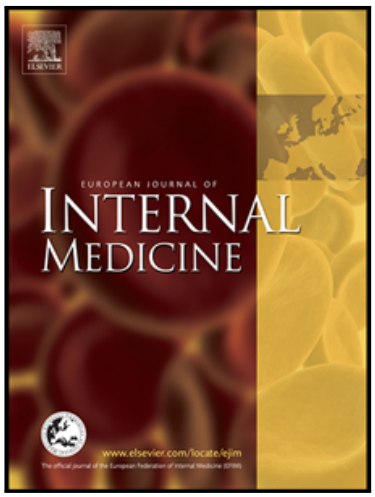

Lessons from pathophysiology: Use of individualized combination treatments with immune interventional agents to tackle severe respiratory failure in patients with COVID-19

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Lessons from pathophysiology: Use of individualized combination treatments with immune interventional agents to tackle severe respiratory failure in patients with COVID-19

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Highlights

- SARS-CoV-2 infection is characterised by complex immune dysregulation
- Specific treatment against SARS-CoV-2 is currently missing and is far from ideal
- Ferritin is an easy to determine marker that could stratify treatment decisions
- Timely personalised combinations of immunomodulatory agents proved efficacious
- This treatment algorithm proved also safe with rare adverse events

Abstract

Aims: Infection by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) may lead to the development of severe respiratory failure. In hospitalized-patients, prompt interruption of the virus-driven inflammatory process by using combination treatments seems theoretically of outmost importance. Our aim was to investigate the hypothesis of multifaceted management of these patients.

Methods: A treatment algorithm based on ferritin was applied in 311 patients (67.2% males; median age 63-years; moderate disease, n=101; severe, n=210). Patients with ferritin <500ng/ml received anakinra 2-4mg/kg/day ± corticosteroids (Arm A, n=142) while those with ≥500ng/ml received anakinra 5-8mg/kg/day with corticosteroids and γ-globulins (Arm B, n=169). In case of no improvement a single dose of tocilizumab (8mg/kg; maximum 800mg) was administered with the potential of additional second and/or third pulses. Treatment endpoints were the rate of the development of respiratory failure necessitating intubation and the SARS-CoV-2-related mortality. The proposed algorithm was also validated in matched hospitalized-patients treated with standard-of-care during the same period.

Results: In overall, intubation and mortality rates were 5.8% and 5.1% (0% in moderate; 8.6% and 7.6% in severe). Low baseline pO_2/FiO_2 and older age were independent risk factors. Comparators had significantly higher intubation (HR=7.4; 95%CI: 4.1-13.4; p<0.001) and death rates (HR=4.5, 95%CI: 2.1-9.4, p<0.001). Significant adverse events were rare, including severe secondary infections in only 7/311 (2.3%).

Conclusions: Early administration of personalized combinations of immunomodulatory agents may be life-saving in hospitalized-patients with COVID-19. An immediate intervention (the sooner the better) could be helpful to avoid development of full-blown acute respiratory distress syndrome and improve survival.

Key words: SARS-CoV-2; COVID-19; anakinra; tocilizumab; IL-1; IL-6.

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2-related; IL, interleukin; TNF-α, tumor necrosis factor-α, sHLH, haemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome; IVIG, intravenous infusions of γ-globulins; JAK, Janus kinase; BMI, body mass index; CRP, C-reactive protein; LDH, lactate dehydrogenase; OR, odds ratio; HR, hazard ratio; RCTs, randomized controlled trials.

Introduction

Severe coronavirus disease-2019 (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and characterized by complex dysregulation of the immune system resulting in hyperinflammation and full-blown viral pneumonia, which in turn, can lead to severe respiratory failure, multiorgan failure, and death [1,2]. Therefore, special attention has been paid to this specific group of patients, which is at high risk of fatal outcome. Indeed, it has been shown that many of the severely ill patients, have an hyperinflammatory condition characterized by high levels of several pro-inflammatory mediators such as, interleukin (IL)-1, IL-7, interferon-γ inducible protein 10, tumor necrosis factor-α (TNF-α) and granulocyte colony stimulating factor [2-5]. Moreover, increased concentrations of ferritin and IL-6 have been identified as predictors of fatality in these patients [2-5]. .

As no treatment has yet been proven specifically effective against COVID-19, the logical next step was to attempt the interruption of the virus-driven inflammatory process in the host in order to prevent the progression of lower respiratory tract infection to severe respiratory

failure necessitating mechanical ventilation. [5-7]. However, the precise timing and the best dosages of these interventions are largely unknown. Indeed, therapeutic options with corticosteroids, intravenous infusions of γ-globulins (IVIG), anti-cytokine interventions such as, anakinra and tocilizumab as well as Janus kinase (JAK) inhibitors have been used in patients with severe COVID-19 resulting in some promising results [8-18].

However, as in the secondary hemophagocytic lymphohistiocytosis (sHLH) or macrophage activation syndrome (MAS), the achievement of efficient blocking of the inflammatory process by using only one specific agent in patients with severe COVID-19 seems exceptionally difficult [19,20]. In this regard, early administration of combinations of immune interventional drugs as soon as there is clinical suspicion, probably in less than 12 hours from admission, could theoretically tackle the process of the disease and prevent the development of severe respiratory failure and the need of mechanical ventilation [2,7,17-23]. Accordingly, we present in this study the results of our hypothesis of multifaceted management of patients with confirmed COVID-19-related pneumonia using a proposed combination treatment algorithm.

Patients and Methods

Hospital admission

In this prospective, open-label observational study, 434 patients (age ≥18 years) with confirmed COVID-19 by real-time PCR (COVID-19 genesig® Real-Time PCR assay, Primerdesign, Camberley, UK) of nasopharynx swab were admitted between March 20 and December 10, 2020 in the Infectious Disease Unit of the Department of Internal Medicine at the General University Hospital of Larissa, Greece, which is the referral center for the management of COVID-19 in our region (Thessaly, Greece). The characteristics of our region have already been published in other patients' groups such as, chronic viral and autoimmune liver diseases [24-26].

