HGF, IL-1α and IL-27 are Robust Biomarkers in Early Severity Stratification of COVID-19 Patients

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Abstract

Background: Pneumonia is the leading cause of hospital admission and mortality in coronavirus disease 2019 (COVID-19), attributed to a cytokine storm. The objective of our study is to characterize this profile to identify the cytokines responsible for lung damage and mortality.

Methods: Plasma samples of 108 prospectively recruited COVID-19 patients were collected between March and April 2020. Patients were divided into four groups according to the severity of respiratory symptoms: 34 mild (no oxygen support), 26 moderate (low oxygen support using nasal cannula), 16 severe (high oxygen support) and 32 critical (mechanical ventilation). A 45-plex Human XL Cytokine Luminex Performance Panel kit was used in duplicate for each plasma sample. Twenty-eight healthy volunteers were used for normalization of the results.

Results: Multiple cytokines showed statistically significant differences when comparing mild and critical patients (HGF, PDGFBB, PIGF-1, IL-1α, MCP-1, VEGFA, IL-15 and IL-2). The best multivariable model included HGF, IL-1α, IL-2 and IL-27. High HGF levels were associated with the critical group (OR = 3.51; $p < 0.001$; 95%CI = 1.95−6.33). Moreover, high IL-1α (OR = 1.36; $p = 0.01$; 95%CI = 1.07−1.73) and low IL-27 (OR = 0.58; $p < 0.005$; 95%CI = 0.39−0.85) greatly increased the risk of ending up in the severe group. This model was especially sensitive in order to predict critical status (AUC = 0.794; specificity = 69.74%; sensitivity = 81.25%). Furthermore, high levels of HGF and IL-1α showed significant results in the survival analysis ($p = 0.033$ and $p = 0.011$, respectively).

Conclusions: Our study showed that HGF, IL-1α and IL 27 at hospital admission were strongly associated with severe/critical COVID-19 patients and therefore are excellent predictors of bad prognosis. Indeed, HGF and IL-1α were also mortality biomarkers.

Introduction

In December 2019, a new strain of coronavirus – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – was recognized to have emerged in Wuhan, China. Along with SARS-CoV[1] and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 is the third coronavirus to cause severe respiratory disease in humans, called coronavirus disease 2019 (COVID-19).[2] The epidemiology of the disease is not completely understood.[3] After a median incubation period of approximately 5 days, around half of patients present mild or no symptoms.[4] The others present moderate or severe respiratory disease, including 20% who present serious illness with high fever and pneumonia,[5] leading to acute respiratory distress syndrome (ARDS).[6]

The pathophysiology is not fully understood.[7] Viral infection leads to rapid activation of innate immune cells, especially in patients who develop severe disease. The infection induces lymphocytopenia that primarily affects CD4^{+} T cells, including effector, memory and regulatory T cells.[8,9] Some biomarkers are related to moderate and severe COVID-19 infection, such as low lymphocyte absolute numbers[10] or increased levels of serum C-reactive protein (CRP), hypoalbuminaemia, alanine aminotransferase, lactate dehydrogenase, ferritin and/or D-dimer.[11,12] Indeed, these patients display increased levels of proinflammatory cytokines in serum, such as IL-1β, IL-6, IL-12, IFN-γ, IP-10 or MCP-1/CCL2,[13,14] which are related to T helper 1 (Th1) cell responses. Moreover, the more severe patients (including those who require intensive care) display higher plasma levels of GCSF, IP-10, MCP-1, MIP-1A and TNF-α, suggesting an association with the degree of severity.[15–17]

Based on this background, studies attribute the systemic impact of COVID-19 to a cytokine storm: a kind of ARDS induced by cytokine release syndrome[18] or haemophagocytic lymphohistiocytosis,[19] similar to that described in SARS-CoV and MERS-CoV patients. In this regard, and in order to confirm this point, most studies on the characterization of the cytokine response in COVID-19 patients are retrospective, present a small series of patients and/or they focus on a limited number of cytokines, making them unsuitable for understanding the pathogeny that characterizes cytokine release syndrome.[5,11,15,17] Moreover, the identification of prognosis biomarkers remains an urgent need.

In this regard, the aim here is to perform a cytokine array on plasma samples from a prospective COVID-19 cohort, aiming not only to characterize the cytokine storm but also to identify early biomarkers of severity and mortality outcome.

Methods

Patient selection

A total of 108 adults over 18 years of age who were diagnosed with COVID-19 and admitted to the University Clinical Hospital (Valladolid, Spain) were prospectively recruited between 24 March and 11 April 2020. A positive result for SARS-CoV-2 infection was confirmed in all patients by polymerase chain reaction on a nasopharyngeal sample. Patients with other acute diseases, infections or chronic terminal illness were not included. In addition, we also included 28 age- and gender-matched healthy volunteers for normalization of the analytical data on cytokines. The study was approved by the hospital's clinical ethics committee (CEIm) and permission was obtained from all study participants (code: PI 20-1717). This study followed the code of ethics of the World Medical Association (Declaration of Helsinki).

