

## ORIGINAL ARTICLE

# Diagnostic Value of Serum Level of Interleukin 33 (IL-33), C-C Motif Chemokine Ligand 17 (CCL17) and Interferon Gamma Inducible Protein-10 (IP-10) in Coronavirus Disease 2019 (COVID-19) Patients

<sup>1,2</sup>Nouran M. Moustafa\*, <sup>2</sup>Rania A. Mohamed, <sup>3</sup>Rania G. Elsaid, <sup>2</sup>Fatma M. Mahmoud

<sup>1</sup>Basic Medical Science Department, Faculty of Medicine, Dar Al Uloom University, Riyadh, Saudi Arabia,

<sup>2</sup>Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

<sup>3</sup>Anesthesia, Intensive Care and Pain Management Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

## ABSTRACT

### Key words:

SARS CoV- 2 , IL-33, CCL17, IP-10, Cytokine Storm , COVID-19

### \*Corresponding Author:

Nouran Magdy Moustafa  
Riyadh, KSA  
Tel.: 0537267420  
[Nouran.m@dau.edu.sa](mailto:Nouran.m@dau.edu.sa)

**Background:** Coronavirus disease 2019 (COVID-19) has a diverse course; It seems to be asymptomatic or may produce mild symptoms which occurs in majority of the cases, however cytokine storm, macrophage activation syndrome (MAS), and acute respiratory distress syndrome (ARDS) could also happen as complications in some patients.

**Objective:** This study aims to evaluate the serum level of IL-33, CCL17 and IP-10 in moderate and severe cases of COVID-19 and its diagnostic value. **Methodology:** IL-33, CCL17 and IP-10 were measured in the serum specimens of 40 cases of COVID-19, including 20 severe and 20 moderate patients, and compared with 20 healthy controls.

**Results:** The values of IL-33, CCL17 and IP-10 were significantly higher in COVID-19 cases over than healthy controls. IL-33, CCL17 and IP-10 were excellent predictors for COVID-19, and the three cytokines together exhibited the greatest area under the curve (0.957). **Conclusions:** In this study, we report biomarkers that are highly associated with COVID-19 and can be used as diagnostic biomarkers. These findings aid to our understanding of the immunopathology of SARS CoV2 infection and provide potential targets and strategies for therapy.

## INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a clinical condition caused by a unique RNA virus called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). It occurred originally in December 2019 in China and outspread everywhere and announced by the World Health Organization (WHO) as a worldwide pandemic in March 11, 2020. The beta-coronavirus SARS-CoV-2, is related to two previous coronaviruses, Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV) and the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) that caused fatal infections in the last two decades <sup>1</sup>.

While SARS-CoV-2 produces asymptomatic or mild infections in nearly all cases, atypical pneumonia and severe acute respiratory distress syndrome might occur in almost 10–20% of the patients, particularly in elderly and in those with co-morbidities <sup>2</sup>. This category of COVID patients is noted to have increased values of D-dimer and serum ferritin, liver dysfunction, propensity to thrombosis, and disseminated intravascular coagulopathy (DIC). In fact, these prominent features

mainly denote the incidence of macrophage activation syndrome (MAS) and cytokine storm<sup>3</sup>. The same information were also documented in patients having SARS-CoV and MERS-CoV viral diseases <sup>4</sup>.

The recent studies found that the alarm protein cytokine interleukin-33 (IL-33) is a key factor that could drive all stages of COVID-19. Following infection and cellular injury, IL-33 is rapidly released by damaged epithelial alveolar cells<sup>5</sup>. IL-33 may enhance differentiation and expansion of Foxp3+ T regulatory cells (Treg), thus enhancing inflammation regression<sup>6</sup>. In susceptible people, IL-33 might provoke regulatory T cells that express dysregulated GATA-binding factor 3, thus breaking immune tolerance and causing SARS CoV2-Induced autoinflammation of the lung. In COVID-19 severe cases, the axis of IL-33–ST2 might act through expanding the T cells expressing pathogenic granulocyte–macrophage colony-stimulating factor, dampening antiviral interferon actions, eliciting hyperinflammation, and favoring thromboses and pulmonary fibrosis <sup>7</sup>.

CCL17, thymus activation regulated chemokine (TARC), is essentially found in thymus, and transiently in stimulated peripheral blood monocytes, macrophages

and dendritic cells<sup>8</sup>. This chemokine stimulates the development of T cells and promotes its activation at inflammatory areas. It is a selective chemoattractant for T cells, especially Th2, and T regulatory cells, that have the chemokine receptor 4 (CCR4)<sup>9</sup>. Recent study found that CCL17 could be valuable marker in the discrimination between the pre-severe, mild, moderate, and critical cases<sup>10</sup>.