Inclusion in the study

Three hundred and fifty-six out of 434 admitted patients (median age: 64 years; 37.1% females) were initially eligible for inclusion in the study as they had confirmed moderate or severe lower respiratory tract infection by chest-x-ray and non-enhanced, high resolution computed

tomography of the chest (Figure 1). According to the National Public Health Organization of Greece moderate infection was defined by the presence of pneumonia and oxygen saturation by pulse oximetry in room-air (SpO_2) ≥94% [27]. Severe disease was defined by the presence of pneumonia and one or more of the following: SpO_2<94% in room air, pO_2/FiO_2<300, breath rate >30/min and pulmonary infiltrates in more than >50% of the lung parenchyma [27].

However, 45 patients were excluded from the study (see Supplementary Table 1 for the exclusion criteria of the study) and thus, 311 out of 356 patients (122 with moderate and 189 with severe disease) enrolled finally to the study protocol after written informed consent obtained by the patients or legal representatives (Figure 1). During hospitalization, 21 patients from the moderate group developed severe disease and therefore, the analysis was also done between 101 patients with moderate and 210 patients with severe disease (Table 1). Several parameters and outcome measures according to the severity of the disease and treatment arms were recorded at baseline, during hospitalization and follow-up after discharge such as, demographics, comorbidities, inflammatory markers [ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-Dimers], complete white blood counts including absolute neutrophil and lymphocyte counts, platelets count, pO_2/FiO_2 ratio, respiratory rate, disease duration, O_2 supplementation, days of O_2 supplementation, duration of hospitalization, rate of intubation, 30-day mortality and safety issues. In particular, all patients who discharged from the hospital were evaluated every 5–7 days at a dedicated COVID-19-related outpatient clinic of our department by clinical examination and laboratory determination of inflammatory markers, complete white blood counts, platelets, pO_2/FiO_2 ratio and respiratory rate. In few patients who were not able to attend our outpatient clinic on day-30, there was a phone call to assess the general well-being, 30-day mortality and available SpO_2 and respiratory rate.

Treatment algorithm

According to our hypothesis, the treatment protocol was based on ferritin levels at baseline. Actually, sampling for ferritin measurement at baseline was taking immediately in less than 2 hours after the presentation of patients in the hospital, either at the emergency room or in our department (Figure 2). As shown in figure 2, patients were immediately (in less than 12 hours)

assigned to different treatment arms based on ferritin levels. More precisely, when ferritin levels were less than 500 ng/mL (Arm A), patients were started immediately anakinra 2-4 mg/kg/day in 1-3 doses subcutaneously or intravenously with or without 50 mg of intravenous hydrocortisone four times daily (total daily dose: 200 mg) or dexamethasone 6 mg/day intravenously for seven days.

When ferritin levels were above 500 ng/mL (Arm B), patients were started immediately anakinra 5-8 mg/kg/day subcutaneously or intravenously divided in 3-4 doses plus intravenous pulses of 500 mg/day methylprednisolone for the first three days in combination with IVIG (2g/kg in 5-7 days). In Arm B, 50 mg of intravenous hydrocortisone four times daily (total daily dose: 200 mg) or dexamethasone 6 mg/day intravenously for seven days was followed after the initial three-days course of intravenous pulses of methylprednisolone. In both treatment arms, the intravenous route of anakinra administration 2-4 times daily by a dedicated line was preferred if high doses were required (i.e. >200 mg/day) in order to avoid pain from multiple injections daily or if the patient has peripheral oedema, anasarka or obesity as adiposity results in slower absorption rate of anakinra after subcutaneous administration [20,28].

The reason for choosing a cut-off of ferritin levels at 500 ng/mL was that according to the published criteria, levels less than 500 ng/mL have a high negative predictive value for the development of sHLH or MAS [29,30]. In both arms, the use of corticosteroids was withheld transiently or permanently in case of lymphopenia (absolute number of lymphocytes < 800/μL) either at initial evaluation of patients or during hospitalization. In addition, the decision for intravenous corticosteroids administration in an index patient and in particular for the pulsed doses of methylprednisolone was based on the treating physicians' discretion after weighing the benefits and harms according to the medical history, the severity of the disease, the laboratory parameters of inflammation and the underlying comorbidities of the patients (see also footnote of Figure 2). For instance, corticosteroids could be withheld in patients aged above 80 years with severe osteoporosis or patients of any age with poorly controlled diabetes. Administration of IVIG was not done in cases with severe underlying cardiac failure in order to avoid the potential risk of volume overload.

In case of no clinical and/or laboratory improvement in 1-2 days after the abovementioned management, a single intravenous pulse dose of tocilizumab (8 mg/kg; maximum 800 mg) was administered with the potential of an additional second and/or third pulse after at least 24-48 hours from the first dose (Figure 2). No improvement was defined by at least two of the following: persistent fever >38.5^{o}C, deterioration of dyspnea confirmed by increased needs of supplemental O_2 compared to baseline, considerable decrease of SpO_2 and pO_2/FiO_2 ratio compared to baseline levels, increase of respiratory rates, development of new pulmonary infiltrates on chest-x-ray and at least 50% increase of any of the inflammatory serological markers. The criteria for the additional (≥2) doses of tocilizumab were similar to that used for the first dose of the drug.

The use of anakinra first instead of tocilizumab was chosen because anakinra has a shorter half-life and subsequently, a faster wash out resulting probably in less myelosuppressive and hepatotoxic side effects, as well as, lower risk of opportunistic infections [20]. Additionally, it does not mask parameters of acute phase response like fever and CRP while in a reanalysis of a phase III trial, anakinra was associated with reduced mortality in patients with sepsis and features of MAS [20,31]. As remdesivir was available in Greece after October 16, 2020, this antiviral drug was also used intravenously in both arms at the initial evaluation of patients and particularly in those with disease onset equal or less than 10 days (200 mg on day 1 followed by 100 mg on days 2-5 in single daily infusions performed over 30 to 60 minutes) [32-34]. All patients received the standard of care with enoxaparin and antibiotics according to the guidelines of the National Public Health Organization of Greece [27].

