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Impact of Corticosteroids and Anticoagulant Combined Treatment on Patients Affected by COVID-19 Pneumonia

Fabio Anastasio[1], Elisa Scarnecchia[1], Sarah Barbuto[1], Fabio Spitaleri[1], Tommaso Saporito[1], Valeria Bisogni[2#] and Giacomo Pucci[2#]*

[1]*ASST Valtellina and Alto Lario, Eugenio Morelli Hospital, Sondalo (SO), Italy*

[2]*University of Perugia, Terni University Hospital, Terni, Italy*

[#]*Authors contributed equally to this study*

Abstract

Background: The pathogenesis of novel coronavirus disease (COVID-19) includes virus-induced systemic endothelial dysfunction, cytokine storm, and complement cascade hyperactivation, creating massive inflammatory and pro-coagulative states with diffuse vascular thrombosis.

Objective: Our observational cohort study analyzed the effectiveness of corticosteroids and anticoagulant combined treatment in patients affected by COVID-19.

Design and patients: 423 patients were retrospectively included in the analysis. Patients were divided into four groups: Group 1 (G1, n = 135): No treated; group 2 (G2, n = 8): Treated by methylprednisolone; group 3 (G3, n = 214): Those received low-molecular-weight heparin (LMWH); group 4 (G4, n = 66): Patients treated with combined methylprednisolone *plus* LMWH. The study outcome was the treatment failure, defined as all-cause of death or Intensive Care Unit (ICU) admission.

Results: Mortality and ICU admission rates were 20.6% and 15.1%, respectively. The per-group analysis showed an increased prognosis for G3 (HR 0.59, 95%CI 0.36-0.95, $p = 0.03$) and G4 (HR 0.47, 95%CI 0.27-0.82, $p = 0.007$) *vs.* non-treated group (G1). Combined therapy was especially successful in those patients with the worst respiratory function (SpO_2/FiO_2 ratio ≤ 220) (HR 0.43, 95%CI 0.24-0.77, $p = 0.004$).

Conclusions: LMWH treatment alone and/or in combination with methylprednisolone seems to be associated with a better outcome, reducing the rate of treatment failure in patients affected by COVID-19 pneumonia.

Keywords

COVID-19, SARS-CoV-2, Pneumonia, Corticosteroids, Low-molecular-weight heparin

Abbreviations

ALP: Alkaline Phosphatase; ALT: Alanine Transferase; APTT: Activated Partial Thromboplastin Time; ARDS: Acute Respiratory Distress Syndrome; AST: Aspartate Aminotransferase; BMI: Body Mass Index; BNP: B-Type Natriuretic Peptide; CI: Confidence Interval; CKD: Chronic Kidney Disease; COPD: Chronic Obstructive Pulmonary Disease; COVID: Coronavirus Disease; CRP: C-Reactive Protein; CVD: Cardiovascular Disease; DM: Diabetes Mellitus; FIO2: Fraction of Inspired Oxygen; Gamma-Gt: Gamma-Glutamyltransferase; HR: Hazard Ratio; HRCT: High Resolution Computed Tomography; ICU: Intensive Care Unit; Il-1β: Interleukin-1β; Il-6: Interleukin-6; Il-8: Interleukin-8; INR: International Normalized Ratio; IQR: Interquartile Range; I.V.: Intravenous; LDH: Lactate Dehydrogenase; LMWH: Low-Molecular-Weight Heparin; MOF: Multi-Organs Failure; N/L: Neutrophils/Leucocytes Ratio; PCR: Polymerase Chain Reaction; PCT: Procalcitonin; PMN: Polymorphonuclear Leukocytes; PT: Prothrombin Time; RR: Respiratory Rate; SARS-Cov-2: Severe Acute Respiratory Syndrome Coronavirus 2; SOFA: Sequential Organ Failure Assessment; SPO2: Peripheral Capillary Oxygen Saturation; SPO2/FIO2: Oxygen Saturation to Fraction of Inspired Oxygen Ratio; TNF-A: Tumor Necrosis Factor-A; VIF: Variance Inflation Factor; WHO: World Health Organization.

Introduction

In March 2020, the World Health Organization (WHO) declared the 2019 novel coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a public health emergency of international concern [1-4]. The viral infection can lead to pneumonia, severe acute respiratory syndrome (ARDS), multi-organ fail-

ure (MOF), and even death in the most severe cases [5]. The pathogenesis of COVID-19 disease is dominated by an acute pneumonic process characterized by diffuse alveolar damage, inflammatory infiltrates, and microvascular thrombosis, configuring an ARDS [6,7]. Consequent MOF would appear secondary to the massive host immune response and the inflammatory organ injury, with markedly and persistently elevated inflammatory markers such as procalcitonin (PCT), serum ferritin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, D-dimer, lactate dehydrogenase (LDH), and tumor necrosis factor-alpha (TNF-α), interleukins 1β, 6, and 8 (IL-1β, IL-6, IL-8) [1,8,9]. These latter are able to promote a procoagulant state by the activation of platelets, vascular endothelial cells, and the expression of tissue factor [10], associated with an increased risk of thromboembolic complications (e.g., micro-thrombosis, large vessel thrombosis) with a progressive elevation of D-dimer in function of the severity and extent of thrombosis [11-13]. Hence, several therapeutic interventions have been proposed to mitigate the inflammatory organ injury in viral COVID-19 disease and, consequently, to reduce the hypercoagulable state [13]. Anticoagulant therapy for the management of patients with pneumonia from SARS-CoV-2 infection is widely discussed [10,14-17]; recent retrospective studies seem to show a positive effect on the prognosis [10,14,18-21]. Moreover, current trials also showed the improvement of clinical outcomes due to the administration of corticosteroids in critical patients [22-27]. However, in viral COVID-19 pneumonia patients, data on the associated and simultaneous treatment with corticosteroids *plus* anticoagulant are still under evaluation [28].

Consistently with the hypothesis of a combined beneficial outcome with regard to the reduction of the state of hypercoagulability and inflammation respectively, the aim of our study was to evaluate the effectiveness of low-molecular-weight heparin (LMWH) associated with the administration of corticosteroid (e.g., methylprednisolone) in the management of COVID-19 infected patients with pneumonia, in terms of reducing all-cause mortality and Intensive Care Unit (ICU) admission.

Materials and Methods

Study design

All symptomatic suspected COVID-19 patients admitted at the "Eugenio Morelli Hospital" of Sondalo (SO), Lombardy, Italy, in a period between March 3rd and June 3rd, 2020 were considered as showed in the Flow-chart of the study (Figure 1). Data were collected from the electronic medical records and retrospectively analyzed. Written informed consent was waived in light of the urgent need to collect data.

Inclusion criteria were: 1) Patients aged > 18-years-old, with COVID-19 diagnosis confirmed by positive results of polymerase chain reaction (PCR) on nasal and pharyngeal swab or on alveolar-bronchiole washing in case of double negative swab [1], and 2) Patients hospitalized to COVID-19 Department for about 24 hours before ICU admission and/or death for all causes, or eventually discharged.

