Interleukin-21: a potential biomarker for diagnosis and predicting prognosis in COVID-19 patients

Abstract

Background/aim: COVID-19 patients have a wide spectrum of disease severity. Several biomarkers were evaluated as predictors for progression towards severe disease. IL-21 is a member of common γ-chain cytokine family and creates some specific effects during programming and maintenance of antiviral immunity. We aimed to assess IL-21 as a biomarker for diagnosis and outcome prediction in patients hospitalized with COVID-19.

Materials and methods: Patients with a preliminary diagnosis of COVID-19 and pneumonia other than COVID-19 admitted to a tertiary care hospital were included consecutively in this comparative study.

Results: The study population consisted of 51 patients with COVID-19 and 11 patients with non-COVID-19 pneumonia. Serum IL-21 concentration was markedly higher and serum CRP concentration was significantly lower in COVID-19 patients compared to non-COVID-19 pneumonia patients.

Within COVID-19 patients 10 patients showed radiological and clinical progression. Patients with clinical worsening had lower lymphocyte count and haemoglobin. In addition to that deteriorating patients had higher urea, LDH levels and elevated concentration of both IL-6 and IL-21. The cut-off value of 106 ng/L for IL-21 has 80.0% sensitivity, %60.9 specificity for discriminating patients with clinical worsening.

Multivariable analysis performed to define risk factors for disease progression identified IL-6 and IL-21 as independent predictors. Odds ratio for serum IL-6 concentrations ≥ 3.2 pg/mL was 8.07 (95% CI: 1.37-47.50, p= 0.04) and odds ratio for serum IL-21 concentrations ≥ 106 ng/L was 6.24 (95% CI: 1.04 – 37.3, p= 0.02).

Conclusion: We identified specific differences in serum IL-21 between COVID-19 and non-COVID-19 pneumonia patients. Serum IL-21 measurement has promising predictive value for disease progression in COVID-19 patients. High serum IL-6 and IL-21 levels obtained upon admission are independent risk factors for clinical worsening.

Key words: COVID-19, interleukin 21, prognosis

1. Introduction

Patients with new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have different clinical features and different prognosis [1]. COVID-19 patients have a wide spectrum of disease severity, Wu et al reported 14% severe and 5% critical cases within 72314 confirmed patients [2]. Even though most patients might have a favorable prognosis patients with risk factors such as old age, underlying hypertension and diabetes, elevated serum total bilirubin, urea, interleukin (IL) 6, d-dimer or decreased lymphocyte count and CD8+ T cells may develop hypoxemia and progress to acute respiratory distress syndrome (ARDS) [3]. Several biomarkers were evaluated as predictors for progression towards severe disease. Henry et al defined higher white blood cell count, lower lymphocyte and platelet count as predictors for severe disease in their meta-analysis. In addition, higher inflammatory biomarkers such as C-reactive protein (CRP), procalcitonin, serum ferritin, lactate dehydrogenase (LDH), IL-2R and IL-6 are found to be associated with severe disease in COVID-19 patients [4]. Chen et al demonstrated that apart from increase of serum cytokines such as IL-2R, IL-6, IL-10 and tumor necrosis factor (TNF) α severe COVID-19 patients had decreased circulating immune cell subsets such as total T lymphocytes, CD4+ T cells and CD8+ T cells [5]. Immunopathogenesis of coronavirus infections is an exuberant immune response resulting in pulmonary cell infiltration, vascular permeability increase and tissue damage

[6]. The primary response is a result of a direct effect of coronavirus, includes cytokine secretion, apoptosis, pyroptosis, RAS dysfunction due to angiotensin-converting enzyme-2 (ACE2) downregulation and shedding [7]. Coronavirus-cell interactions result in increased production of immune mediators, especially cytokines secreted by T helper (Th) 17 cells such as IL-1, IL-6, IL-8, IL-21, TNF-β, monocyte chemoattractant protein-1 (MCP-1) [6]. The outcome of primary response is pulmonary cell infiltration, increase in vascular permeability and lymphopenia [7]. The secondary response created by virus and antibody complex results in complement system activation thus cellular damage. Antibody-dependent cell mediated cytotoxicity and antibody-dependent enhancement are possible cellular damage mechanisms [7, 8].

IL-21 is a member of common γ-chain cytokine family and is produced by activated CD4+ T cells, natural killer (NK) T cells and Th17 cells. IL-21 has effects on both innate and adaptive immune response. IL-21 activates dendritic cells, induces NK cells to produce more interferon (IFN) γ and therefore increases cytotoxic activity. IL-21 inhibits differentiation to Th1 cells and induces CD8+ T cell proliferation. IL-21 has upregulating effects on CD27 and CD28 expression thus has control over viral-specific effector CD8+ T cells [9]. IL-21 also stimulates B cell proliferation and promotes B cell maturation playing an important role for long-term immunity [10].