Biological samples

We prospectively collected plasma samples from each patient at 9am immediately after their first night in the hospital in order to prevent circadian variations. Blood was collected in 3.2% sodium citrate tubes and centrifuged at 2000 g for 20 minutes at room temperature. The resulting plasma was aliquoted and directly frozen at −80°C until used.

Degree of severity

Patients were divided into four groups based on their subsequent clinical outcome according to the severity of the respiratory symptoms: mild (no oxygen support, $n = 34$); moderate (low oxygen support using nasal cannula, $n = 26$); severe (high oxygen support, $n = 16$); and critical (mechanical ventilation, $n = 32$). This classification is based on the Centers for Disease Control guide.[20]

Cytokine and chemokine analysis

Plasma aliquots were analysed in duplicate for quantification of soluble mediators using the 45-plex Human XL Cytokine Luminex Performance Panel kit (R&D) and following the manufacturer´s guidelines and recommendations. Cytokines or chemokines included in the assay were BDNF, EGF, eotaxin (also known as CCL11), FGF-2, GM-CSF, GRO-α (CXCL1), HGF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-10, IL-12 p70, IL-13, IL-15, IL-17a (CTLA-18), IL-18, IL-1RA, IL-2, IL-21, IL-22, IL-23, IL-27, IL-31, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, MIP-1β (CCL4), IP-10 (CXCL10), LIF, MCP-1 (CCL2), MIP-1α (CCL3), NGF-β, PDGF-BB, PlGF-1, RANTES (CCL5), SCF, SDF-1α, TNF-α, TNF-β, VEGFA and VEGFD.

Variables

Demographic, clinical and analytical data (leukocytes, lymphocytes, neutrophils, platelets, bilirubin, creatinine, glucose, troponin T, CRP, lactate dehydrogenase, ferritin, procalcitonin and D-dimer) of each patient were also recorded to describe the clinical phenotype.

Hospital protocol treatment

The hospital protocol for the treatment of COVID-19 pneumonia in March and April 2020 included lopinavir/ritonavir (Kaletra®, Abbott), 200/50 mg/ml solution twice a day, and hydroxychloroquine (Dolquine®, Rubió), 400 mg twice a day. According to inflammatory criteria, the standard of care would also add: interferon 1β (Betaferon®, Bayer), 0.25 mg every 48 hours; corticosteroids, 240 mg every day for three days; and tocilizumab (RoActemra®, Roche), baricitinib (Olumiant®, Lilly) or anakinra (Kineret®, Amgen). In the case of suspected bacterial superinfection, antibiotic treatment is required. Oxygen support (nasal cannula, high-flow nasal cannula and non-invasive or invasive mechanical ventilation) was administered to patients based on the severity of hypoxaemia.

Statistical analysis

Statistical analysis was performed by a PhD-licensed statistician (co-author I.F.) using the R software package, version 4.0.2 (R Core Team; Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/). Statistical significance was set at $p \leq 0.05$.

To impute cytokine values below the assay detection limit, robust regression on order statistics was used: this method performs a regression to impute low values assuming log-normal quantiles for samples with a detection rate of at least 20%, after checking that the data follow a log-normal distribution. To accomplish this, the NADA (Nondetects And Data Analysis) R package was used.[21] Molecules detected in less than 20% of the samples were not statistically analysed any further. Cytokine expression data were transformed using the logarithmic base 2 scale. Continuous variables are represented as median and interquartile range (IQR) whereas categorical variables are represented as percentage and number.

The strength of each biomarker was evaluated at the individual level to determine the pulmonary severity of the patient. The main variable was severity, which is an ordinal variable with four levels. The first model to be fitted was an ordinal logistic regression model or a proportional odds model. These models require compliance with the proportional odds assumption. To confirm this, the proportional odds model was compared with a multinomial logistic regression model through the likelihood ratio test. However, in none of the cases was it possible to assume this hypothesis, so multinomial models were fitted. In these models, the chosen reference level was mild and the probability of belonging to the 'moderate', 'severe' and 'critical' groups was modelled. All models have been corrected for age and gender.

Biomarkers associated with severity at the 10% significance level were identified as potential biomarkers and they were evaluated simultaneously to fit a multivariable model. The first step to fit this model was to identify the optimal subset of potential biomarkers. An exhaustive search was performed, fitting a multinomial model based on every possible combination of potential biomarkers, with age and gender as confounding variables. The optimal model was the one with the minimum Akaike information criterion (AIC) value.

The leave-one-out cross-validation (LOOCV) procedure was used to estimate the prediction accuracy of the final fitted models, and receiver operating characteristic (ROC) curve analysis was used to assess their discriminate ability. The final models were evaluated according to the area under the ROC curve (AUC). In addition, sensitivity and specificity were obtained by setting an optimal threshold.

