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More Evidence of the Link of Interleukin-6 and Interleukin-10 with Critical COVID-19: A Report in Mexican Patients

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ABSTRACT

Background: According to the World Health Organization, Mexico presents one of the highest mortality rates due to coronavirus disease 2019 (COVID-19). The "cytokine storm" phenomenon has been proposed as a pathological hallmark of severe COVID-19.

Objective: To determine the association of serum cytokine levels with COVID-19 severity.

Methods: We studied the cytokines IL-2, IL-4, IL-6, IL-10, TNF-α, and the IFN-γ serum levels through flow cytometry in 56 COVID-19 patients (24 critical and 32 non-critical) from Northwest Mexico.

Results: We observed a significant increase inthe IL-6 and the IL-10 levels in the sera of critical patients. These cytokines were also associated with mechanical ventilation necessity and death, IL-6 showing AUC values above 0.7 for both variables; and correlated with Na+, creatinine, and platelet levels. On the other hand, no association was found between IL-2, IL-4, TNF-α, and IFN-γ with tested variables.

Conclusion: Our results corroborate previous observations regarding IL-6 and IL-10 involvement in the severity of COVID-19.

Keywords: COVID-19, Cytokine, IL-6, IL-10, Mechanic ventilation

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (1). In Mexico, the first COVID-19 case was detected in late February 2020 and, up to January 2021, a cumulative of 1,534,039 confirmed cases with 133,706 deaths have been registered (1), positioning it among the countries with the highest mortality rates due to COVID-19.

Even though there are several COVID-19 epidemiological and clinical description studies, the exact cause of its clinical variability remains a topic under debate. However, the evidence shows that disease worsening in some COVID-19 patients is closely associated with dysregulated and excessive cytokine release, the so-called cytokine storm, which plays a central role in pneumonia, vascular endotheliitis, coagulopathy, and acute respiratory distress syndrome (2, 3).

According to recent research data, similar and contrasting results have been reported about the implication of inflammatory cytokines in the pathogenesis of COVID-19. One of the first studies observed a correlation between the disease severity and interleukin (IL)-2, IL-7, IL-10, and tumor necrosis factor (TNF)-α levels (4). Diao et al. and Qin et al. corroborated the finding for IL-10 and TNF-α and also reported elevated serum IL-6 levels (5, 6). On the other hand, Luo et al. did not detect IL-2, IL-4, TNF-α, and interferon (IFN)-γ in the serum of COVID-19 patients in the intensive care unit (ICU), whereas Song et al. found no differences in IL-4, IL-5, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ levels between the mild and the severe patients (7, 8).

To contribute to clarifying the cytokine role in COVID-19, we evaluated the serum levels of the cytokines IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ in Mexican COVID-19 patients in association with the clinical severity and definitive outcomes.

MATERIAL AND METHODS

For this ambispective (with retrospective and prospective components) and descriptive study, we recruited 56 patients hospitalized at Hospital General de Culiacán in the period from April 1 to May 7, 2020. A COVID-19 patient was defined based on a positive result in the SARS-CoV-2 RT-PCR test of nasal and pharyngeal swab samples (9).

This research protocol was approved by the Medical Research Ethics Committee of Hospital General de Culiacán. Informed consent was obtained in either verbal or written manner from the patients or their relatives. Procedures were performed following the ethical standards in accordance with the Helsinki Declaration.

Of the 56 COVID-19 patients, 32 were considered non-critical and 24 critical, risk stratification of disease severity that was based on Yang et al. criteria as follows (9). Briefly, patients presenting fever, headache, cough, respiratory symptoms, or chest pain without respiratory difficulties were considered as moderate COVID-19. Severe cases also presented a respiratory rate ≥30 breaths per minute and an oxygen saturation percentage <94. Both the moderate and the severe were considered as non-critical, whereas critical definition includes the aforementioned signs plus the need for mechanical ventilation, and/or the development of shock or multiple organ dysfunction syndrome.

Patient data (demographics, symptomatology, laboratory tests, comorbidities, and clinical outcomes) were collected from the electronic medical record system. Samples were taken on admission, corresponding to a median of 7 days (range: 2-9 days) after the clinical symptoms onset. Concentrations of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ were estimated from serum samples through flow cytometry using the Human Th1/Th2 Cytokine Kit II, BD™ Cytometric Bead Array (CBA) (Becton-Dickinson, San Jose, CA, USA), according to the manufacturer's instructions, in a BD Accuri™ C6 flow cytometer. Flow cytometry results were analyzed using the FCAP Array Software v3.0 (Becton-Dickinson, San Jose, CA, USA).