IL-21 creates some specific effects during programming and maintenance of antiviral immunity. IL-21 concentration is higher in patients with acute viral respiratory tract infections compared to healthy controls. While IL-21 levels show difference between different respiratory viruses, IL-21 concentration doesn't show any relation with clinical parameters such as fever, cough and myalgia [11]. Studies on chronic hepatitis B infection suggest IL-21 is a component of antiviral immune response controlling viral replication

and viral clearance [12, 13]. Serum IL-21 levels are found to be correlated with disease severity and showed changes during disease course in Hantavirus infected patients [14]. Identifying COVID-19 patients with risk of clinical worsening is very important to select these cases to administer appropriate care and treatment. We aimed to assess IL-21 as a biomarker for diagnosis and outcome prediction in patients hospitalized with COVID-19.

2. Materials and methods

2.1. Study design and participants

Patients with a preliminary diagnosis of COVID-19 admitted to a tertiary care hospital between 15 May and 15 June 2020 were included consecutively in this comparative study. Patients admitted to the department of pulmonology with a preliminary diagnosis of pneumonia other than COVID-19 were also included consecutively. Patients aged younger than 18 years old and patients with acute hepatitis, congestive heart failure or lung cancer were excluded from the study.

2.2. Definitions

COVID-19 is defined as SARS-CoV-2-induced coronavirus disease. Definition of possible COVID-19 cases according to national guideline conducted by the Turkish Ministry of Health has four categories [15]. Proven COVID-19 is patients with positive real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV-2.

Patients with respiratory rate >30 per min or oxygen saturation ≤ 90% or age > 50 years or lymphocyte count < 800/μl or CRP > 50 mg/L or ferritin > 500ng/ml or d-dimer > 1000 ng/ml and with bilateral infiltration in chest computed tomography (CT) are being considered to be treated in a hospital [15]. Clinical worsening is described as progression in radiological findings, clinical parameters and inflammatory markers. Radiological

findings compatible with progression were determined by chest x-ray and/or chest CT. New ground-glass opacities, consolidation, crazy-paving sign and/or reverse halo that weren't present at the initial examination were considered as the progression of the disease. Clinical parameters evaluated for progression consisted ongoing fever over 37.8 °C, increased respiratory rate and decreased oxygen saturation compared to initial examination. Increased CRP and ferritin levels compared to the first result at admission were measurements of inflammatory response indicating clinical worsening.

Pneumonia patients were defined as; a demonstrable infiltrate by chest radiograph or other imaging technique in addition to suggestive clinical symptoms, with or without microbiological data [16]. Patients with pneumonia were differentiated from COVID-19 patients by negative RT-PCR results and chest CT findings not compatible for SARS-CoV-2 infection. Patients with pneumonia didn’t have any contact with COVID-19 patients, suggesting risk of transmission. Laboratory measurements such as neutrophil percentage, CRP and procalcitonin were suggestive of bacterial etiology. Patient classifications and definition of clinical worsening were done by two experienced pulmonologists.

The gold standard for diagnosis of COVID-19 is a positive RT-PCR test for SARS-CoV-2. However, it is shown that chest CT has higher sensitivity for diagnosis of RT-PCR test [17, 18]. Typical chest CT patterns and distributions are carefully described by the Radiological Society of North America (RSNA) expert consensus statement. According to RSNA bilateral and peripheral distributed ground-glass opacities with or without consolidation or crazy-paving sign are typical appearances of early COVID-19 pneumonia. Reverse halo sign or organizing pneumonia patterns can be seen later in the disease course [19]. Definitions according to RSNA are shown to have very good

interobserver agreement among chest radiologists [20]. In our study chest CT of all suspected COVID-19 patients were evaluated by a chest radiologist and two experienced pulmonologists.

2.3. Hospital care and treatment of patients

Clinical decisions such as hospital admission and treatment were in accordance with national guideline conducted by the Turkish Ministry of Health [15].

2.4. Data collection

Demographic data, clinical presentation, laboratory tests and chest CT data were collected from inpatient medical records by a trained team of pulmonary physicians. Laboratory data included complete blood count, liver and renal functions, infection biomarkers, coagulation profile, LDH and serum ferritin measured upon admission.

Nasopharyngeal swabs were collected once upon admission to be tested by RT-PCR. If the patient was evaluated as high probability for COVID-19 but had one negative result of RT-PCR a second nasopharyngeal swab was performed. The decision for a third respiratory tract specimen RT-PCR test was decided by the attending physician. Blood and sputum cultures were taken in order to detect possible bacterial pathogens.