Treatment endpoints

The treatment endpoints were first, the rate of the development of severe respiratory failure necessitating intubation and mechanical ventilation and second, the SARS-CoV-2-related mortality. Patients were monitored every day for the presence of severe respiratory failure and survival. Adverse events from baseline until the last evaluation of the patients were also recorded. Severe respiratory failure was defined as the presence all of the following: severe

decrease of the respiratory ratio (pO_2/FiO_2) in equal or less than 150 and the need for mechanical ventilation.

All patients consented to participate in this study. The Research Ethics Committee of the University of Thessaly and the respective committee of the General University Hospital of Larissa approved the protocol which conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Statistics

Normality of the distribution of variables was assessed by Kolmogorov-Smirnov test. Quantitative values are expressed as median (IQR). Data were analyzed by Mann-Whitney U-test, x^2 (two-by-two with Yate's correction), Fisher's exact test and binary logistic regression analysis where applicable. Univariate and multivariate Cox regression analyses were performed to assess the impact of various baseline factors on the rates of mortality or intubation and mechanical ventilation. The cumulative risk for mechanical ventilation and survival were evaluated with Kaplan-Meir analysis up to the end of follow-up or when patients reached one of the endpoints (intubation or death).

In order to validate the efficacy of our treatment protocol, 213 of our patients ("Larissa patients") were matched (1:1) and compared with 213 patients with SARS-CoV-2-related pneumonia hospitalised at Greek hospitals during the same period ("Comparators"). "Comparators" were selected by using propensity score matching according to the baseline characteristics and severity of the disease at presentation in order to avoid selection bias and control for potential confounding factors. Variables of matching were: age, sex, comorbidities (diabetes mellitus, renal disease, chronic obstructive pulmonary disease, arterial hypertension, coronary artery disease), body mass index (BMI), WHO severity scores, respiratory ratio and treatment with corticosteroids. The seasonable distribution of hospital admissions in the two groups are shown in Supplementary Figure 1. In order to avoid significant bias, the exclusion criteria used in the study (Supplementary Table 1) were also taken into consideration in the "Comparators" group. The comparisons between "Larissa patients" and "Comparators" regarding the endpoints were performed by the log-rank test.

Two-sided *P*<0.05 were considered statistically significant. All data analyses were performed using the statistical software SPSS version 20.0.

Results

Characteristics of patients at initial evaluation (n=311)

Data on demographics, clinical and laboratory parameters of the patients at initial evaluation are shown in Table 1. Almost two thirds of patients were males (62.7%; Table 1). The median age (IQR) of patients was 63 (20) years (range: 18-91 years). The BMI was 27.8 (5.8) kg/m^2. A considerable proportion of patients had underlying important comorbidities such as, diabetes, arterial hypertension, chronic obstructive pulmonary disease and coronary artery disease (Table 1). History of previous or current smoking was recorded in 127 (40.8%) patients.

As expected, patients with severe disease at baseline (n=189) were significantly older (p<0.001), had higher BMI (p<0.001), higher prevalence of cardiovascular disease (p<0.001), lower pO_2/FiO_2 ratio (<0.001), and higher ferritin (p<0.001), CRP (p<0.001), D-Dimers (p<0.001) and LDH levels (p<0.001) compared to those with moderate disease (n=122; Table 1).

Multivariate analysis revealed that disease severity at baseline was independently associated with older age [odds ratio (OR) 1.055, 95%CI: 1.030-1.081; p<0.001)], increased BMI (OR=1.165, 95%CI: 1.088-1.248, p<0.001) and higher CRP (OR=1.065, 95%CI: 1.017-1.115; p=0.007) and LDH levels (OR=1.007, 95%CI: 1.004-1.010; p<0.001) (Supplementary Table 2).

Treatment

According to our treatment algorithm, 142 (45.7%) patients were enrolled in Arm A and 169 (54.3%) in Arm B (Table 2). The precise combination treatments are shown in Figure 3. The univariate analysis showed that patients in Arm B had significantly higher ferritin (p<0.001), CRP (p<0.001), D-Dimers (p<0.02) and LDH levels (p<0.001) compared to Arm A patients (Table 2). In addition, patients in Arm B were characterized by increased prevalence of male sex (p<0.001), longer disease duration (p<0.02), lower pO_2/FiO_2 ratio (p<0.001) and higher frequency of severe disease (p<0.001) compared to those in Arm A (Table 2). However, after multivariate analysis,

Arm B patients were independently associated only with male sex (OR=4.183, 95%CI: 2.435-7.186, p<0.001), lower pO_2/FiO_2 ratio (OR=0.995, 95%CI: 0.991-0.998; p<0.001) and increased CRP (OR=1.063, 95%CI: 1.019-1.109; p=0.005; Supplementary Table 3).

Outcome of patients

a. Intubation

In overall, 18 patients (5.8%) intubated. All of these patients had severe disease at baseline (18/210, 8.6% vs. 0/101, 0%; p=0.006; Table 3). Of note, none of the patients who progressed from moderate to severe disease during hospitalization (n=21) needed mechanical ventilation (Table 3).