The chemokine interferon- $\gamma$  (IFN- $\gamma$ ) inducible protein or IP-10/CXCL10 is produced by many cells including neutrophils, fibroblasts, and dendritic cells. It binds to chemokine receptor 3 (CXCR3) on T cells, monocytes, and NK cells causing their activation and recruitment thus making a great tissue damage<sup>11</sup>. It is informed that increased IP-10 is accompanied with severe H5N1, H1N1, SARS-CoV, and MERS-CoV infections<sup>12</sup>. Recently, IP-10 elevation in COVID-19 patients and the higher elevation in ICU patients, suggest its association with severity and progression of disease<sup>4</sup>.

This study aims to evaluate the serum level of IL-33, CCL17 and IP-10 in moderate and severe cases of COVID-19 and its diagnostic value.

## METHODOLOGY

A total of 40 blood samples were collected from RT-PCR confirmed COVID-19 patients admitted to Ain Shams University Hospital from October 2020 to January 2021. These patients were categorized, according to WHO recommendations for COVID-19 clinical management, into 20 severe cases and 20 moderate cases<sup>13</sup>. Blood samples were collected also from 20 healthy controls.

Complete history was taken, and the patients were divided according to the predisposing diseases into; 10 diabetes mellitus (DM) (25.0%), 16 hypertension (HTN) (40.0%), 7 chronic kidney diseases (CKD) (17.5%), 5 Hepatic disease (12.5%), 3 ischemic heart disease (IHD) (7.5%), 2 bronchial asthma (5.0%) and one chronic obstructive pulmonary disease (COPD) (2.5%).

Clinical information and blood samples for laboratory tests were obtained early after hospitalization. The serum level of IL-33, CXCL-10 and CCL17 in the three groups was identified through the use of the enzyme-linked immunosorbent assay (ELISA) kit. The laboratory results and the patients' outcome were evaluated. The approval was taken for the study protocol by the Ethics Committee of Ain Shams University Hospitals (No FWA000017585 taken on 6 April 2021). Informed consents were got from all patients or their family members.

### Specimen collection:

Under complete aseptic procedure, 3 ml blood were taken from each participant, in a tube without additives for ELISA. The samples were transported to the

laboratory of Ain Shams University Hospitals for further steps.

### Separation of the serum for ELISA

Blood samples were allowed to clot for 10-20 minutes at room temperature, then the samples were centrifuged at 500- 1000 x g for 20 minutes. The serum was collected in a tube and stored at (-80°C) till investigations could be done by means of the IL-33, CXCL10 and CCL17 ELISA kits (Biotech., LTD, China). These kits use a double-antibody sandwich ELISA to assay the IL-33, CXCL10 and CCL17.

### Detection of IL- 33, CXCL10 and CCL17

The serum samples were added to the wells pre-coated with the specific monoclonal antibodies and then incubated. After that, anti-antibodies labeled with biotin and combined with streptavidin-HRP were added to form immune complex. Unbound enzymes after incubation were removed through washing. Chromogen Substrate Solution A and B were added. The liquid turned blue and changed into yellow with the effect of acid in the positive cases. The chroma of color and the concentration of IL-33, CXCL10 and CCL17 were positively correlated.

### Data Management and Analysis

SPSS program was used for statistical analysis. The quantitative parametric data were presented as mean, standard deviations and ranges, while non-parametric data were presented as median, inter-quartile range (IQR). Also, qualitative variables were presented as number and percentages. To compare qualitative data between two different groups, Chi-square test was utilized. As well, to compare quantitative parametric data between two groups, we can use Independent t-test, while in non-parametric distribution Mann-Whitney test was used. The comparison between more than two groups regarding numerical parametric data was done by using One Way ANOVA test while with non-parametric distribution was done by using Kruskal-Wallis test. Correlations between paired data were investigated using the Spearman rank correlation coefficient. The ROC AUC of IL-33, CCL17 and IP10 serum levels was estimated for normal group and COVID-19 cases. A P value < 0.05 indicates statistical significance.

## RESULTS

### Epidemiological and clinical characteristics

This study was conducted on 40 patients who were diagnosed as COVID-19 patients and admitted to isolation rooms in Ain Shams University Hospitals, 19 of them were females (47.5%) and 21 were males (52.5%) with mean age  $49.38 \pm 15.65$ . The control group was 20 healthy control subjects, 10 of them were females (50%) and 10 were males (50%) with mean age of  $44.35 \pm 14.47$ . The study was done in the period from

July 2020 to October 2021. All patients in the study were exposed to collection of blood samples.

