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Impact of serum IL-10 level on the clinical outcome of COVID-19 patients and the development of post-COVID pulmonary fibrosis

Mariam M. Amin[1], Aliaa S. Sheha[2], Mahetab Moustafa[1], Eman N. Osman[1], and Hossam M. Elkady[1]

[1]Department of Internal Medicine, Allergy & Clinical Immunology, Faculty of Medicine, Ain Shams University. Cairo, Egypt.
[2]Department of Diagnostic Radiology Faculty of Medicine, Ain Shams University. Cairo, Egypt.

Corresponding author: Mahetab Moustafa, Department of Internal Medicine, Allergy & Clinical Immunology, Faculty of Medicine, Ain Shams University. Cairo, Egypt.
Email: mahetab.hany2@yahoo.com

Abstract

A characteristic feature of the cytokine storm in coronavirus disease 2019 (COVID-19) is the dramatic elevation of serum interleukin 10 (IL-10). This may be a negative feedback mechanism to suppress inflammation. However, this IL-10 elevation may contribute to COVID-19 severity. In our study, we aimed to evaluate the effect of serum IL-10 level on patients' clinical outcome and the incidence of post-COVID19 pulmonary fibrosis. This was a prospective observational study, included 100 patients, confirmed to have COVID-19, Of these, 50 patients had COVID-19 without evidence of pneumonia in computed tomography (CT) scans (group I) and the other 50 patients had COVID-19 pneumonia (group II). Our results showed a significant increase in serum ferritin level in patients with COVID pneumonia. However, no difference was found in serum C-reactive protein (CRP) nor D-Dimer between both groups. There was a statistically significant increase in serum IL-10 in patients with COVID pneumonia compared with COVID patients without pneumonia (p<0.001). Fibrosis was developed in 35 patients (70%) with COVID pneumonia after 3 months and 4 of them died, however, all patients without pneumonia survived. Among age, serum IL-10, aspartate aminotransferase (AST), alanine transaminase (ALT), elevated serum IL-10 was found to be an independent predictor of pneumonia (p=0.32). However, there was no significant effect for IL-10 on patients' clinical outcome. There was a statistically significant correlation between serum IL-10 levels and oxygen (O_2) demand, CRP and D-Dimer (p= 0.015, p=0.034 and p=0.042, respectively). The higher the level of IL-10 the less fibrosis detected in follow up CT scans (p=0.038). In conclusion, even though IL-10 was significantly associated with disease severity (higher in pneumonia), elevated serum Il-10 has an independent role in decreasing the incidence of post-COVID-19 pulmonary fibrosis.

Keywords: Interleukin 10 (IL-10), post-COVID pulmonary fibrosis, transforming growth factor Beta.

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Introduction

In December 2019, China reported a cluster of pneumonia cases caused by a novel coronavirus related to the agent of severe acute respiratory syndrome (SARS) and was termed Coronavirus-19 (COVID-19) (SARS-CoV-2)[1]. The infection spread to all continents and was declared as a

pandemic by the World Health Organization (WHO).[1] Based on the severity of presenting illness, the United States of America (USA), National Institutes of Health (NIH) issued guidelines that classify COVID-19 into five distinct types: asymptomatic, mild, moderate, severe and critical illness.[2]

Patients with severe COVID-19 develop a pathological state termed cytokine release syndrome that is characterized by dysregulated immune response.[3] Cytokine release syndrome may induce acute respiratory distress syndrome and secondary hemophagocytic lympho-histiocytosis, which may predispose to multi-organ failure and death.[4]

Interleukin-10 (IL-10) acts as an anti-inflammatory cytokine and is essential for immune system homeostasis and inflammatory response modulation.[5,6] High serum IL-10 can predict poor outcomes in COVID-19 patients.[7] Hence, IL-10 might play a role in COVID-19 disease progression.[8] Early induction of IL-10 upon SARS-CoV-2 infection during the initiation phase in the lung might represent a negative feedback mechanism that serves as a counter measure to inflammation caused by other proinflammatory mediators.[8] However, as IL-10 production increases, it might function as an immune activating/proinflammatory agent that stimulates the production of other mediators of the cytokine storm.[8] IL-10 might also amplify the viral sepsis-related hyperinflammation in critically ill COVID-19 patients and contribute to exacerbating disease severity.[8]

One of the most important complications of COVID-19 infection is the development of pulmonary fibrosis.[9] During post-viral conditions, dysregulated inflammation is assumed to have a role in lung damage and fibrosis.[9] IL-10 also has a potent anti-fibrotic activity.[9] It may compete with other pro-fibrotic cytokines such as transforming growth factor Beta to inhibit fibrosis as well as suppression of the activity of fibroblasts and amelioration of pulmonary fibrosis.[9]

In our study, we aimed to evaluate the effect of serum IL-10 on patients' clinical outcome, severity of COVID-19 related symptoms and the incidence of post-COVID19 pulmonary fibrosis. This may open the gates towards the development of new treatment options to improve the outcome of COVID-19 patients, in terms of decreasing the severity of infection and lowering the incidence of post-COVID pulmonary fibrosis.