In the univariate analysis, the baseline factors which were associated with the need of mechanical ventilation were: older age [hazard ratio (HR) 1.049, 95%CI: 1.009-1.088, p<0.02), lower pO_2/FiO_2 ratio (HR=0.989, 95%CI: 0.984-0.994; p<0.001) and medical history of hypertension (HR=4.174, 95%CI: 1.208-14.42; p=0.024). The treatment arm allocation and the administration of remdesivir or corticosteroids did not affect the intubation rates. After multivariate analysis, the pO_2/FiO_2 ratio (HR=0.989, 95%CI: 0.983-0.994; P<0.001) at baseline was identified as the only independent prognostic factor for intubation (Table 4). The Kaplan-Meier curves of the cumulative incidence of intubation in the total group of patients and in patients with severe disease at baseline or during hospitalization are shown in Figure 4A.

b. Mortality

In overall, 16 patients (5.1%) died including 4 before intubation. All of these patients had severe disease (16/210, 7.6% vs. 0/101, 0%; p=0.01; Table 3). Of note, none of the patients who progressed from moderate to severe disease during hospitalization (n=21) died (Table 3).

In the univariate analysis, the baseline factors that associated with high mortality were: older age (HR=1.074, 95%CI: 1.026-1.124; p=0.002) and low pO_2/FiO_2 ratio (HR=0.990, 95%CI: 0.985-0.996; p=0.001). No impact was found on mortality in relation to the treatment arm, administration of remdesivir or corticosteroids. Multivariate analysis rendered both older age (HR=1.075, 95%CI: 1.024-1.128; p=0.003) and decreased pO_2/FiO_2 ratio at baseline (HR=0.990,

95%CI: 0.984-0.996; p=0.002) as negative prognostic factors (Table 5). The Kaplan-Meier curves of the cumulative incidence of death in the total group of patients and in patients with severe disease at baseline or during hospitalization are shown in Figure 4B.

c. Oxygen supplementation and duration of hospitalization

During hospitalization, 202/311 patients -all from the severe disease group- required oxygen supplementation (202/210; 96.2%, p<0.001; Table 3). As expected, the duration of oxygen supplementation was significantly higher in patients with severe compared to those with moderate disease and in patients of treatment Arm B compared to those treated with Arm A (p=0.009 and p=0.03, respectively; Table 3).

Duration of hospitalization was longer in patients with severe disease at baseline or during hospitalization compared to patients with moderate disease (p<0.001 for both comparisons) and also in patients in Arm B compared to those in Arm A (p<0.001; Table 3).

d. Validation of the treatment algorithm by using "Comparators"

The selected concurrent "Comparators" (n=213) and "Larissa patients" (n=213) had similar baseline characteristics and disease severity except for lymphocytes count and D-Dimers (Supplementary Table 4). In overall, 13/213 (6.1%) "Larissa patients" needed intubation compared to 73/213 (34.3%) of "Comparators" (p<0.001). Additionally, mortality was significantly lower in "Larissa patients" group (10/213; 4.7%) compared to the "Comparators" group (36/213; 16.9%; p<0.001). Kaplan-Meir analysis revealed significantly higher cumulative rates of intubation (HR=6.7; 95%CI: 4.4-10.3; p<0.001) and death (HR=4.0; 95%CI: 2.2-7.1; p<0.001) in the "Comparators" group compared to the "Larissa patients" group (Figures 5A and 5B). Of note, the risk of intubation and death remained significantly higher in "Comparators" compared to "Larissa patients" after adjustment for lymphocytes count and D-Dimers (HR=7.4; 95%CI: 4.1-13.4; p<0.001 and HR=4.5; 95%CI: 2.1-9.4; p<0.001, respectively). The superiority of our treatment algorithm regarding the mortality rates remained stable compared to the standard of care at each trimester, despite a relative disproportion of hospital admissions between the two groups (Supplementary Figure 1).

In addition, in order to avoid the possibility of selection bias, we performed a sub-analysis regarding the mortality rates after excluding 7 patients from the "Comparators" group who died before intubation. The Kaplan-Meier analysis revealed again significantly higher cumulative rates of death in the "Comparators" (HR=3.5, 95%CI: 1.9-6.5; p<0.001), which remained significantly higher even after adjustment for lymphocytes count and D-Dimers (HR=3.9; 95%CI: 1.8-8.2; p<0.001).

Safety

Adverse events in total and also according to the treatment arm and disease severity are shown in Table 6. Mild reactions at the injection sites of anakinra were occurred in 8 patients (2.6%) but without any need of treatment discontinuation. Serious infections of grade≥3 occurred in only 7/311 (2.3%) patients; 2 patients with secondary bacterial pneumonia and 5 patients with hospital-acquired blood stream infections including 4 with central line-related infection. Only 2/36 (5.6%) patients who developed neutropenia contracted severe infection (1 with central line-related blood stream infection due to *Candida* and *Stenotrophomonas maltophilia* and 1 with primary bacteremia due to *Staphylococcus epidermidis*). Two out of the 7 patients with infections died before intubation, 1 patient intubated and eventually discharged, while the rest 4 patients were successfully treated and discharged without necessitating mechanical ventilation. The development of these infections was not associated with tocilizumab administration (data not shown).

Thrombotic events detected more frequently in patients with severe disease treated with Arm B (Table 6) but not in those who received tocilizumab (data not shown). In fact, 10 patients (9 with pulmonary embolism and 1 with both pulmonary embolism and mesenteric vein thrombosis) developed thrombotic events. Two of these patients died including one after intubation.

