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ORIGINAL ARTICLE

The intercorrelations between blood levels of ferritin, sCD163, and IL-18 in COVID-19 patients and their association to prognosis

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Abstract

Coronavirus disease 2019 (COVID-19) is associated with immune dysregulation, severe respiratory failure, and multiple organ dysfunction caused by a cytokine storm involving high blood levels of ferritin and IL-18. Furthermore, there is a resemblance between COVID-19 and macrophage activation syndrome (MAS) characterized by high concentrations of soluble CD163 (sCD163) receptor and IL-18. High levels of ferritin, IL-18, and sCD163 receptor are associated with "hyperferritinemic syndrome", a family of diseases that appears to include COVID-19. In this retrospective cohort study, we tested the association and intercorrelations in the serum levels of ferritin, sCD163, and IL-18 and their impact on the prognosis of COVID-19. We analyzed data of 70 hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The levels of sCD163, ferritin, and IL-18 were measured and the correlation of these parameters with the respiratory deterioration and overall 30-day survival was assessed. Among the 70 patients, 60 survived 30 days from hospitalization. There were substantial differences between the subjects who were alive following 30 days compared to those who expired. The differences were referring to lymphocyte and leukocyte count, CRP, D-dimer, ferritin, sCD163, and IL-18. Results showed high levels of IL-18 (median, 444 pg/mL in the survival group compared with 916 pg/mL in the mortality group, p-value 8.54×10–2), a statistically significant rise in levels of ferritin (median, 484 ng/mL in the survival group compared with 1004 ng/mL in the mortality group p-value, 7.94×10–3), and an elevated value of in sCD163 (mean, 559 ng/mL in the survival group compared with 840 ng/mL in the mortality group, p-value 1.68×10–2). There was no significant correlation between the rise of ferritin and the levels sCD163 or IL-18. Taken together, sCD163, ferritin, and IL-18 were found to correlate with the severity of COVID-19 infection. Although these markers are associated with COVID-19 and might contribute to the cytokine storm, no intercorrelation was found among them. It cannot be excluded though that the results depend on the timing of sampling, assuming that they play distinct roles in different stages of the disease course. The data represented herein may provide clinical benefit in improving our understanding of the pathological course of the disease. Furthermore, measuring these biomarkers during the disease progression may help target them at the right time and refine the decision-making regarding the requirement for hospitalization.

Keywords Hyperferritinemic syndrome · Ferritin · Cytokine storm · sCD163 · IL-18 · Macrophage activation syndrome · COVID-19 · SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the virus known as SARS-CoV-2 and has been declared a worldwide pandemic by the World Health Organization in February 2020. Until now, the infection was confirmed in more than 617 million cases, with a mortality record of over 6.53 million deaths [1, 2]. Hence, there is an ongoing need for better understanding and identifying its pathogenesis in parallel to the attempts in developing novel therapeutics. SARS-CoV-2 infects the respiratory tract resulting in pneumonia and acute respiratory distress syndrome (ARDS) in about 15% of the cases [3]. Patients with a severe course often show high concentrations of ferritin in their blood which correlate with the severity of their disease [3, 4]. Furthermore, a growing body of clinical data suggests that

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the severity, prognosis, and mortality in COVID-19 patients may be linked to the presence of the so-called "cytokine storm" induced by the virus [5–10]. Cytokine storm is an umbrella term encompassing several disorders of immune dysregulation and was recently redefined on the basis of the following criteria: elevated circulating cytokine levels, acute systemic inflammatory symptoms, and secondary organ dysfunction beyond that which could be attributed to a normal response to a pathogen, if a pathogen is present [7]. Patients, who had been shown to have laboratory findings compatible with a cytokine storm, also experienced deleterious clinical manifestations and higher mortality rates [6]. Furthermore, COVID-19 patients, who required mechanical ventilation, developed consequent lung injury that is also associated with the release of the inflammatory factors, apoptosis, endothelial dysfunction, and activation of the coagulation system [11]. CT tests of COVID-19 lung involvement showed a certain degree of similarity to findings seen in MAS. Both diseases manifested similarity in ground-glass opacities (GGOs) extension and pattern of parenchymal consolidation, except for apical consolidations which were more frequent in COVID-19 than MAS [12].

Interestingly, the cytokine storm observed in COVID-19 patients is similar to that presented in other hyperferritinemic conditions [13]. In addition to ferritin, additional cytokines classically seen in hyperferritinemic syndrome are also associated with COVID-19, e.g., IL-6 and IL-18 [9, 10, 14–16]. Among the markers classically seen in hyperferritinemic syndrome, we focused on two main candidates that possibly may link to the hyperferritinemic syndrome and COVID-19, IL-18 and sCD163 receptor, and we investigated their correlation with the severity of the disease.

IL-18 was first identified as interferon gamma inducing factor that enhances inflammation [17]. It belongs to the IL-1 family [18] and had been associated with various cytokine disorders [7]. This cytokine has effector and regulatory roles in a variety of early inflammatory responses. IL-18 level in the blood is strongly associated with septicemia and ARDS risk and often predicts mortality [19]. Furthermore, recent studies show that IL-18 rises not only in hyperferritinemic hyperinflammatory conditions such as macrophage activating syndrome (MAS), septicemia, and ARDS but also in COVID-19 along with IL-6 [9, 10, 20].

CD163 is a member of the scavenger receptor cysteine-rich (SRCR) superfamily, and is exclusively expressed in monocytes and macrophages. It functions as an acute phase-regulated receptor involved in the clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages [16]. CD163 is commonly denoted as soluble-CD163 (sCD163), which is generated by ectodomain shedding of the membrane bound receptor, as a result of enzymatic cleavage by ADAM17/TACE metalloproteinase [21, 22]. sCD163 is released into the sera during macrophage activation, therefore considered a marker of macrophage activation [16]. Over-expression of sCD163 has been observed in several inflammatory diseases such as MAS and the adult-onset Still's disease [16]. Over the course of MAS, the sCD163 was already positively correlated with ferritin serum levels, suggesting a possible pathogenic relationship between these molecules [16].