2.5. IL-6 and IL-21 measurements

Peripheral venous blood (~ 5 mL) was collected from study participants by venipuncture. Blood samples were taken once at the time of hospitalization before treatment procedures. Blood samples were centrifuged at 3000 × g for 15-20 min. Obtained serum samples were stored at - 80 °C until analyzed. Briefly, before starting the assay procedure, the supernatants and ELISA reagents were brought to room temperature and both IL-6 and IL-21 ELISA assays were performed according to the manufacturer's recommendations. Microplates were processed with an optical microplate reader at 450 nm and 570 nm. The

absorbance at 570 nm was subtracted from the absorbance at 450 nm. OD values were calculated accordingly by drawing the standard curve. Serum IL-6 measurement was done by enzyme-linked immunosorbent assay (ELISA) for quantitative detection of human IL-6 (Invitrogen, Ebioscience-Thermo) according to the instructions of the manufacturer. The assay range for IL-6 was 1.56 - 100 pg/mL and sensitivity was 0.92 pg/mL. Serum IL-21 measurement was done by ELISA sandwich kit (Bioassay Technology Laboratory) according to the instructions of the manufacturer. The assay range for IL-21 was 5ng/L - 1500ng/L was and sensitivity was 2.46 ng/mL.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 software program (IBM Corp., Armonk, NY, USA) and MedCalc statistical software 2021. Variables were investigated using histogram and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine distribution. Continuous data are described as the mean ± standard deviation or median (interquartile range) if normally distributed or non-normally distributed, respectively. Categorical characteristics are described as numbers (%). Correlation coefficients and their significance were calculated using the Spearman test. Continuous outcome variables were compared between groups by two-sample t-test for normally distributed data and by Mann–Whitney U-test for non-normally distributed data. The ability of serum IL-6 and IL-21 levels to discriminate patients with clinical worsening was analyzed using receiver operating characteristics (ROC) curve analysis. Optimal cutoff maximizing sensitivity and specificity was selected. Candidate risk factors related with clinical outcome were evaluated firstly by univariate analysis and then possible risk factors with p values below 0.15 were evaluated by logistic regression analysis to determine independent predictors of patient clinical outcome. Hosmer-

Lemeshow goodness of fit statistics was used to assess model fit. An overall 5% type-1 error level was used to infer statistical significance.

2.7. Ethical approval

The study was approved by the ethics committee of the Uludağ University Faculty of Medicine (date: 29/04/2020 number: 2020-7/25). Written informed consent was obtained from every participant prior to their inclusion in the study.

3. Results

3.1. Patient demographics and characteristics of the study population

A total of 62 patients were included in this study. Study population consisted 51 (82.2%) patients with COVID-19, 11 (18.8%) patients with non-COVID-19 pneumonia. Within COVID-19 patients 25 (49%) patients were PCR positive, 26 (51%) were diagnosed by clinical and radiologic features. The mean age was 54.9 ± 16.5 and 29 (46.7%) were male. The symptoms started 3 [2 - 6] days before admission, the most common self-reported symptoms were cough and fever. Frequent comorbidities were as follows; hypertension, diabetes mellitus and immunosuppression. Chest CT showed 31 (50%) patients have bilateral lung involvement. Peripheral distribution was observed in 55 (88.7%) chest CT with lower lobe dominance in 50 (80%) patients. The most common patterns seen on chest CT were ground-glass opacity and consolidation. The characteristics and clinical features of the study subjects are outlined in Table-1.

3.2. Differences between COVID-19 and non-COVID-19 pneumonia patients

The patient's demographics and characteristics were similar in terms of age, symptoms upon admission and comorbidities. COVID-19 patients tended to have less frequent consolidation and central distribution. Whole blood count and alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, d-dimer, LDH on admission

were similar between groups. Serum IL-21 concentrations were markedly higher and serum CRP concentration were significantly lower in COVID-19 patients compared to non-COVID-19 pneumonia patients. However, serum IL-6 levels showed no difference between patient groups (Table 1).

3.3. Risk factors for disease progression

Within COVID-19 patients 10 (19.6%) patients showed radiological and clinical progression. Clinical worsening was observed on the 6^{th} [3 - 8] day. Immunocompromised patients made up half of the deteriorating patients however other coexisting conditions showed no difference between patients. Patients with clinical worsening had lower lymphocyte count and haemoglobin. In addition to that deteriorating patients had higher urea and LDH levels. Elevated concentrations of both IL-6 and IL-21 were observed in patients with clinical worsening (Table 2).

In order to identify the optimum serum IL-21 concentration threshold which would discriminate patients with clinical worsening from those who didn't show deterioration, a ROC curve analysis was performed. Area under curve (AUC) for IL-21 was 0.713 [0.523 – 0.904] and represented fair discrimination. Cut-off value of 106 ng/L for IL-21 has 80.0 [44.3 - 97.4] % sensitivity, 60.9 [44.5 - 75.8] % specificity, 33.3 [24.4 -.45.0] % positive predictive value and 92.5 [77.9 -.97.7] % negative predictive value. Positive likelihood ratio is calculated to be 2.05. A separate ROC curve analysis was performed to evaluate the diagnostic value of serum IL-6 levels in distinguishing deteriorating patients from patients who didn’t show disease progression. Discrimination of IL-6 was fair with AUC 0.726 [0.533 – 0.918]. Calculated cut-off value for serum IL-6 concentration 3.24 pg/mL has 80 [44.3 – 97.4] % sensitivity, 51.2 [35.1 – 67.1] % specificity, 28.5 [20.4 – 38.3] % positive predictive value and 91.3 [74.5 – 97.4] % negative predictive value with 1.64

positive likelihood ratio. Youden index for IL-21 and IL-6 were 0.434 and 0.431, respectively. Pairwise comparison of ROC curves showed no difference between serum IL-21 and serum IL-6 levels in discriminating patients with disease progression (p=0.93) (Figure).

