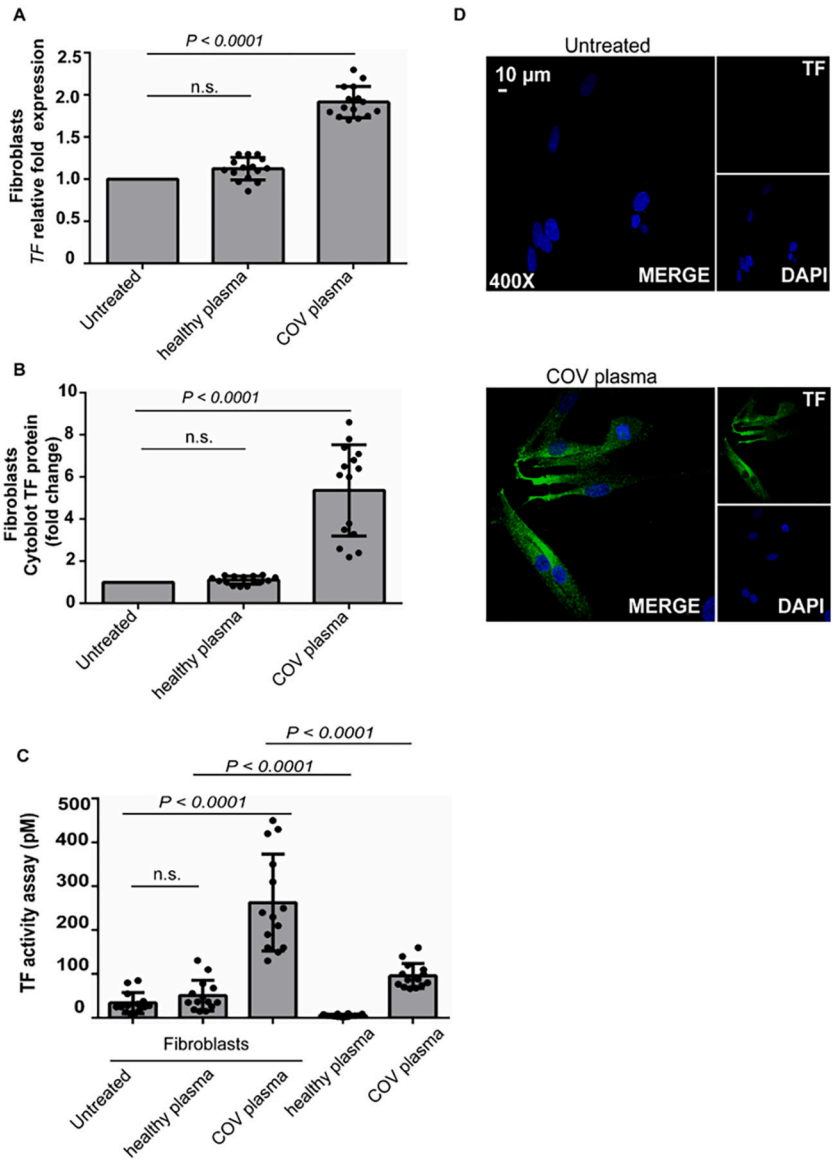

Fig. 4. COVID-19 plasma triggers lung fibroblasts to produce tissue factor (TF) *in vitro*. Lung fibroblasts (LFs) were stimulated with 2% plasma, derived either from healthy subjects (healthy plasma) or COVID-19 patients (COV plasma). Tissue factor (TF) expression in LFs as assessed by (A) qPCR and (B) In-Cell ELISA (Cytoblot). All conditions were compared to untreated cells. (C) TF activity was evaluated in culture supernatants of LFs as well as in COVID-19 plasma samples as control, diluted directly with the culture medium in a final dilution that was used in experimental conditions. In (A)-(C), $n = 15$, bars represent mean ± SD, (n.s.: not significant); in case of (C), alpha was set to 0.0125 after Bonferroni correction. (D) Confocal fluorescence microscopy showing TF in LFs (green: TF, blue: DAPI). A representative example of three independent experiments is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TF/thrombin axis have multiple functions leading to thrombosis, amplification of inflammation, cell proliferation, and fibrosis [25–27], we prompted to study the *in vitro* effect of the applied therapeutic regimens.