Survival analysis was performed with the final panel of cytokines identified by the multivariable models. The outcome was tested related to T2 (time since hospitalization until death/end of the survey). For survivals, the days of follow-up were hospitalization time or 28 days in outpatients after leaving the hospital. The Kaplan−Meier survival function was used in the log-rank test to determine differences in survival rates (considered different when $p < 0.05$). The cut-off point is established in each cytokine, selecting the one with the greatest AUC in the individual model.

Results

Our cohort had a median age of 67 years, mostly male (63.26%). The control group of healthy volunteers had a median age of 61 years and most of them (57.1%) were also male. Patients were divided into four severity levels based on the subsequent outcome during their hospital stay: mild (*n* = 34, 31.5%; 95%CI = 23.07–41.23), moderate (*n* = 26, 24.1%; 95%CI = 16.59–33.43), severe (*n* = 16, 14.8%; 95%CI = 8.96–23.24) and critical (*n* = 32, 29.6%; 95%CI = 21.43–39.3), defined by their need for oxygen supplementation.

Patient clinical and analytical profiles at hospital admission are shown in Table 1. Patient group did not differ regarding age, gender or comorbidities. However, ferritin, D-dimer, leukocytes, neutrophils, procalcitonin and glycaemia displayed higher levels with greater severity. In contrast, lymphocytes, platelets and PaO2/FiO2 were decreased in critical patients. Length of hospital stay increased according to severity (8, 8, 13.5 and 26.5 days, respectively). Mortality was also higher in severe (50%, 8 patients) and critical (43.8%, 14 patients) groups compared with moderate (3.8%, 1 patient) and mild (2.9%, 1 patient) groups.

Table 1
Clinical characteristics of the patients.

	Mild (N = 34)	Moderate (N = 26)	Severe (N = 16)	Critical (N = 32)	P value
Age [median (IQR)]	68 (18)	65 (17)	75 (14)	70 (16)	0.121
Male [%(n)]	45.2 (14)	61.5 (16)	62.5 (10)	54.8 (17)	0.568
-Comorbidities, [%(n)]					
Use of tobacco	8.80 (3)	3.80 (1)	6.3(1)	12.5 (4)	0.679
Coronary cardiopathy	8.8 (3)	11.5 (3)	12.5 (2)	6.30 (2)	0.870
Valvular disease	5.90 (2)	0 (0)	12.5 (2)	0 (0)	0.104
Atrial fibrillation	17.6 (6)	3.80 (1)	18.8(3)	6.3 (2)	0.206
Diabetes	11.8 (4)	11.5 (3)	18.8 (3)	25 (8)	0.435
Hypertension	50 (17)	34.6 (9)	56.3 (9)	46.9 (15)	0.521
Liver disease	0 (0)	0 (0)	0 (0)	6.3 (2)	
COPD	0 (0)	7.7 (2)	18.8 (3)	6.3 (2)	0.094
Kidney disease	2.90 (1)	0 (0)	0 (0)	6.3 (2)	0.452
Asthma	11.8 (4)	3.80 (1)	0 (0)	3.1 (1)	0.268
-Laboratory, [median (IQR)]					
Glucemia (mg/dL)	90 (13)	109 (56)	120 (59)	209 (99)	< 0.001
Leukocytes (n°/ml)	4620 (2880)	6990 (3020)	6630 (3480)	7900 (8680)	< 0.001
Lymphocytes (n°/ml)	1000 (430)	1000 (1000)	1120 (531)	440 (455)	< 0.001
Neutrophil (n°/ml)	3215 (2420)	4945 (2380)	5315 (3450)	7045 (7800)	< 0.001
Procalcitonin (ng/ml)	0.06 (0)	0.05 (0)	0.15 (1)	0.24 (0)	< 0.001
CRP (mg/L)	76.5 (88)	73.5 (106)	127.0 (113)	97.0 (153)	0.250
Creatinine (mg/dL)	0.81 (0)	0.78 (0)	0.88 (0)	0.89 (1)	0.242
Total bilirubin (mg/dL)	0.40 (0)	0.5 (0)	0.65 (0)	0.50 (1)	0.187
Platelet (cell/mm3)	(82000)	232500 (171000)	198500 (108500)	216500 (108000)	0.005
Ferritin (ng/ml)	587 (600)	674 (906)	1025 (938)	1700 (1093)	< 0.001
D-dimer (ng/ml)	547 (333)	693 (702)	1083 (1398)	1847 (1823)	< 0.001
PaO2/FiO2	371 (48)	304 (94)	238 (102)	127 (44)	< 0.001
-Hospital meters, [median (IQR)]					
Length of hospital stay (days)	8 (4)	8 (6)	13.5 (10)	26.5 (39)	< 0.001

	Mild (N = 34)	Moderate (N = 26)	Severe (N = 16)	Critical (N = 32)	P value
Length of ICU stay (days)	0 (0)	0 (0)	0 (0)	18.5 (14)	0.172
Intubation time (days)	0 (0)	0 (0)	0 (0)	14 (12)	0.172
-Mortality, [(n)]					
90-days mortality	2.9 (1)	3.8 (1)	50(8)	43.8(14)	< 0.001
28-days mortality	0 (0)	3.8 (1)	43.8(7)	37.5(12)	< 0.001

Continuous variables are represented as [median, (interquartile range, IQR)]; categorical variables are represented as [, (n)]. COPD, chronic obstructive pulmonary disease; CRP, C-Reactive protein.