We did not consider in our collection data from (exclusion criteria): 1) Patients still hospitalized at the time of the analysis (i.e., 3rd June 2020); 2) Subjects with incomplete clinical and/or biochemical data; 3) Patients who were directly admitted to the ICU for the severity of clinical conditions; 4) Patients who received other treatments, such as tocilizumab, anakinra, and convalescent plasma therapy, this latter not available in our Hospital.

Data source and variables assessed

We collected demographic and anthropometric variables, comorbidities, past medical and pharmacological history for all patients meeting the inclusion criteria. Clinical symptoms or signs of COVID-19 disease and laboratory findings at baseline were also evaluated. Laboratory assessments consisted of a complete blood count, blood chemical analysis, coagulation testing, assessment of liver and renal function, measures of electrolytes, CRP, PCT, LDH, cardiac enzymes, high-sensitivity (HS) troponin I, B-type natriuretic peptide (BNP), and urine sample. Radiologic assessments included chest radiography and/or high-resolution computed tomography (HRCT).

For all patients, at the baseline, we also calculated the Charlson index score [29], the Sequential Organ Failure Assessment (SOFA) score [30], haemogasanalytic parameters, including the oxygen saturation to fraction of inspired oxygen (SpO_2/FiO_2) ratio [31,32].

During the hospitalization, we extracted and analyzed data on administered therapies such as LMWH and methylprednisolone. LMWH was administered at prophylaxis dose for deep vein thrombosis (4,000 IU subcutaneously per day) [25] for at least 14 days. The intravenous (i.v.) administration of methylprednisolone dose, alone or combined with LMWH, was between 0.5 and 1 mg/kg per day for at least 7 days.

Study outcome

Considering the paucity of data published so far on the combined treatment with corticosteroids *plus* anticoagulant in the management of patients affected by COVID-19 pneumonia, the main scope of our study was to evaluate the effectiveness of LMWH associated with the administration with methylprednisolone in terms of reduced all-cause mortality and ICU admission rates of COVID-19 pneumonia cases.

The primary endpoint was the treatment failure rate, defined as ICU admission with invasive ventilatory support and/or death for all causes, comparing four groups of patients: group 1 (G1): No treated (neither corticosteroid nor LMWH was administered); group 2 (G2): In which only methylpred-

***Corresponding author:** Fabio Anastasio, MD, ASST Valtellina and Alto Lario, "Eugenio Morelli Hospital", Sondalo (SO), Italy, Via Zubiani 33, 23035 Sondalo (SO), Italy

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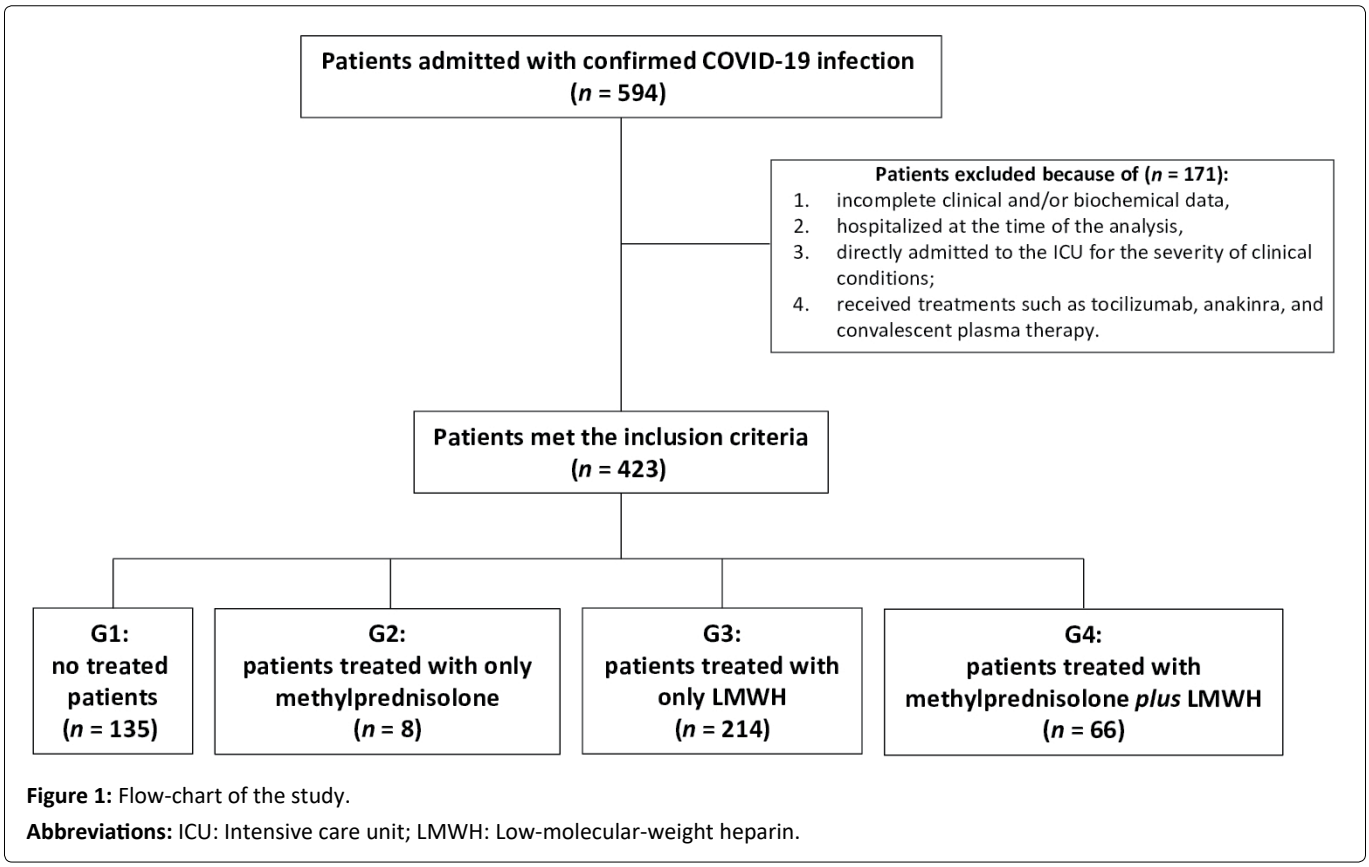

Figure 1: Flow-chart of the study.

Abbreviations: ICU: Intensive care unit; LMWH: Low-molecular-weight heparin.

nisolone was administered; group 3 (G3): Including patients who received only LMWH treatment; group 4 (G4): Patients treated with combined methylprednisolone *plus* LMWH.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) when not-normally distributed; categorical variables were presented as absolute value and percentage. Differences between groups were assessed by median test and Kruskal-Wallis test applying pairwise comparison and Bonferroni correction. Chi-square statistics were used to assess differences between categorical variables. The multicollinearity was examined using KMO and Bartlett's Test and the variance inflation factor (VIF): Variables with VIF > 3 were excluded by the same multivariable model. All baseline significant variables ($p < 0.05$) were included in adjusted multivariate Cox regression model with time-dependent covariates. Results were reported as hazard ratios (HR) with associated 95% confidence intervals (CI).