Table 1 Clinical evaluations, complete blood count, coagulation factors, and liver and kidney function factors of the patients taken from admission. Values are depicted for two groups — patients who survived 30 days from hospitalization (survival group) and patients who did not (mortality group)

Characteristic	30-day mortality, $N=10$ [1]	30-day survival, $N=60$ [1]	p-Value [2]
Temperature (°C)	37.2 (36.8, 37.6)	37.2 (36.8, 37.7)	0.906
Systolic pressure (mmHg)	117 (106, 122)	124 (111, 139)	0.102
Diastolic pressure (mmHg)	73 (66, 79)	73 (67, 80)	0.65
Respiratory rate (B/m)	24 (16, 30)	18 (16, 22)	0.419
Saturation (%)	93 (92, 95)	95 (92, 97)	0.445
RBC (cells/mcL)	5 (4, 5)	5 (4, 5)	0.311
PLT (K/mL)	244 (183, 300)	186 (132, 251)	0.156
WBC (K/μL)	9.9 (8.1, 13.3)	6.8 (5.1, 9.6)	0.0285
Lymphocytes (K/μL)	57 (34, 75)	140 (84, 210)	0.00451
CRP (mg/L)	215 (134, 292)	87 (46, 136)	0.00081
Ferritin (ng/mL)	767 (590, 1,058)	418 (225, 805)	0.0834
D-dimer (ng/mL)	1,333 (827, 4,752)	613 (504, 1,041)	0.0208
INR	1.2 (1.1, 1.2)	1.1 (1.0, 1.2)	0.198
ALT (IU/I)	26 (18, 56)	28 (21, 42)	0.945
Creatinin (mg/dL)	1.2 (0.9, 1.4)	0.8 (0.7, 1.1)	0.0155

[1] Statistics presented: median (IQR) [2].Statistical tests performed: Wilcoxon rank-sum test. Clinical and laboratory values of the 70 COVID-19 patients elicited from blood samples taken at admission. Higher levels of WBC, CRP, D-dimer, and creatinine were significantly associated with 30-day mortality. Lower levels of lymphocytes were significantly associated with 30-day mortality

Table 2 Peak levels of clinical evaluations, complete blood count, coagulation factors, and liver and kidney function factors of the patients throughout hospitalization. Values are depicted for two groups — patients who survived 30 days from hospitalization (survival group) and patients who did not (mortality group)

Characteristic	30-day mortality, $N=10$ [1]	30-day survival, $N=60$ [1]	p-Value [2]
Temperature (°C)	37.9 (37.7, 38.5)	38.0 (37.5, 38.9)	0.86
Systolic pressure (mmHg)	122 (82, 161)	136 (98, 156)	0.551
Diastolic pressure (mmHg)	67 (48, 78)	71 (60, 81)	0.302
Respiratory rate (B/m)	34 (26, 36)	25 (23, 30)	0.0675
Saturation (%)	78 (70, 84)	89 (84, 93)	0.0135
RBC (cells/mcL)	3 (3, 3)	4 (3, 5)	0.298
PLT (K/mL)	131 (71, 409)	173 (108, 456)	0.469
WBC (K/μL)	25.4 (13.7, 33.9)	12.3 (7.2, 16.3)	0.00591
Lymphocytes (K/μL)	16 (14, 22)	75 (36, 134)	0.000188
CRP (mg/L)	315 (192, 352)	186 (109, 303)	0.0358
Ferritin (ng/mL)	1,560 (1,071, 4,521)	860 (395, 1,512)	0.00719
D-dimer (ng/mL)	6,991 (4,396, 54,524)	2,076 (679, 8,285)	0.0192
INR	1.3 (1.2, 1.3)	1.2 (1.2, 1.4)	0.395
ALT (IU/I)	78 (48, 165)	64 (39, 105)	0.421
Creatinin (mg/dL)	4.0 (1.8, 4.7)	1.0 (0.7, 1.3)	0.000106

[1] Statistics presented: median (IQR) [2].Statistical tests performed: Wilcoxon rank-sum test. Clinical and laboratory peak levels values of the 70 COVID-19 patients throughout hospitalization time, in patients who survived 30 days from admission to patients who did not. Values are presented by median and interquartile range. Higher respiratory rate, WBC, CRP, ferritin, D-dimer, and creatinine were significantly associated with 30-day mortality. Lower saturation and lymphocytes were significantly associated with 30-day mortality

Table 3 sCD163, IL-18, and ferritin levels from blood samples taken on the same day short after admission

Characteristic	30-day mortality, $N=10$ [1]	30-day survival, $N=60$ [1]	p-Value [2]
Ferritin (ng/mL)	1,004 (851, 1,405)	484 (235, 1,023)	0.00794
IL-18 (pg/mL)	916 (431, 1,104)	444 (297, 714)	0.0854
sCD163 (ng/mL)	840 (673, 1,227)	559 (388, 739)	0.0168

[1]Statistics presented: median (IQR); $n(\%)$[2].Statistical tests performed: Wilcoxon rank-sum test; chi-square test of independence. Levels of ferritin, sCD163, and IL-18 of the 70 COVID-19 patients from blood samples taken at the same day near admission (5.1 days after in average), in patients who survived 30 days from admission to patients who did not. Values are presented by median and interquartile range. Higher levels of ferritin and CD163 were significantly associated with 30-day mortality

We hypothesize that COVID-19 has a common pathophysiological mechanism with the hyperferritinemic syndrome in general; therefore, we tested the levels of these three markers — ferritin, IL-18, and sCD163, and calculated the correlations among them.

Methods

This research is a retrospective cohort study based on 70 hospitalized patients verified for COVID-19 by Sheba Medical Center. The research has an IRB approval (number 7132–20-SMC) granted from the Sheba Medical Center. All 70 patients admissions had taken place between 8th of March and the 28th of April 2020.

Clinical evaluations and laboratory values of blood samples throughout their hospitalization were taken from the hospital records. The clinical assessment included fever, blood pressure, necessity of invasive ventilation, saturation, and need of ventilation support. Laboratory values included levels of ferritin, RBCs, leukocytes, lymphocytes, platelets, CRP, D-dimer, INR, ALT, creatinine, TSH, IL-1β, IL-6, IL-8, and TNFα. IL-18 plasma levels were measured with the DL180 Human Total IL-18/IL-1F4 Quantikine ELISA Kit according to the manufacturer instructions.

The levels of sCD-163 and IL-18 were measured from blood tests a few days following admission (5.1 days in average), by next-generation enzyme-linked immunosorbent assay (ELISA) (Simple PlexTM Ella microfluidic platform, Protein Simple, CA, USA). Briefly, 25 μL diluted serum samples was added to a microfluidic cartridge, automatically separated into triplicates, and coated with sCD-163 or IL-18 specific capture monoclonal antibodies. Detection of the antibodies and streptavidin-DyLight650 conjugates, as well as all washing steps,