Multivariable analysis performed to define risk factors for disease progression identified IL-6 and IL-21 as independent predictors. Odds ratio for serum IL-6 concentrations ≥ 3.2 pg/mL was 8.07 (95% CI: 1.37 – 47.53, p= 0.04) and odds ratio for serum IL-21 concentrations ≥ 106 ng/L was 6.24 (95% CI: 1.04 – 37.35, p= 0.02). (Table 3)

3.4. Clinical and laboratory correlations with IL-21 and IL-6

Serum IL-21 level was weakly negatively correlated with blood neutrophil count (r=-0.310, p= 0.02) while serum IL-6 level was moderately correlated with lymphocyte count (r= - 0.404, p= 0.003), troponin (r= 0.429, p= 0.003) and weakly correlated with CRP (r= 0.298 p= 0.03), procalcitonin (r= 0.343, p= 0.02), d-dimer (r= 0.353, p= 0.02).

Patients who had fever at day 3 after admission had higher serum IL-21 concentration (409.2 [107.3 - 658.6] ng/L vs 101.0 [90.4 – 150.5] ng/L, p=0.01). Serum IL-6 measurement was correlated with hospital length of stay (r= 0.310, p= 0.02).

4. Discussion

Over-activation of T cells, increased T helper 17 (Th17) and CD8 T cell activity can play a role in immune injury in severe COVID-19 patients [21]. Clinical findings and studies about inflammatory biomarkers showed that exuberant inflammatory responses result in uncontrolled pulmonary inflammation thus leading to mortality [22]. IL-21 plays critical roles in both cytotoxic and humoral arms of the immune response. IL-21 and TGF-β are responsible for the differentiation of naïve CD4+ T cells to TH17 cells. TH17 cells, in turn, produce IL-21, IL-22, IL-17A, IL-17F and CCL20 targeting immune cells,

fibroblasts, endothelial and epithelial cells. These TH17 derived cytokines play a role in the pathogenesis of many autoimmune, allergic and inflammatory diseases [23]. IL-21 is also important for the generation and maintenance of germinal center, differentiation of B cells and immunoglobulin (Ig) especially IgG1 production [24, 25]. In addition, IL-21 increases the proliferation and cytotoxicity of CD8+ T cells [26].

Serum IL-21 and CRP measurements were significantly different between COVID-19 and non-COVID-19 pneumonia patients. Previous studies evaluated cytokine levels in different pneumonia groups. IL-6, IL-10, IL-17A and IFN-γ showed significant differences between viral community-acquired pneumonia (CAP), pneumococcal CAP and combined viral-bacterial CAP groups and the study created a cytokine-based prediction model. However, serum IL-21 levels were not detectable in all samples thus couldn't be evaluated [27]. In a study evaluating 76 patients with pneumonia caused by different respiratory viruses showed patients infected with influenza A had higher serum IL-21 levels than patients infected with respiratory syncytial virus [11]. Lieberman et al concluded serum IL-1 beta and IL-6 levels can be used to differentiate S. pneumoniae-CAP and M. pneumoniae-CAP during the acute phase [28]. Similarly in a study including 201 pneumonia patients showed that serum IL-1RA and IL-6 measurements upon admission were higher in patients with pneumococcal pneumonia [29]. However, in our study IL-6 levels showed no difference between COVID-19 and non-COVID-19 pneumonia patients.

In our study lymphopenia, anemia, higher LDH, urea, IL-6 and IL-21 levels are observed in patients with clinical worsening. Other studies evaluating prognostic factors also associated increased serum d-dimer, CRP, IL-6, procalcitonin, ferritin, troponin, creatine kinase measurements with worse clinical outcomes [30]. Serum IL-6 concentrations are

significantly related with complicated disease (ARDS, requiring ICU or severe/critical presentation) and mortality [31].

Cytokine storm is a result of high virus titers and dysregulation of cytokine/chemokine response which results in pathological changes of the lung [22]. Cytokines secreted by Th17 cells are increased in viral infections. In our study, serum IL-21 and serum IL-6 levels are independent predictors of clinical worsening. There are few studies evaluating IL-21 in COVID-19 patients. A study evaluating adaptive immune response with plasmablasts from patients with severe COVID-19 requiring ICU revealed that in early stages of severe disease switch instruction of B cells are dominated by IL-21 and TGF-β thus leading to expression of IgG1 and IgA1. Severely affected COVID-19 patients have significant populations of CD4+ T cells programmed to express IL-21 and TGF-β. Despite increased secretion of IgA from B cells, interestingly this created IgA doesn't bind SARS-CoV-2 spike or nucleocapsid protein. In addition to that beyond day 7, patients showed a continued immune reaction with IL-2/IL-21 activated B cells. These results lead to the conclusion of the importance of IL-21 and TGF-β in adaptive and chronic immune reactions not targeting the SARS-CoV-2 virus itself [32]. Results of high levels of IL-21 in severe COVID-19 patients are in accordance with the results of our study. Results of a clinical trial with IL-15 therapy for COVID-19 [33] and prior studies indicating IL-21 promoting effects of IL-15 lead to a conclusion that particularly in patients with diminished B and/or CD8+ T cell counts treatment with IL-15 and IL-21 might be effective [34]. More studies evaluating IL-21 and IL-6 in different viral infections are present. Studies with chronic hepatitis B infections suggested that IL-21 is a component of antiviral immune response controlling viral replication and viral clearance [12] and low serum levels of IL-21 might have a role in the persistence of viral