There was no significant difference in age, gender and predisposing diseases between healthy, moderate and severe groups except for chronic kidney disease (p

value: 0.046) (table.1) .While there was a highly significant difference in CRP, D dimer, total T cell count, CD4 & CD8 count where CRP, D-dimer were increased in moderate and severe groups while total T cell count, CD4 and CD8 are decreased (table.2).

**Table1. Comparison of epidemiological and clinical characteristics between healthy control, moderate and severe groups**

		Healthy control group	Moderate group	Severe group	Test value	P-value	Sig.
		No. = 20	No. = 20	No. = 20			
Age	Mean $\pm$ SD	44.35 $\pm$ 14.47	49.65 $\pm$ 16.11	49.10 $\pm$ 15.59	0.716•	0.493	NS
	Range	26 – 72	26 – 77	27 – 77			
Sex	Females	10 (50.0%)	9 (45.0%)	10 (50.0%)	0.133*	0.935	NS
	Males	10 (50.0%)	11 (55.0%)	10 (50.0%)			
Predisposing factors	No	10 (50.0%)	8 (40.0%)	5 (25.0%)	2.679*	0.262	NS
	Yes	10 (50.0%)	12 (60.0%)	15 (75.0%)			
Predisposing factors	DM	3 (15.0%)	4 (20.0%)	6 (30.0%)	1.375*	0.503	NS
	HTN	7 (35.0%)	7 (35.0%)	9 (45.0%)	0.564*	0.754	NS
	CKD	0 (0.0%)	2 (10.0%)	5 (25.0%)	6.146*	0.046	S
	Hepatic disease	0 (0.0%)	4 (20.0%)	1 (5.0%)	5.673*	0.059	NS
	IHD	2 (10.0%)	1 (5.0%)	2 (10.0%)	0.436*	0.804	NS
	Asthmatic	1 (5.0%)	1 (5.0%)	1 (5.0%)	0.000*	1.000	NS
	COPD	1 (5.0%)	1 (5.0%)	0 (0.0%)	1.034*	0.596	NS

DM diabetes mellitus, HTN hypertension, CKD chronic kidney diseases, IHD ischemic heart disease, COPD chronic obstructive pulmonary disease.

No significant difference in age, gender and predisposing diseases (except CKD) between healthy, moderate and severe groups. P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant.

\*: Chi-square test; •: One Way ANOVA test

**Table 2. Comparison of laboratory findings between healthy control, moderate and severe groups**

		Healthy control group	Moderate group	Severe group	Test value	P-value	Sig.
		No. = 20	No. = 20	No. = 20			
CRP	Median (IQR)	–	15 (12 – 23.3)	81.75 (25 – 146.75)	-3.761##	0.000	HS
	Range	–	7.8 – 65	7.4 – 309			
D.dimer	Median (IQR)	–	0.65 (0.45 – 1.8)	2.75 (1.43 – 8.6)	-3.236##	0.001	HS
	Range	–	0.3 – 11.2	0.4 – 202			
Total T cell count	Mean $\pm$ SD	1348.22 $\pm$ 281.92	687.78 $\pm$ 326.03	337.15 $\pm$ 101.06	80.687•	0.000	HS
	Range	808.6 – 1677.5	303.6 – 1468.7	184.8 – 513.9			
CD4 count	Mean $\pm$ SD	797.78 $\pm$ 239.90	402.33 $\pm$ 181.70	191.10 $\pm$ 64.56	60.069•	0.000	HS
	Range	445.9 – 1159.2	155.6 – 866	71.1 – 291			
CD8 count	Mean $\pm$ SD	501.74 $\pm$ 137.06	254.08 $\pm$ 142.04	153.05 $\pm$ 70.93	43.901•	0.000	HS
	Range	327.6 – 736.8	77.5 – 491.4	31.4 – 275.2			
Post hoc analysis							
		Control Vs moderate group	Control Vs severe group	Moderate Vs severe group			
Total T cell count		0.000	0.000	0.000			
CD4 count		0.000	0.000	0.000			
CD8 count		0.000	0.000	0.011			