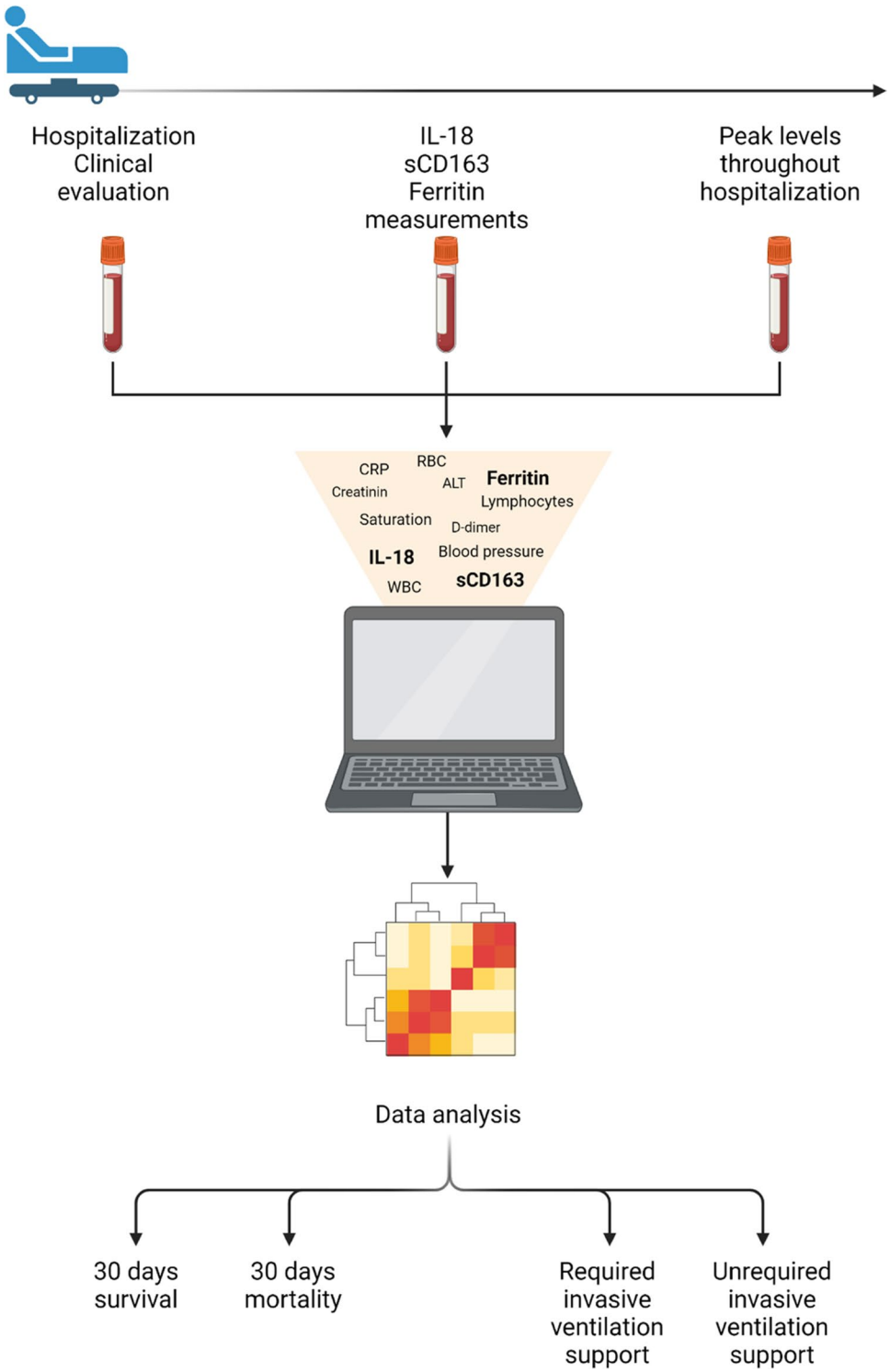

Fig. 1 General scheme of the study

was automatically performed. Raw data were analyzed using the SimplePlex Explorer software. All values other than sCD163 and IL-18 were taken twice — from time of admission of peak levels throughout hospitalization. In addition, a third value of ferritin was taken from the same day in which blood samples were taken to assess the levels of sCD163 and IL-18.

Population study The study cohort included 70 hospitalized patients which were randomly selected in the internal medicine division converted to COVID-19 department in Sheba