To impute low values assuming log-normal quantiles for samples, a detection rate of at least 20 is required. Under these conditions, eight cytokines (FGF-2, IL-12, IL-21, IL-23, IL-31, IL-9, NGF-β and TNF-β) were excluded from analysis (Additional file 1). Median values of each cytokine according to the degree of severity are shown in Additional file 2. Based on a likelihood ratio test (Additional file 3), the most plausible model in all cases is the multinomial model. Hence, we performed individual multinomial models using the mild group as a reference (Figs. 1a, 1b and 1c).

Comparison of mild with moderate (Fig. 1a) or severe (Fig. 1b) patients was not statistically significant for any of the studied cytokines, although eotaxin, IL-1α, IL-27, IL-5 and PIGF-1 were borderline in the severe patients. Nevertheless, the comparison of mild patients with those who ended up critical displayed statistical differences for several cytokines: HGF (hepatocyte growth factor), PDGFBB, PIGF-1, IL-1α, MCP-1 and VEGFA were over-expressed at hospital admission in the critical group by 3.83, 1.38, 1.15, 1.13, 1.5 and 1.31 times, respectively, whereas IL-15 and IL-2 were under-expressed by 1.56 (1/0.64) and 1.47 (1/0.68).

The best multivariable model based on these molecules is the one with four cytokines: HGF, IL-1α, IL-2 and IL-27 (Table 2). The gender- and age-adjusted odds ratios (OR) are shown in Table 3. This analysis revealed an association between high levels of HGF and IL-1α coupled with low levels of IL-27 at hospital admission as bad prognosis predictors because these patients ended up in the severe or critical group. In this regard, patients with twice the expression of HGF at admission had a 3.51 higher chance of being critical than mild (OR = 3.51; $p < 0.001$; 95CI = 1.95−6.33). In a similar manner, if IL-1α (OR = 1.36; $p = 0.01$; 95CI = 1.07−1.73) or IL-27 (OR = 0.58; $p < 0.005$; 95CI = 0.39−0.85) was over- or under-expressed at admission, the risk of being in the severe group was 1.36 and 1.74, respectively (1/0.5753), compared to the mild group.

Table 2
Identification of the best multivarible model following AIC ("Akaike's Information Criterion").

	Int.	Age	Sex	HGF	IL-1α	IL-15	IL-2	IL-27	IL-5	MCP1	PDGFBB	PIGF1	VEGFA	AIC
M0	✓	✓	✓											301.7077
M1	✓	✓	✓	✓										268.1021
M2	✓	✓	✓	✓					✓					268.3859
M3	✓	✓	✓	✓		✓			✓					265.8642
M4	✓	✓	✓	✓	✓		✓	✓						**264.8347**
M5	✓	✓	✓	✓		✓		✓			✓	✓		265.6192
M6	✓	✓	✓	✓		✓	✓	✓			✓	✓		267.9954
M7	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓		271.669
M8	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	274.8803
M9	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	278.3977
M10	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	282.1787

Int, Intercept.

Table 3
Different multivariable model according to the degrees of severity.

Severity	Effect	p-value	OR	CI 95	
				Low	High
Moderate	Age	0.573	0.9883	0.9486	1.0296
	Sex = Female	0.1648	0.4618	0.1553	1.3735
	HGF	0.7528	1.0853	0.652	1.8066
	IL1a	0.4346	1.081	0.8891	1.3144
	IL2	*0.067*	*0.57*	*0.3124*	*1.0401*
	IL27	0.487	1.1148	0.8206	1.5144
Severe	Age	0.0452	1.0687	1.0014	1.1405
	Sex = Female	0.1504	0.3517	0.0847	1.4611
	HGF	0.2144	1.5301	0.7818	2.9946
	IL1a	0.0109	1.3634	1.0741	1.7308
	IL2	0.4125	1.4144	0.6172	3.2414
	IL27	0.0057	0.5753	0.3888	0.8511
Critical	Age	0.13	0.9615	0.9139	1.0116
	Sex = Female	0.758	0.8242	0.241	2.8192
	HGF	< 0.0001	3.5122	1.9495	6.3276
	IL1a	0.1977	1.134	0.9365	1.3731
	IL2	0.1105	0.5776	0.2943	1.1334
	IL27	0.8571	0.9677	0.6772	1.383
CI, confidence interval; OR, Odds ratio					