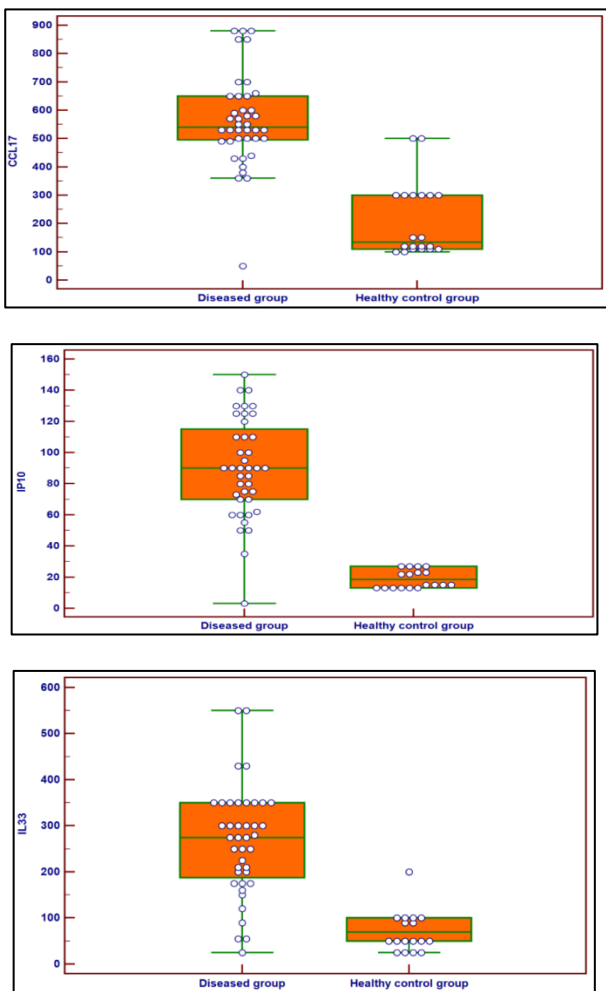
There is highly significant difference in CRP, D-dimer, total T cell count, CD4<sup>+</sup> T cell&CD8<sup>+</sup> T cell counts where CRP, D-dimer are increased in moderate and severe groups while total T cell counts, CD4<sup>+</sup> T cell counts&CD8<sup>+</sup> T cell counts are decreased.

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

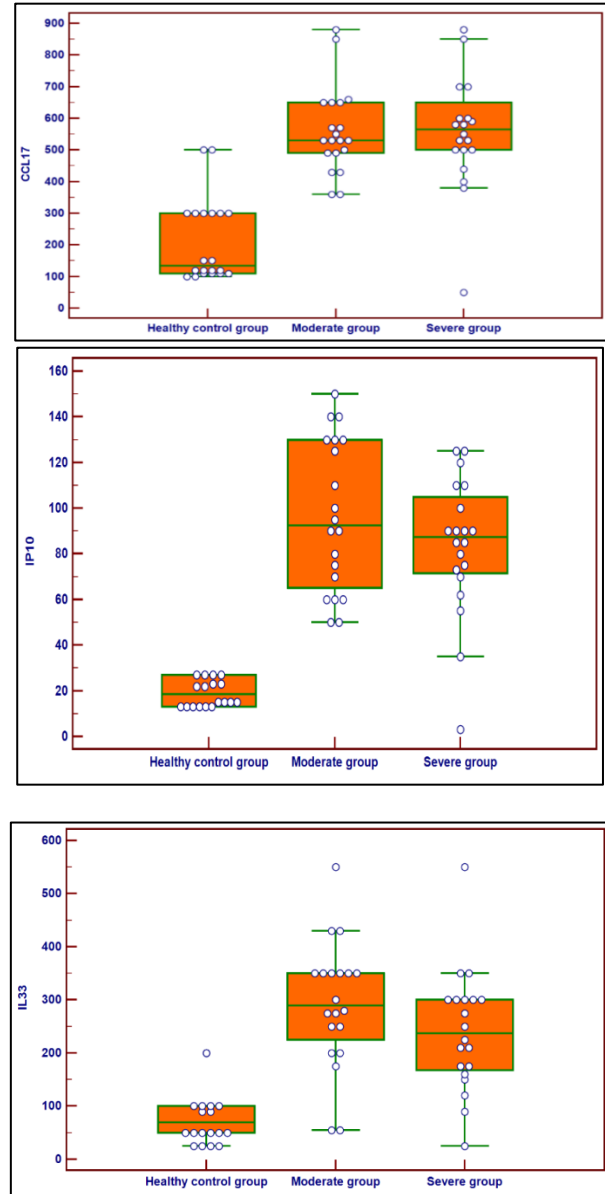
•: One Way ANOVA test; ##: Mann-Whitney test