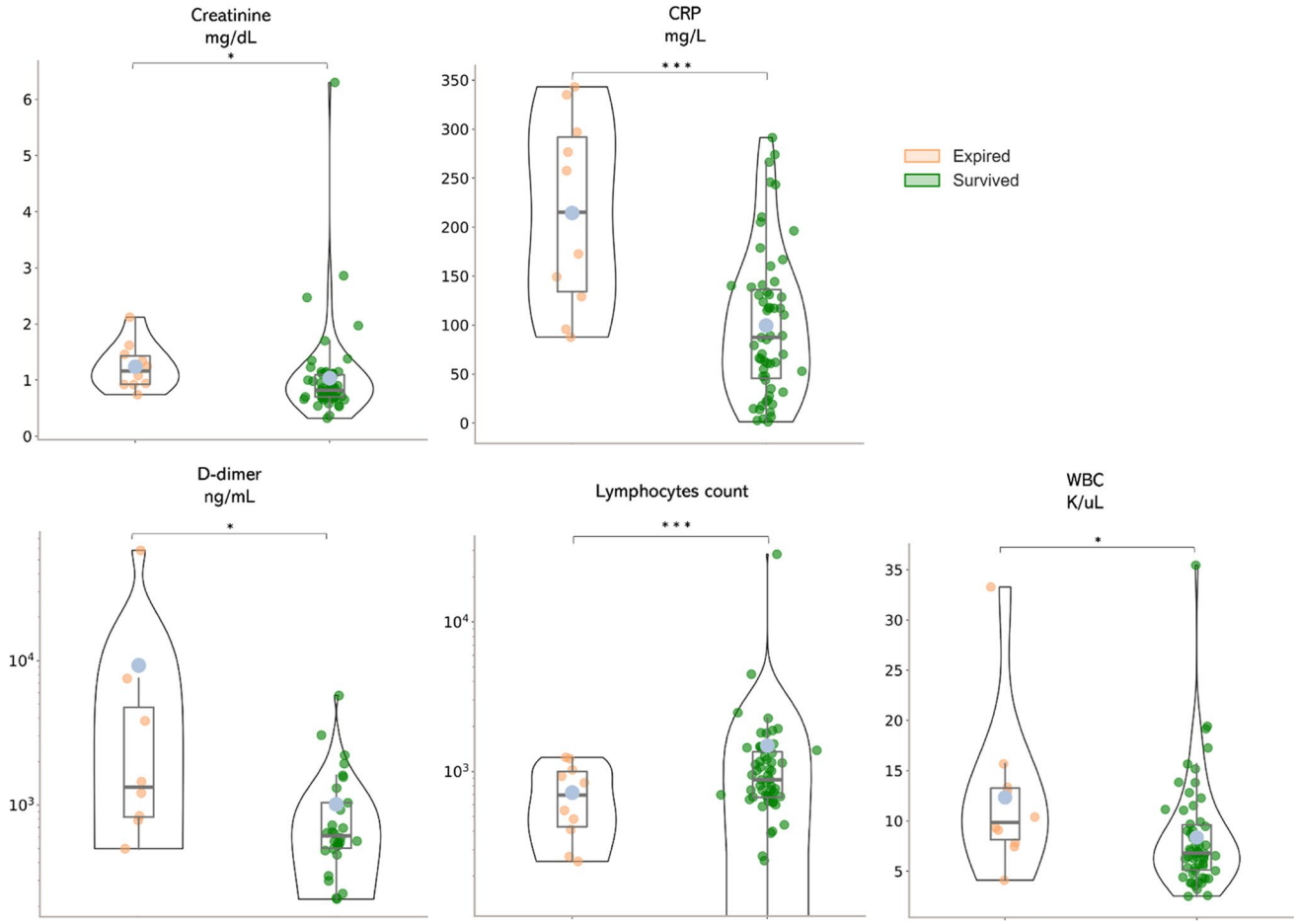

Fig. 2 CRP, D-dimer, creatinine, lymphocytes count, and white blood cells count of the patients taken from admission. Laboratory values of the 70 COVID-19 patients elicited from blood samples taken at admission depicted via violin plots. Green dots represent the patients who survived 30 days from admission, and the orange dots represent the patients who did not. The middle horizontal line in each box plot represents the median value of the group (either survival or mortality) and the light blue circle represents the group average. Patients who admitted with lower levels of CRP or lower levels of D-dimer had a better survival rate. To assess the increase/decrease in each marker from its levels in the survival and mortality groups, unpaired two-tailed *t*-test was performed between each two groups. Statistical significance: **p* value < 0.05, ***p* value < 0.005, ****p* value < 0.0005, otherwise non-significant

Medical Center. Inclusion criteria were positive RT-PCR test for SARS-CoV-2 and the need for hospitalization (as both are admission criteria into Sheba COVID-19 division). Among those patients, there were no exclusion criteria.

The cohort of 70 patients was classified into two groups, those who survived 30 days from the hospitalization (60 patients) and those who did not (10 patients). Follow-up time was during 2 months from hospitalization.

In the further part of the study, we categorized the cohort study population into two groups: "severe group" as patients who needed invasive ventilation support during hospitalization and "mild group" which included the rest of the patients.

Categorical variables were presented as n (%) and compared using chi-square test of independence; continuous variables were presented as median (IQR) and compared using Wilcoxon rank-sum test. Correlations were assessed using the Spearman's method. Logistic regression models were used to assess the odds ratio between predictors and the outcome. *p*-Value < 0.05 was considered statistically significant.

Results

The two groups of patients were evaluated twice for their clinical parameters, once at admission (Table 1) and once during the hospitalization (Table 2). In addition, levels of IL-18 and sCD163 were measured short after admission (with average of 5.14 days after) (Table 3). Ferritin levels were measured 3 times, at admission (Table 1), peak levels during hospitalization (Table 2), and short after admission

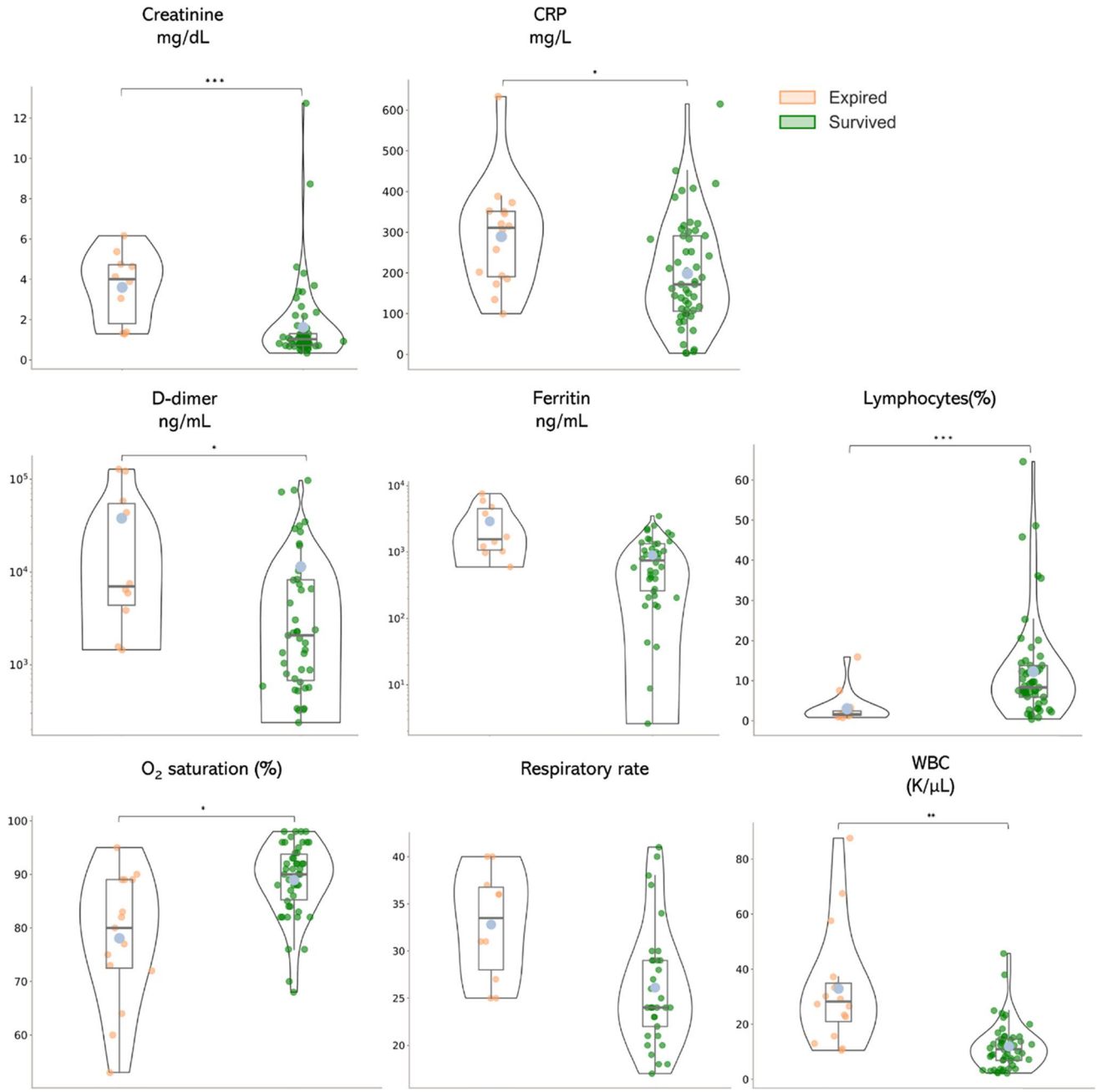

Fig. 3 Peak levels of creatinine, CRP, D-dimer, ferritin, lymphocytes, respiratory rate, saturation, and white blood cells count of the patients throughout hospitalization. Clinical and laboratory peak levels values of the 70 COVID-19 patients throughout hospitalization time, depicted via violin plots. Green dots represent the patients who survived 30 days from admission, and the orange dots represent the patients who did not. The middle horizontal line in each box plot represents the median and the light blue circle represents the average of each group (either survival or mortality). Patients who reached lower levels of lymphocytes or high levels of ferritin had considerable worse outcome. To assess the increase / decrease in each marker from its levels in the survival and mortality groups, unpaired two-tailed t-test was performed between each two groups. Statistical significance: *=*P* value <0.05, **=*P* value <0.005, ***= *P* value <0.0005, otherwise non-significant

along with IL-18 and sCD163 for intercorrelation analysis purposes (Table 3).