infections [13]. Chen et al showed higher serum IL-21 levels are related with disease progression and disease severity in hemorrhagic fever with renal syndrome caused by Hantavirus infection [14]. In a study conducted by Almansa et al patients with severe respiratory disease due to pandemic influenza had higher viral load and higher cytokines such as IL-6, IL-8, IL-10, IL-1ra upon admission indicating exuberant cytokine response. Within this study cytokine levels were correlated with viral load [35]. Serum IL-6 measurements in pandemic H1N1 influenza pneumonia patients predicted worse clinical parameters such as fever, tachypnea, deoxygenation and ICU admission [36].

In our study serum IL-6 level is also positively correlated with length of hospital stay. Lee et al also defined serum IL-6 to be correlated with length of stay in pandemic influenza patients [17]. Serum IL-21 concentration is found to be higher in patients who had fever at day 3 during the illness course. However, Antalis et al didn't show any relation of serum IL-21 with clinical parameters such as fever, cough and myalgia within patients infected with various respiratory viruses other than SARS-CoV-2 [11].

Cytokine measurements and understanding particular roles of cytokines in COVID-19 disease might help clinicians to identify patients at risk for worse outcomes and elucidate potential treatment modalities.

5. Limitations

This study has some limitations. First, patients were not tested for other respiratory viruses. As shown in recent studies other viruses might affect serum IL-21 measurements. However, patients admitted within influenza season were treated with oseltamivir empirically. Therefore clinical worsening attributed to undetected influenza is less likely. Secondly, this study population has few numbers of patients with non-COVID-19 pneumonia. This limits the power of statistical analysis such as the predictive value of

biomarkers because they are affected by prevalence. Thirdly, within COVID-19 patients some patients are RT-PCR negative. However, chest CT findings are found to be more sensitive for COVID-19 diagnosis compared to RT-PCR results [17-18]. Inter-observer agreement for diagnosis by chest CT is found to be almost perfect [20] and in our study chest CT of suspected COVID-19 patients were evaluated by a chest radiologist and two experienced pulmonologists. In addition to that, false negative rate of RT-PCR test ranges from 2% to 54% in different studies [37]. RT-PCR test results are also affected by time since exposure and time since symptom onset [38, 39]. Pooled analysis performed by Kucirka et al. revealed 38% false negative on the day of symptom onset, 20% false negative on the 3rd day and increased false negative results after the 4th day [38]. Our study population was tested 3 [2 - 7] days after symptom onset. Therefore taken into consideration the radiological, clinical and laboratory parameters, RT-PCR negative COVID-19 patients were included in the study population. Results from statistical analysis repeated for only RT-PCR positive patients, showed no difference in terms of difference of serum IL-21 measurements between stable patients and patients with clinical worsening (Table-S1).

6. Conclusion

We identified specific differences in serum IL-21 between COVID-19 and non-COVID-19 pneumonia patients. Serum Il-21 measurement has promising predictive value for disease progression in COVID-19 patients with 80.0% sensitivity, 60.9% specificity and 92.5% negative predictive value. High serum IL-6 and IL-21 levels obtained upon admission in COVID-19 patients are risk factors for clinical worsening independent of lymphocyte count and immunosuppression. These findings joined with previous studies

support the need for further research on the role of IL-21 in disease progression and possible treatment modalities related to IL-21 in COVID-19 patients.

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Table 1: Demographics, characteristics and initial laboratory measures of COVID-19 patients and pneumonia patients

	Study population (n=62)	COVID-19 patients (n=51)	Pneumonia patients (n=11)	p
Age, years	54.9 ± 16.5	54.3 ± 15.2	57.5 ± 22.3	0.56
Male gender, n(%)	29 (46.7)	27 (52.9)	7 (63.6)	0.41
Symtoms upon admisson, n(%)				
Fever, n(%)	28 (45.2)	23 (45.1)	5 (45.4)	0.61
Cough, n(%)	39 (62.9)	33 (64.7)	6 (54.5)	0.38
Dyspnea, n(%)	21 (33.9)	18 (35.3)	3 (27.2)	0.44
Sputum production, n(%)	17 (27.4)	14 (27.5)	3 (27.2)	0.65
Fatigue, n(%)	14 (22.6)	13 (25.5)	1 (9.1)	0.22
Duration of symptoms before admission, days	3 [2-6]	3 [2-6]	2 [1-4]	0.31
Coexisting conditions				
Hypertension, n(%)	21 (33.9)	16 (31.4)	5 (45.4)	0.28
Diabetes mellitus, n(%)	17 (27.4)	15 (29.4)	2 (18.1)	0.36
Immunosuppression, n(%)	13 (21)	11 (21.6)	2 (18.1)	0.62