As expected, neutropenia was observed more frequently in patients with severe disease who were treated with the high anakinra dose (Arm B; Table 6) or tocilizumab (30/177, 16.9% vs. 6/134, 4.5%; p=0.001). However, all 36/311 (11.6%) patients who developed neutropenia, were successfully managed with subcutaneous administration of a single dose of granulocyte colony-

stimulating factor without requiring permanent discontinuation of treatment. Actually, in all these 36 patients, a transient withdrawal of anakinra for 1 day was applied, followed by a sharp and rapid restoration of neutrophils count in less than 24 hours after the administration of the growth factor. These results led us to reintroduce anakinra after 24 hours of restoration under close monitoring in all affected patients without observing any additional event during the study period and follow-up. Emergence of thrombocytopenia (platelets count ≤140000/μL) occurred in 22 patients (7.1%) including 5 (1.6%) with severe thrombocytopenia (<50000/μL) who treated successfully with corticosteroids and IVIG as either drug-induced or SARS-CoV-2-related thrombocytopenia. Thrombocytopenia was not associated with tocilizumab administration (16/177, 9% vs. 6/134, 4.5%; p=0.183). Significant increase of alanine aminotransferase levels (ALT>200 IU/L) was observed during hospitalization in 24/311 (7.7%) patients including 3 who had already increased ALT (>200 IU) at baseline. This adverse event was associated with severe disease, Arm B of treatment algorithm (Table 6) and administration of tocilizumab (19/177, 10.7% vs. 5/134, 3.7%; p=0.03). Only one out of these 24 patients intubated and died. In the rest patients, ALT increase eventually resolved.

Discussion

In general, the management of COVID-19 depends on the severity of the disease [35-37]. People with mild disease usually recover at home without any specific treatment while in hospitalized patients the aim is to accelerate recovery and avoid complications and death. It is believed that coagulopathy and a hyperinflammatory condition can lead finally to respiratory failure, multiorgan disease and death in a considerable proportion of hospitalized patients and therefore, antivirals, anti-inflammatory drugs, anticoagulants and immune modulators have been used mainly as monotherapies albeit conflicting results [9,11-19,23,32-34,38,39].

However, the use of best intervention in these patients and particularly in those with severe disease by administering the right drug in the right patient at the right time remains “a million dollar’s answer”. In this context, antiviral treatment with remdesivir in patients with moderate or severe infection has shown significant superiority to the placebo arm regarding the time to

recovery although, mortality at day 29 was not different [34]. Of note, the most significant clinical benefit of remdesivir administration in that study was observed in patients who were receiving low-flow oxygen (not critically ill individuals) [34]. Therefore, the drug seems to be most effective when it is used early, just before or soon after symptoms. However, unfortunately, this is not always the case in everyday clinical practice as most of hospitalized patients are seeking help after several days of the onset of the disease. In contrast, dexamethasone administration showed a reduced 28-day mortality in hospitalized patients with COVID-19 compared to patients who received standard of care (22.9% vs. 25.7%) [9]. However, this benefit was mainly restricted to subjects who had advanced disease (either under mechanical ventilation or supplemental oxygen) [9]. The rational next step is to assess whether multifaceted management of patients with combination treatments including antivirals, therapies that enhance antiviral immune response and immune modulators could be an option in this multidimensional disease [23].

In this regard, our study in hospitalized patients with SARS-CoV-2 infection showed the following main findings: a). the proposed treatment algorithm seems to bear very good efficacy regarding intubation and 30-day mortality rates either in overall (5.8% and 5.1%, respectively) or in the moderate (n=101; 0% and 0%, respectively) and severe disease (n=210; 8.6% and 7.6%, respectively) b). all 21 patients who on admission had moderate disease and progressed to the severe form had excellent survival, as well (100%) c). severe adverse events were uncommon including secondary bloodstream infections which were rare (2.3%) d). our algorithm showed significantly higher efficacy rates compared to “Comparators” and most importantly, e). although, known prognostic markers such as, ferritin, CRP, D-Dimers and LDH were verified to associate with disease severity, they had no impact on treatment outcomes, suggesting that the prompt and timely initiation of the algorithm counterbalances their prognostic effect on outcome. Unfortunately, the study was designed and started in March 2020, well before the WHO clinical progression scale was introduced as an endpoint for trials of COVID-19, so such an assessment was not feasible.

In a recent elegant study in 1484 COVID-19 patients, Del Valle et al [5] showed after adjustment for disease severity, markers of inflammation, hypoxia, demographics, and several

comorbidities, that IL-6 and TNF-α in serum were independent and significant predictors of disease severity and death. However, in another recent study, TNF-α levels -although increased in patients with SARS-CoV-2 infection compared to healthy- were not associated with disease severity and survival [40]. Moreover, the relative role of abnormal IL-1b responses in the immunopathology of severe COVID-19 remains controversial [4,5,40-42]. Indeed, in the very recent study by Abers et al [40], the IL-1b levels although higher than healthy, did not correlate either at baseline or after serial determinations during the disease process with the severity of COVID-19 and patient mortality. In parallel with the previous study, Bell et al [41] have shown that the elevated expression of IL-1b and IL-6 response modules was a characteristic feature of SARS-CoV-2 infection but was not associated with the disease severity, length of stay or mortality.

Taking together, these contradictory data suggest that stratification of patients in treatment schedules according to the determination of proinflammatory cytokines in serum could be premature. This is why in our study we decided not to determine the levels of these cytokines at baseline or during hospitalization and we preferred a much more standard and easily to measure marker like ferritin, taking into account our general experience in sHLH or MAS [21,22]. Indeed, the serum levels of the above cytokines may be in the normal range in patients with sHLH-like syndromes, though they might be elevated at local sites of injury, and frequently do not correlate with the outcome following therapy with specific IL-1b or IL-6 inhibitors [20]. Another point concerning the determination of these cytokines in patients' sera, might be the technical difficulties because of assays variability making comparisons among laboratories inefficient and the fact that determination of individual cytokines at the protein level may not reflect precisely their biological activity [20,41].

Since all patients who received high-dose intravenous pulse corticosteroids (Arm B) treated also with other immunomodulatory drugs, it is not possible to specifically evaluate the response of COVID-19 patients to the administration of pulse corticosteroids. However, it is worthy to state that to the best of our knowledge, this is the first study where high-dose intravenous pulse corticosteroids were administered in patients with COVID-19. These doses were used according to our experience and the rational of treatment in other 'catastrophic' diseases and/or

syndromes with increased mortality such as, vasculitis, catastrophic antiphospholipid syndrome or sHLH [21,22,43,44].