At first, we measured and compared laboratory values which are commonly taken in practice (Fig. 1). While comparing values taken at admission, there was a substantial difference in the lymphocytes count (median, 59 K/μL in the mortality group compared with 140 K/μL in the survival group), which is typical to viral infections and

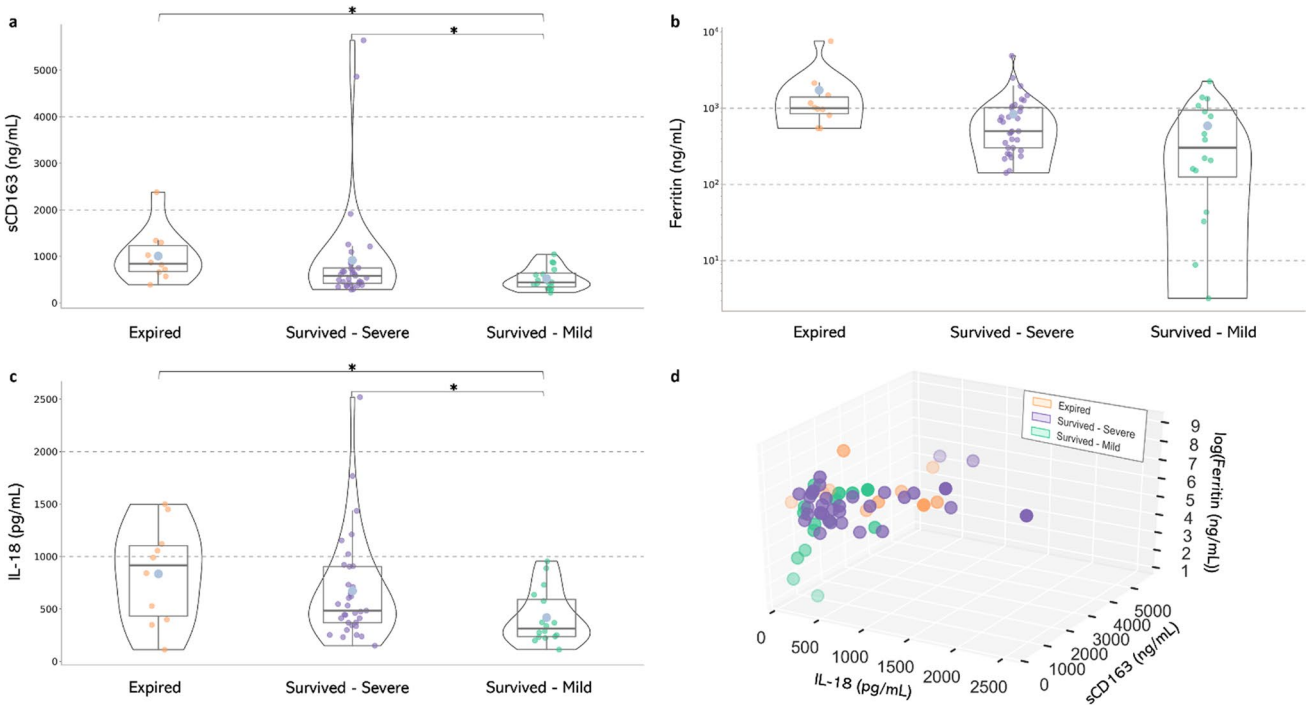

Fig. 4 Levels of (**a**) sCD163, (**b**) ferritin and (**c**) IL-18 of the 70 COVID-19 patients from blood samples taken at the same day close to admission (5.1 days after in average), depicted via violin and box plots. Light green dots represent the mild patients, purple dots represent the severe patients, and the orange dots represent the patients who did not survive 30 days from admission. High levels of IL-18 and sCD163 show significant association with worse outcome, while the same trend was observed in ferritin. (**d**) 3D scatterplot representation of the intercorrelation of the three markers in the three patients' groups. To assess the increase / decrease in each marker from its levels in the survival and mortality groups, unpaired two-tailed t-test was performed between each two groups. Statistical significance: *=*P* value <0.05, otherwise nonsignificant

particularly in COVID-19 (Table 1). In addition, there were differences in the CRP (median, 215 mg/L in the mortality group compared with 87 mg/L in the survival group), leukocytes (median, 9.9 K/μL in the mortality group compared with 6.8 K/μL in the survival group), and D-dimer (median, 1333 ng/mL in the mortality group compared with 613 ng/mL in the survival group) which were all higher in the mortality group (Table 1). Notably, all the patients who admitted with CRP levels lower than 90 mg/L and D-dimer level lower than 600 ng/mL survived (Fig. 2, depicted in violin plots [23]). Additionally, we examined the correlation between distinct markers found to be associated with the prognosis of COVID-19 (Figs. 3, 4, 5, and 6): high CRP and low lymphocytes appear to be risk markers for 30 days mortality.

Besides values taken at admission, we collected clinical and laboratory peak level records throughout hospitalization and compared the records of the mortality group with the survival group (Table 2). Similarly to values at admission (Table 1), lower lymphocytes count (median, 16 K/μL in the mortality group compared with 75 K/μL in the survival group), and higher levels of WBC (median, 25.4 K/μL in the mortality group compared with 12.3 K/μL in the survival group), CRP (median, 315 mg/L in the mortality group compared with 186 mg/L in the survival group) and creatinine (median, 4 mg/dL in the mortality group compared with 1 mg/dL in the survival group), were significantly associated with 30-day mortality. Furthermore, all patients with lymphocytes count higher than 40 K/μL throughout hospitalization survived (Fig. 3). Another considerable finding is that patients who reached higher levels of ferritin had worse survival outcome (Fig. 3). High ferritin levels showed a statistical significance in the mortality group with a median of 1569 ng/mL compared with 860 ng/mL in the survival group, which strengthens the association between COVID-19 and hyperferritinemic syndrome (Table 2). In the analysis of ferritin levels with peak lymphocytes count, 3 out of 4 quarters survived, showing that 100% of the patients with ferritin levels lower than 500 ng/ml, and/or lymphocytes higher than 40 K/μL survived. Furthermore, 10 out of 19 patients with lowest lymphocytes value below 40 K/μL and highest ferritin above 500 ng/ml died within 30 days (53%) (Fig. 6a).