The fitted models are used to estimate predicted probabilities and their associated confidence bands for the groups. These estimated probabilities are visualized as effect plots in Figs. 2a, 2b and 2c. We clearly see how the chances of ending up in a critical condition were directly related to higher HGF levels at admission. Hence, HGF levels above 128 pg/ml (2^7) imply a 25 chance of being critical whereas levels above 223 pg/ml increase that critical risk to 50. In contrast, patients with HGF levels below 64 pg/ml (2^6) have no risk (practically 0) of ending up critical. In the same manner, low IL-1α levels at admission had a probability of over 37 of being mild whereas IL-1α levels above 1024 pg/ml (2^{10}) had a 50 chance of being in the severe group. Lastly, lower levels of IL-27 at admission were associated with the severe group because levels below 1 are reflected in a 50 chance of belonging to the severe group whereas levels above 64 pg/ml (2^6) decrease that risk to practically 0.

Internal validation by the LOOCV procedure shows that the AUC is significantly greater than 0.5 in all the groups (Table 4), especially in the severe (AUC = 0.730) and critical (AUC = 0.794) groups. This model is especially sensitive in order to classify patients who end up as critical (sensitivity = 81.25). Lastly, survival analysis taking into account the

three statistically significant cytokines included in the multivariable model was significant for HGF and IL-1α (Figs. 3a and 3b) but not for IL-27 (Fig. 3c).

Table 4
Internal validation in each degree of severity using the AUC (Area Under the ROC Curve).

| | Mild | Moderate | Severe | Critical |
	Threshold: 0.3597126	Threshold: 0.2513263	Threshold: 0.1438022	Threshold: 0.2084408
	CI 95	CI 95	CI 95	CI 95
AUC	**0.647** (0.535−0.759)	**0.602** (0.477−0.727)	**0.730** (0.624−0.837)	**0.794** (0.701−0.888)
Sensitivity ()	58.82 (42.28−75.37)	53.85 (34.68−73.01)	62.5 (38.78−86.22)	**81.25** (67.73−94.77)
Specificity ()	70.27 (59.86−80.68)	65.85 (55.59−76.12)	73.91 (64.94−82.89)	69.74 (59.41−80.07)
Accuracy ()	66.67 (57.78−75.56)	62.96 (53.86−72.07)	72.22 (63.77−80.67)	73.15 (64.79−81.51)
CI, Confident Interval.				

Additional files show this in more detail (see Additional file 1, 2 and 3)

Discussion

After performing a 45-plex cytokine array on plasma samples from 108 patients at hospital admission, five cytokines displayed statistically significant differences according to degree of severity in COVID-19. Indeed, high levels of HGF and IL-1α coupled with low levels of IL-27 at admission can predict bad clinical outcome compared to the patient subset with better prognosis; these cytokines are therefore particularly important as predictors of admission to intensive care. Moreover, this multivariate model was especially sensitive in order to identify those patients who end up as critical (AUC = 0.794; specificity = 69.74; sensitivity = 81.25) following hospital admission. Lastly, we have also described how the combination of high levels of HGF and IL-1α at admission can predict mortality, showing significant results in the survival analysis (p = 0.033 and p = 0.011, respectively).

During recent months, several studies have tried to understand the cytokine profile in patients with COVID-19. Most of them relate the severity of lung disease to high levels of multiple cytokines in blood according to what has been defined as a cytokine storm. Indeed, some authors even describe three different clinical phenotypes of COVID-19 based on cytokine levels.[22] In this regard, Huang et al. suggest that the cytokine storm is associated with severity after analysing 27 cytokines in 41 patients: those in the intensive care unit (ICU) had higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP-10, MCP-1, MIP-1A and TNF-α.[17] In a similar manner, Liu et al. studied 40 patients, 13 of them severe, and found increased plasma levels of IL-6, IL-10, IL-2 and IFN-γ in severe compared to mild cases.[23] Zhao et al. studied 71 patients (53 mild and 18 severe) and 18 healthy volunteers, describing that IL-1RA and IL-10 correlated with disease severity[24], and Zhang et al. analysed 326 patients, finding higher levels of IL-6 and IL-8 in severe or critical patients.[25] Nevertheless, these studies display several limitations, such as small sample size, the study of low numbers of cytokines and the lack of a well-defined degree of severity. Moreover, patients who required mechanical ventilation were not usually differentiated from patients with severe disease, despite this aggressive intervention increasing cytokine levels. Lastly, these studies usually applied basic statistical approaches. Therefore, and in order to overcome these limitations, he have analysed in duplicate the plasma levels of 45 cytokines from an extremely well categorized cohort of 108 COVID-19 patients, who were classified into severity groups based on their clinical evolution defined by objective criteria, at which time we also performed an exhaustive statistical analysis. Hence, we have considered all confounders by using both univariate and multivariate regression analysis, showing at least an internal validation.