Statistical analysis was done in Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS, Chicago, IL, USA). Graphs were elaborated using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Quantitative data values with normal distribution were expressed as the mean (M) ± standard deviation (SD) and analyzed with the T-test and not normal data as median with

interquartile ranges (IQR) and analyzed with Mann-Whitney test. Categorical data were analyzed by χ^2 and binary logistic regression. Receiver operating characteristic (ROC) curves were performed to assess if the cytokines could predict patients' need for mechanical ventilation, and/or death. Additionally, comparisons between cytokine levels and other quantitative variables were performed by linear regression analysis. Values of the $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The inflammatory response is a normal defense mechanism of the immune system against a diversity of harmful agents. The term 'cytokine storm', first used in the early 90s, applied to maladaptive cytokine release both at local and systemic levels, in response to infection and other stimuli (2), and currently, for COVID-19 pandemic, it has become synonymous with damage and cause of death.

In the present study, we enrolled 56 COVID-19 of which 39 were male (with mean age of 52.29 years) and 18 females (with mean age of 55.39 years) distributed into 32 non-critical and 24 critical. Table 1 shows the clinical and laboratory features of these patients. It is shown that critical cases

Table 1. Demographic, clinical, and laboratory features of Mexican patients with Coronavirus disease 2019 (COVID-19).

Variable	Total n=57 (%)	Non-critical n=32 (%)	Critical n=25 (%)	P value*
Gender (Male)	39/57 (68.4)	20/32 (62.5)	19/25 (76)	0.273
Age (years, M±SD)	52.96±12.6	52.13±13.3	54.04±11.8	0.573
Body mass index	31.1 (28.8-34.8)	30.4 (28.1-33.9)	33.8 (30.8-35.8)	0.203
Type 2 diabetes	19/57 (33.3)	12/32 (37.5)	7/25 (28)	0.450
Hypertension	33/57 (57.9)	15/32 (46.9)	18/25 (72)	0.057
Cough	39/45 (86.7)	25/29 (86.2)	14/16 (87.5)	0.902
Headache	28/45 (62.2)	19/29 (65.5)	9/16 (56.2)	0.539
Fever	37/45 (82.2)	23/29 (79.3)	14/16 (87.5)	0.492
Myalgia/arthralgia	13/45 (28.9)	7/29 (24.1)	6/16 (37.5)	0.344
Thoracic pain	12/45 (26.7)	10/29 (34.5)	2/16 (12.5)	0.110
Dyspnea	53/57 (93)	28/32 (87.5)	25/25 (100)	0.028
Tachypnea (bpm ≥30)	13/56 (23.2)	4/32 (12.5)	9/24 (37.5)	0.035**
Oximetry <94%	33/53 (62.3)	16/30 (53.3)	17/23 (73.9)	0.126
Mechanic ventilation	23/57 (40.3)	0/32 (0)	23/25 (92)	<0.001
Organic failure	11/57 (19.3)	0/32 (0)	11/25 (44)	<0.001
Death	19/57 (33.3)	2/32 (6.2)	17/25 (68)	<0.001**
Hospitalization (days)	11 (8-16)	11.5 (8-16)	10.5 (7.2-17.5)	0.934
Glucose (mg/dl)	118 (91-153)	111 (86.5-135)	138 (107-214)	0.040
Creatinine (mg/dl)	0.79 (0.7-1.1)	0.77 (0.6-1)	0.95 (0.7-1.4)	0.075
LDH (IU/l)	377 (260-495)	367 (243-432.5)	482 (338-609.5)	0.074
Albumin (g/dl, M±SD)	2.6 ± 0.6	2.69 ± 0.7	2.24 ± 0.3	0.035
Na^+ (mEq/l, M±SD)	138.42 ± 5	137.47 ± 5.3	139.88 ± 4.3	0.096
Platelets (10^3/µl, M±SD)	322.63 ± 126.5	340.13 ± 130.1	300.92 ± 120.9	0.252
Leukocytes (10^3/µl)	11.01 (7.7-13.6)	8.73 (6.7-12)	13.1 (10.6-20.4)	<0.001
Neutrophils (10^3/µl)	9.56 (6.1-12.3)	7.17 (5.2-10.1)	12.1 (9.4-19.8)	0.001
Lymphocytes (10^3/µl, M±SD)	0.9 ± 0.4	0.98 ± 0.4	0.78 ± 0.2	0.046
Prothrombin time (seconds)	13.5 (12.9-14.8)	13.3 (12.8-13.8)	15.05 (13.5-18.1)	0.020

