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

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Perspectives

Soluble CD137 as a potential biomarker for severe COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly pathogenic infection responsible for the world pandemic in 2020. COVID-19 is characterized by an increased number of critically ill patients with a high risk of health care system collapse. Therefore, the search for severity biomarkers and potential therapies is crucial.

In this study, we evaluated SARS-CoV-2 -induced cytokines, cytokines receptors and growth factors profile, in critical COVID-19 patients admitted in intensive care unit (ICU) aiming to identify potential biomarkers and therapeutic targets.

We designed a prospective study enrolling 62 adults with severe COVID-19 during the first two Brazilian COVID-19 waves (from May to July 2020 and December 2020 to May 2021), convenience samples recruitment in first 24 hours and then, every 4 days until day 20 of ICU admission from a tertiary hospital in São Paulo, Brazil. Controls were healthy blood donors. Whole blood was used to evaluate 17 cytokines, cytokines receptors and growth factors. Due to low mortality rate, we used the need of mechanical ventilation as primary endpoint.

In our analysis, we found a different pattern in soluble CD137 (sCD137) in critically ill patients with COVID-19, with a direct relationship between increased levels and worse clinical outcome. sCD137 was related with increased risk of mechanical ventilation and World Health Organization (WHO) clinical score for disease severity.

CD137 is a tumor necrosis factor receptor (TNF) family member, mainly responsible for T-cell activation. Soluble isoforms of immune checkpoints competitively regulate function of their membrane-bound counterparts. Our study demonstrated the onward increase in sCD137 levels during severe SARS-CoV-2 infection and its correlation with worse outcomes, suggesting sCD137 as a potential reliable severity biomarker.

Introduction

The first known infection of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) was described in Wuhan, China in 2019. [1,2]. SARS-COV-2 is a highly contagious RNA virus and causes respiratory tract infections that can range from mild to lethal, also is denominated COVID-19 [1,3]. In previous reports, 40% of symptomatic cases had dyspnea; 14% develops severe disease and 5% become critical [3]. Indeed, in severe cases, an unbalanced immune response may lead to host tissues damage and increased viral replication. [4] From this background, early identification of prognostic biomarkers is imperative, and so is the search for potential therapeutic targets.

We performed a prospective study to evaluate immune proteins profile, through serial serum samples collection, from patients with severe COVID-19 admitted in intensive care unit (ICU) of a Brazilian tertiary hospital. From 17 cytokines, receptors and growth factors analyzed, we identified soluble CD137 (sCD137) as a biomarker of disease severity and need for mechanical ventilation.

Methods

The present study aims to describe findings of a prospective analysis enrolling severe COVID-19 subjects which required ICU in a tertiary hospital in São Paulo city, Brazil. Inclusion criteria were adults admitted in the first 24 hours in a clinical ICU with confirmed COVID-19. Further, the recruitment period was during the first two Brazilian COVID-19 waves (from May to July 2020 and December 2020 to May 2021). Clinical, laboratory data and outcomes were retrieved, and blood samples collected on the day of ICU admission and then, every 4 days, until day 20 or death. Healthy blood donors were selected as controls.

Samples were obtained after centrifugation of no coagulated whole blood and then frozen in small aliquots (500 μL) at -80°C until use. Seventeen plasma cytokines, receptors and growth factors analyzed (GMCSF, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, sCD137, INFγ, Granzyme A, Granzyme B, sFAS, sFASl, MIP-1α, MIP-1β, TNFα, Perforin) were studied using a multiplexed immunoassay system, according to the manufacturer's instructions (Luminex, Austin, TX). Immune proteins levels were measured using a Milliplex Map kit of Human CD8+ T-cell magnetic bead panel (Millipore, Billerica, MA) and the analysis was performed using the Luminex 100 IS version 2.3 software. Samples were processed

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in duplicate, and the results were determined using the mean value obtained from both measures. If discrepancies occurred (> 20%), the sample analysis was repeated or discarded. This research was submitted and approved on Institutions ethical review committee and national ethical committee (CONEP) and informed consent was obtained from all participants.

Summary statistics, namely frequencies and median (interquartile range, IQR) were reported. Mechanical/invasive ventilation rates during ICU stay were presented with Clooper-Pearson 95% confidence intervals. sCD137 levels were scaled and centered applying log10 transformation before data exploration and visualization. Group comparisons were carried on using Wilcoxon or Kruskal-Wallis tests. Correlation analyses were performed by computing Pearson or Spearman coefficients. All statistical tests were two-sided with p-values below 0.05 demonstrating statistical significance. All analysis were attained with Rstudio 1.3.959 statistical software (https://www.rstudio.com).