Other studies have performed a similar approach to the one described here, such as that by Han et al., which classified 102 patients into moderate, severe and critical groups according to their symptoms and also presented a control group of healthy volunteers. However, that study showed higher serum levels of TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-10 and CRP compared to the controls; using a logistic regression analysis, IL-6 and IL-10 were found to predict disease severity and internal validation could further confirm this result.[26] However, they only analysed six cytokines and a duplicate analysis was not performed on each sample. In a similar manner, Meizlish et al. analysed a cohort with 49 adult patients (40 in the ICU and 9 in other units), as well as 13 healthy volunteers. They analysed 78 circulating proteins with immunological functions. Their study identified a neutrophil activation signature composed of neutrophil activators (G-CSF, IL-8) and effectors (resistin, lipocalin-2 and HGF), which had greater power to identify critically ill patients.[27] As a default, the small number of patients and the different degrees of pulmonary severity do not differ in the non-critical patients.

Based on the results displayed by these two studies, and in agreement with ours, we can conclude that there is no specific cytokine pattern correlating with disease severity. On the one hand, high levels of HGF were associated with a risk of up to 3.5 times higher of being critical with mechanical ventilation. This growth factor, which has already been related to severity in other studies, primarily elicits its effects on epithelial cells. In a similar manner, IL-1α, which is a pro-inflammatory cytokine from the innate immune system mainly produced by macrophages but also epithelial cells, can also predict a bad prognosis and disease outcome. Hence, both cytokines could be reflecting the tissue damage elicited by the macrophage infiltration to the lungs.[28,29] Nonetheless, low levels of IL-27, which belongs to the IL-12 family and is therefore involved in Th1 differentiation, is a good prognosis biomarker in COVID-19 patients. Together, these results suggest that, although in our hands the cytokine storm may not be the trigger of the bilateral pneumonia, there is certainly a mixed and altered cytokine profile that drives disease progression and inflammation, as highlighted by the fact that high HGF levels combined with low IL-27 levels are revealed as early mortality markers.

Since the beginning of this health crisis, treatment strategies in the most severe cases were aimed at blocking interleukins such as IL-6 (tocilizumab), IL-1 (anakinra) and TNF-α (infliximab, adalimumab, etc.). Nevertheless, there is no clear evidence about their utility. Indeed, tocilizumab has been reported to be ineffective in preventing intubation or death in hospitalized patients with moderate COVID-19 infection.[30] Only the use of dexamethasone (two drops daily for 10 days) decreased mortality at Day 28 in patients who were receiving invasive mechanical ventilation.[31] However, even with a corticosteroid, this approach did not identify the key immune components involved in this process. Accordingly, and with the results reported here, it is to be expected that these strategies do not cause a reduction in mortality because increased levels of IL-6, IL-1 or TNFα are not directly responsible for driving disease severity in these patients. Therefore, even though increased levels of plasma cytokines in COVID-19 patients have been largely reported, the identification of disease progression and severity biomarkers remains an urgent need. In this regard, we hereby report that HGF, IL-1α and IL-27 contribute to the deterioration of the disease and the adverse outcome of COVID-19, revealing these three compounds as novel biomarkers and as future therapeutic targets in COVID-19.

Conclusion

Our study characterized the plasma cytokine profile of COVID-19 patients at hospital admission, based on their subsequent clinical evolution into 4 well-defined degrees of severity, revealing that HGF, IL-1α, and IL27 were strongly associated with disease severity and could be used as excellent predictors of bad prognosis. Indeed, HGF and IL-1α are also mortality biomarkers.

Therefore, the early detection of HGF, IL-1α and IL-27 plasma levels in patients in COVID-19 patients can provide useful information for getting quickly and intensive treatment as well as providing possible therapeutic targets.

Declarations

Aknowledgments

We appreciate the collaboration of the Nursing staff and the Research Unit from the Hospital Clínico Universitario de Valladolid.

Authors´ contributions

Literature search: AT-V, EG-S; ET; Study Design: AT-V, ET, HG-B, PM-P,MM-F, EG-P; Figures: IF, IdF, SP-G; Data collection: AT-V, EG-S, LR, MTJ-G, AS-R, MH-V, EG-P, ML-L; Data analysis: MFM-M, AT-V; Data interpretation: MH-R, OG-G, IC-F; Writing: AT-V, ET, FJA, MJP-P, CD, ML-L DB; Supervision and visualization: ET, EG-S, PM-P.

Availability of data and materials

The datasets analyzed during the current study are available from the cor-responding author on reasonable request

Ethics approval and consent to participate

The study was approved by the hospital's clinical ethics committee (code: PI 20-1717).

Consent to participate

Permission was obtained from all study participants

Consent for publication

Not applicable

Code availability

Not applicable.

Competing interests: The authors declare no conflict of interest.