**Cytokines expression profile in COVID 19 patients with distinct disease severity**

We analysed the cytokines expression profile in COVID-19 patients upon admission. Results revealed that IL-33, IP-10 and CCL17 were significantly elevated in COVID-19 patients upon admission, in comparison to the healthy control group (figure1). After that, the differential expression profiles of patients having different disease severity were further analyzed but there was no statistically significant increase in IL-33, IP-10 and CCL17 level between the moderate and severe group (figure2)



**Fig. 1.** Comparison of CCL17, IP-10 and IL-33 respectively between diseased and healthy group. There is statistically significant difference in CCL17, IP-10 and IL-33 level between healthy and diseased group

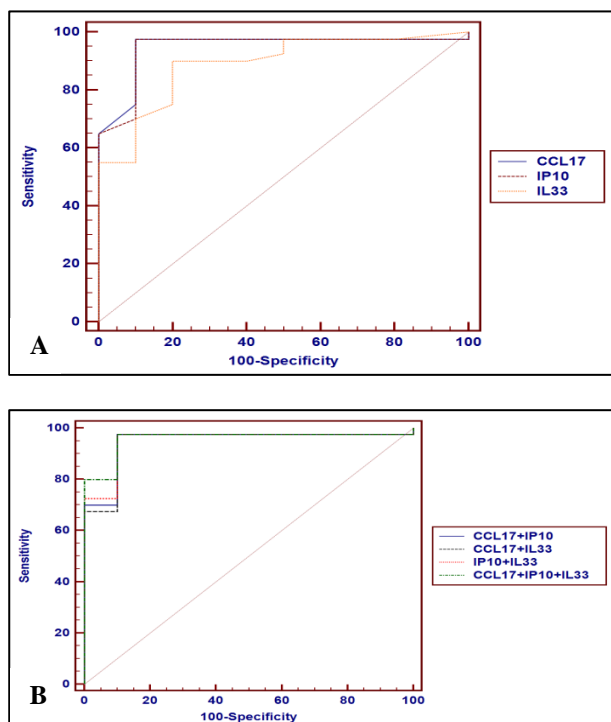


**Fig. 2.** Comparison of CCL17, IP-10 & IL-33 between healthy control, moderate and severe groups. Although there is a statistically significant difference in CCL17, IP-10 and IL-33 level between healthy and diseased group, there is no statistically significant difference between the moderate and severe groups in the three cytokines levels.

### IP-10, CCL17, and IL-33 are predictors for COVID-19

After that, we analyzed if these cytokines could be utilized as predictors for COVID-19. The ROC curve of every cytokine was calculated using the expression levels upon admission. Results revealed that the AUC of the ROC curve was 0.947 for CCL17, 0.945 for IP-10 and 0.889 for IL33. Then we examined the different combinations of these cytokines with each other for the prediction of disease. Combinations of the three cytokines exhibited the highest AUC of 0.957, after that the combination of IP-10 and IL-33 (0.950), then the combination of IP-10 and CCL17 (0.947) and finally combination of CCL17 and IL33 (0.945) (figure3).

IP-10, CCL17 and IL-33 were excellent predictors for the COVID-19 but couldn't be used as predictors of disease severity and progression.



**Fig. 3.** COVID-19 patients' receiver operating characteristic (ROC) curve for plasma cytokine levels. (A) The ROC curve of each single cytokine: Area under the curve (AUC) for CCL17 (0.947), IP-10 (0.945) and IL33(0.889). (B) The ROC curve of different combinations of the cytokines, AUC for CCL17 and IP-10 (0.947), CCL17 and IL33 (0.945), IP-10 and IL-33 (0.950). Combinations of the three cytokines exhibited the highest AUC of (0.957)

### DISCUSSION

Rigorous efforts are ongoing to reveal the immunopathogenesis of COVID-19, resulted from SARS-CoV-2 infection, and to control the pandemic. Considered as public health emergency with insufficient antiviral treatment, and rapid progression of lung disease, COVID-19 patients are mostly treated as if they had secondary haemophagocytic lymphohistiocytosis or MAS. These treatments depend on therapies that neutralize the major cytokines such as interleukin-6 (IL-6; eg, tocilizumab) or interferon gamma (IFN $\gamma$ ; eg, emapalumab) driving classical MAS<sup>14</sup>.

COVID-19, however, does not look like typical MAS in several aspects. The unique COVID-19 cytokine signature could provide keys to the disease immunopathogenesis and its potential therapies in the future<sup>7</sup>. We introduce here a pathogenic model where the cytokine alarmin, IL-33, is considered as a crucial player which drives all COVID-19 stages of disease. We found a high statistically significant increase in serum levels of IL-33 (p-value: 0.000) between the healthy control and diseased groups but no significant difference was found between moderate and severe groups.