Subjects and Methods

This prospective observational study included 50 individuals confirmed to have COVID-19 pneumonia and another group of 50 individuals matched for age and gender, having COVID-19 without pneumonia, as a control group. Patients above 18 years old who were confirmed to have COVID-19 clinically, radiologically and by the polymerase chain reaction (PCR) were recruited from Ain shams University hospitals between the period 2021 to 2022.

Pregnancy or breastfeeding women, patients with severe renal impairment (creatinine clearance <30 ml / min) or severe hepatic impairment [aspartate aminotransferase (AST) or alanine transaminase (ALT) >5 times the normal limits in International Units], or having any previous underlying chest conditions, or patients with severe cardiac insufficiency or cancer were excluded from the study. We also excluded patients with immunodeficiency or patients receiving immune modulators or individuals already enrolled in other clinical trials.

All study participants were managed according to the local COVID-19 protocol. Patients were followed up by high resolution computed tomography (CT) scans for exploring the development of pulmonary fibrosis at 3 months post infection.

A complete history of symptomatology and clinical examination was carried out for all participants. We assessed the participants' severity according to the USA Center for Disease Control (CDC) and NIH (2020) into mild, moderate, severe, or critical disease.[10] Mild disease included mild symptoms like diarrhea, fever, cough, up to mild pneumonia. Moderate disease included clinical or radiological evidence of lower respiratory tract affection, oxygen (O_2) saturation ≥ 94%, severe disease included dyspnea, hypoxia, or >50% lung involvement on

imaging, and critical disease included respiratory failure, shock, or multiorgan system dysfunction.[10]

The clinical status was assessed by the Seven-category ordinal scale (WHO R&D Blueprint expert group). These included (1) not hospitalized with resumption of normal activities; (2) not hospitalized, but unable to resume normal activities; (3) hospitalized, not requiring supplemental oxygen; (4) hospitalized, requiring supplemental oxygen; (5) hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; (6) hospitalized, requiring extracorporeal membrane oxygenation, invasive mechanical ventilation, or both; and (7) death.[11]

Complete blood count, inflammatory markers including C Reactive Protein (CRP), erythrocyte sedimentation rate (ESR), Ferritin by the enzyme linked immunosorbent assay (ELISA), Lactate Dehydrogenase (LDH), and D-dimer were investigated. Renal function tests (Urea and creatinine) and liver enzymes (ALT, AST) were also included. Data for all these parameters were collected from the patients' hospital records.

A venous blood sample (2 ml) was collected from each study participant using aseptic venipuncture technique. Serum samples were separated by centrifugation and kept frozen at -20°C until used. Serum IL10: was assessed using commercial kits (Catalogue No. 201-12-0103, Human Interleukin 10 ELISA Kit, SunRed Biotechnology Company, *Shanghai*, China), according to the manufacturer's instructions.

Computerized tomography

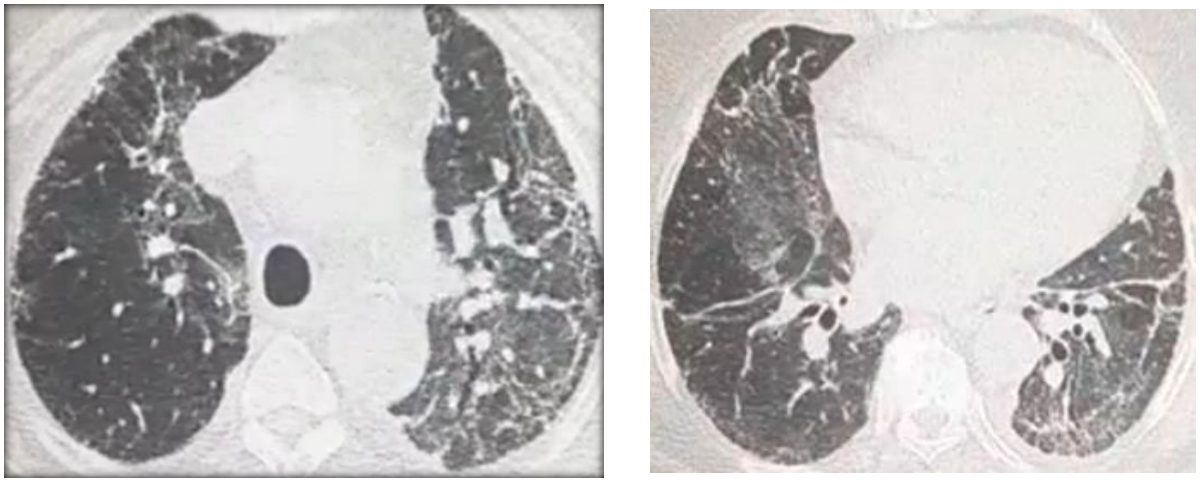
The development of COVID-19 pneumonia was assessed radiologically by an experienced author. Non-contrast high resolution CT scans were done at 8-14 days from the onset of symptoms. The degree of involvement of the lungs was assessed using a scoring system according to the study of Chung et al., 2020 by a CT machine (80-slice CT machine, Prime Aquilion, Toshiba, USA).[12] Each of the five lung lobes was assessed for the degree of involvement and classified as none (0%), minimal (1%–25%), mild (26%–50%), moderate (51%–75), or severe (76%–100%). No involvement corresponded to a lobe score of 0, minimal involvement to 1, mild involvement to 2, moderate involvement to 3, and severe involvement to 4.[12] An overall lung "total severity score" was reached by summing the five lobe scores (range of possible scores, 0–20).[12]