- Alvaro Tamayo-Velasco declares no conflict of interest.
- Pedro Martínez-Paz declares no conflict of interest.
- María Jesús Peñarrubia declares no conflict of interest.
- Ignacio de la Fuente declares no conflict of interest.
- Sonia Pérez González declares no conflict of interest.
- Itziar Fernández declares no conflict of interest.
- Carlos Dueñas declares no conflict of interest.
- Esther Gómez-Sánchez declares no conflict of interest.
- Mario Lorenzo-López no conflict of interest.
- Estefanía Gómez-Pesquera no conflict of interest.
- María Heredia-Rodríguez declares no conflict of interest.
- Irene Carnicero-Frutos declares no conflict of interest.
- Maria Fe Muñoz-Moreno declares no conflict of interest.
- Óscar Gorgojo-Galindo declares no conflict of interest.

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Figures
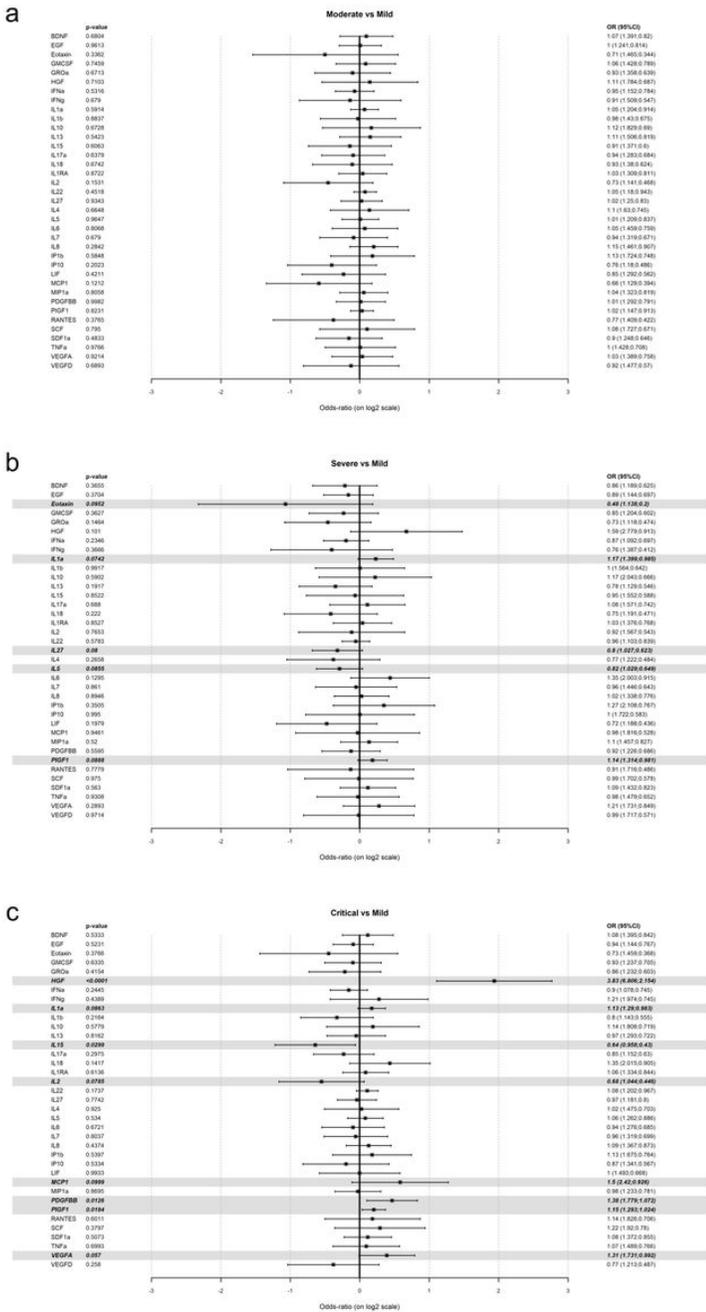
Figure 1

Individual multinomial models using the mild group as a reference. 1a: moderate. 1b: severe. 1c: critical.
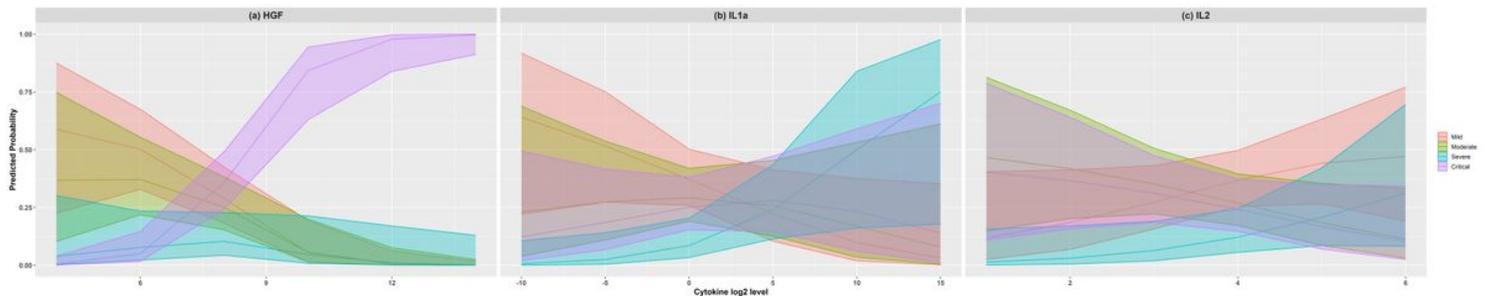
Figure 2