Results

Sixty-two patients were enrolled, with a male predominance (74.2%) and median age of 58.7 years. Previous illness included mainly Hypertension 30 (48.4%), Obesity 31 (50%), Diabetes mellitus 12 (19.3%), Chronic Pulmonary disorders 10 (16.1%), Cardiovascular disease 6 (9.7%) and Cancer 3 (4.8%). (Table 1) Regarding to clinical findings in ICU admission, 45% had tachypnea; oxygen supplementation was through nasal catheter, face mask, non-invasive ventilation, or high flow nasal cannula despite merely one, that was in mechanical ventilation support need; Around a quarter, 25.8%, of patients had more than 50% of lung affected by chest-computed tomography.

Table 1
Baseline characteristics of the cohort. Characteristics of the role population; according to invasive ventilation need; and who developed thrombotic events.

	Total (n=62)	Invasive Ventilation (n=34)	Thrombotic Event (n=18)
Male, n (%)	46 (74.2)	23 (67.6)	12 (66.7)
Female, n (%)	16 (25.8)	11 (32.4)	6 (33.3)
Age, median (IQR)	58.7 (21)	64.3 (16) / 52.8 (20)	65.7 (13) / 53.7 (22)
Comorbidities, n (%)			
Hypertension	30 (48.4)	20 (58.8)	13 (72.2)
Obesity	31 (50.0)	17 (50.0)	11 (61.1)
Diabetes mellitus	12 (19.3)	7 (20.6)	3 (16.7)
Respiratory diseases	10 (16.1)	6 (17.6)	5 (27.8)
Heart disease	6 (9.7)	4 (11.8)	2 (11.1)
Malignancy	3 (4.8)	0 (-)	0 (-)
Days from symptoms onset, median (IQR)	10.5 (3.0)	10.0 (4) / 11.0 (4)	11.0 (4) / 10.0 (4)
Lung injury in chest CT, n (%)			
< 25%	25 (40.3)	17 (50.0)	9 (50.0)
25%-50%	21 (33.9)	8 (23.5)	6 (33.3)
> 50%	16 (25.8)	9 (26.5)	3 (16.7)
SOFA at ICU admission, median (IQR)	3 (2)	3.5 (2) / 3.0 (2)	3.5 (2) / 3 (2)
SAPS III at ICU admission, median (IQR)	41 (11)	42 (9) / 40 (8)	43.0 (9) / 40.0 (8)
Laboratory tests at admission, median (IQR)			
D-dimer (ng/mL)	1057 (1246)	1188 (1254) / 1045 (1022)	1435 (1650) / 997 (984)
LDH (U/L)	571 (314)	571 (264) / 556 (254)	634 (420) / 567 (275)
CRP (mg/dL)	18.1 (15)	21.0 (13) / 15.4 (14)	15.4 (10) / 20.0 (16)
Lymphocyte count (cells x10^9/L)	0.72 (0.442)	0.72 (0.4) / 0.77 (0.465)	0.725 (0.278) /0.71 (0.508)
Neutrophils count (cells x10^9/L)	7.75 (5.342)	7.41 (4.62) / 9.59 (6.225)	9.025 (3.79) / 6.845 (6,402)
sCD137 (ng/mg), median (IQR)	0.022 (0.03)	0.027 (0.03) / 0.019 (0.02)	0.018 (0.04) / 0.022 (0.02)

*CRP, C-reactive protein; CT, chest tomography; ICU, intensive care unit; IQR, interquartile range; LDH, lactate dehydrogenase; sCD137, soluble CD137.

Increased median levels of D-dimer 1057 (IQR 1246) ng/mL; Lactate dehydrogenase 571 (IQR 314)U/L (normal range up to 480); and C-reactive protein 18.1 (IQR 15)mg/dL were found in our cohort, together with low lymphocytes count with median 0.72 (IQR 0.44) cellsx10^9/L and high neutrophil-to-lymphocyte ratio of 9.8. (Table 1)

The overall ICU mortality rate was 8% (95%CI 2.7-17.8). Mechanical ventilation was required in 55.0% of all patients (95%CI 41.6-67.9), furthermore 3.3% required extracorporeal membrane oxygenation (ECMO). Thromboembolic events were documented in 29.0% (x/62) of all patients (95%CI 18.2-41.9) including pulmonary thromboembolism, acute venous deep thrombosis and two arterial events. All enrolled patients have received corticosteroids, usually dexamethasone, and thromboembolism prophylaxis.

From all cytokines, receptors and growth factors evaluated, sCD137 demonstrated impressive results as shown below. This soluble biomarker was significantly increased at ICU admission in contrast with healthy controls. This difference was more overt in early COVID-19 phase (up to 10 days from symptoms onset). sCD137 levels were also related to COVID-19 severity, whereas they were significantly raising at time-points with higher WHO clinical scores [2]. The admission levels of sCD137 were directly related with C-reactive protein levels. (Fig. 1)

Longitudinal analyses revealed a promising prognostic biomarker during severe COVID-19 infection. Patients who required mechanical ventilation showed, in all assessed time-points, a continuous sCD137 increase especially, in the first 12 days of ICU admission with a distinct evolution since symptoms onset. Regarding thromboembolic events, no differences were observed in the evolution of sCD137 levels. (Fig. 2; Table 1).