In our treatment algorithm, we did not use at all JAK inhibitors. The reason of not choosing this drug category was the theoretical safety concerns about probable deleterious effects of simultaneous, instead of selective blockade of multiple cytokines, although these drugs have the advantages of oral formulation and short half-life. Besides, in a recent comparative analysis of immunomodulatory therapies, it has been shown that combination treatments of corticosteroids with tocilizumab or anakinra have resulted in superior survival of COVID-19 patients [19]. Indeed, recent data from systematic reviews and meta-analyses including randomized controlled trials (RCTs) and observational studies of low/moderate risk of bias, showed promising favorable results such as, reduced rates of mechanical ventilation and mortality by using anakinra, corticosteroids and/or tocilizumab in a prompt and timely fashion during the disease process [45-51].

Last but not least, safety was not a major problem in our study, as the frequency of hospitalized-acquired and secondary blood stream infections either bacterial or fungal was very low, even though in other series, up to a third of hospitalized patients with severe COVID-19 developed infections [52]. In addition, the significantly higher frequency of thrombotic events and transaminasemia observed in the treatment Arm B was rather attributed to the more severe disease than the treatment arm itself.

Our study has some limitations: a) it is a single-center non-RCT based on a case-by-case management, b) although the kind of treatment arms seems not to affect the treatment response, there was a lack of homogeneity in treatment schedules within each arm (Figure 3) and c) intravenous administration of corticosteroids was relied on a case-by-case clinical decision after taking into consideration the potential benefits and harms according to the medical history, the severity of the disease, the laboratory markers and the underlying comorbidities of the patients. However, the use of our criteria shows that if immunomodulatory intervention starts promptly enough, can prevent deterioration of the disease and the need of mechanical ventilation in the vast majority of patients.

In conclusion, our study suggests that the early administration of combined immunomodulatory agents may be life-saving in hospitalized patients with SARS-CoV-2 infection and therefore, it should be started as soon as possible (the sooner the better) before full-blown acute respiratory distress syndrome is developed. At least in our hands, this prompt initiation appears to prevent disease deterioration, while it does not seem to increase mortality and complications in the vast majority of patients. Our findings need of course validation from other groups in the field in order to definitely address the urgent unmet need of SARS-CoV-2 management although RCTs using our treatment algorithm vs. conventional treatment schedules with enoxaparin, remdesivir plus/minus corticosteroids in the real-life setting of this urgent situation could be unethical.

Conflict of interest: George N Dalekos is an advisor or lecturer for Ipsen, Pfizer, Genkyotex, Novartis, Sobi, received research grants from Abbvie, Gilead and has served as PI in studies for Abbvie, Novartis, Gilead, Novo Nordisk, Genkyotex, Regulus Therapeutics Inc, Tiziana Life Sciences, Bayer, Astellas, Pfizer, Amyndas Pharmaceuticals, CymaBay Therapeutics Inc., Sobi and Intercept Pharmaceuticals. E.J. Giamarellos-Bourboulis has received honoraria from Abbott CH, Angelini Italy, InflaRx GmbH, MSD Greece, XBiotech Inc., and B·R·A·H·M·S GmbH (Thermo Fisher Scientific); independent educational grants from AbbVie Inc, Abbott CH, Astellas Pharma Europe, AxisShield, bioMérieux Inc, Novartis, InflaRx GmbH, and XBiotech Inc; and funding from the FrameWork 7 program HemoSpec (granted to the National and Kapodistrian University of Athens), the Horizon2020 Marie-Curie Project European Sepsis Academy (granted to the National and Kapodistrian University of Athens), and the Horizon 2020 European Grant ImmunoSep (granted to the Hellenic Institute for the Study of Sepsis). The rest authors declare they have no conflict of interest.

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Legend to the figures

Figure 1. Flow-chart of the patients included in the study.

Figure 2. Proposal of the therapeutic algorithm used in COVID-19 patients with moderate or severe pneumonia. COVID-19, coronavirus disease 2019; SpO_2, oxygen saturation as measured by pulse oximetry in room air; SC, subcutaneously; d, day; IV, intravenously. *Since October 16, 2020, intravenous administration of remdesivir was available in Greece and since then it was given in both groups at the initial evaluation when disease duration was less than 10 days. [1]When more than 200 mg/d of Anakinra were used, as well as in obese patients or patients with oedema and critically ill patients the IV administration was preferred. [2]Corticosteroids were not administered in the presence of lymphopenia (absolute number of lymphocytes <800/μL) at first evaluation or were stopped if lymphopenia was developing during management. [3]Corticosteroids administration was also decided at physicians' discretion according to other parameters of inflammation and severity such as, C-reactive protein ≥ 5 mg/dL, D-Dimers > 400 ng/mL, the extent of pulmonary infiltrates, SpO_2 < 94% in room air and the presence of tachypnoea (breath rate >30/min). [4]An additional second and/or third pulse therapy after at least 24-48 hours from the first dose was used according to the physicians' discretion.

Figure 3. The detailed combination therapies of patients in both treatment arms. 166 patients received corticosteroids (72 in Arm A and 94 in Arm B). Corticosteroids were discontinued permanently in 12 and transiently in 5 patients (Arm A) as well as, in 16 and 29 patients, respectively (Arm B). IVIG was administered in 135/169 (79.9%) of Arm B patients. Tocilizumab was given in 177 patients (Arm A, n=51; Arm B, n=126). Additional pulses (≥2) of tocilizumab were needed in 8 patients (Arm A) and 53 patients (Arm B). 77 patients received also remdesivir (31 in Arm A and 46 in Arm B).

Figure 4. Kaplan-Meier analyses of efficacy outcomes. (A) Intubation: Analysis includes 18 events-intubations. Patients who died before intubation were excluded (n=4). (B) Mortality: Analysis includes 16 events-deaths in the total group of patients (n=311). Data from patients who were event-free at the end of follow-up were censored at 30 days (one death occurred at 58th day of hospitalization in ICU).