The development of post-COVID19 pulmonary fibrosis was assessed radiologically three months from hospitalization. Radiographic patterns were categorized and quantitated using a severity scoring system developed by acute respiratory distress syndrome net and used in acute respiratory distress syndrome survivors and classified into two groups (non-fibrotic or fibrotic).[13] Fibrotic-like patterns included those with reticulations, traction bronchiectasis or honeycombing (Figure 1). The extent of fibrosis was determined and assessed in both lungs for different patterns of fibrosis and the number of involved lung lobes to evaluate the radiological severity.[13]

Statistical Analysis

Data were fed to the computer and analyzed using the statistical package for the social sciences (SPSS) software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data are described as numbers and percentages. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data are described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). We used the Chi-square test for categorical variables, to compare between different groups, Fisher's Exact or Monte Carlo correction for correction for chi-square when more than 20% of the cells have expected count less than 5. The student t-test was utilized for normally distributed quantitative variables, to compare between two studied groups. The Mann Whitney test was chosen for abnormally distributed quantitative variables, to compare between two studied groups. To correlate between two distributed abnormally quantitative variables, we used the Spearman coefficient. Logistic Regression was used to detect the most affecting factor for affecting pneumonia in COVID patients. The

significance of the obtained results was judged at the 5% level.



A. B.

Figure 1. (A and B.) 62-year-old diabetic hypertensive patient presented with fever, dyspnea and desaturation. 3 months follow up CT showed diffuse bilateral lung fibrotic changes with ground glass opacities, reticulations, traction bronchiectasis and honeycombing. Ground Glass Opacities 1.2, reticulations 7, traction bronchiectasis 3.4, honeycombing 2.8.

Results

Comparison of age and gender between Group I (COVID-19 pneumonia) and Group II (COVID-19 without pneumonia), showed no statistically significant difference in gender. However, there was a significant increase in COVID pneumonia with increasing age, the mean age among group I was 59.92 ± 12.60 years compared to 52.50 ± 19.68 years in group II. Of the 50 patients in group I, 16 patients were smokers, compared to 9 patients in group II, with no statistical significance difference (Table 1).

Table 1. Comparison of the demographic data between the two study groups.

	Group I (COVID pneumonia) (n = 50)		Group II (COVID-19 without pneumonia) (n = 50)		*p* value
	No.	%	No.	%	
Gender					
Male	26	52.0	24	48.0	NS
Female	24	48.0	26	52.0	
Age (years)					
Minimum – Maximum.	32.0 – 85.0		18.0 – 98.0		0.028
Mean ± SD.	59.92 ± 12.60		52.54 ± 19.60		
Smoking habit					
No	34	68.0	41	82.0	NS
Yes	16	32.0	9	18.0	

p > 0.05 is not significant (NS). SD: Standard deviation;

Significant increase in fever, cough, dyspnea, and bony aches was detected in patients with COVID pneumonia compared to patients with COVID without pneumonia with (p=0.011, p=0.009, p<0.001 and p=0.001, respectively) (Table 2). Also, there was a significant increase in the oxygen demand and significant decrease in the oxygen saturation in patients with COVID pneumonia compared with patients without pneumonia. (p<0.001) (Table 3).

Table 2. Comparison of COVID-19 symptoms between the two study groups

Symptoms of COVID-19	Group I (COVID-19 pneumonia) (n = 50)		Group II (COVID-19 without pneumonia) (n = 50)		*p* value
	No.	%	No.	%	
Fever	43	86.0	32	64.0	0.011
Cough	41	82.0	29	58.0	0.009
Dyspnea	37	74.0	19	38.0	<0.001
Bony aches	19	38.0	5	10.0	0.001
Loss of taste and smell	0	0.0	2	4.0	^{FE}NS
GIT Symptoms	15	30.0	15	30.0	NS

p > 0.05 is not significant (NS). FE: Fisher Exact.

Table 3. Comparison of Oxygen ($O_{2)}$ demand and O_2 saturation between the two studied groups.