Discussion

In severe COVID-19 cases, long stay in ICU and potential health system collapse is a threat; therefore, early identification of reliable biomarkers, especially in ICU patients, is of paramount importance.[3] In this brief report, we identify sCD137 as a biomarker of disease severity evaluating mechanical ventilation risk.

In severe COVID-19 cases, an unbalanced immune response is seen, with pro and anti-inflammatory cytokines playing a crucial role in disease progression and prognosis.[5] Zhang *et al*, described the association with high anti-inflammatory cytokine levels, Il-6 and IL-8, and low lymphocytes counts, including B, T and NK cells, in severe COVID-19.[1]

In peripheral blood, neutrophil-to-lymphocyte ratio is associated with worse outcome; with a ratio over 3.63 correlating with higher mortality rate.[6] In our study, median neutrophil-to-lymphocyte ratio was 9.8, highlighting the disease severity in our cohort. In addition, sCD137 was associated with elevated serum C-reactive protein.

CD137 (IBB-4 or TNFRSF9), is a member of TNF receptor family, CD137 and CD137 ligand (lCD137) are activated when they are linked, having a role in immune response regulation in different cells subsets, especially CD8+T cells. Functionally, they can enhance defense system against pathogens. [7,8] sCD137 is released as a regulator in excessive T-cell state excitation. [9,10] Soluble forms competitively control function of their membrane-bound counterparts, hence sCD137 blocks CD137 function.

In severe influenza infection, lack of CD137 was associated with decreased CD8 T cells in infected pulmonary cells, consequently reducing viral clearance, causing lung function impairment and higher mortality rate.[8] Kong *et al* investigated soluble forms in COVID-19 and found that those were persistently increased in severe cases.[9]

Ajami et al, developed tests with a recombinant CD137-Fc protein evaluating immune responses in vitro, showing potential effect in immune modulation during coronavirus infection, especially in cytokine-release syndrome. This therapy approach probably should be tested in