Statistical analysis was performed with SPSS software version 26.0 (Statistical Package for Social Sciences, software; SPSS Inc, Chicago, Illinois, USA), and a *p*-value of 0.05 or less was considered statistically significant.

Results

Characteristics of patients

594 consecutive patients with confirmed COVID-19 infection were hospitalized in COVID-19 Department. Of those, 171 were excluded from the analysis because did not meet the inclusion criteria. In more detail, we excluded patients with incomplete clinical and/or biochemical information, those who were still hospitalized at the time of the analysis, and those who were directly admitted to the ICU for the severity of clinical conditions. Lastly, we excluded also patients who received treatments such as to cilizumab or anakinra (Figure 1).

Table 1, Table 2 and Supplemental Table 1 (see Supplemental Appendix) include the baseline data from a total of 423 analyzed patients and the same characteristics divided for each subgroup 276 (65.2%) were males and 147 (34.8%) females; the median (IQR) age was 71 (59-80). The distribution of age classes divided for sex is represented in the Supplemental Appendix-Supplemental Figure 1. The median body mass index (BMI) was 26.4 (23.9-30.3 kg/m^2); 73 (17.3%) patients were obese (BMI > 30 kg/m^2 [33,34]). The Charlson index showed a median score of 4 (2-6) and the median SOFA score was 1 (1-2). At the time of the hospitalization, 87% of patients needed oxygen support with SpO_2/FiO_2 ratio ranging > 300 in 36% of patients, between 220 and 300 in 21%, between 140 and 220 in 13%, and < 140 in 17% of cases. The median of SpO_2/FiO_2 ratio was 296 (175-442). Particular focus was given to the climax point of lung failure corresponding to the worst clinical respiratory condition from the admission, based on the worst SpO_2/FiO_2 ratio. A median of 2 (1-5) days was registered between the hospitalization and the detection of the lowest SpO_2/FiO_2 ratio.

135 patients did not receive any therapy (G1), 8 patients received only methylprednisolone (G2), 214 patients received LMWH (G3), and 66 patients received LMWH *plus* methylprednisolone (G4). Table 1 and Table 2 show the differences between groups. The SpO_2/FiO_2 ratio at the admission was

Table 1: Baseline characteristics of patients affected by COVID-19 pneumonia divided by type of received treatment.

	Total (n = 423)	**G1 (n = 135)**	**G2 (n = 8)**	**G3 (n = 214)**	**G4 (n = 66)**	***P***
Age (years)	71 (59-80)	68 (57-77)*	74 (66-82)	70 (59-80)	78 (69-84)*	**< 0.001**
Sex (male) (n, %)	276 (65.2%)	91 (67.4)	6 (75.0)	132 (61.7)	47 (71.2)	0.42
BMI (Kg/m²)	26.4 (23.9-30.3)	26.1 (23.8-30.1)	28.4 (24.2-30.2)	26.8 (23.8-30.3)	26.2 (24.3-31.2)	0.86
Obesity (BMI > 30 Kg/m²) (n, %)	73 (17.3%)	21 (15.6)	2 (25.0)	34 (15.9)	16 (24.2)	0.37
Charlson index	4 (2-6)	3 (1-5)*	5 (4-7)	4 (2-6)§	5 (4-8)*§	**< 0.001**
Comorbidities (n, %):						
• **Hypertension**	222 (52.5)	71 (52.6)	5 (62.5)	107 (50.0)	39 (59.1)	0.57
• **CVD#**	82 (19.4)	25 (18.5)	1 (12.5)	36 (16.8)	20 (30.3)	0.10
• **DM**	86 (20.3)	21 (15.6)	1 (12.5)	50 (23.4)	14 (21.2)	0.65
• **Neoplasia**	61 (14.4)	14 (10.3)	2 (25.0)	23 (13.1)	17 (25.8)	**0.02**
• **COPD**	50 (11.8)	13 (9.6)	1 (12.5)	20 (9.3)	16 (24.2)	**0.01**
• **Asthma**	16 (3.8)	5 (3.7)	0 (0)	6 (2.8)	5 (7.6)	0.32
• **Hepatic disease**	5 (1.2)	0 (0)	0 (0)	3 (1.4)	3 (3.0)	0.29
• **CKD**	38 (9.0)	11 (8.1)	0 (0)	19 (8.9)	8 (12.1)	0.63
Smoke (n, %)	50 (11.8)	13 (9.6)	0 (0)	25 (11.7)	12 (18.2)	0.24
Heart rate (bpm)	80 (74-90)	84 (76-95)	73 (70-89)	80 (72-90)	80 (71-90)	0.13
RR (breath per minute)	22 (20-26)	22 (19-28)	23 (19-25)	22 (20-26)	22 (20-26)	0.55
Fever (n, %)	273 (64.5)	69 (51.1)*	4 (50)	148 (69.2)§	52 (78.8)*§	**< 0.001**
Temperature (°C)	38.0 (37.2-38.7)	37.8 (37.1-38.6)	37.6 (26.8-38.3)	38.0 (37.2-38.7)	38.0 (37.2-38.7)	0.80
SOFA score	1 (1-2)	1 (1-2)	2 (1-3)	1 (1-2)	2 (1-2)	0.06
SpO_2/FiO_2 ratio at the admission	296 (175-442)	312 (149-442)*	342 (138-442)	328 (245-447)§	228 (123-310)*§	**< 0.001**
Lowest peak of SpO_2/FiO_2 ratio	201 (97-332)	211 (94-339)*	97 (88-208)	237 (104-235)§	100 (94-179)*§	**< 0.001**
Onset of symptoms (days)	7 (4-10)	7 (5-10)^	4 (3-5)^**	7 (4-10)**	6 (4-10)	**0.02**
Oxygen support (n, %)	347 (82)	97 (71.9)*°	6 (75.0)	178 (83.6)°§	66 (98.5)*§	**< 0.001**
Day from hospitalization to treatment	1 (0-3)	/	3 (1-6)	0 (0-1)§	3 (1-6)§	**< 0.001**
Day from hospitalization to climax	2 (0-5)	2 (0-4)*	5 (1-10)	2 (1-4)§	5 (2-8)*§	**< 0.001**
ICU with mechanical ventilation (n, %)	64 (15.1)	40 (29.6)*°	1 (12.5)	16 (7.5)°	7 (10.6)*	**< 0.001**
Death for all causes (n, %)	87 (20.6)	24 (17.8)*	4 (50.0)	38 (17.7)§	21 (31.8)*§	**0.01**
Treatment failure (n, %)	151 (35.7)	64 (47.4)°	5 (62.5)	54 (25.2)°§	28 (42.4)§	**< 0.001**

Data are expressed as median ± interquartile range (IQR) when not specified.

#CVD included acute coronary syndrome, chronic heart failure, arrhythmias.