	Group I (COVID-19 pneumonia) (n = 50)	Group II (COVID-19 without pneumonia) (n = 50)	*p* value
O_2 demand (mg/L)			
Minimum – Maximum	0.0 – 10.0	0.0 – 3.0	<0.001
Median (IQR)	4.0 (2.0 – 6.0)	0.0 (0.0 – 0.0)	
O_2 saturation (%)			
Minimum – Maximum	83.0 – 98.0	94.0 – 99.0	<0.001
Mean ± SD.	94.34 ± 2.07	96.28 ± 1.37	

p ≤ 0.05 is significant. SD: Standard deviation;

Comparing the laboratory investigations between the two studied groups, there was a statistically significant increase in AST, ALT and hemoglobin in patients with COVID pneumonia (group I) (Table 4). Also, comparing inflammatory markers between the two study groups, there was a statistically significant increase in serum ferritin level in patients with COVID pneumonia. However, there was no difference in serum CRP or D-Dimer in group I compared to group II (Table 5). Table 6 illustrates the statistically significant increase in IL-10 in patients with COVID pneumonia compared to patients without COVID pneumonia. (*p*<0.001).

Table 4. Comparison of laboratory investigations between the two studied groups.

	Group I (COVID-19 pneumonia) (n = 50)	Group II (COVID-19 without pneumonia) (n = 50)	*p* value
Total leucocytic count (cells/ microliter)			
Minimum – Maximum	2.40 – 19.50	3.10 – 35.40	NS
Median (IQR)	7.30 (6.0 – 9.30)	7.95 (5.20 – 11.50)	
Lymphocytes (cells/ microliter)			
Minimum – Maximum	0.30 – 11.60	0.20 – 2.60	NS
Median (IQR)	1.20 (0.80 – 1.50)	1.20 (0.85 – 1.80)	
Hemoglobin (g/dL)			
Minimum – Maximum	8.40 – 15.20	5.70 – 15.80	0.048
Mean ± SD.	12.57 ± 1.52	11.79 ± 2.30	

Table 4. Continued.

	Group I (COVID-19 pneumonia) (n = 50)	Group II (COVID-19 without pneumonia) (n = 50)	*p* value
Platelets (cells/ microliter)			
Minimum – Maximum	123.0 – 464.0	84.0 – 495.0	NS
Mean ± SD.	252.3 ± 80.84	276.0 ± 105.1	
AST (in U/L)			
Min. – Max.	7.0 – 170.0	8.0 – 139.0	0.015*
Median (IQR)	32.0 (21.0 – 50.0)	24.50 (19.0 – 34.0)	
ALT (in U/L)			
Min. – Max.	6.0 – 159.0	4.10 – 60.0	0.001
Median (IQR)	33.0 (20.0 – 54.0)	20.50 (14.0 – 28.0)	
Total Bilirubin (mg/dL)			
Min. – Max.	0.20 – 1.40	0.10 – 2.10	NS
Median (IQR)	0.60 (0.30 – 0.70)	0.40 (0.30 – 0.70)	
Direct Bilirubin (mg/dL)			
Min. – Max.	0.10 – 0.80	0.0 – 1.80	NS
Median (IQR)	0.20 (0.10 – 0.30)	0.20 (0.10 – 0.30)	
Albumin (in g/dL)			
Min. – Max.	2.70 – 5.70	2.30 – 5.20	NS
Mean ± SD	3.74 ± 0.52	3.78 ± 0.55	

IQR: Inter quartile range; SD: Standard deviation; ALT (alanine transaminase); AST (aspartate aminotransferase).
$p > 0.05$ is not significant (NS).

Table 5. Comparison of different inflammatory markers between the two studied groups.

Inflammatory markers	Group I (COVID-19 pneumonia) (n = 50)	Group II (COVID-19 without pneumonia) (n = 50)	*p* value
CRP (mg/l)			
Minimum – Maximum	0.17 – 796.0	1.34 – 206.0	NS
Median (IQR)	51.0 (15.98 – 96.0)	44.35(13.10 – 72.0)	
Ferritin (µg/l)			
Minimum – Maximum	9.0 – 2708.2	18.40 – 7700.0	0.003
Median (IQR)	594.5 (214.0 – 1122.0)	270.5 (1122.0 – 510.0)	
D. Dimer (ng/ml)			
Minimum – Maximum	239.0 – 9246.0	0.60 – 10000.0	NS
Median (IQR)	1030.0 (552.0 – 1600.0)	645.0 (404.0 – 1519.0)	

IQR: Inter quartile range; CRP (C-Reactive Protein) $p > 0.05$ is not significant (NS).

Table 6. Comparison of serum IL-10 level between the two studied groups.

Serum IL-10 level	Group I (COVID-19 pneumonia) (n = 50)	Group II (COVID-19 without pneumonia) (n = 50)	*p* value
Minimum – Maximum.	210.0 – 4000.0	80.0 – 980.0	<0.001
Median (IQR)	400.0 (300.0 – 630.0)	230.0 (180.0 – 300.0)	

IQR: Inter quartile range. $p \leq 0.05$ is significant.