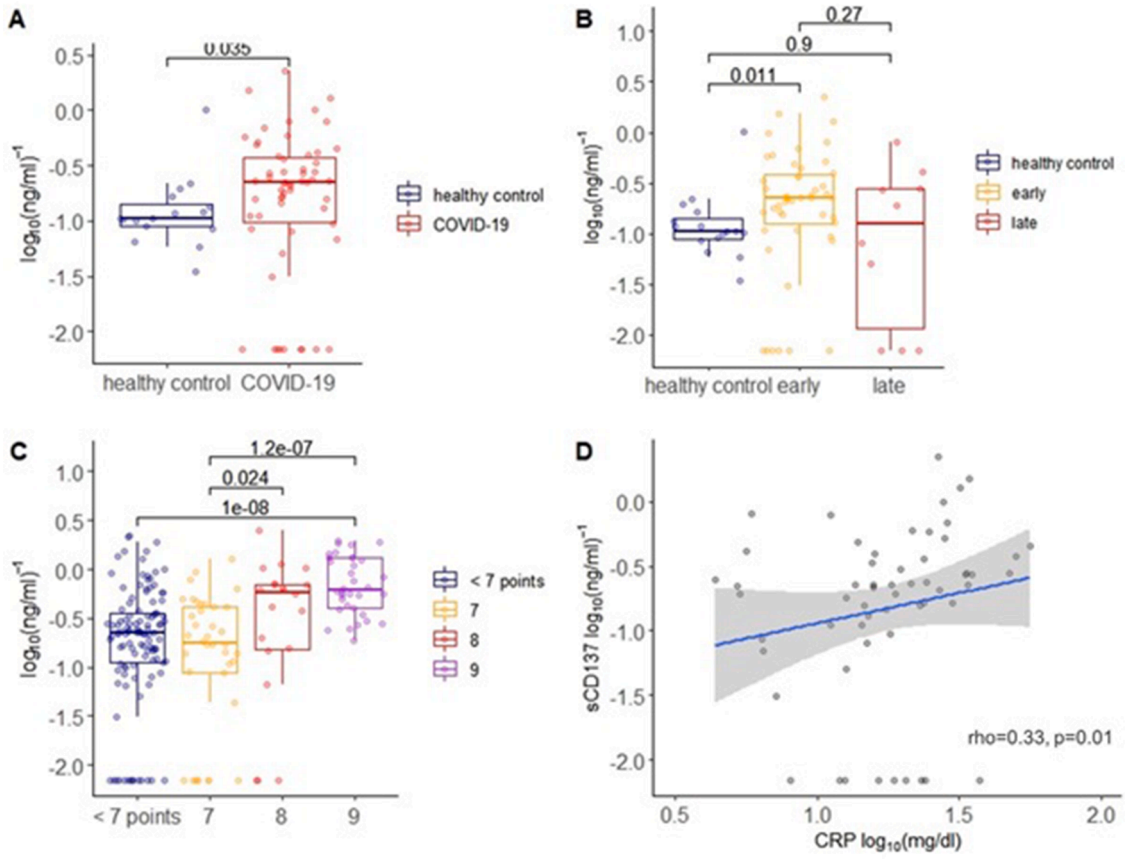

Fig. 1. Soluble CD137 levels in patients with moderate or severe COVID-19 requiring ICU admission. (A) Soluble CD137 levels in healthy controls subjects (n=16) and COVID-19 patients at ICU admission (n=58). (B) Soluble CD137 levels in healthy controls subjects (n=16) and COVID-19 patients according to the COVID-19 phase of time from symptoms onset. Early phase (up to 10 days) and late phase (more than 10 days) until ICU admission (n=58). (C) Soluble CD137 levels according to the WHO COVID-19 clinical score. Each dot represents an individual patient measure per within time point (n=245). (D) Spearman correlation of soluble CD137 and C-reactive protein levels at ICU admission (n=58).

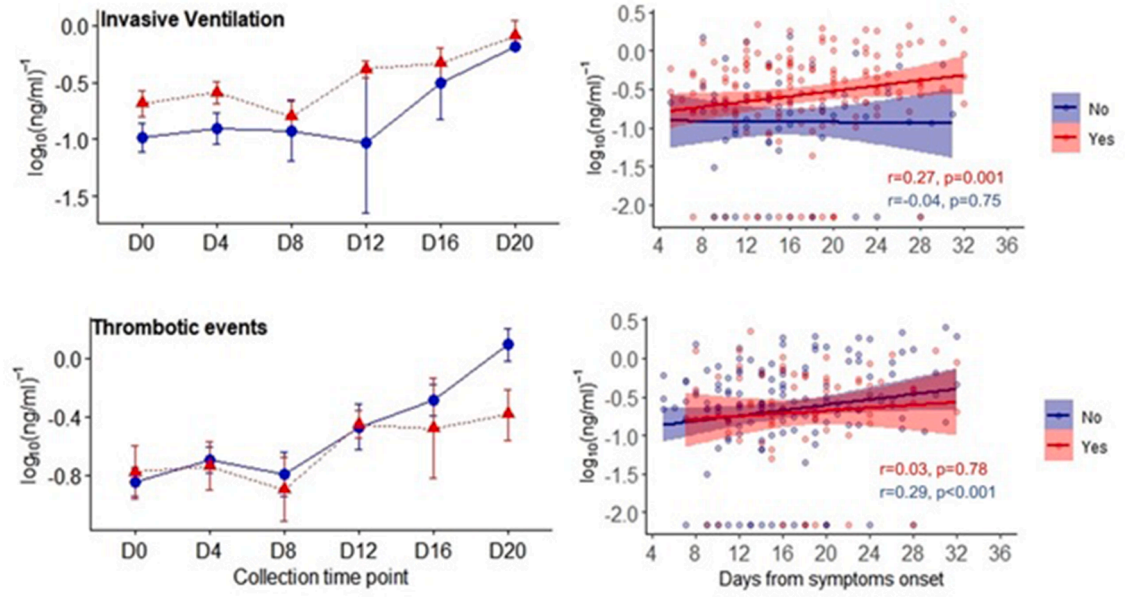

Fig. 2. Longitudinal analyses of soluble CD137 levels in COVID-19 patients during ICU hospitalization according to clinical outcomes. Error bars represent the means and standard error at each collection time point. Each dot of two axis dispersion graphs represents an individual patient measure per within time point. Pearson regression coefficient and lines are presented by the clinical outcome invasive ventilation (n=34) and thrombotic events (n=18).

association with antiviral treatment. [11]

In our study, sCD137 were significantly higher in patients compared to healthy controls, additionally we evaluate samples into COVID-19 infection course in six different time points. Furthermore, a significant relation between COVID-19 severity and sCD137, using WHO clinical scores, during the infection, was shown. [2] We also showed that increased sCD137 levels was related with the need of mechanical ventilation and worse outcome. Our findings point towards a more anti-inflammatory response in severe cases, as sCD137 affect CD8 cytotoxic response and viral clearance.

To date, this is the first report addressing sCD137 as a promising biomarker for COVID-19 severity and risk of mechanical ventilation. Moreover, could be substantial to decision making for early intervention therapy or for clinical trials design. Noteworthy, we could not evaluate the sCD137 mortality rate impact due to our small sample size, further multicentric studies and larger cohorts are warranted.

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