Consistent with our result, it was found that SARS-CoV-2 peptide provoked expression of IL-33 in seropositive people. IL-33 is associated with activation of CD4+ T cell in PBMCs from convalescent subjects and was mostly due to the effects of T cell on IL-33-producing cells. An increase in population of cells that produce IL-33 was revealed during evaluation of published scRNAseq data on bronchoalveolar lavage fluid (BALF) from mild to severe COVID-19 patients. All these data illustrate that production of IL-33 is related to infection with SARS-CoV-2<sup>15</sup>. High level of IL-33, TNF, IL-1 $\beta$ , IL-6 and IL-8 was reported to be significantly related to the adverse effects across all age groups, with IL-33 and IL-1 $\beta$  having the greatest effect. The larger effect of IL-33 and IL-1 $\beta$  could be justified by membership of IL-33 as one of IL-1 superfamily, as well being an alarmin<sup>5</sup>.

Another study<sup>16</sup> has revealed that IL-33 was variable by Transcriptomic analysis of bronchoalveolar lavage fluid among patients with COVID-19.

In contrary to our result, the median serum levels of IL-33 in patients with COVID-19 were found at a very low level in control subjects and COVID-19 patients without significant difference, proposing that IL-33 function may be inhibited or may not be activated after SARS-CoV-2 infection<sup>17</sup>.

All these information indicate that IL-33 plays an essential role in pathogenesis and immune response to COVID-19. Well consideration of the major forces in an



every patient's inflammatory reaction to COVID-19 provides the probable for exact therapy, as proposed by Behrens et al<sup>18</sup>.

We also propose that CCL17 is important in driving moderate and severe stages of COVID-19 as we detected a high statistically significant increase in serum levels of CCL17 (p-value: 0.000) between the healthy and diseased groups with no significant difference between moderate and severe groups. So, it can be recognized as one of chemokines responsible for pathogenicity of the disease and a biomarker for diagnosis.

Another study identified CCL17 as a potential factor for myeloid recruitment in the lung during SARS-CoV-2 and there was an association with absolute numbers of monocytes in blood, viral load measured in bronchial brushes and CCL17 in non-human primate models. They also observed that the number of Th2 lymphocytes and their cytokines such as IL-5 and IL-13 gradually increase in the airway. These data showed a chain of immune events that occur in the lung as a host immune response to the virus, and that could progress into other less efficient antiviral responses<sup>19</sup>.

However, other studies reported different results<sup>9</sup>. They have measured serum level of CCL17 in 28 COVID-19 patients in serial manner. They reported that CCL17 showed greater levels in the mild and moderate group than in the severe and critical group but the cause of low level of CCL17 in severe cases is not known. At the early phase of COVID-19 disease, CCL17 value can differentiate between the groups with different severity levels and could be a promising prognostic marker, allowing separation between cases, thus avoiding overwhelming medical facilities. However, these data were not matched with healthy controls. Difference in results may be due to low number of COVID-19 patients, characters of patients included in both studies and times of samples collection.

Others have investigated the transcriptome profiles of immune cells in COVID-19 patients with pneumonia and recognized expression of CCR4 as an upregulated target in comparison to the healthy controls<sup>20</sup>. It was reported that hydroxychloroquine interacts with CCR4 and inhibits interaction of CCL17, trafficking of lymphocyte and release of cytokines<sup>21</sup>. These data propose a possible role for using CCR4 antagonists and CCL17 blocking antibodies in the management of COVID-19 patients. Also, CCR4 receptor antagonists and hydroxychloroquine have demonstrated efficacy in the prevention and treatment of septic shock<sup>22,23</sup>.

CCL 17 production from activated blood monocytes and macrophages is linked to SARS-CoV-2 infection. Binding of CCL17 to its receptor CCR4 expressed on Th2 and T regulatory cells confirms the role of Th2 cells in pathogenesis of COVID 19. This will provide

unique visions into the immunologic effectors that may help in the immunopathogenesis of COVID-19.

We found also that IP10 could drive moderate and severe stages of COVID-19 as we detected a high statistically significant increase in serum levels of IP-10 (p-value: 0.000) between the healthy control and diseased groups with no significant difference between moderate and severe groups.