As regard severity in patients with COVID pneumonia, 58% of the patients had severe disease, 24 % moderate and 18 % mild disease, while all the COVID patients without pneumonia had mild disease (p<0.001). The Seven-category ordinal score demonstrated that most of the patients with COVID pneumonia were hospitalized and 34% required nasal oxygen therapy, 54% required noninvasive mechanical ventilation, or 2% required invasive ventilation. Meanwhile, it was demonstrated that most patients of COVID without pneumonia (80%) were hospitalized but with no need for supplemental oxygen. (Table 7).

Table 7. Comparison of the COVID-19 severity and the seven-category ordinal Score between the two studied groups.

Studied parameter	Group I (COVID-19 pneumonia) (n = 50)		Group II (COVID-19 without pneumonia) (n = 50)		*p* value
	No.	%	No.	%	
Score of COVID-19 disease severity					
Mild	9	18.0	50	100.0	
Moderate	12	24.0	0	0.0	<0.001
Severe	29	58.0	0	0.0	
Seven category ordinal score					
1	0	0.0	0	0.0	
2	0	0.0	6	12.0	
3	4	8.0	40	80.0	
4	17	34.0	4	8.0	^{MC}p<0.001
5	27	54.0	0	0.0	
6	1	2.0	0	0.0	
7	1	2.0	0	0.0	
Minimum – Maximum	3.0 –7.0		2.0 –4.0		<0.001
Mean ± SD.	4.56 ±0.76		2.96 ±0.45		

IQR: Inter quartile range; SD: Standard deviation; MC: Monte Carlo. $p \leq 0.05$ is significant.
1: Not hospitalized with resumption of normal activities.
2: Not hospitalized, but unable to resume normal activities
3: Hospitalized, not requiring supplemental oxygen
4: Hospitalized, requiring supplemental oxygen.
5: Hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both
6: Hospitalized, requiring ECMO, invasive mechanical ventilation, or both
7: Death

Regarding the severity of lung involvement in patients with COVID pneumonia (Group I) at admission, 17 patients (34%) had grade I, 32 patients (64%) grade II lung involvement, and one patient (2%) grade III lung involvement (Table 8). As shown in (Table 9), IL-10 level was not different in patients with severe COVID infection compared to patients with mild and moderate COVID infection.

Table 8. Distribution of the 50 studied cases according to grade of lung involvement among Group I (COVID-19 pneumonia).

Grade of lung involvement	No. of cases	%
Grade I	17	34.0
Grade II	32	64.0
Grade III	1	2.0

Table 9. Relation between COVID-19 disease severity and serum IL-10 level (pg/ml) in the 50 patients of group I (COVID-19 pneumonia).

Serum IL-10 level (pg/ml)	COVID-19 disease Severity			*p* value
	Mild (n= 3)	Moderate (n= 12)	Severe (n = 29)	
Mean ± SD.	338.89 ± 58.62	473.33 ± 203.31	650.69 ± 682.79	NS
Median (Minimum – Maximum)	310.0 (250.0 – 410.0)	380.0 (300.0 – 900.0)	500.0 (210.0 – 4000.0)	

SD: Standard deviation. $p > 0.05$ is not significant (NS).

The relation between serum IL-10 levels and the Seven category score in group I (patients with COVID pneumonia) is highlighted in Table 10 which showed no differences in IL-10 levels among the 7-category ordinal score. However, IL-10 levels were highest in groups 5 and 7.

Table 10. Relation between serum IL-10 level and the Seven category score in the 50 patients of group I (COVID-19 pneumonia).

Seven category score	No of patients	Serum IL-10 level		*p* value
		Mean ± SD	Median (Minimum – Maximum)	
3	4	350.0 ± 57.74	350.0 (300.0 – 400.0)	NS
4	17	431.18 ± 184.35	380.0 (250.0 – 900.0)	
5	27	668.89 ± 702.96	500.0 (210.0 – 4000.0)	
6	1[#]		210.0	
7	1[#]		600.0	

#: Excluded from the comparison due to small number of case (n = 1). $p > 0.05$ is not significant (NS).

The relation between grades of pneumonia and serum IL-10 levels (pg/ml) in group I (n = 50) is demonstrated in Table 11. It showed no difference in serum IL-10 levels between the different groups. However, serum IL-10 levels were highest in grade II, considering that there was only one patient with grade III pneumonia as shown by CT scans.

Table 11. Relation between grades of pneumonia and Serum IL-10 level (pg/ml) in the 50 patients of group I (COVID-19 pneumonia).

Serum IL-10 level (pg/ml)	Grade				*p* value
	Grade 0 (n= 3)	Grade I (n= 17)	Grade II (n = 29)	Grade III (n = 1[#])	
Mean ± SD.	370.0 ± 60.83	445.88 ± 180.87	644.83 ± 684.33	210.0	NS
Median (Minimum – Maximum)	400.0 (300 – 410.0)	380.0 (250.0 – 800.0)	500.0 (210.0 – 4000.0)		

SD: Standard deviation

#: Excluded from the comparison due to small number of case (n = 1)

$p > 0.05$ is not significant (NS).