*Significant difference between G1 and G4; §Significant difference between G3 and G4; ^Significant difference between G1 and G2; **Significant difference between G2 and G3; °Significant difference between G1 and G3

Abbreviations: BMI: Body mass index; CVD: Cardiovascular disease; DM: Diabetes mellitus; COPD: Chronic obstructive pulmonary disease; CKD: Chronic kidney disease; RR: Respiratory rate; SOFA: Sequential organ failure assessment; SpO_2/FiO_2: Oxygen saturation to fraction of inspired oxygen ratio; ICU: Intensive care unit.

worse in G4 patients ($p < 0.001$) and the rate of oxygen support was significantly higher in patients of G4 (compared to all other groups, p 0.001).

The international statements in the first months of the pandemic advised against the use of corticosteroids in the COVID-19 pneumonia patient's management [35-37]. For this reason, corticosteroids were not administered during

Table 2: Main biochemical data of patients affected by COVID-19 pneumonia divided by type of received treatment.

N/L	5.6 (3.5-9.6)	6.3 (3.5-10.4)	7.8 (2.6-24.7)	4.9 (3.3-9.0)	6.4 (3.9-11.6)	0.11
Platelets (1000/µl)	206 (152-275)	192 (148-259)	222 (143-248)	211 (161-292)	210 (141-269)	0.19
aPTT (sec)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.0)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	0.71
INR	1.4 (1.2-2.6)	1.5 (1.3-2.8)	/	1.3 (1.2-2.5)	1.3 (1.2-4.6)	0.79
D-dimer (ng/ml)	1081 (620-2102)	1245 (736-2411)	1142 (658-3089)	986 (536-2098)	1174 (749-2026)	0.25
HS troponin I (µg/l)	12.8 (4.8-37.7)	24.7 (5.1-56.0)°	4.0 (3.0-8.7)	8.0 (3.2-23.4)§°	23.7 (12.1-47.9)§	**< 0.001**
BNP (pg/ml)	76 (31-180)	67 (13-195)	53 (31-68)	74 (30-157)	132 (44-227)	0.46
PCT (ng/ml)	0.14 (0.07-0.45)	0.18 (0.06-0.81)	0.31 (0.07-1.82)	0.12 (0.06-0.29)§	0.18 (0.09-0.52)§	**0.008**
CRP (mg/l)	80.9 (31.5-144.2)	91.7 (35.4-145.8)	102.7 (36.7-191.8)	72.9 (24.5-130.4)§	91.7 (51.2-157.1)§	**0.05**
Ferritin (ng/ml)	811 (368-1903)	1704 (629-3584)°	/	743 (273-1450)°	793 (538-2624)	**0.04**
Cholinesterase (U/L)	7741 (6132-9367)	8083 (6718-9378)*	6851 (5868-7731)	7945 (6211-9468)§	6402 (5534-8552)*§	**0.01**
Serum creatinine (mg/dl)	0.9 (0.8-1.3)	1.0 (0.7-1.3)	1.1 (0.9-1.8)	0.9 (0.8-1.2)	1.0 (0.7-1.4)	0.37
Proteinuria (mg/l)	300 (150-1000)	300 (37-1000)	650 (300-2500)	300 (150-500)§	300 (150-1000)§	**0.02**
Total bilirubin (mg/dl)	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.7 (0.4-1.0)	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.92
ALT (U/L)	29 (19-48)	32 (22-54)°	24 (15-33)	26 (17-47)°	29 (18-45)	**0.04**
AST (U/L)	36 (27-57)	40 (29-62)	45 (41-53)	34 (25-51)	40 (30-57)	**0.02**
Gamma-GT (U/L)	51 (27-93)	60 (29-120)	40 (22-58)	43 (25-85)	54 (36-84)	**0.05**
LDH (U/L)	320 (242-417)	333 (245-487)	385 (267-503)	298 (228-397)§	348 (269-466)§	**0.01**

Data are expressed as median ± interquartile range (IQR) when not specified.

*Significant difference between G1 and G4; §Significant difference between G3 and G4; °Significant difference between G1 and G3.

Abbreviations: N/L: Neutrophils/leucocytes ratio; aPTT: Activated partial thromboplastin time; INR: International normalized ratio; PCT: Procalcitonin; CRP: C-reactive protein; BNP: B-type natriuretic peptide; ALT: Alanine transferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; gamma-GT: Gamma-glutamyltransferase.

the hospitalization, but only when the clinical and lung parameters were critical (i.e., SpO_2/FiO_2 ratio < 220 - diagnosis of ARDS), causing differences in administration timing in our four groups. Therefore, treatment was started at different times for our four groups: A median of 3 (1-6) days after hospitalization in G2 and G4, and 0 (0-1) days in G3. Climax point of clinical status (i.e., the worst SpO_2/FiO_2 ratio) was registered after 2 (0-4) days in G1, 5 (1-10) days in G2, 2 (1-4) days in G3, and 5 (2-8) days in G4.

Study endpoint

Ina total of 151 patients (35.7%) the composite study endpoint (e.g., treatment failure defined as ICU admission and/or death for all-cause) has been observed. After a median of 2 (1-4) days of hospitalization, 64 patients were admitted to the ICU (15.1%) and supported by invasive mechanical ventilation. All-cause of death were observed in 87 subjects (20.5%).

Treatment failure was significantly lower for LMWH-treated (G3) compared to G1 (25.2% *vs.* 47.4%, $p < 0.001$) and to G4 (25.2% *vs*. 42.4%, $p < 0.001$).

In more detail, the rate of patients admitted to the ICU and supported by invasive mechanical ventilation was higher in G1 compared to G3 (29.6% *vs*.7.5%, $p < 0.001$) and compared to G4 (29.6% *vs*. 10.6%, $p = 0.007$). The rate of death for all causes was increased in patients who received only methylprednisolone (G2) compared to G1, G3, and G4 ($p = 0.01$). Moreover, we also observed significantly increased rates of all-cause death between G1 and G4 (31.8% *vs*. 17.8%, $p = 0.02$), and between G3 and G4 (31.8% *vs*. 17.7%, $p = 0.02$).

The main characteristics of patients' ongoing treatment success or failure are shown in Supplemental Table 2 of Supplemental Appendix. As expected, the treatment success was reached by younger people, most of them were non-obese, females, with a significantly lower percentage of comorbidities such as hypertension, diabetes, smoking habit, and dementia. They showed also significantly lower Charlson index and SOFA scores. The SpO_2/FiO_2 ratio was higher in survived patients (340 [261-447] *vs*. 166 [166-305], p < 0.001) (Supplemental Table 2).

Survival predictors

The Cox regression analysis showed a significantly positive correlation between treatment failure and age (HR 4.90, 95%CI 1.93-12.46, $p = 0.001$), male sex (HR 2.13, 95%CI 1.45-3.12, $p < 0.001$), obesity (HR 1.69, 95%CI 1.16-2.46, $p = 0.006$), SOFA score (HR 1.89, 95%CI 1.33-2.69, $p < 0.001$), and SpO_2/FiO_2 ratio (HR 0.35, 95%CI 0.28-043, $p < 0.001$) (Table 3). Amongst the laboratory tests, in the multivariate analysis, treatment failure was statistically associated with a higher value of neutrophils/lymphocytes (N/L) ratio (HR 1.36, 95%CI 1.09-1.69, $p = 0.006$), PCT (HR 1.23, 95%CI 1.07-1.41, $p = 0.004$), CRP (HR 1.28, 95%CI 1.04-1.59, $p = 0.02$), LDH (HR

Table 3: Univariate and multivariate analyses.