Alongside the clinical and laboratory assessment of marker commonly taken in practice, we collected a blood sample of each patient obtained short after admission

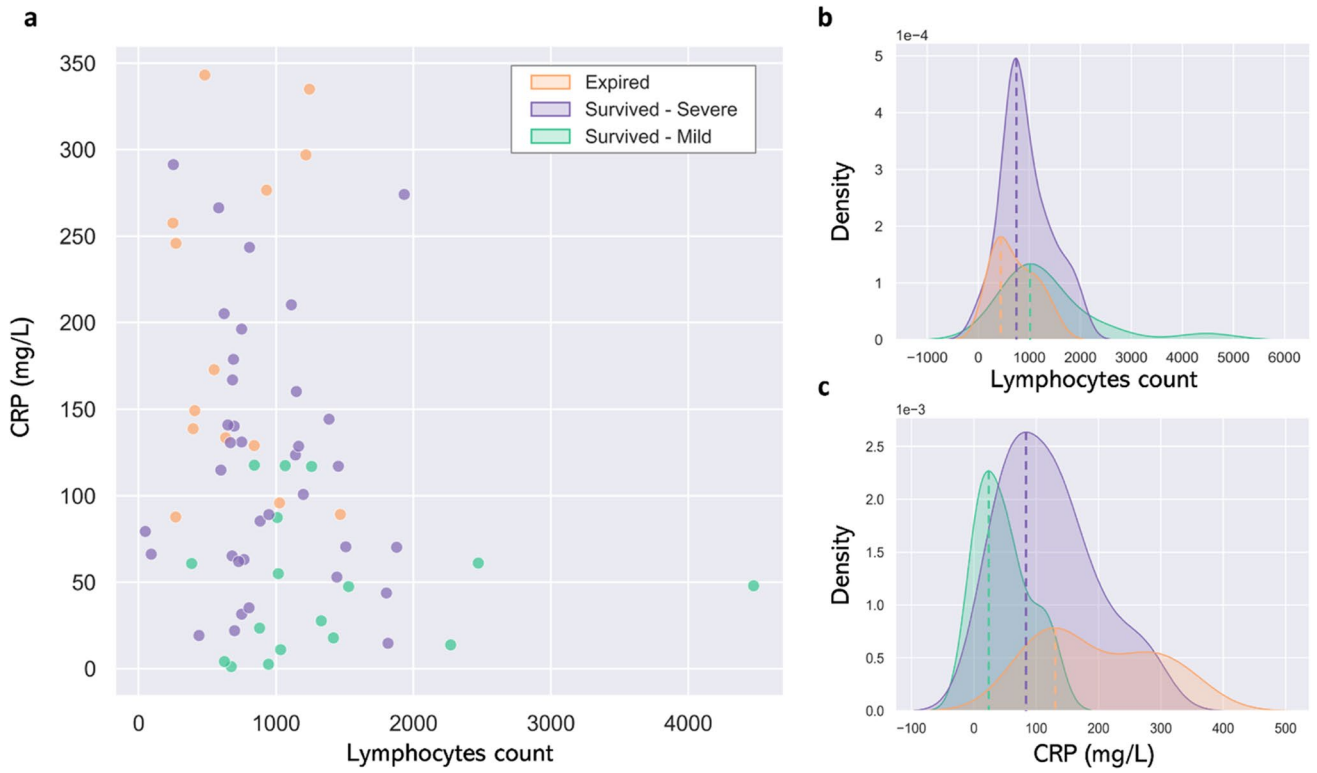

Fig. 5 CRP and lymphocytes blood test values of the patients taken from admission. (**a**) Values of CRP compared with lymphocytes count in the 70 COVID-19 patients from blood samples taken at admission. Light-green dots represent the mild patients, purple dots represent the severe patients, and the orange dots represent the patients who did not survive 30 days from admission. High levels of CRP and low lymphocytes are risk-markers for 30-day mortality. (**b**) Density plot of the lymphocytes count for each group. (**c**) Density plot of CRP for each group. The dashed lines represent the peak of each density curve

(5.1 days in average). The samples collected were tested for levels of sCD163, IL-18, and ferritin (Table 3). As expected from the previous studies, IL-18 levels were higher in the mortality group (median, 916 pg/ml compared with 444 pg/ml in the survival group), but interestingly, the differences in ferritin levels and sCD163 levels were even more significant in the mortality group (median, 1004 ng/ml compared with 484 ng/ml in the survival group, and 840 ng/ml compared with 559 ng/ml in the survival group, respectively), indicating a correlation between COVID-19, hyperferritinemic syndrome in general, and macrophage activation syndrome in particular (Table 3, Fig. 4).

In the last part of our research, we compared the usage of invasive ventilation or extracorporeal membrane oxygenation (ECMO) between the mortality and the survival groups. Expectedly, invasive ventilation and ECMO were significantly associated with 30-day mortality (Table 4). Assessing for total mortality further than 30 days mortality, 13% of the patients in the 30-day survival group died during the post 30 days 2 months of follow-up. Afterwards, we classified the patients into two other groups — patients who needed invasive ventilation or ECMO (severe group, $n=27$) and patients who discharged without it (mild group, $n=43$). For both groups, we assessed the aforementioned clinical and laboratory markers (Table 5). High levels of CRP, D-dimer, lymphopenia, sCD163, and IL-18 levels were associated with invasive ventilation support, with median value differences of 709 pg/ml in the severe group compared to 393 pg/ml in the mild group referring to IL-18, and 716 ng/ml in the severe group compared to 453 ng/ml in the mild group regarding sCD163 (Table 5).

Discussion

Our study focuses on COVID-19, hyperferrtinemia, and a cytokine storm which is common to both conditions and might share a common pathophysiology. Cytokine storm appears in hyperferritinemic syndromes, such as MAS, adults onset Still's disease (AOSD), catastrophic antiphospholipid syndrome, and septic shock [24]. These disorders are characterized by high levels of serum ferritin and high levels of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-18, TNFα, and sCD163 receptor as part of the aforementioned cytokine storm [7–10, 24, 25]. Ferritin appears to be a key marker of and a pathogenic player in inflammatory