Figure 5. Kaplan-Meier analyses of efficacy outcomes of “Larissa patients” and “Comparators”. (A) Intubation: Analysis includes 86 events-intubations in total. Patients who died before intubation were excluded (2 from “Larissa patients” and 7 from “Comparators”). (B) Mortality: Analysis includes 46 events-deaths in total. Data from patients who were event-free at the end of follow-up were censored at 30 days (one death occurred at 58th day of hospitalization in ICU).

Table 1. Baseline characteristics according to disease severity at baseline.

	Total (n=311)	**Moderate (n=122)**	**Severe (n=189)**	**P-value**
Age, median (IQR), years	63 (20)	58.5 (26)	65 (18)	<0.001
Male sex, n (%)	195 (62.7%)	75 (61.5%)	120 (63.5%)	0.811
BMI, median (IQR), kg/m^2	27.8 (5.8)	26.4 (4.8)	28.4 (6.8)	<0.001
Diabetes, n (%)	63 (20.3%)	21 (17.2%)	42 (22.2%)	0.353
COPD, n (%)	26 (8.4%)	7 (5.7%)	19 (10.1%)	0.257
Cardiovascular Disease, n (%)	175 (56.3%)	54 (44.3%)	121 (64%)	0.001
Hypertension	173 (55.6%)	51 (41.8%)	122 (64.6%)	<0.001
Coronary artery disease	25 (8%)	9 (7.4%)	16 (8.5%)	0.896
Smoking, n (%)				
previous/active	127 (40.8%)	49 (40.2%)	78 (41.3%)	0.940
Never	184 (59.2%)	73 (59.8%)	111 (58.7%)	
Disease duration, median (IQR), days[a]	7 (5)	6 (7)	7 (4)	0.079
PO_2/FiO_2 ratio, median (IQR)	295 (106)	390 (104)	271 (52)	<0.001
Respiratory rate, median (IQR), /min	22 (8)	18 (6)	24 (10)	<0.001
Lymphocytes, median (IQR), /μL	940 (600)	995 (633)	900 (605)	0.151
Ferritin, median (IQR), ng/ml	543 (822)	411 (481)	666 (848)	<0.001
CRP, median (IQR), mg/dL	3.8 (7.9)	2.1 (4.5)	5.3 (9.7)	<0.001
D-Dimers, median (IQR), ng/mL	363 (314)	309 (310)	414 (395)	<0.001
LDH, median (IQR), UI/L	265 (153)	215 (90)	302 (181)	<0.001
Treatment Arm				
Arm A	142 (45.7%)	78 (63.9%)	64 (33.9%)	<0.001
Arm B	169 (54.3%)	44 (36.1%)	125 (66.1%)	

[a]Disease duration was determined by the date of first symptoms till the date of admission. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LDH, lactate dehydrogenase.

Table 2. Baseline characteristics according to treatment arm.

	Total (n=311)	**Arm A (n= 142)**	**Arm B (n=169)**	**P-value**
Age, median (IQR), years	63 (20)	64 (24)	62 (19)	0.410
Male sex, n (%)	195 (62.7%)	67 (47.2%)	128 (75.7%)	<0.001
BMI, median (IQR), kg/m^2	27.8 (5.8)	27.7 (5.9)	27.8 (5.7)	0.415
Diabetes, n (%)	63 (20.3%)	31 (21.8%)	32 (18.9%)	0.623
COPD, n (%)	26 (8.4%)	13 (9.2%)	13 (7.7%)	0.796
Cardiovascular Disease, n (%)	175 (56.3%)	80 (56.3%)	95 (56.2%)	1.000
Hypertension	173 (55.6%)	75 (52.8%)	98 (58%)	0.424
Coronary artery disease	25 (8%)	11 (7.7%)	14 (8.3%)	1.000
Smoking, n (%)				
previous/active	127 (40.8%)	56 (39.4%)	71 (42%)	0.731
Never	184 (59.2%)	86 (60.6%)	98 (58%)	
Disease duration, median (IQR), days	7 (5)	6 (6)	7 (5)	0.014
PO_2/FiO_2 ratio, median (IQR)	295 (106)	340 (123)	280 (97)	<0.001
Respiratory rate, median (IQR), /min	22 (8)	20 (8)	24 (9)	0.011
Lymphocytes, median (IQR), /μL	940 (600)	1000 (588)	900 (630)	0.051
Ferritin, median (IQR), ng/ml	543 (822)	235 (203)	912 (800)	<0.001
CRP, median (IQR), mg/dL	3.8 (7.9)	2.4 (4.6)	5.6 (10)	<0.001
D-Dimers, median (IQR), ng/mL	363 (314)	336 (322)	410 (311)	0.019
LDH, median (IQR), UI/L	265 (153)	230 (114)	295 (169)	<0.001
Disease severity				
Moderate	122 (39.2%)	78 (54.9%)	44 (26%)	<0.001
Severe	189 (60.8%)	64 (45.1%)	125 (74%)	

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LDH, lactate dehydrogenase.

Table 3. Outcome of patients according to disease severity at baseline or during hospitalization and treatment arm.