Covariates	Univariate			Multivariate model 1			Multivariate model 2		
	P	HR	95%CI	*P*	HR	95%CI	*P*	HR	95%CI
Log Age	**0.003**	3.76	1.55-9.10	**0.001**	4.90*	1.93-12.46	**0.04**	3.34*	1.07-10.4
Sex Male	**0.001**	1.91	1.31-2.78	**< 0.001**	2.13*	1.45-3.12	**0.05**	1.54*	1.00-2.38
Obesity	**0.01**	1.61	1.10-2.34	**0.006**	1.69*	1.16-2.46	**0.002**	1.88*	1.25-2.83
Charlson index	**0.003**	1.08	1.03-1.14	0.44			/		
Number of comorbidities	**0.002**	1.12	1.04-1.21	0.23			/		
Any comorbidity	**0.02**	1.81	1.12-2.93	0.47			/		
Connective disease	**0.05**	2.15	1.01-4.60	0.57			/		
Hypertension	**0.03**	1.43	1.03-1.98	0.85			/		
Log SOFA score	**< 0.001**	1.72	1.28-2.31	**0.009**	1.52	1.11-2.08	**< 0.001**	1.89*	1.33-2.69
Log initial SpO_2/FiO_2 ratio	**< 0.001**	0.32	0.26-0.39	**< 0.001**	0.36	0.29-0.44	**< 0.001**	0.35*	0.28-0.43
Log N/L ratio	**< 0.001**	1.99	1.63-2.45	**< 0.001**	1.85	1.51-2.28	**0.006**	1.36	1.09-1.69
Log D-dimer	**< 0.001**	1.43	1.25-1.63	**< 0.001**	1.42	1.23-1.64	0.74		
Log PCT	**< 0.001**	1.51	1.37-1.66	**< 0.001**	1.46	1.32-1.62	**0.004**	1.23	1.07-1.41
Log CRP	**< 0.001**	1.78	1.48-2.16	**< 0.001**	1.67	1.37-2.02	**0.02**	1.28	1.04-1.59
Log Ferritin	**0.001**	1.56	1.20-2.01	**0.005**	1.52	1.14-2.03	0.26		
Log HS troponin I	**< 0.001**	1.25	1.14-1.36	**< 0.001**	1.25	1.13-1.39	**0.03**	1.13	1.01-1.27
Log Proteinuria	**< 0.001**	1.77	1.32-2.37	**< 0.001**	1.75	1.29-2.37	**0.004**	1.62	1.17-2.24
Log Creatinine	**0.03**	1.35	1.02-1.77	0.44			/		
Log LDH	**< 0.001**	3.85	2.77-5.34	**< 0.001**	3.56	2.52-5.01	**< 0.001**	2.31	1.54-3.46
Log Albumin	**0.02**	0.31	0.12-0.82	0.06	0.38	0.14-1.05	0.44		

Not-normal distributed variables were transformed into a logarithmic scale.

Multivariate Model 1: Cox proportional-hazard model adjusted for: age, sex and obesity.

Multivariate Model 2: Cox proportional-hazard model adjusted for: age, sex, obesity, SpO_2/FiO_2 ratio and SOFA score.

Abbreviations: N/L: Neutrophils/leucocytes ratio; PCT: Procalcitonin; CRP: C-reactive protein; LDH: Lactate dehydrogenase; SpO_2/FiO_2: Oxygen saturation to fraction of inspired oxygen ratio; SOFA: Sequential organ failure assessment.

2.31, 95%CI 1.54-3.46, $p < 0.001$), HS troponin I (HR 1.13, 95%CI 1.01-1.27, $p = 0.03$), and proteinuria (HR 1.62, 95%CI 1.17-2.24, $p = 0.004$) (Table 3).

Effect of treatment

At the univariate analysis, the LMWH (HR 0.61, 95%CI 0.41-0.89, $p = 0.01$), but not methylprednisolone treatment ($p = 0.32$), exhibited a significant negative correlation with treatment failure. The different timing of methylprednisolone administration, related to the climax point of clinical and radiological conditions, was included in the analysis, showing an inverse significant correlation between methylprednisolone administration and treatment failure (HR 0.48, 95%CI 0.30-0.75, $p = 0.001$). Response to treatment was then corrected, in a multivariate analysis, for significant prognostic variables, for clinical conditions at the hospitalization (i.e., age, sex, BMI, SOFA score, and SpO_2/FiO_2 ratio), and for the different clinical conditions at the time of methylprednisolone administration (i.e., worst SpO_2/FiO_2 ratio). Both of LMWH (HR 0.51, 95%CI 0.33-0.77 $p = 0.001$) and methylprednisolone treatments (HR 0.53, 95%CI 0.33-0.84, $p = 0.007$) showed a significant prognostic value.

According to the hypothesis of our study, that is the combined effect of LWMH *plus* corticosteroids could reduce the severity of COVID-19 pneumonia, analyzing the different therapeutic strategies between the four groups, we observed that G4 (LMWH *plus* methylprednisolone) showed the best prognosis compared to non-treated group (G1) (HR 0.47, 95%CI 0.27-0.82, $p = 0.007$). G3 showed an increased prognosis compared to G1 (HR 0.59, 95%CI 0.36-0.95, $p = 0.03$). G2 didn't show improvement ($p = 0.92$) (see Supplemental Table 3 of Supplemental Appendix). Additionally, we observed a better prognosis for G4 patients *vs*. G3 (HR 0.59, 95%CI 0.35-0.97, $p = 0.04$), especially versus G1 + G2 + G3 (HR 0.44, 95%CI 0.28-0.71, $p < 0.001$). Differences between G2 and G3 or G4 were not significant due to the small sample size of G2.

The cumulative risk of treatment failure after multivariate analysis in the four groups is showed in Figure 2 and the cumulative risk of G4 *vs*. G1 + G2 + G3 in Figure 3.