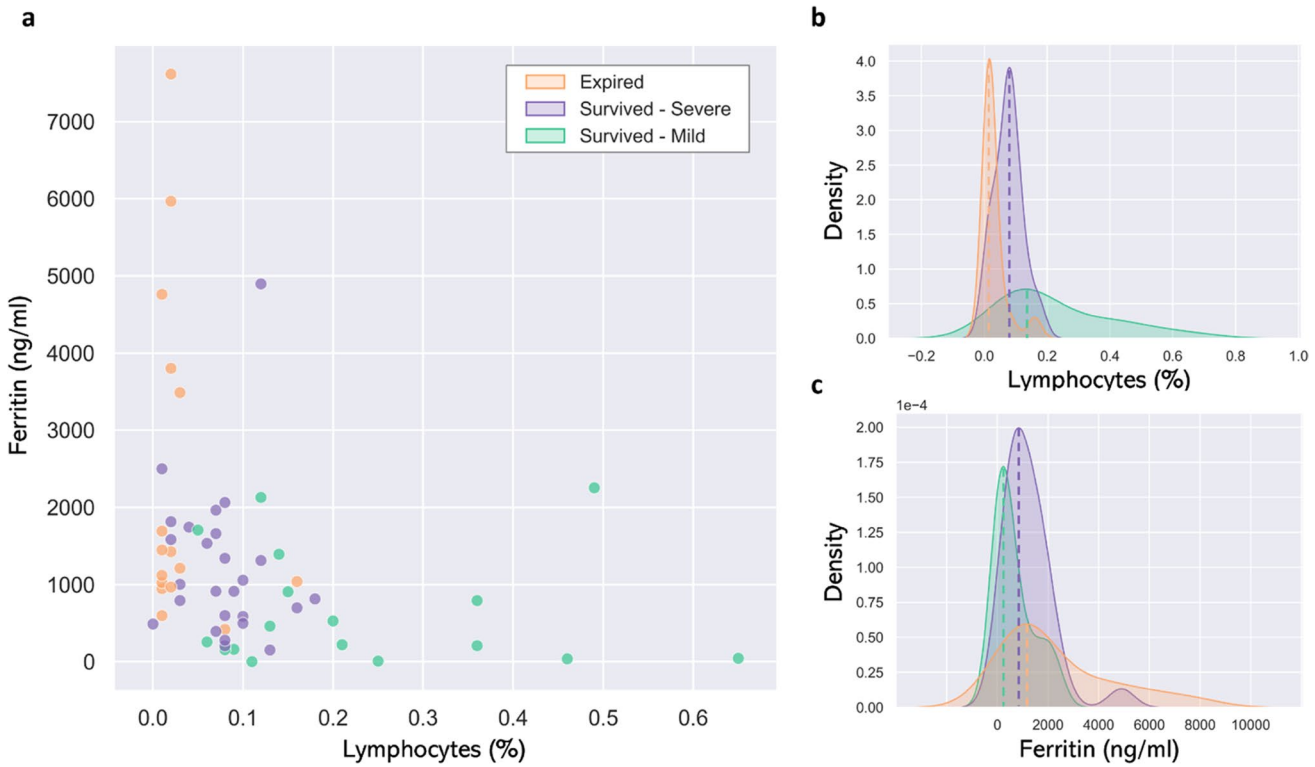

Fig. 6 Peak levels of ferritin and lymphocytes of the patients throughout hospitalization. (**a**) Peak levels of ferritin compared with peak lymphocytes (%) in the 70 COVID-19 patients throughout their hospitalization time. Light-green dots represent the mild patients, purple dots represent the severe patients, and the orange dots represent the patients who did not survive 30 days from admission. 10 out of 14 patients with lowest lymphocytes value below 40 k/μL and ferritin above 500 ng/mL died within 30 days (71%). No patient with lowest lymphocyte value above 40 k/μL or with ferritin below 500 ng/mL died. (**b**) Density plot of the lymphocytes (%) for each group. (**c**) Density plot of ferritin for each group. The dashed lines represent the peak of each density curve

processes through signaling pathways involving innate immune response and modulation of lymphocyte function [26]. According to the previous studies, ferritin can act as a pro-inflammatory but also as an anti-inflammatory molecule. Regarding the question how ferritin signals in those two opposite pathways, the consensus is that it depends on which subunit of the molecule is activated, namely, the ferritin H subunit or L subunit [13, 24, 27]. Ferritin H subunit (FeH) has shown an iron-independent stimulatory effect on NF-κB, leading to the production of pro-inflammatory molecules [13]. On the other hand, the ferritin L subunit (FeL) has shown an opposite anti-inflammatory effect by inhibiting NF-κB [13]. Interestingly, the pro-inflammatory cytokines which are elevated in hyperferritinemic syndrome have also been described in severe COVID-19 [13] and may preferentially induce the expression of FeH via FER2, a regulatory element acting as a binding site to NF-κB. The latter, in turn, stimulates the synthesis of further FeH and pro-inflammatory cytokines, thus perpetuating a vicious inflammatory loop [13]. Accordingly, in our study, there was a strong association between ferritin and COVID-19 along the progression of the disease, with a worse outcome and higher mortality rate. Therefore, we suggest that in COVID-19 patients, there is a cytokine storm involving the same FeH inflammatory loop seen in hyperferritinemic syndrome, manifested in the disease progression and prognosis.

IL-18 is a pro-inflammatory cytokine expressed at sites of chronic inflammation, in autoimmune diseases, a variety of cancers, and in numerous infectious diseases [18, 28]. Like IL-1β, IL-18 is synthesized as an inactive precursor missing a signal peptide and the caspase-1-mediated cleavage is needed for its activation [29, 30]. The transcription of IL-18 precursor is further increased during infection after toll-like receptor (TLR) binding of PAMPs. As a result, an activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway occurs [29]. IL-18 synergizes with interleukin-12 (IL-12) or interleukin-15 (IL-15) to stimulate secretion of interferon-γ from T cells and NK cells, and thus promotes Th1-type inflammatory responses [7]. IL-18 is regulated by the naturally occurring and very potent antagonist, the IL-18 binding protein (IL-18BP) [17]. A drug which blocks IL-18 activity, named Tadekinig alfa (a recombinant IL-18BP), is currently in clinical studies targeting IL-18 in various conditions of hyperinflammation

Table 4 Usage percentage of different kinds of ventilation support throughout hospitalization. Values are depicted for two groups — patients who survived 30 days from hospitalization (survival group) and patients who did not (mortality group)

Characteristic	30-day mortality, $N=10$ [1]	30-day survival, $N=60$ [1]	p-Value [2]
Ventilatory support			0.0185
ECMO	1 (10%)	3 (5.0%)	
Invasive	8 (80%)	15 (25%)	
Nasal cannula	1 (10%)	16 (27%)	
Non rebreather	0 (0%)	8 (13%)	
None	0 (0%)	17 (28%)	
Vapotherm	0 (0%)	1 (1.7%)	
Ventilatory support grading			0.00112
Severe	9 (90%)	18 (30%)	
Mild	1 (10%)	42 (70%)	
Mortality total	10 (100%)	8 (13%)	<0.00005