Table 12 shows that among the different studied parameters (age, serum IL-10, AST, and ALT), elevated serum IL-10 was the strongest predictor of pneumonia (p=0.32). Table 13 illustrates that there was a statistically significant correlation between serum IL-10 level and O_2 demand, CRP and D-Dimer (p= 0.015, p=0.034 and p=0.042, respectively).

Table 12. Univariate and multivariate Logistic regression analysis for the parameters affecting pneumonia in the COVID patients (50 with pneumonia vs. 50 without pneumonia).

	Univariate		#Multivariate	
	p value	OR (95% CI)	*p* value	OR (95% CI)
Males	NS	1.174 (0.536 – 2.572)		
Females	NS	0.852 (0.389 – 1.867)		
Age (years)	0.031	1.028 (1.003 – 1.054)	NS	1.016 (0.944 – 1.094)
Diabetes mellitus	NS	0.711 (0.316 – 1.601)		
Hypertension	NS	1.941 (0.867 – 4.346)		
Ischemic heart disease	NS	1.872 (0.512 – 6.848)		
Total leucocytic count (cells/microliter)	NS	0.941 (0.858 – 1.032)		
Neutrophils (cells/ microliter)	NS	0.923 (0.832 – 1.024)		
Lymphocytes (cells/ microliter)	NS	1.119 (0.811 – 1.545)		
Hemoglobin (g/dL)	NS	1.237 (0.997 – 1.533)		
Platelets (cells/ microliter)	NS	0.997 (0.993 – 1.002)		
BUN (mg/dL)	NS	1.008 (0.990 – 1.026)		
Creatinine (mg/dL)	NS	2.849 (0.897 – 9.046)		
Sodium (mEq/L)	NS	1.011 (0.917 – 1.115)		
Potassium (mmol/L)	NS	0.610 (0.290 – 1.286)		
AST (U/L)	0.040	1.021 (1.001 – 1.041)	NS	1.020 (0.969 – 1.074)
ALT (U/L)	0.001	1.045 (1.017 – 1.073)	NS	1.043 (0.998 – 1.089)
Total Bilirubin (mg/dL)	NS	1.137 (0.347 – 3.729)		
Direct Bilirubin(mg/dL)	NS	0.567 (0.086 – 3.743)		
Albumin (g/L)	NS	0.872 (0.414 – 1.837)		
INR	NS	6.036 (0.348 – 104.57)		
C-reactive protein (mg/L)	NS	1.004 (0.999 – 1.009)		
Ferritin (µg/L)	NS	1.000 (1.000 – 1.001)		
D. Dimer (ng/ml)	NS	1.000 (1.000 – 1.000)		
Serum IL-10 level (pg/ml)	<0.001	1.010 (1.006 – 1.015)	0.032	1.007 (1.001 – 1.013)
Seven category score	<0.001	47.996 (11.784 – 195.49)	NS	9.232 (0.749 – 113.738)

OR: Odd`s ratio; C.I: Confidence interval; ALT (alanine transaminase); AST (aspartate aminotransferase); INR (international normalized ratio). $p > 0.05$ is not significant (NS).
#: All variables with $p<0.05$ were included in the multivariate

Table 13. Correlation between serum Interlunkin-10 (IL-10) level and different parameters among group I (COVID-19 pneumonia).

Different parameters	Serum IL-10 level	
	r_s	*p* value
Age (years)	0.251	NS
O_2 demand (mg/L)	0.341	0.015
O_2 saturation (%)	-0.173	NS
Total leucocytic count (cells/ µl)	0.157	NS
Neutrophils (cells/ µl)	0.181	NS
Lymphocytes (cells/ µl)	0.094	NS
Hemoglobin (g/dL)	-0.004	NS
Platelets (cells/ µl)	0.128	NS
BUN (mg/dL)	-0.029	NS
Creatinine (mg/dL)	-0.137	NS
Sodium (mEq/L)	0.135	NS
Potassium (mmol/L)	0.050	NS
AST (U/L)	-0.028	NS
ALT (U/L)	-0.114	NS
Total Bilirubin (mg/dL)	-0.089	NS
Direct Bilirubin (mg/dL)	0.027	NS
Albumin (g/L)	0.046	NS
INR	0.016	NS
C-reactive protein (mg/L)	0.300	0.034
Ferritin (microgram/L)	0.008	NS
D. Dimer (ng/ml)	0.289	0.042

r_s: Spearman coefficient; ALT (alanine transaminase); AST (aspartate aminotransferase); INR (international normalized ratio); BUN (blood urea nitrogen). $p > 0.05$ is not significant (NS).

All patients with COVID infection without pneumonia survived, while 4 patients with COVID infection with pneumonia died because of severe COVID infection and other comorbidities, with no statistically significant difference between the two studied groups. Also, there was no significant effect for IL-10 levels either on intensive care unit (ICU) admission or on patients' outcome (Table 14). As seen in Table 15, the higher the level of IL-10 the less the fibrosis in the CT scan follow up (p=0.038).