Furthermore, after stratifying patients in four groups according to the SpO_2/FiO_2 ratio thresholds (i.e., a) 300-400; b) 220-300; c) 140-200; d) < 140), we found a significant effect of combined LMWH *plus* methylprednisolone administration on treatment failure in patients with SpO_2/FiO_2 ratio between

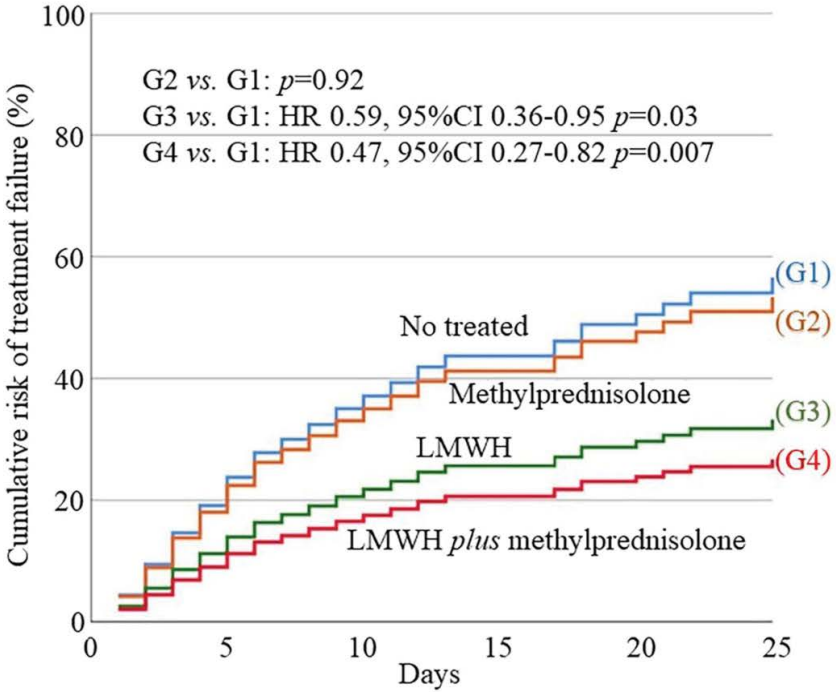

Figure 2: Cumulative risk (%) of treatment failure according to the treatment strategies (group 1 (G1, n = 135): Non-treated patients; group 2 (G2, n = 8): Patients treated by methylprednisolone; group 3 (G3, n = 241): Subjects receiving low-molecular-weight heparin (LMWH); group 4 (G4, n = 66): Patients treated with combined therapy LMWH *plus* methylprednisolone) for a median follow-up of 10 days.

Abbreviations: LMWH, low-molecular-weight heparin.

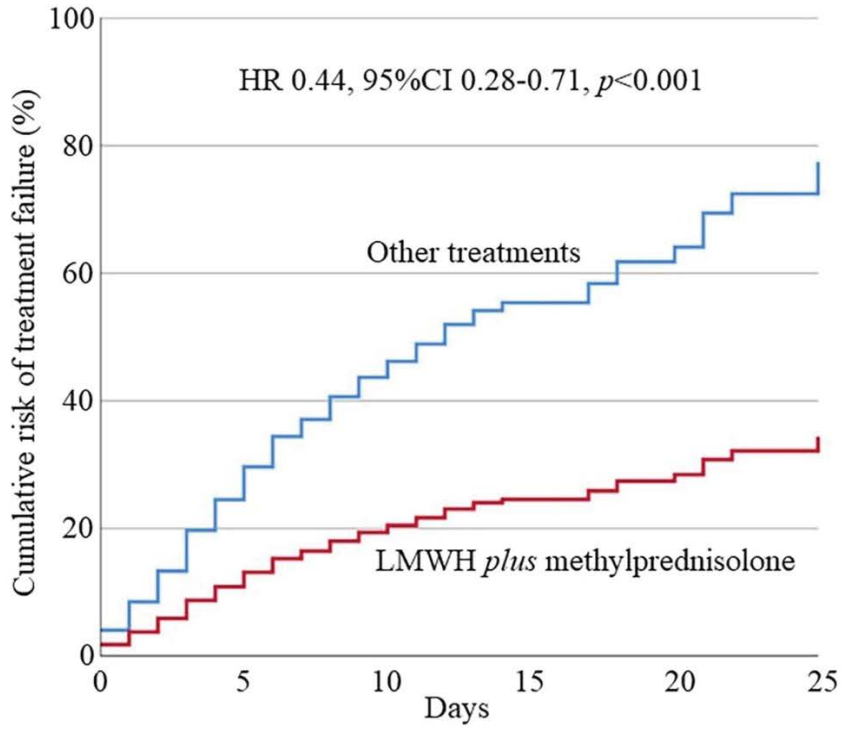

Figure 3: Cumulative risk (%) of treatment failure comparing the subgroup G4 (patients treated with combined therapy LMWH *plus* methylprednisolone) compared to all other therapeutic strategies for a median follow-up of 10 days.

Abbreviations: LMWH: Low-molecular-weight heparin; HR: Hazard ratio; CI: Confidence interval.

140-220 (HR 0.25, 95%CI 0.07-0.87, $p = 0.03$) and in those with < 140 (HR 0.49, 95%CI 0.27-0.91, $p = 0.02$) (see Supplemental Table 4 of Supplemental Appendix), suggesting a better effect of this combined treatment in those cases with worse respiratory conditions in terms of gas exchange.

Discussion

By our knowledge, this study documented, for the first time, that in patients affected by COVID-19 pneumonia combined therapy with LMWH *plus* methylprednisolone is associated with a better outcome with a significant reduction of all-cause death rate and/or ICU admission needing of invasive mechanical ventilation.

SARS-CoV-2 infection is dominated by an acute, often bilateral, pneumonic involvement characterized by diffuse alveolar damage with inflammatory infiltrates and microvascular thrombosis, configuring an ARDS [6,7,38]. The subsequent MOF would appear secondary to the massive host immune response and the inflammatory organs injury. Hence, based on this hyperinflammation state caused by an increase in proinflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNF-α, triggered by SARS-CoV-2 infection, different studies have been performed with the aim to test the efficacy of IL-6 receptor blockade (e.g., tocilizumab) in hospitalized patients with COVID-19 pneumonia, but the results are still contradictory [39-41], just as the findings on the administration of convalescent plasma [42,43]. Moreover, convalescent plasma contains procoagulant factors, thus, administering it to patients with COVID-19 means introducing procoagulant factors into their bloodstream [44]. Recently, the IL-1 receptor antagonist, anakinra, was used to treat patients affected by severe COVID-19 disease forms with significantly reduced both need for invasive mechanical ventilation in the ICU and mortality and without serious side-effects [45]. However, beyond the efficacy and safety of these very expensive therapeutic strategies, which has yet to be definitively tested, their use is off-label and not always available, particularly in small peripheral care centers and hospitals.

On the other hand, numerous studies have been published evaluating the individual effectiveness of corticosteroid therapy [23-25]. Amongst these, the RECOVERY trial [46] was one of the most robust analysis demonstrating a moderate but significant reduction in mortality with corticosteroids administration (dexamethasone, 6 mg per day). More recently, pooled data from seven randomized clinical trials, including the RECOVERY trial [46], were analysed in a meta-analysis promoted by WHO [25] and reported an improvement prognosis related to the administration of corticosteroids in critically ill COVID-19 patients with significant reduction in the mortality rate. Nevertheless, results of this meta-analysis are still challenged [38] because of, when data from the RECOVERY trial [46] were excluded from the analysis, this positive effect disappeared suggesting an overweight of this trial. Other studies showed contrasting results; in the CAPE COVID [23] no benefit of corticosteroids was found, as well as in the CoDEX trial [30], where corticosteroids significantly increased ventilator-free days during the first 28 days, but there was no benefit on 28-day mortality or length of stay in ICU in the Metcovid study [47]. Conversely, the REMAP trial [48], which included 903 treated patients, hydrocortisone (40 mg intravenous every 6 h) significantly reduced mortality from severe COVID-19.