	Cancer, n(%)	11 (17.7)	9 (17.6)	0 (0)	0.15
	COPD, n(%)	6 (9.7)	5 (9.8)	1 (9.1)	0.71
	Asthma, n(%)	5 (8.1)	4 (7.8)	1 (9.1)	0.63
	Chronic kidney disease, n(%)	6 (9.7)	4 (7.8)	2 (18.1)	0.28
	Transplant recipient	2 (3.2)	2 (3.9)	0 (0)	0.67
Distribution of Chest CT abnormalities					
Bilateral involvement of Chest CT, n(%)		31 (50)	27 (52.9)	4 (36.3)	0.97
	Upper lobe, n(%)	26 (41.9)	22 (43.1)	4 (36.3)	0.45
	Lower lob, n(%)	50 (80)	40 (78.4)	10 (90.9)	0.36
	Peripheral, n(%)	55 (88.7)	44 (86.3)	11 (100)	0.28
	Central, n(%)	15 (24.2)	10 (19.6)	5 (45.5)	0.08
Patterns on Chest CT, n(%)					
	Ground glass opacity	53 (85.5)	43 (84.3)	10 (90.9)	0.55
	Air bronchogram	10 (16.1)	7 (13.7)	3 (27.2)	0.25
	Consolidation	19 (30.6)	13 (25.5)	6 (54.5)	0.07
	Crazy paving	3 (4.8)	3 (5.9)	0 (0)	0.54
	Reticular pattern	5 (8.1)	4 (7.8)	1 (9.1)	0.64
Laboratory measures at admission					

Leukocyte count, K/µL	8.59 [5.94-10.71]	8.06 [5.60-1.06]	9.09 [8.51-12.52]	0.11
Lymphocyte count, K/µL	1.59 [1.052.23]	1.57 [1.00-2.48]	1.66 [0.79-2.07]	0.80
Neutrophil count, K/µL	5.67 [3.02-8.11]	4.83 [3.02-8.05]	6.07 [5.37-11.37]	0.22
Haemoglobin, g/dL	12.3 ± 2.6	12.4 ± 2.73	12.08 ± 2.17	0.57
CRP, mg/L	9.6 [2.5-42.6]	6.2 [2.0-25.4]	20.6 [9.3-95.9]	0.01
Procalcitonin, µg/L	0.05 [0.02-0.15]	0.04 [0.02-0.16]	0.06 [0.03-0.75]	0.42
LDH, U/L	211 [168-303]	211 [178-309]	137 [34-240]	0.55
Ferritin, µg/L	135 [47.3-420.2]	147.0 [50.4-695.6]	69.5 [27.7-307.1]	0.34
d-dimer, mg/L	0.72 [0.31-1.48]	0.66 [0.3-1.5]	1.01 [0.69-1.31]	0.45
Urea, mg/dL	31 [24-42]	29.5 [23.0-39.5]	42 [30.5-52.5]	0.11
Creatinine, mg/dL	0.81 [0.7-1.06]	0.81 [0.68-1.07]	0.79 [0.75-0.94]	0.25
ALT, U/L	19 [11-32]	19.0 [14.2-31.7]	10 [8.5-28.5]	0.18
AST U/L	21 [16-31]	21.0 [16.0-32.2]	19 [14-24]	0.25

IL-6, pg/mL	3.52 [2.95-4.57]	3.45 [2.93-4.31]	3.80 [3.07-5.61]	0.17
IL-21, ng/L	100.9 [90.2-169.0]	105.0 [93.9-210.7]	92.0 [47.6-100.8]	0.01
Length of stay, days	7 [6-9]	7 [6-10]	6 [5-7]	0.01

Notes: Data are mean ± standard deviation or median (IQR 25-75), as appropriate. Abbreviations: CT, computed tomography; CRP, C-reactive protein; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL-6, interleukin 6; IL-21, interleukin 21.