Table 14. Relation between serum IL-10 level and disease outcome in the 50 patients of group I (COVID-19 pneumonia).

	Number	Serum IL-10 level		*p* value
		Mean ± SD.	Median (Minimum – Maximum)	
ICU admission and ventilation				
No	45	557.1 ± 565.6	400.0 (210.0 – 4000.0)	NS
Yes	5	506.0 ± 231.4	600.0 (210.0 – 710.0)	
Disease outcome				
Survived	46	549.6 ± 561.6	400.0 (210.0 – 4000.0)	NS
Death	4	580.0 ± 186.7	650.0 (310.0 – 710.0)	

SD: Standard deviation; ICU: intensive care unit; ICU (intensive care unit). $p > 0.05$ is not significant (NS).

Table 15. Relation between fibrosis score and serum IL-10 level in 46 patients of group I (COVID-19 pneumonia).

Serum IL-10 level	Fibrosis		*p* value
	Negative (n = 11)	Positive (n = 35)	
Median (Minimum – Maximum)	600 (300 – 1180)	380 (210 – 4000)	0.038

$p \leq 0.05$ is significant.

Discussion

A common cause of death among patients with COVID-19 is the overproduction of pro-inflammatory cytokines.[14] The dramatic early rise in IL-10 – canonically classified as an anti-inflammatory cytokine – appears to be a distinguishing feature of hyperinflammation in patients with COVID-19,[8] and several studies indicated that IL-10 levels can predict the poor disease outcome.[7,15] Based on its well-established role as an anti-inflammatory and immunosuppressive cytokine, serum IL-10 elevation could be considered as an attempt to temper hyperinflammation and prevent tissue damage.[16]

However, the concurrent elevations in IL-10 and various pro-inflammatory cytokines, and the observed relationship between elevated IL-10 levels and COVID-19 disease severity, suggest that IL-10 is either failing to appropriately suppress inflammation or acting in a manner that deviates from anti-inflammatory role.[17,18] However, the seemingly paradoxical observation of concurrently elevated IL-10 and pro-inflammatory cytokine levels can be explained by the ability of IL-10 to act as a pro-inflammatory and immunostimulatory molecule under certain contexts.[8]

Another suggested explanation is the potential escape of activated immune cells from IL-10's anti-inflammatory action (IL-10 "resistance") leading to overexuberant pro-inflammatory cytokine responses.[19] Although typically classified as anti-inflammatory and immunosuppressive cytokine, the effects of IL-10 are highly context-dependent. There are several scenarios where IL-10 enhances immune cell activation and proliferation causing the release of pro-inflammatory cytokines.[19]

What is well known is the multi-faceted immune regulatory role of IL-10, both in protecting the lung from injury and in defense against infections, as well as its potential cellular source.[20] While the absence of an IL-10 response in SARS is thought to contribute to early deterioration, IL-10 can protect the lung from early immune-mediated damage and interfere with viral clearance in COVID-19.[20]

Our study aimed to illustrate the impact of serum IL-10 level on COVID-19 patients in terms of developing fibrosis post COVID-19 infection in addition to its effect on patients' clinical outcome. This study included 100 patients who proved to have COVID-19 infection by PCR, of

whom, 50 patients had pneumonia (group 1) and the other 50 patients without pneumonia (group 2), as proved by radiological investigations. Our patients' ages ranged between 18 and 98 years. We detected a significant increase in COVID pneumonia with increasing age, the mean age among group I (COVID with pneumonia) was 59.92 ± 12.60 years compared to 52.50 ± 19.68 years in group II (COVID without pneumonia). This is in line with what was reported by Zhao et al., 2020, who stated that increasing age was associated with progression of COVID-19 disease.[7] They stated that pre-existing hypertension cardiovascular and respiratory conditions were also associated with progression of the disease. However, in our study there was no significant difference between the two groups as regard past medical history. This difference between the two study findings could be because their study included healthy individuals with no significant comorbidities.[7]

We detected a significant increase in oxygen demand and significant decrease in oxygen saturation in patients with COVID pneumonia compared with patients without pneumonia (p<0.001). This was in line with the findings of the study by Zhao and colleagues, 2020 who concluded that blood oxygen saturation measured in the severe patients was much lower than that in mild patients (p<0.001).[7]

Meanwhile the same study of Zhao and colleagues, 2020, concluded that the absolute number of white blood count, neutrophils, and monocytes in the severe group were much higher than that in the mild group (p< 0.05). However, there was no significant difference of lymphocyte between the mild and severe groups.[7] On the contrary, our study showed no significant difference between laboratory investigations in both studied groups, except for elevated AST and ALT in patients with COVID pneumonia. This difference between the two studies could be related to the larger number of mild cases in their study (53 patients) compared to severe patients (18 patients).[7] In the present study, regarding the inflammatory markers, a significant increase in serum ferritin level was detected in patients with COVID pneumonia, and this finding was observed across multiple studies.[21-23] However, no significant difference in serum CRP nor D-Dimer between both groups was found.[21-23]

The study by Han et al., 2020 concluded that IL-10 and CRP were increased along with COVID-19 disease severity.[15] In our study, IL-10 levels were also increased with disease severity, however, the CRP rise in patients with severe COVID-19 was not statistically significant.[15]

In the correlation between IL-10 and CRP, the results of the study by Han et al., 2020 showed that CRP was significantly positively correlated with IL-10 ($r = 0.41$, $p < 0.01$).[15] In the same line, our study illustrated that there was a statistically significant positive correlation between serum IL-10 level and CRP (p= 0.034).