SARS-CoV-2 infection seems to activate the NF-kB signaling pathway, which may subsequently induce the secretion of several inflammatory cytokines, including IL-1, IL-6 and tumor necrosis factor-α (TNF-α) [28]. Since both IL-1 and IL-6 are elevated in patients with COVID-19 [14,15,29], LFs were pretreated with either a recombinant human IL-1 receptor antagonist (anakinra) or an anti-IL-6 receptor monoclonal antibody (tocilizumab), in an attempt to disrupt the autoinflammatory loops driven by these cytokines. However, TF expression (Fig. 5A-B, Supplementary Fig. 2A-D) and activity (Fig. 5C) were not significantly attenuated in COVID plasma-stimulated LFs upon these *in vitro* inhibitions.

On the other hand, pre-incubation of cells with a selective JAK1/JAK2 inhibitor (baricitinib) [16], or pre-treatment of plasma samples with DNase I, an enzyme able to dismantle NETs [17,18], markedly reduced TF expression (Fig. 5A-B, Supplementary Fig. 2E-F) and activity (Fig. 5C) in plasma-stimulated LFs.

In view of these data and considering that COVID-19 is mediated by plenty of plausible mechanisms, cells were simultaneously blocked against IL-6, JAK1/JAK-2 and NET signaling, leading to TF suppression in plasma-stimulated LFs (Fig. 5A-D).

Collectively, both the separate use of DNase or JAK1/JAK2 inhibitor and the concomitant treatment of LFs with an IL-6 inhibitor, a JAK1/JAK2 inhibitor and DNase, *i.e.* the agents of COMBI protocol, could disrupt multiple pathways leading to TF release from LFs, preventing thromboinflammatory responses in COVID-19.

4. Discussion

A large amount of evidence in the pathogenesis of severe COVID-19 has implicated the role of various amplification loops in a vicious cycle pattern. It is suggested that viral proteins trigger the activation of NFκB pathway [30] and complement [11,31]. The activation of NFκB, induces the secretion of multiple chemokines and cytokines such as IL-1, IL-6, IL-8 and TNFα leading to a true "cytokine storm" and influx of macrophages and neutrophils damaging the lung tissue [28]. Additionally, mediators of the inflammatory environment of COVID 19, such as complement anaphylatoxins, platelet-derived factors, thrombin, IL-8 and G-CSF, trigger the NET-driven thromboinflammation [23,31–33]. Within NET structures, proteases and cytokines are preserved in their active form, further amplifying the thrombotic potential, inflammation,