Table 2: Demographics, characteristics and initial laboratory measures of COVID-19 patients with different clinical course

		Stable Patients (n=41)	Patients with clinical worsening (n=10)	p
Age, years		54.0 ± 16.7	53.4 ± 5.0	0.92
Male gender, n(%)		23 (56)	4 (40)	0.67
Symptoms upon admission, n(%)				
	Fever, n(%)	20 (48.7)	3 (30)	0.23
	Cough, n(%)	26 (63.4)	7 (70)	0.50
	Dyspnea, n(%)	15 (36.5)	3 (30)	0.50
	Sputum production, n(%)	11 (26.8)	3 (30)	0.56
	Fatigue, n(%)	10 (24.3)	3 (30)	0.49
Duration of symptoms before admission, days		3 [2-5.5]	3 [1.5-7]	0.80
Coexisting conditions				
	Hypertension, n(%)	12 (29.2)	4 (40)	0.38
	Diabetes mellitus, n(%)	11 (26.8)	4 (40)	0.32
	Immunosuppression, n(%)	6 (14.6)	5 (50)	0.03
	Cancer, n(%)	8 (19.5)	3 (30)	0.23
	COPD, n(%)	4 (9.75)	4 (40)	0.68
	Asthma, n(%)	4 (9.75)	0 (0)	0.40

	Chronic kidney disease, n(%)	2 (4.87)	2 (20)	0.16
Distribution of CT abnormalities				
Bilateral involvement of Chest CT, n(%)		23 (56.0)	4 (40)	0.73
	Upper lobe, n(%)	18 (43.9)	4 (40)	0.53
	Lower lob, n(%)	32 (78)	8 (80)	0.65
	Peripheral, n(%)	35 (85.3)	9 (90)	0.65
Patterns on Chest CT, n(%)				
	Ground glass opacity	34 (82.9)	9 (90)	0.57
	Air bronchogram	4 (9.75)	3 (30)	0.13
	Consolidation	9 (21.9)	4 (40)	0.22
	Crazy paving	3 (7.3)	0 (0)	0.50
	Reticular pattern	3 (7.3)	1 (10)	0.60
Laboratory measures at admission				
Leukocyte count, K/µL		8.29 [6.22-10.9]	6.73 [5.03-9.65]	0.21
Lymphocyte count, K/µL		1.65 [1.32-2.50]	0.78 [0.58-2.27]	0.05
Neutrophil count, K/µL		5.26 [3.29-7.84]	4.17 [2.67-8.35]	0.55
Haemoglobin, g/dL		12.6 ± 2.9	11.4 ± 1.9	0.09
CRP, mg/L		4.9 [2.0-17.0]	18.5 [5.0-213.1]	0.31
Procalcitonin, µg/L		0.03 [0.02-0.13]	0.12 [0.03-0.33]	0.10

LDH, U/L	203.0 [154.0-245.7]	321.5 [231.0-407.5]	0.08
Ferritin, μg/L	147 [50.4-695.6]	183.5 [61.7-1559.2]	0.76
d-dimer, mg/L	0.64 [0.29-1.44]	0.87 [0.57-1.88]	0.33
Urea, mg/dL	29 [23-36]	37 [28-50]	0.06
Creatinine, mg/dL	0.82 [0.70-1.06]	0.69 [0.65-1.28]	0.37
ALT, U/L	19 [15-34]	19 [12.5-21]	0.21
AST U/L	21 [16-35]	20 [13.5-30]	0.49
IL-6, pg/mL	3.17 [2.89-3.91]	4.40 [3.21-20.0]	0.02
IL-21, ng/L	101.0 [89.9-161.6]	295.9 [103.8-611.0]	0.03
Outcome			
Length of stay, days	7 [6-9]	9.5 [9-11]	0.006
In hospital mortality, n(%)	1 (2.43)	2 (20)	0.09

Notes: Data are mean ± standard deviation or median (IQR 25-75), as appropriate. Abbreviations: CT, computed tomography; CRP, C-reactive protein; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL-6, interleukin 6; IL-21, interleukin 21.

Table 3: Multivariable analysis of possible risk factors for clinical worsening in COVID-19 patients

	OR	95% CI	p-value	OR	95% CI	p-value
Age, years	1.01	0.96-1.06	0.63			
Gender, male	1.83	0.44-7.51	0.40			
DM	1.81	0.43-7.68	0.41			
HT	1.61	0.38-6.75	0.51			
Immunosuppression	5.66	1.24-25.73	0.02	2.35	0.25-16.99	0.40
Smoking	1.77	0.13-23.39	0.66			
Bilateral involvement in Chest CT	3.18	0.30-33.25	0.33			
Lymphocyte count	0.99	0.99-1.00	0.11	1	0.99-1.00	0.93
Haemoglobulin	0.85	0.65-1.10	0.21			
Urea	1.00	0.98-1.01	0.78			
LDH	1.00	0.99-1.01	0.26			
Ferritin	1.00	0.99-1.00	0.52			
CRP	1.05	0.99-1.01	0.31			
Procalcitonin	3.79	0.56-25.2	0.16			
IL-6 ≥ 3.2 pg/mL	4.20	0.79–22.2	0.09	8.07	1.37–47.53	0.04
IL-21 ≥ 106 ng/L	6.25	1.17–33.2	0.03	6.24	1.04–37.35	0.02

Abbreviations: HT, Hypertension; DM, Diabetes mellitus; IL-6, interleukin 6; IL-21, interleukin 21.