In our study, there was a statistically significant increase in IL-10 in patients with COVID pneumonia compared with patients without COVID pneumonia (p<0.001). This finding comes in parallel with the findings of most of the previous studies.[7, 24, 25] For instance, Zhao and colleagues, 2020, concluded that IL-10 was significantly associated with disease severity.[7] Also, the study by Le Bert, 2021, showed that symptomatic patients with severe disease had higher levels of IL-10.[24] Moreover, the study by Mulchandani and coworkers, 2021, stated that the inflammatory cytokines, especially circulating IL-10, were significantly elevated among severe cases, as compared to the mild-to-moderate COVID-19 patients.[25]

As shown in the study by Han, 2020, high IL-10 expression was found to predict poor outcomes in COVID-19 patients. However, in our study there was no significant relation between IL-10 and the patients' outcome. This difference in study findings could be related to the difference in sample size between the two studies.[15]

In our study, there was no significant effect for IL-10 on ICU admission and mechanical ventilation. However, in the study by Blot and coworkers, 2020, it was shown that IL-10 was independently associated with the duration of mechanical ventilation.[26] This difference between the two studies could be related to the finding that the greater percentage of our patients were moderate cases, as a smaller number of severe cases was recruited.

However, they also declared that there was no statistically significant association between the COVID-19 status and IL-10, which may be explained by that IL-10 is not the only or main driver of the length of mechanical ventilation in COVID-19 patients.[26]

In our study, the relation between grades of pneumonia and serum IL-10 levels (pg/ml) in group I (patients with COVID pneumonia) demonstrated that levels of serum IL-10 were not statistically significantly different between the different study groups. However, serum IL-10 levels were highest in grade II, considering that there was only one patient with grade III pneumonia as shown in CT scans.

In our study, the relation between serum IL-10 levels and the Seven category score in group I (patients with COVID pneumonia) showed no statistically significant differences between IL-10 levels in the different 7-category ordinal score groups. However, IL-10 levels were highest in group 5 and 7.

In a study by Hend et al., 2022, serum IL-10 was significantly higher in the severe and moderate groups compared to the mild group (p<0.001 and p=0.003, respectively).[27] Although higher levels were detected in the severe group compared to the moderate group, the difference was not statistically significant (p=1.00).[27] Such results were in line with our findings that IL-10 was higher in patients with severe COVID infection compared to patients with mild and moderate COVID infection. However, the results were not statistically significant.

For the correlation between IL-10 and CRP, the results of the study by Han et al, 2020, showed that CRP was significantly positively correlated with IL-10 ($r = 0.41$, $p < 0.01$).[15.]Meanwhile, our study illustrated that there was a statistically significant positive correlation between serum IL-10 level and CRP (P= 0.034).

In our study, we observed that the higher the level of IL-10 the less the fibrosis in the follow up CT scan, (p=0.038). This was in line with the finding of the study by Nakagome et al., 2006, performed on mice, where IL-10 was shown to suppress the development of pulmonary fibrosis in a TGF-β1-dependent manner, where in TGF-β1 production by alveolar macrophages was suppressed by IL-10 both in-vivo and ex-vivo.[28] IL-10 suppresses the production and activation of TGF-β in the lung and thus attenuates pulmonary fibrosis, even when delivered in chronic phase.[28]

In conclusion, IL-10 was significantly associated with disease severity (higher in pneumonia), elevated serum Il-10 has an independent role in decreasing the incidence of post-COVID-19 pulmonary fibrosis. Although IL-10 is classified as an anti-inflammatory and immunosuppressive cytokine it can increase the rate of COVID induced pneumonia. Elderly people are more liable to develop COVID pneumonia and other complications. Among the studied parameters, (age, serum IL-10, AST, ALT) elevated serum IL-10 was the strongest predictive of the development of pneumonia in COVID positive patients.

Author Contributions

MM, MA, HE, AS and EN proposed the topic of this research and designed the study. MM collected the data. All the authors contributing to preparing the final draft of the manuscript, revised the manuscript and critically reviewed the intellectual contents. In addition, they have all read and approved the final manuscript and are responsible for its accuracy and integrity.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the local Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 179/2021).

Informed consent

An informed written consent was obtained from each participant before being included in the study.

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