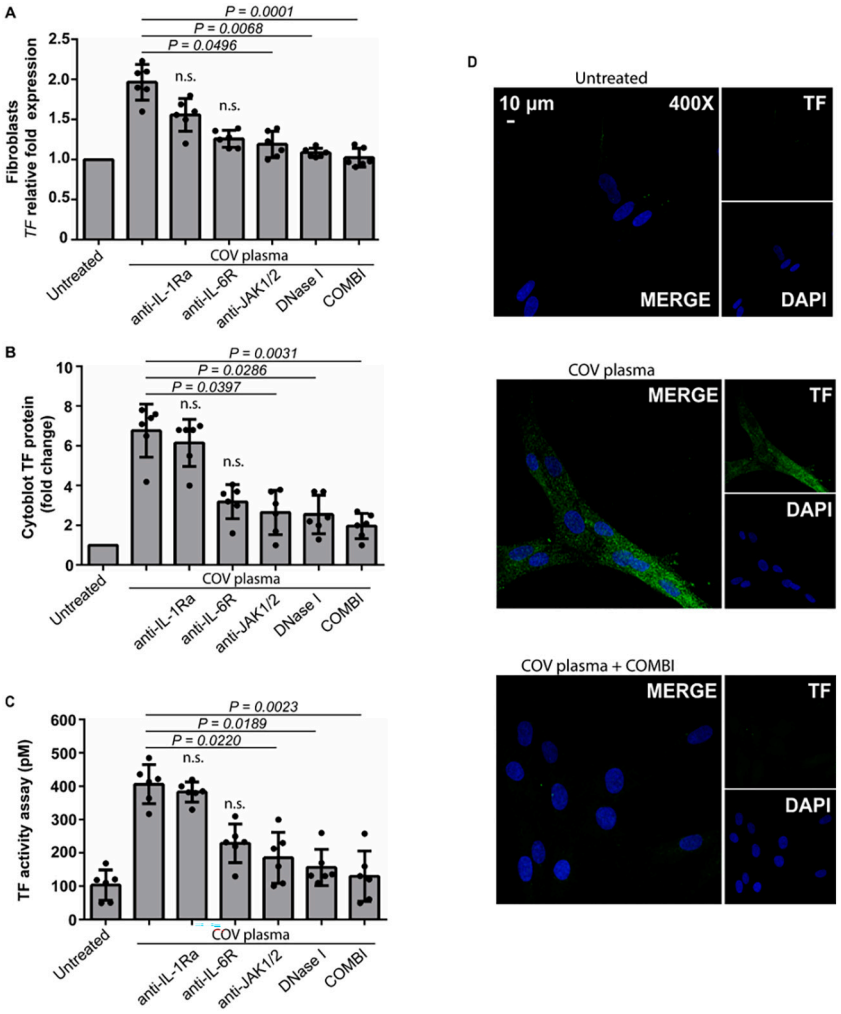

Fig. 5. Agents of combined treatment result in reduction of tissue factor (TF) expression and activity in cultures of lung fibroblasts. Relative fold expression of (A) tissue factor (TF) mRNA and (B) In-Cell TF ELISA (Cytoblot) in lung fibroblasts (LFs) treated with 2% COVID-19-derived plasma (COV plasma) and inhibited with a recombinant IL-1 receptor antagonist (anakinra), an anti-IL-6 receptor monoclonal antibody (tocilizumab), a selective JAK1/JAK2 inhibitor (baricitinib), DNase I or combination of therapeutic agents (tocilizumab, baricitinib and DNase I). (C) TF activity in cell supernatants in conditions as previously described. In (A)-(C), the effect of therapeutic agents was compared to COV plasma condition, $n = 6$, bars represent mean ± SD, statistical significance was set at $p < 0.05$ (n.s.: not significant). (D) Confocal fluorescence microscopy showing TF staining in stimulation and inhibition studies of LFs (green: TF, blue: DAPI). A representative example of three independent experiments is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and fibrosis [11,34,35]. Moreover, IL-6 triggers JAK/STAT signaling, driving the transcription of an extensive range of acute phase proteins such as CRP. Furthermore, IL-6 may activate and promote differentiation or proliferation of several non-immune cells, including fibroblasts [36,37]. The application of combined therapeutic regimens against COVID-19-related SRF is an emerging challenge against this complex and heterogeneous basis of COVID-19 pathophysiology.

Heparin, as a part of SOC regimen, inhibits the hypercoagulable condition and the generation of fibrin microthrombi in the lung by potentiating antithrombin III (ATIII) activity [38,39]. In the same context, dexamethasone, also part of the SOC as the first agent proven to reduce mortality in a RCT [5], has anti-inflammatory/anti-cytokine effects contributing to the control of NFκB-induced hyperinflammation [40]. Recently it has been suggested that dexamethasone favors the immune response by modulating COVID-19 immature neutrophils [41].

In this study, we initially added immunomodulatory monotherapies, such anakinra or tocilizumab, on top of SOC. Anakinra and tocilizumab have been tested in several RCTs that enrolled patients with COVID-19-related SRF. Their results provided evidence that tocilizumab, when administered with corticosteroids, offers a modest mortality benefit in certain patients (including ICU patients) with COVID-19 [42–44]. In our study, which analysed most severely affected patients (PaO2/FiO2 < 100 mmHg), the addition of anakinra or tocilizumab on top of SOC did not add significant benefit regarding mortality, need for IMV and time to discharge. As the mortality rates observed remained high, we considered that the mechanisms involved in the inflammatory environment of COVID-19 are probably interdependent and create a vicious cycle of positive feedback.