Quantitative variables are expressed as median (interquartile range) unless indicated otherwise. Frequencies show the number of individuals with the variable/total of individuals with available data. *Result of χ^2 or Fisher's exact tests, and Mann-Whitney or T-tests. **Logistic regression: Odds Ratio [confidence interval]=4.2 [1.1-15.9] for tachypnea and 31.87 [6.1-167.6] for death. bpm: breaths per minute; M±SD: mean±standard deviation; LDH: lactic dehydrogenase

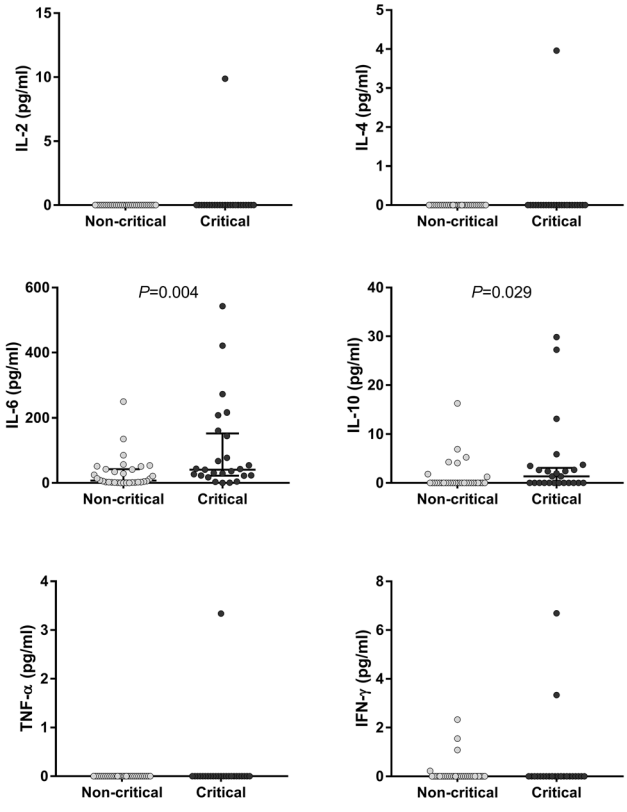

Figure 1. Serum cytokine levels in critical (n=24) and non-critical (n=32) COVID-19 patients. Individual values are shown, bars denote medians with interquartile ranges.

presented a higher frequency of dyspnea, tachypnea, and death; and higher values of total leukocytes, neutrophils, PT, and glucose (Table 1). Most of these clinical and laboratory findings have been observed to increase according to COVID-19 severity in previous reports, except for glucose (4, 6). Nevertheless, the latter has been considered as a bad prognosis factor in COVID-19 patients in a United States study (10).

In the analysis of serum cytokines, we observed that the critical patient group presented significantly higher IL-6 and IL-10 levels than the non-critical group, while IL-2, IL-4, TNF-α, and IFN-γ levels were detectable in only a few samples without presenting differences between the groups (Figure 1) and were, therefore, discarded for further analysis. Similar to our results, Luo et al. detected the IL-6 and IL-10 levels above reference ranges in the serum of COVID-19 patients in ICU but did not find detectable levels of IL-2, IL-4, TNF-α, and IFN-γ, although correlation analysis with severity was not reported (7). Additionally, several authors have found increased circulating levels of the IL-6, IL-10, and the TNF-α in critically ill patients, but did not report variations in IL-2, IL-4, and IFN-γ levels (4-6). Nevertheless, recent studies have reported higher serum IL-2 levels in asymptomatic/mild patients (11), a clinical category not considered for the present investigation; and increased levels of the IFN-γ and IL-4 in early and late stages of COVID-19 infection, respectively, regardless of severity (12); remarking the importance of longitudinal cytokine measurements in COVID-19 patients, which unfortunately were not possible for our study.