		Disease severity at baseline			Disease severity during hospitalization			Treatment arm		
	Total (n=311)	**Moderate (n=122)**	**Severe (n=189)**	**P-value**	**Moderate (n=101)**	**Severe (n=210)**	**P-value**	**Arm A (n=142)**	**Arm B (n=169)**	**P-value**
Intubation[a]	18 (5.8%)	0 (0%)	18 (9.5%)	<0.001	0 (0%)	18 (8.6%)	0.006	6 (4.2%)	12 (7.1%)	0.402
Mortality[b]	16 (5.1%)	0 (0%)	16 (8.4%)	0.002	0 (0%)	16 (7.6%)	0.01	7 (4.9%)	9 (5.3%)	1.000
O_2 supplementation	202 (65%)	21 (17.2%)	181 (95.8%)	<0.001	0 (0%)	202 (96.2%)	<0.001	72 (50.7%)	130 (76.9%)	<0.001
Days of O_2 supplementation, median (IQR)[c]	6 (6)	4 (5)	6 (6)	0.009	na	6 (6)	na	6 (5)	6 (7)	0.030
Days of Hospitalization, median (IQR)	7 (6)	5 (3)	9 (7)	<0.001	4 (4)	8 (6)	<0.001	5 (5)	8 (7)	<0.001

[a] Among 18 patients that were intubated: 12 patients died at ICU Department, 5 patients were discharged, and 1 patient was extubated and remains hospitalized at ICU. [b] Four out of 16 deceased patients died before intubation. [c] Days of O_2 supplementation have been calculated for patients that were discharged without intubation. [d] Days of hospitalization have been calculated for patients that were eventually discharged from the hospital. na, not applicable.

Table 4. Baseline factors associated with mechanical ventilation (n=18) in the total group of patients (n=307).

	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Age	1.049	1.009-1.088	0.015	1.034	0.988-1.082	0.154
Male sex	1.536	0.547-4.307	0.415			
BMI	1.043	0.971-1.120	0.247			
Diabetes	2.115	0.794-5.634	0.134			
COPD	0.637	0.085-4.788	0.661			
Cardiovascular disease	4.063	1.176-14.03	0.027			
Hypertension	4.174	1.208-14.42	0.024	2.431	0.661-8.943	0.181
Coronary	0.675	0.086-4.881	0.675			
Smoking (previous/active)	1.435	0.570-3.616	0.443			
Disease duration	0.973	0.855-1.107	0.679			
pO_2/FiO_2 ratio	0.989	0.984-0.994	<0.001	0.989	0.983-0.994	<0.001
Lymphocytes	0.999	0.998-1.000	0.226			
Ferritin	1.000	1.000-1.000	0.958			
CRP	1.022	0.966-1.081	0.455			
D-Dimers	1.000	0.999-1.001	0.777			
LDH	1.000	0.998-1.003	0.772			
Treatment Arm B vs. A	1.698	0.637-4.524	0.290			
Remdesivir	0.375	0.086-1.632	0.191			
Corticosteroids	1.352	0.524-3.488	0.533			

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LDH, lactate dehydrogenase. Analysis includes 18 events-intubations. Patients who died before intubation were excluded (n=4). Data from patients who were event-free at the end of follow-up were censored at 30 days.

Table 5. Baseline factors associated with mortality (n=16) in the total group of patients (n=311).

	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Age	1.074	1.026-1.124	0.002	1.075	1.024-1.128	0.003
Male sex	0.888	0.316-2.496	0.822			
BMI	1.028	0.945-1.118	0.518			
Diabetes	2.744	0.977-7.709	0.055			
COPD	0.788	0.104-5.995	0.788			
Cardiovascular disease	3.654	0.817-9.90	0.062			
Hypertension	3.908	0.842-10.36	0.060			
Coronary	0.812	0.197-6.173	0.840			
Smoking (previous/active)	0.518	0.165-1.628	0.260			
Disease duration	0.984	0.856-1.133	0.826			
pO_2/FiO_2 ratio	0.990	0.985-0.996	0.001	0.990	0.984-0.996	0.002
Lymphocytes	0.999	0.998-1.001	0.311			
Ferritin	1.000	1.000-1.000	0.652			
CRP	1.044	0.990-1.102	0.113			
D-Dimers	1.000	0.999-1.001	0.847			
LDH	1.001	0.999-1.003	0.392			
Treatment Arm B vs. A	1.273	0.453-3.576	0.647			
Remdesivir	1.106	0.352-3.472	1.106			
Corticosteroids	1.281	0.456-3.598	0.639			

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; LDH, lactate dehydrogenase. Analysis includes 16 events-deaths in the total group of patients (n=311). Data from patients who were event-free at the end of follow-up were censored at 30 days.

Table 6. Adverse events in the treated patients.

		Treatment Arm			Disease severity during hospitalization		
	Total (n=311)	**Arm A (n=142)**	**Arm B (n=169)**	**P-value**	**Moderate (n=101)**	**Severe (n=210)**	**P-value**
Neutropenia (<1000/µL)	36 (11.6%)	11 (7.7%)	25 (14.8%)	0.08	6 (5.9%)	30 (14.3%)	0.049
Injection site reaction	8 (2.6%)	4 (2.8%)	4 (2.4%)	1.00	2 (2%)	6 (2.9%)	1
Serious infection (grade≥3)	7 (2.3%)	2 (1.4%)	5 (3%)	0.46	0 (0%)	7 (3.3%)	0.101
Central line blood stream infection	4* (1.3%)	1 (0.7%)	3 (1.8%)	0.63	0 (0%)	4 (1.9%)	0.31
Secondary bacterial pneumonia	2 (0.6%)	0 (0%)	2 (1.2%)	0.50	0 (0%)	2 (1%)	1.00
Subcutaneous hematoma	1 (0.3%)	1 (0.7%)	0 (0%)	0.46	0 (0%)	1 (0.5%)	1.00
Pulmonary embolism	10 (3.2%)	1 (0.7%)	9 (5.3%)	0.024	0 (0%)	10 (4.8%)	0.034
Mesenteric vein thrombosis	1 (0.3%)	1 (0.7%)	0 (0%)	0.46	0 (0%)	1 (0.5%)	1.000
ALT > 5x UNL (200 UI/L)	24 (7.7%)	1 (0.5%)	23 (13.6%)	<0.001	1 (0.7%)	23 (13.6%)	<0.001
Severe thrombocytopenia (<50000/µL)	5 (1.6%)	3 (2.1%)	2 (1.2%)	0.66	2 (2%)	3 (1.4%)	0.66

* One additional patient suffered from primary bacteremia. ALT, alanine aminotransferase.