Effect plots of the estimated probabilities of belonging to each severity group according to the level of HGF (a), IL-1α (b) and IL-27 (c). The log2 level of each cytokine is measured in pg/ml.
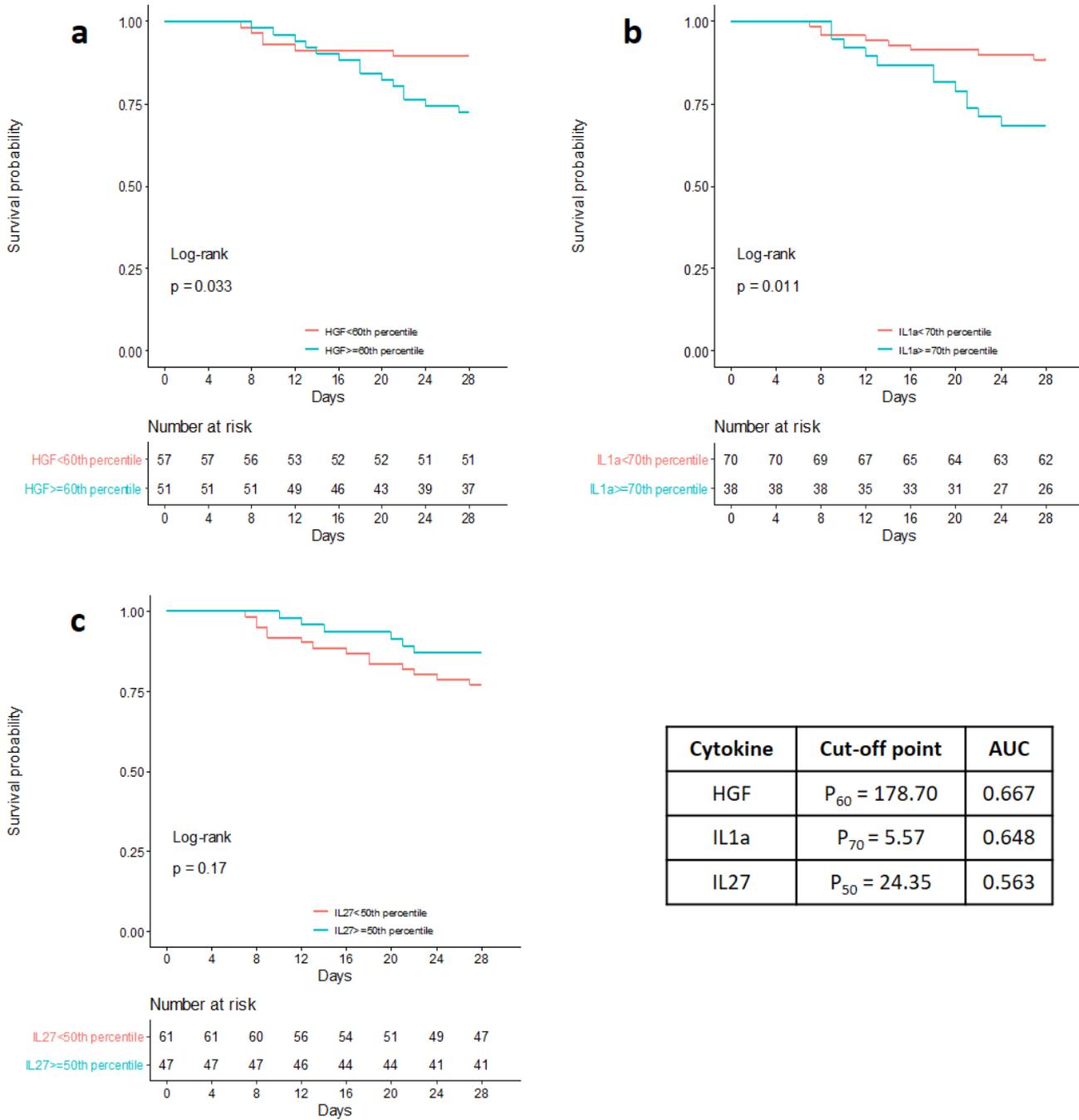

Cytokine	Cut-off point	AUC
HGF	$P_{60} = 178.70$	0.667
IL1a	$P_{70} = 5.57$	0.648
IL27	$P_{50} = 24.35$	0.563

Figure 3

Kaplan-Meier survival curves for HGF (a), IL-1α (b), IL-27 (c).

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