Table S1: Demographics, characteristics and initial laboratory measures of COVID-19 patients

		Stable Patients (n=19)	Patients with clinical worsening (n=7)	p
Age		48.9 ± 15.7	58.0 ± 11.0	0.17
Male gender, n(%)		9 (47.3)	1 (14.2)	0.23
Symptoms upon admission, n(%)				
	Fever, n(%)	10 (52.6)	3 (42.8)	0.67
	Cough, n(%)	12 (70.5)	5 (71.4)	1
	Dyspnea, n(%)	7 (36.8)	2 (28.5)	1
	Sputum production, n(%)	2 (10.5)	3 (42.8)	0.11
	Fatigue, n(%)	5 (26.3)	3 (42.8)	0.64
Duration of symptoms before admission, days				
Coexisting conditions				
	Hypertension, n(%)	4 (21.0)	3 (42.8)	0.35
	Diabetes mellitus, n(%)	4 (21.0)	4 (57.1)	0.15
	Immunosuppression, n(%)	1 (5.2)	2 (28.5)	0.19
	Cancer, n(%)	1 (5.2)	1 (14.2)	0.49
	COPD, n(%)	1 (5.2)	0 (0.0)	1
	Asthma, n(%)	2 (10.5)	0 (0.0)	1

	Chronic kidney disease, n(%)	0 (0.0)	2 (28.5)	0.07
Distribution of CT abnormalities				
Bilateral involvement of Chest CT		11 (57.8)	3 (42.8)	0.90
	Upper lobe	7 (36.8)	3 (42.8)	1.00
	Lower lob	11 (57.8)	5 (71.4)	1.00
	Peripheral	12 (70.5)	6 (85.7)	0.62
Patterns on Chest CT				
	Ground glass opacity	12 (70.5)	6 (85.7)	0.62
	Air bronchogram	2 (10.5)	2 (28.5)	0.55
	Consolidation	4 (21.0)	3 (42.8)	0.35
	Crazy paving	2 (10.5)	0 (0.0)	1.00
	Reticular pattern	1 (5.2)	1 (14.2)	0.49
Laboratory measures at admission				
Leukocyte count, K/μL		6.81 [4.76-8.66]	6.35 [5.65-9.00]	0.95
Lymphocyte count, K/μL		1.76 [1.08-2.48]	1.35 [0.78-2.55]	0.73
Neutrophil count, K/μL		4.21 [2.61-5.70]	4.05 [2.54-7.99]	0.95
Haemoglobin, g/dL		13.0 ± 2.4	10.9 ±1.4	0.02
CRP, mg/L		9.4 [2.5-31.4]	18.5 [3.0-33.0]	0.57
Procalsitonin, μg/L		0.03 [0.02-0.10]	0.20 [0.02-1.01]	0.19

LDH, U/L	245.0 [178.0-403.0]	321.5 [231.0-407.5]	0.35
Ferritin, μg/L	158.6 [39.4-474.5]	130 [84.5-183.5]	0.55
d-dimer, mg/L	0.38 [0.25-1.23]	1.22 [0.34-10.2]	0.32
Urea, mg/dL	25.0 [19.2-36.0]	33.5 [27.0-37.7]	0.07
Creatinine, mg/dL	0.7 [0.6-1.0]	0.6 [0.6-0.9]	0.73
ALT, U/L	21.0 [18.0-43.7]	20.5 [16.2-73.5]	0.13
AST U/L	23.0 [17.2-45.5]	27.0 [20.0-34.7]	0.69
IL-6, pg/mL	3.46 [3.04-3.95]	2.91 [2.87-3.11]	0.18
IL-21, ng/L	128.3 [84.8-429.3]	602.8 [326.9-619.2]	0.04
Outcome			
Length of stay, days	7.0 [6.0-10.0]	10.0 [9.0-13.0]	0.02
In hospital mortality, n(%)	0 (0.0)	2 (28.5)	0.07

Notes: Data are mean ± standard deviation or median (IQR 25-75), as appropriate. Abbreviations: CT, computed tomography; CRP, C-reactive protein; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL-6, interleukin 6; IL-21, interleukin 21.

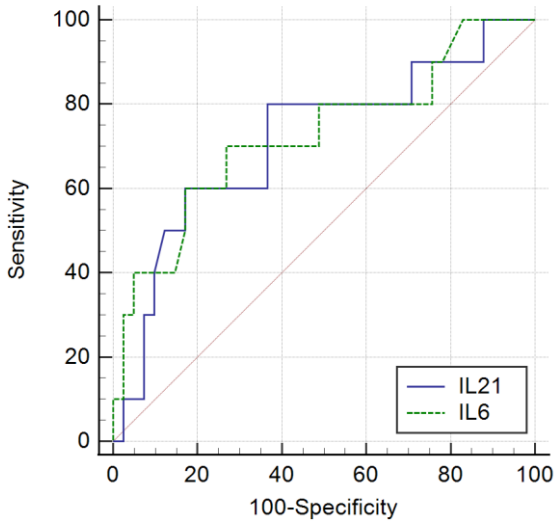

Figure: Receiver operator characteristic curve for serum IL-21 and serum IL-6 discriminating stable patients and patients with disease progression within the COVID-19 population