[1] Statistics presented: median (IQR); *n*(%) [2].Statistical tests performed: Wilcoxon rank-sum test; chi-square test of independence. Comparison of invasive ventilation support usage throughout hospitalization. Invasive ventilation and ECMO were significantly associated with 30-day mortality

such as MAS associated with AOSD and MAS resulting from a mutation in the inflammasome [31, 32]. In our study, IL-18 has been shown to correlate with higher mortality rate in COVID-19 patients (Fig, 4), together with ferritin and sCD163. Therefore, rise in IL-18 levels, which is known to occur due to NF-κB activation, might be a product of the aforementioned FeH-NF-κB inflammatory loop and might be a factor in the disease progression as well. Thus, Tadekinig alfa might serve as a potential therapeutic candidate also for COVID-19.

sCD163 receptor is upregulated in numerous inflammatory diseases, including MAS, RA, lupus nephritis, sepsis and also in cirrhosis, type 2 diabetes, Gaucher's disease, HIV infection, and Hodgkin lymphoma [32–34]. Furthermore, serum sCD163 levels profoundly increase with the progress of MAS and correlate positively with the disease activity of systemic juvenile idiopathic arthritis (s-JIA), even in patients receiving immunosuppressive drugs such as tocilizumab [33]. During MAS, levels of sCD163 seem to be strongly related to the degree of macrophage activation [16].

sCD163 is also known to be related to ferritin via ferritin H subunit which was found to be an activator of macrophages and as part of this activation levels of sCD163 increase [34].

Table 5 Invasive ventilation as outcome among hospitalized patients clinical evaluations, complete blood count, coagulation factors, and liver and kidney function factors of the patients taken from admission. Values are depicted for two groups — patients who required invasive ventilation support (severe group) and patients who did not (mild group)

Characteristic	Severe, $N=27$ [1]	Mild, $N=43$ [1]	p-Value [2]
Temperature (°C) admission	37.2 (37.0, 37.8)	37.1 (36.8, 37.5)	0.411
Systolic pressure (mmHg) admission	123 (112, 136)	123 (110, 140)	0.612
Diastolic pressure (mmHg) admission	73 (66, 80)	72 (69, 80)	0.633
Respiratory rate admission (B/m)	17 (16, 22)	20 (17, 22)	0.365
Saturation (%) admission	94 (92, 95)	95 (90, 97)	0.323
RBC admission (cells/mcL)	5 (4, 5)	5 (4, 5)	0.282
PLT admission (K/mL)	213 (129, 272)	187 (142, 256)	0.684
WBC admission (K/μL)	9.1 (7.0, 13.8)	6.5 (5.0, 8.7)	0.00847
Lymphocytes admission (K/μL)	71 (53, 123)	156 (102, 214)	0.00344
CRP admission (mg/L)	132 (88, 255)	70 (40, 130)	0.00399
Ferritin admission (ng/mL)	657 (319, 886)	418 (225, 757)	0.211
D-dimer admission (ng/mL)	1,452 (739, 2,882)	563 (490, 1,040)	0.0105
INR admission	1.1 (1.0, 1.2)	1.1 (1.1, 1.2)	1.00
ALT admission (IU/I)	31 (21, 52)	27 (20, 40)	0.255
Cr admission (mg/dL)	1.0 (0.8, 1.3)	0.8 (0.7, 1.1)	0.0428
IL-18 (pg/mL)	709 (430, 1,039)	393 (252, 598)	0.00426
CD163 (ng/mL)	716 (647, 1,021)	453 (371, 665)	0.000415
mortality_30d			0.00112
30-day mortality	9 (33%)	1 (2.3%)	
30-day survival	18 (67%)	42 (98%)	

[1] Statistics presented: median (IQR); *n*(%) [2].Statistical tests performed: Wilcoxon rank-sum test; chi-square test of independence. Comparison of patients who needed invasive ventilation (classified as severe) to patients who did not (classified as mild), in relation to clinical and laboratory values presented in their admission. Lymphopenia, high CRP, D-dimer, sCD163, and Il-18 levels were statistically significant in the patients eventually needed invasive ventilation support

sCD163 in correlation to COVID-19 was first described this February by Maria Antonella Zingaropoli et al., in a research which included 102 COVID-19 patients in admission in Italy and was found to be a potential marker to assess the progression of the disease [35]. In their study, sCD163 showed a statistical significance in COVID-19 patients compared with a control group of healthy blood donors. In addition, sCD163 levels in the blood of COVID-19 patients who developed ARDS were higher compared with a non-ARDS COVID-19 patients group [35].

In our study, we tested levels of sCD163 in a mortality group compared to a survival group, in which both groups were diagnosed with COVID-19. We found that levels of sCD163 were strongly correlated with severity and mortality in COVID-19 patients together with ferritin and IL-18. However, even though all three markers (ferritin, IL-18, and sCD163) were associated with ominous prognosis, there was no intercorrelation between sCD163 to either, IL-18 or ferritin (Fig. 4d). We suggest that each of the markers is associated to the progression of the disease, but is more prominent in a different phase along a common pathophysiology. We conclude from our study, and in view of the previous publications, that IL-18 might be associated to an earlier phase of the disease, while ferritin to a more advance phase and/or its peak, and sCD163 to its advance to late phase. We hypothesize that as part of macrophage activation in COVID-19, IL-18 and ferritin levels rise together with CD163 receptors on M2 macrophages. IL-18, together with FeH, might take a prominent part in the cytokine storm, while sCD163, presumably with FeL, in the advance to late anti-inflammatory phase of the disease. In addition, in recent years, studies have demonstrated that as part of the inflammatory process induced by FeH in MAS, FeH induced a significant increase in IL-1β and IL-12, which appear to also play a role in the inflammatory vicious cycle and might fill some gaps in understanding the intercorrelations processes [36]. Furthermore, IL-12 has shown a synergistic effect with IL-18 to stimulate secretion of interferon-γ which promotes Th1-type inflammatory responses [7].

In summary, while assessing the prognosis of COVID-19 in hospitalized patients, high levels of ferritin, sCD163, and IL-18 have been found to be important markers for predicting patients' prognosis, along with CRP, lymphocytes, and D-dimer

IL-18 and mainly sCD163 have played significant roles as prognostic markers among hospitalized patients, and in predicting the need of invasive ventilation and therefore can serve as therapeutic targets.

The precise role of sCD163 in the mechanism of the disease has not been discovered yet and might be a good candidate as a target to treat COVID-19, hyperferritinemic syndrome, and cytokine storm-associated diseases in general.

Author contribution Yuval Volfovitch: conceptualization, investigation, and writing — original draft. Avishai M Tsur: formal analysis and methodology. Michael Gurevitch: investigation and methodology. Daniela Novick: conceptualization, methodology, writing — original draft, and resources. Roy Rabinowitz: data analysis and visualization. Menachem Rubinstein: conceptualization. Mathilda Mandel: conceptualization. Anat Achiron: conceptualization. Yehuda Shoenfeld: conceptualization, writing — original draft, and supervision. Howard Amital: conceptualization, writing — original draft, project administration, funding acquisition, and supervision.

Declarations

Conflict of interests The authors declare no competing interests.

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