Following the association with severity, we found an association of increased levels of the IL-6 and the IL-10 with mechanical ventilation and death (Table 2). However, only the IL-6 showed a fair predictive power (AUC>0.7) for the aforementioned COVID-19 infection outcomes (Table 2). In this respect, Han et al. have proposed the increase of the IL-6 and the IL-10 levels as a predictor of

Table 2. ROC analysis of IL-6 and IL-10 serum levels in COVID-19 patients with unfavorable outcomes.

	Yes	No	P value*	AUC (95% CI)	Std error	P value**
Mechanic ventilation						
IL-6	42.72 (22.8-160.6)	7.13 (1.3-41.7)	0.001	0.752 (0.62−0.88)	0.065	0.001
IL-10	1.35 (0-3.5)	0 (0-0.3)	0.028	0.648 (0.5−0.8)	0.077	0.060
Death						
IL-6	42.72 (22.1-208.4)	13.39 (1.3-42.7)	0.003	0.740 (0.61−0.87)	0.066	0.003
IL-10	1.35 (0-3.7)	0 (0-0.3)	0.015	0.670 (0.51−0.82)	0.079	0.038

Cytokine values are expressed in pg/ml as median (interquartile ranges). *Result of Mann-Whitney test. **Result of ROC analysis.

Table 3. Linear regression analysis of the IL-6 and the IL-10 with laboratory results of COVID-19 patients*

Variable	B	Std error	Beta	t	P value
IL-6**					
Creatinine	25.525	10.718	0.325	2.382	0.021
Na^+	6.878	2.86	0.325	2.405	0.020
IL-10**					
Platelets	-0.013	0.006	-0.274	-2.096	0.041

*Only significant correlations are shown. **Dependent variable.

disease worsening (13). Additionally, IL-6 has been previously linked to the necessity of mechanical ventilation but IL-10 has not (14). In this respect, Diao et al. reported that the high levels of IL-6 and IL-10, observed in patients with severe COVID-19, correlate with a decrease in numbers and function of T helper (Th) 1 and T $CD8^+$ cells promoting an impaired adaptive response against SARS-CoV-2 infection (5). This finding was recently corroborated by the research of Gil-Etayo et al. in which it is also reported that the IL-6 and IL-10 could relate to an overactivation of the Th2 cell subset leading to disease worsening or even death (15).

Regarding symptoms and comorbidities, non of them (seen in Table 1) were found to be associated with the studied cytokines other than a trend favoring higher IL-6 levels in patients with hypertension (P=0.073)(data not shown). This opposes previous studies which showed diabetic and hypertensive COVID-19 patients exhibit higher serum IL-6 levels (16, 17), a difference which might be due to the study design and/or limitations since elevated IL-6 levels have been observed in diabetic and hypertensive patients regardless of COVID-19 (16, 18).

In the present work, a positive correlation of creatinine and Na^+ levels with IL-6 was observed (Table 3). These variables are related to kidney injury but, unfortunately, the kidney involvement was not monitored in our patients. Nevertheless, it has been hypothesized that during the course of COVID-19, low Na^+ status makes kidney involvement more likely due to the upregulation of membrane-bound ACE2 in the kidneys (19). Moreover, we observed a negative correlation of low platelet counts with IL-10 (Table 3), which might correspond to the previously reported association of thrombocytopenia with severe disease and increased mortality in COVID-19 patients (20).

Given the ambispective nature of our study, there is a lack of information in the laboratory results of some participants, which led to uneven values analyzed between the variables and might account for the lack of difference observed in variables previously reported and associated with the disease.

In conclusion, our research adds knowledge

on the participation of the IL-6 and the IL-10 in COVID-19 worsening but does not support a relevant role of the rest of the evaluated cytokines (IL-2, IL-4, TNF-α, and IFN-γ) in the pathology. The significant variability observed between the different studies invite us to reconsider the cytokine storm analysis within COVID-19 pathophysiology context that includes issues such as time of sample taking, type of sample, type of cytokine, the technique used for detection, cut-off points for each cytokine, and probable variability of cytokine expression according to the population genetic background or the geographical region.

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Conflicts of Interest: None declared.

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