Different analyses concerning the role of anticoagulant therapy in patients with COVID-19 disease have been published as well [10,14,18-21,49,50]. The rationale of anticoagulant administration, such as the LMWH, in COVID-19 disease is justified by the need to control the hypercoagulable state that has been proved in this patients and could be characterized by a wide clinical manifestation, form a local thrombosis in the pulmonary vasculature to pulmonary embolism, and vascular thromboembolism (VTE), until the onset of severe disseminated intravascular coagulation and thrombotic microangiopathy [4,51-55]. However, to date, only retrospective studies analyzed the effect of LMWH therapy on the survival rate in COVID-19 patients, most of them strongly suggested that anticoagulant therapy improves the prognosis of SARS-CoV-2 infection [10,14,18-20]. In the study by Tang and colleagues [14], the LMWH treatment appeared to be associated with a better prognosis in severe COVID-19 infection with sepsis-induced coagulopathy, reducing 28-day mortality rate. On the contrary, these results have not been confirmed in patients without coagulopathy [14].

Accordingly, both of these treatments seem to show a positive effect on the prognosis of COVID-19 patients, especially in the severe form of the disease with lungs involvement but, at present, no studies are available on the effectiveness of the LMWH associated with corticosteroids in the management of SARS-CoV-2 infection, compared to the single treatment administration. Simultaneous combined treatment with corticosteroid *plus* anticoagulant is under evaluation in only one trial [28].

In our retrospective observational study, conducted on COVID-19 pneumonia patients from the North of Italy, we reported a treatment failure (e.g., ICU admission and/or death for all-cause) in 35.7% of cases; after a median of 2 (1-4) days of hospitalization, 64 patients were admitted to the ICU for development of severe respiratory failure/ARDS (15.1%). All patients in ICU were supported by invasive mechanical ventilation. The death for all-cause were observed in 87 (20.5%) of subjects. Older age, male sex, obesity, a worse SpO_2/FiO_2 ratio at the admission, and higher SOFA score, showed a significant negative impact on outcome of COVID-19 patients, as previously highlighted in the literature [1]. However, unlike for what has been described, in our sample of subjects arterial hypertension and diabetes mellitus were not associated with a higher mortality risk [1,56]. Our findings show first that the combined therapy with prophylactic dose of LMWH *plus* i.v. administration of a weight-based dose of methylprednisolone significantly reduced the rate of treatment failure. This effect seems to be more significant for the G4 group (LMWH *plus* methylprednisolone) compared to non-treated patients (G1) (HR 0.47, 95%CI 0.27-0.82, $p = 0.007$) and to patients with single LWMH therapy (G3) (HR 0.59, 95%CI 0.35-0.97, $p = 0.04$). Furthermore, the significant effect of combined LMWH *plus* methylprednisolone administration (G4) on treatment failure appears to be more consistently in patients with $SpO_2/$

FiO_2 ratio from 140 to 220 (HR 0.25, 95% CI 0.07-0.87, p = 0.03) and in those with < 140 (HR 0.49, 95% CI 0.27-0.91, p = 0.02), corresponding approximately to a FiO_2 needing oxygen support > 40%. Patients with slight pulmonary involvement may not have a truly evident improvement in prognosis, in line with the current knowledge [16,46].

The strength of our results, which suggest the effectiveness of combined LMWH *plus* corticosteroid therapy in a cohort of COVID-19 pneumonia patients, mostly in severe respiratory failure cases, was evident after adjustment for confounding factors. In addition, the solidity of our results lies the inclusion in the analysis of all demographic and anthropometric variables, and a complete laboratory assessment, for each patient enrolled from the start to the end of the study.

Nevertheless, our study has some limitations that must be pointed out. First, due to the retrospective design of the analysis, the study can only report associations, cannot investigate causality, and is susceptible to multiple sources of bias such as indication bias and hidden confounders. Second, the small sample size of each of the four therapy-based groups reduces the power of our findings; for example, the analysis on the G2 group, receiving methylprednisolone without LMWH, was not relevant, due to the sample exiguity (only 8 cases). Our analysis, indeed, concerns information on the first cases of COVID-19 pneumonia with rapid worsening, which has been collected retrospectively. Likely, for these cases, there was not yet sufficient evidence on the procoagulant state and the possible increased risk of thromboembolic complications of COVID-19 diseases, and on the use of LMWH as a critical therapeutic strategy [10]. Lastly, the time to methylprednisolone administration was delayed due to the lack of recommendations of corticosteroids treatment in the first weeks of the COVID-19 pandemic. However, we performed a statistical analysis able to reduce bias due to administration of therapy in patients after clinical improvement and we demonstrated a significant reduction in cumulative risk in the subgroup of LMWH *plus* methylprednisolone compared to other treatment strategies.

Conclusion

In conclusion, this retrospective observational cohort study shows that combined treatment with LMWH *plus* methylprednisolone during hospitalization was associated with a lower treatment failure rate in COVID-19 pneumonia patients. The effect appeared to be more relevant in patients with SpO_2/FiO_2 ratio ≤ 220 and in those suffering from a more severe form of lung failure. These findings need to be confirmed by further double-blind randomized and with greater sample-sized clinical trials.

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Author contributions

FA, ES, SB and FS contributed to the acquisition of data. FA, VB and GP contributed to analysis and interpretation of data for the work.

All authors contributed to design of the work, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Prior presentations

None.

Declaration of interest

The authors have no conflicts of interest to declare.

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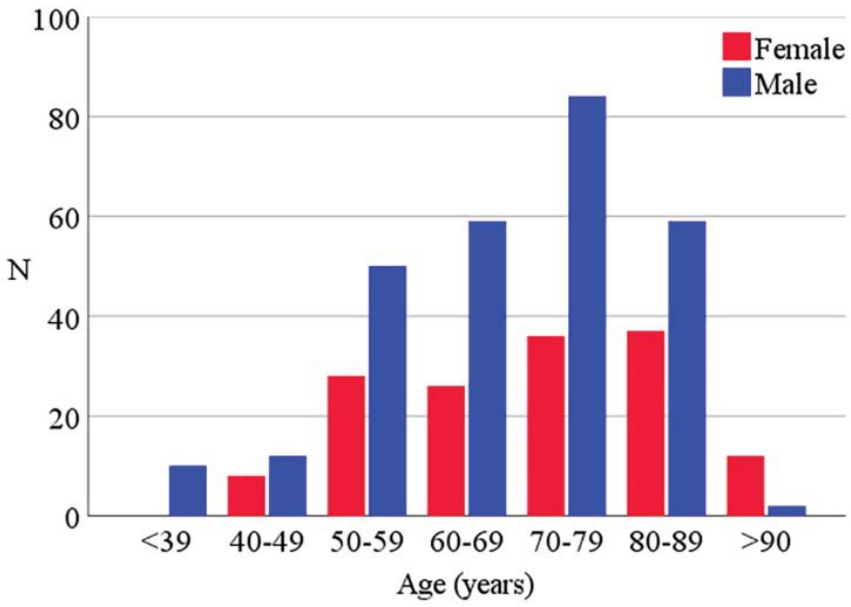

Supplemental Figure 1: Sex distribution for age-group.
Abbreviation: N: Number.