In the next (fourth) consecutive therapeutic protocol, we added a combined immunomodulatory regimen upon SOC treatment, which included inhaled DNase to dismantle NETs and locally suppress the activity of their proteins, tocilizumab to inhibit the signaling of pre-existing IL-6 load, interrupting an initial link between JAK/STAT and NFκB, and baricitinib to further prevent the JAK/STAT-dependent transcription [15,16,28,34,45–47]. Using this protocol (COMBI), the in-hospital mortality rate of this group of COVID-19 patients with SRF was reduced to 9.1%. In line with our clinical observations, COV-BARRIER RCT recently reported an additional survival benefit of baricitinib, when added to corticosteroids in hospitalized, non-IMV patients with COVID-19 pneumonia. The effect was most pronounced in the subgroup of patients receiving high-flow oxygen or non-invasive ventilation at baseline [48]. Additionally, it has been suggested that baricitinib has antiviral activity through interference with SARS-CoV-2 endocytosis [49].

Currently, clinical data regarding the inhaled DNase in COVID-19 are very limited, deriving only from two small, non-randomized, case-control trials enrolling ICU patients with ARDS [17,18]. Treatment with nebulized DNase in most of those patients was associated with improved oxygenation and outcome, especially when used earlier in the disease course [17,18]. Decreased NET remnants in bronchoalveolar lavage fluid was also described [18]. Patients received also corticosteroids, and in some cases convalescent plasma and anticoagulation [17,18].

Administration of COMBI led to a rapid reduction of CRP and restoration of lymphopenia, both systemic biomarkers of COVID-19

disease severity and progression [20–22]. This is in agreement with the favorable disease outcome of those patients. Furthermore, recovery of the lymphocyte count probably implies a reversal of the impaired adaptive cellular immune response described in severe COVID-19 patients [50].

Reduced mortality of SRF patients in the COMBI group, prompted us to investigate the *in vitro* inhibitory effect of applied immunomodulatory agents, namely DNase, tocilizumab, anakinra, and baricitinib in COVID-19 culture conditions. Lung fibroblasts have been found to significantly accumulate and proliferate, repopulating the damaged alveolar wall as the disease progresses. However, their functional role in COVID-19 is still elusive [9,51,52]. Recently, Rendeiro et al. described the spatial landscape of COVID-19 lung pathology in COVID-19-decedents by correlating clinical and pathological variables with high-parameter imaging mass cytometry data. Fibroblasts were closely associated with alveolar type-2 cells, large thrombi, and interventions such as intubation and treatment [8]. Moreover, S and N proteins of SARS-CoV-2 are able to induce *in vitro* the expression of TF on human fibroblasts [53]. Taken together, it appears that fibroblasts emerge as major players of lung tissue thromboinflammation in progressive COVID-19.

We observed an inhibitory effect on TF/thrombin axis expression and activity by primary lung fibroblasts after their treatment with the above immunomodulatory agents, used separately or combined, as in the COMBI clinical protocol. Dismantling of NETs and inactivation of the mediators they contain, as well as JAK inhibition, seem to have a central role in countering NET-driven thromboinflammation. The observed clinical outcome in all groups and the improved regulation of TF activity in supernatants obtained after treatment of cells with DNase, or/and IL-6 and JAK1/2 inhibition, indicate the involvement of various inflammatory triggers and pathways in TF/thrombin axis activation. This suggests the potential value of novel strategies based on combined immunomodulation. Several studies provide consistent evidence that TF/thrombin pathway, apart from thrombosis, is involved in the inflammatory and fibrotic processes [27,54]. Mechanistically, fibroblast-derived extracellular vesicles/exosomes or microparticles could transfer active TF to the lung tissue environment [55–57]. According to recent studies [11,13,31], combined targeting against other involved mediators, specifically in early stages, such as complement anaphylatoxins, may further diminish the mortality rate.

In conclusion, in this study, including non-ICU patients with SRF secondary to COVID-19 pneumonia, we found that combined compassionate therapy using inhaled DNase, tocilizumab, and baricitinib on top of SOC resulted in a lower mortality and intubation rate, as well as a shorter hospitalization time, compared to the use of SOC alone or together with IL-1 or IL-6 blockers only. Inhibition of TF/thrombin axis in lung fibroblast offers a plausible mechanistic explanation for these clinical observations. These results, although in the context of single-center non-RCT, encourage the design of RCTs using combined immunomodulatory therapies to target in a multilevel manner interconnected mechanisms of COVID-19 pathogenesis, in patients with the highest disease severity, before they turn the ICU road.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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