In recently published studies, specifically intended to evaluate the role of biomarkers detections in COVID-19 severity and progression and in consistence with our results, they reported that IP-10 was significantly raised in COVID-19 patients in comparison to healthy controls. But more specifically, they found that the IP-10 expression levels had a strongly significant positive association with viral load and disease severity and was considered as an excellent predictor for the progression of COVID-19, showing different expression profiles according to different disease severity<sup>11,24,25</sup>.

It was also reported that patients infected with SARS-CoV-2 especially patients admitted to ICU showed a great increase in levels of proinflammatory cytokine, especially IP-10 and MCP-1, which may result in the T-helper-1 (Th1) cell activation. They assumed that IP-10 and MCP-1 might be associated with death in patients with COVID-19<sup>4</sup>.

Moreover, other findings may provide new visions into the distinctive pathogenesis of COVID-19 ARDS<sup>26</sup>. CXCL10 may cause dysregulation of immune response that determines the duration of mechanical ventilation (MV) in COVID-19 ARDS patients. CXCL10 seems to be a potential biomarker for the duration of MV, and the signaling axis of CXCL10-CXCR3 could be a probable target for therapy in ARDS due to COVID-19.

Moreover, it was demonstrated that IP-10, away from any other inflammatory factor, could impeded endothelial recovery. Anti-CXCL10 antibody is under trial to be used to prevent cardiovascular incidents as higher serum IP-10 level is present in severe COVID-19 patient. Thus, anti-IP-10 antibody treatment could be considered a new approach in COVID-19 and could add to SARS-CoV-2 treatment<sup>27</sup>.

Bioinformatics study on the sequencing data of COVID-19 was performed to show the changes of immune genes' expression in the body after COVID-19 infection, and to detect the key chemokines that cause the cascade of cytokine storm in the body. This study showed that the core gene was CXCL10 and it was indeed highly expressed in infected patients. They found also that samples with increased expression of CXCL10 were considerably enriched with CD4 and CD8 immune related signature, and the samples with decreased expression of CXCL10 were significantly enriched with monocytes and DC immune related signature. It was concluded that CXCL10 might be a major gene associated with the cytokine storm of COVID-19

infection, and it is anticipated to be the therapeutic target<sup>28</sup>.

All these findings together suggest that IP10 has a significant role in immunopathogenesis of COVID-19 and could be related to disease severity and progression.

## CONCLUSION

We compared variable profiles of cytokine expression among healthy controls, moderate and severe COVID-19 cases and discovered that they were highly increased in COVID-19 cases and might be considered as diagnostic markers. So, detecting the levels of IP-10, CCL17 and IL-33 in patients with early stage COVID-19 may give valuable information for the preparation of specific treatment strategy. Our results helped in the recognition of the SARS-CoV-2 immunopathological mechanisms and provided likely goals and approaches for therapy.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

**Author contributions:** Nouran M Moustafa significantly participated to the study's design and manuscript writing. Rania G Elsaid helped in the samples and data collection. Fatma Mostafa and Rania A Mohamed assisted in the laboratory work.

**Acknowledgements:** The authors extend their appreciation to the Deanship of Postgraduate and Scientific Research at Dar Al-Uloom University for funding this work.

**Ethics statement:** This study was approved by the Ethical Committee of Scientific Research of the Faculty of Medicine, Ain Shams University, Cairo, Egypt. It's no is FWA000017585