Supplemental Table 1: Main biochemical data of patients affected by COVID-19 pneumonia divided by type of received treatment.

Neutrophils (n/mm³)	4.9 (3.4-6.7)	4.9 (3.4-7.0)	4.2 (2.3-8.9)	4.8 (3.3-6.2)	5.1 (3.8-7.9)	0.33
Lymphocytes (n/mm³)	0.8 (0.6-1.2)	0.8 (0.6-1.1)	0.6 (0.2-0.9)	0.9 (0.6-1.2)	0.7 (0.5-1.2)	0.11
Hemoglobin (g/dl)	14.0 (12.8-15.2)	14.2 (13.2-15.4)	13.2 (11.7-15.3)	13.9 (12.7-15.1)	13.7 (12.7-15.2)	0.19
Albumin (g/l)	25.7 (22.3-29.7)	25.3 (21.7-29.7)	/	26.6 (23.3-30.6)	23.7 (22.0-26.6)	0.09
Creatine kinase (U/L)	108 (59-209)	118 (64-240)	287 (126-467)	109 (56-196)	99 (53-186)	0.08
ALP (U/L)	63 (51-84)	62 (50-93)	55 (49-72)	65 (52-84)	62 (47-69)	0.49

Data are expressed as median ± interquartile range (IQR) when not specified. **Abbreviations:** ALP: Alkaline phosphatase.

Supplemental Table 2: Baseline characteristics of patients affected by COVID-19 divided by treatment success or failure.

	Total (423)	**Treatment success (272)**	**Treatment failure (151)**	***P***
Age, years	71 (59-80)	69 (56-79)	74 (66-82)	**< 0.001**
Sex male (n,%)	276 (65.2)	161 (59.2)	115 (76.2)	**< 0.001**
Obesity (n,%)	73 (17.3)	37 (13.6)	36 (23.8)	**0.008**
Charlson index score	4 (2-6)	3 (2-5)	4 (3-7)	**< 0.001**
SpO_2/FiO_2 ratio	296 (175-442)	340 (261-447)	166 (166-305)	**< 0.001**
SOFA score	1 (1-2)	1 (1-2)	2 (1-2)	**< 0.001**
Hypertension (n,%)	222 (52.5)	130 (47.8)	92 (60.9)	**0.009**
Diabetes (n,%)	86 (20.3)	47 (17.3)	39 (25.8)	**0.05**
Smoking history (n,%)	50 (11.8)	26 (9.6)	24 (15.9)	**0.05**
Dementia (n,%)	53 (12.5)	26 (9.6)	27 (17.9)	**0.01**

Data are expressed as median ± interquartile range (IQR) when not specified.
Abbreviations: SpO_2/FiO_2: Oxygen saturation to fraction of inspired oxygen ratio; SOFA: Sequential organ failure assessment.

Supplemental Table 3: Multivariate analysis between treatment strategies and treatment failure after correction for confounding factors (see the main text for additional explanations).

	G1 (n = 135)	G2 (n = 8)	G3 (n = 214)	G4 (n = 66)	*P*
Treatment failure (n, %)	64 (47.4)[*]	5 (62.5)	54 (25.2)[*§]	28 (42.4)[§]	**< 0.001**
ICU with mechanical ventilation (n, %)	40 (29.6)[*°]	1 (12.5)	16 (7.5)[*]	7 (10.6)°	**< 0.001**
Death for all causes (n, %)	24 (17.8)°	4 (50.0)	38 (17.7)[§]	21 (31.8)[°§]	**0.01**
HR (*vs*. G1)	-	-	0.59	0.47	
95%CI (*vs*. G1)	-	-	0.36-0.95	0.27-0.82	
***P* (*vs*. G1)**	-	0.92	**0.03**	**0.007**	
HR (*vs*. G2)					
95%CI (*vs*. G2)					
***P* (*vs*. G2)**			0.92	0.15	
HR (*vs*. G3)				0.59	
95%CI (*vs*. G3)				0.35-0.97	
***P* (*vs*. G3)**				**0.04**	

[*]Significant difference between G1 and G3; °Significant difference between G1 and G4; [§]Significant difference between G3 and G4.

[*]Significant difference between SpO_2/FiO_2 ratio 300-400 and SpO_2/FiO_2 ratio < 140; [^]Significant difference between SpO_2/FiO_2 ratio 300-400 and SpO_2/FiO_2 ratio 140-220; [#]Significant difference between SpO_2/FiO_2 ratio 220-300 and SpO_2/FiO_2 ratio 140-220; °Significant difference between SpO_2/FiO_2 ratio 220-300 and SpO_2/FiO_2 ratio < 140; [§]Significant difference between SpO_2/FiO_2 ratio 140-220 and SpO_2/FiO_2 ratio < 140.

Supplemental Table 4: Multivariate analysis between SpO_2/FiO_2 ratio at baseline and combined therapy (LMWH *plus* methylprednisolone) *versus* no treatment.

	SpO_2/FiO_2 ratio 300-400 (n = 197)	**SpO_2/FiO_2 ratio 220-300 (n = 88)**	**SpO_2/FiO_2 ratio 140-220 (n = 69)**	**SpO_2/FiO_2 ratio < 140 (n = 69)**	*P*
LMWH *plus* methyl prednisolone	17 (8.6%)	18 (20.4%)	15 (21.7%)	21 (30.4%)	**< 0.001**
Treatment failure	35 (17.8%)[^*]	21 (23.8%)°	31 (44.9%)[^§]	55 (79.7%)[*°§]	**< 0.001**
ICU	9 (4.6%)[^*]	6 (6.8%)[°#]	17 (24.6%)[^#]	23 (33.3%)[*°]	**< 0.001**
Death	26 (13.2%)[*]	15 (17.0%)°	14 (20.3%)[§]	32 (46.4%)[*°§]	**< 0.001**
HR	0.99	0.47	0.25	0.49	
95%CI	0.42-2.35	0.14-1.55	0.07-0.87	0.27-0.91	
P	0.98	0.21	**0.03**	**0.02**	

[*]Significant difference between SpO_2/FiO_2 ratio 300-400 and SpO_2/FiO_2 ratio < 140; [^]Significant difference between SpO_2/FiO_2 ratio 300-400 and SpO_2/FiO_2 ratio 140-220; [#]Significant difference between SpO_2/FiO_2 ratio 220-300 and SpO_2/FiO_2 ratio 140-220; °Significant difference between SpO_2/FiO_2 ratio 220-300 and SpO_2/FiO_2 ratio < 140; [§]Significant difference between SpO_2/FiO_2 ratio 140-220 and SpO_2/FiO_2 ratio < 140.

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