## REFERENCES

1. Paules CI, Marston HD, Fauci AS. Coronavirus infections—more than just the common cold. *Jama*. 2020; 323(8), 707-708.
2. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clinical rheumatology*. 2020;39, 2085-2094.
3. Crayne CB, Albeituni S, Nichols KE, Cron, RQ. The immunology of macrophage activation syndrome. *Frontiers in immunology* .2019; 10, 119.
4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020; 395(10223), 497-506.
5. Gabryelska A, Kuna P, Antczak A, Białasiewicz P, Panek M. IL-33 mediated inflammation in chronic respiratory diseases—understanding the role of the member of IL-1 superfamily. *Front Immunol*. 2019; 10:692.
6. Yuan Li, Lujun Chen. COVID-19 Associated Inflammatory Pathways. *Am J Biomed Sci & Res*. 2021-11(5). AJBSR. MS.ID.001679. DOI: 10.34297/AJBSR.2021.11.001679.
7. Zizzio G, Cohen PL. Imperfect storm: Is interleukin 33 the Achilles heel of COVID 19?. *Lancet Rheumatol*. 2020; (12)e779-e790.
8. Foti M, Locati M. Cytokine Effector Functions in Tissues. Academic Press; 2017 Jul 17. 199-200. ISBN978-0-12-804214-4.
9. Korbecki J, Grochans S, Gutowska I, Barczak K, & Baranowska-Bosiacka I. CC Chemokines in a Tumor: A Review of Pro-Cancer and Anti-Cancer Properties of Receptors CCR5, CCR6, CCR7, CCR8, CCR9, and CCR10 Ligands. *International Journal of Molecular Sciences*. 2020;21(20), 7619.(8-Korbeck J, Kojder K, Simińska D, Bohatyrewicz R, Gutowska I, Chlubek D and Baranowska-Bosiacka I. CC Chemokines in a Tumor: A Review of Pro-Cancer and Anti-Cancer Properties of the Ligands of Receptors CCR1, CCR2, CCR3, and CCR4. *Int. J. Mol. Sci.*, 2020.21(21), 8412; <https://doi.org/10.3390/ijms21218412>)
10. Sugiyama M, Kinoshita N, Ide S, Nomoto H, Nakamoto T, Saito S., et al. Serum CCL17 level becomes a predictive marker to distinguish between mild/moderate and severe/critical disease in patients with COVID-19. *Gene*. 2021 Jan 15;766:145145.
11. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine & growth factor reviews*. 2020 Jun 2.
12. Faure E, Poissy J, Goffard A, et al. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? *PLoS One* 9, e88716, doi:10.1371/journal.pone.0088716 (2014).
13. COVID-19 Clinical management: living guidance. January 2021. <https://www.who.int>

14. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmunity reviews*.2020; 19(6),102537.
15. Stanczak MA, Sanin DE, Apostolova P, Nerz G, Lampaki D, Hofmann M. IL-33 expression in response to SARS-CoV-2 correlates with seropositivity in COVID-19 convalescent individuals. *Nature communications*. 2021; 12(1), 1-9.
16. Xiong Y, Liu Y, Cao L, Wang D , Guo M, Jiang A. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerging microbes & infections*.2020; 9(1), 761-770.
17. Zeng Z, Hong XY, Li Y, Chen W, Ye G, Li Y, Luo Y. Serum-soluble ST2 as a novel biomarker reflecting inflammatory status and illness severity in patients with COVID-19. *Biomark Med*. 2020 Dec;14(17):1619-1629. doi: 10.2217/bmm-2020-0410. PMID: 33336592.
18. Behrens EM, Koretzky GA. Review: cytokine storm syndrome: looking toward the precision medicine era. *Arthritis Rheum*.2017;69(6):1135-43.
19. Fahlberg MD, Blair RV, Doyle-Meyers LA, Midkiff CC, Zenere G, Russell-Lodrigue KE. Cellular events of acute, resolving or progressive COVID-19 in SARS-CoV-2 infected non-human primates. *Nature communications*. 2020; 11(1), 1-14.
20. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nature communications*. 2020; 11(1), 1-17.
21. Beck TC, Beck KR, Holloway CB, Hemings Jr RA., Dix TA, Norris RA. The CC Chemokine Receptor Type 4 Is an Immunomodulatory Target Hydroxychloroquine. *Frontiers in pharmacology*. 2020; 11, 1253.
22. Power CA, Chvatchko Y, and Conquet, F. CCR4 Antagonists in Sepsis. WO2000067791A1 World Intellectual Property Organization, 2000. 16 (13).
23. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science*. 2020; 368(6490), 473-474, eabb8925. doi: 10.1126/science. abb89.
24. Chen Y, Wang J, Liu C, Su L, Zhang D, Fan J. IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. *Molecular Medicine* .2020; 26(1), 1-12.
25. Liu Y, Zhang C, Huang F, Yang Y, Wang F, Yuan J. Elevated plasma levels of selective cytokines in COVID-19 patients reflect viral load and lung injury. *National Science Review*.2020; 7(6), 1003-1011.
26. Blot M, Jacquier M, Glele LSA, Beltramo G, Nguyen M, BonniaudP. CXCL10 could drive longer duration of mechanical ventilation during COVID-19 ARDS. *Critical Care*.2020; 24(1), 1-15.
27. Lupieri A, Smirnova NF, Solinhac R, Malet N, Benamar M, Saoudi, A. Smooth muscle cells-derived CXCL10 prevents endothelial healing through PI3K $\gamma$ -dependent T cells response. *Cardiovascular research*. 2020;116(2), 438-449.
28. Zhang N, Zhao YD, Wang XM. CXCL10 an important chemokine associated with cytokine storm in COVID-19 infected patients. *Eur Rev Med Pharmacol Sci*. 2020; 24(13), 7497-7505.