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Plasma IP-10 could identify early lung disease in severe COVID-19 patients

Dear Editor,

The global pandemic of SARS-Coronavirus-2 (SARS-CoV-2) infection has imposed tremendous strain on healthcare resources worldwide, as a significant proportion of patients require intensive care. Although the majority have mild infections, up to 20% are estimated to become critically ill from severe disease.[1] Age, concurrent comorbidities, more severe disease, respiratory failure, higher levels of D-dimer and C-reactive protein (CRP), more severe lymphopaenia, and secondary infections are associated with risk of mortality.[2]

Innate and adaptive immune responses to COVID-19 contribute to disease pathology. Elevated levels of circulating interleukin 1 beta, interferon gamma, CXCL10/interferon gamma inducible protein-10 (IP-10) and CCL2/monocyte chemoattractant protein 1 have been associated with mild disease. Deterioration of disease leading to intensive care and adverse outcome of COVID-19 has been associated with persistently high levels of chemokines such as IP-10.[3] Thus we explored the potential utilisation of plasma IP-10 in conjunction with clinical, imaging and laboratory parameters in assisting risk stratification for disease progression of COVID-19, because of the role this chemokine has in regulating the interferon response and innate immunity.

A total of 72 de-identified patients at the National Centre of Infectious Diseases, Singapore with confirmed COVID-19 by nasopharyngeal swab SARS-CoV-2 real-time reverse transcriptase–polymerase chain reaction (RT-PCR), were recruited over the period 28 March to 1 April 2020. Cytokine assays were performed on frozen sera from blood samples collected for standard clinical evaluation, with waiver of consent approved by institutional review board (DSRB 2020/00910). Data on demographics, comorbidities, and laboratory results were obtained from electronic medical records. The Charlson comorbidity index was calculated.[4] Plasma IP-10 levels were determined by ELISA after viral inactivation by incubation to a final concentration of 1% Triton X-100. Levels from 50 healthy controls were used for comparison.

Correlation of clinical, laboratory, imaging and cytokine data was performed with non-parametric Fisher exact test and Mann-Whitney U test for univariate comparisons. Correlation of levels of plasma IP-10 with levels of CRP, lactate dehydrogenase (LDH), platelets, lymphocytes, neutrophils and RT-PCR cycle threshold values was assessed using Spearman correlation. Diagnostic usefulness in predicting intensive care unit (ICU) admission was evaluated by formulating a logistic model using variables showing statistical significance in univariate analysis. The receiver operator characteristic (ROC) curve of the model was then generated to determine the predictive performance of the model as reflected by the area under the ROC curve.

The patients' clinical characteristics, disease severity, relevant laboratory data, imaging and course, including stay in intensive care, are shown in Table 1. Disease severity varied. Most had fever (37/72, 51%) and cough (31/72, 43%); other symptoms included myalgia (6/72, 8%), sore throat (5/72, 7%), anosmia (4/72, 6%), rhinorrhoea (4/72, 6%), diarrhoea (2/72, 3%) and headache (1/72, 1%). Thirty patients (41.6%) exhibited abnormal chest X-ray changes ranging from opacities to consolidation.

Plasma samples that were assayed for IP-10 levels were drawn from 1 to 22 (mean 7.8±4.5) days after symptom onset. Significantly higher median plasma levels of IP-10 were found in SARS-CoV-2 patients than in healthy controls: 95.8 (interquartile range [IQR] 45.7–195.1) pg/mL versus 22.1 (IQR 9.4–38.1) pg/mL (P<0.001). The correlations between IP-10 levels and the laboratory parameters are summarised in Table 1. We observed that plasma IP-10 levels correlated positively with CRP (r=0.809, P<0.005), LDH (r=0.341, P<0.005), neutrophil count (r=0.520, P<0.001) and negatively with lymphocyte count (r= -0.292, P=0.013). SARS-CoV-2 patients with chest X-ray changes exhibited higher plasma IP-10 levels than those without. In addition, a positive correlation was observed between IP-10 levels and multiple comorbidities based on the Charlson Comorbidity Index (r=0.491, P<0.001, Table 1). IP-10 levels did not correlate with viral load based on SARS-CoV-2 RT-PCR cycle threshold values, likely as a result of the RT-PCR assays being done later in the disease course, while the CRP, LDH and blood counts were done contemporaneously with the IP-10 assays.

We next evaluated predictors of ICU admission. ICU admission was found to be significantly correlated in univariate analysis with the Charlson Comorbidity Index (P<0.01), thrombocytosis (platelet >300x10^9/L,

Table 1. Patient demographics and clinical characteristics, IP-10 plasma levels and clinical correlations

Demographics and clinical characteristics	**SARS-CoV-2 patients (N=72) Mean±SD (IQR)**	
Age, years	45.5±16.5 (30.8−58.0)	
Male/Female, no. (%)	41 (57) / 31 (43)	
Day of illness	7.8±4.5 (4−10)	
Abnormal CXR (opacities/consolidation), no. (%)	30/72 (41.6)	
ICU admission, no. (%)	7/72 (9.7)	
CRP mg/L	27.0±50.4 (3.0−23.6)	
LDH U/L	479.0±355.2 (359.8−487.3)	
Lymphocyte count, 10^9/L	1.3±0.5 (0.9−1.6)	
Neutrophil count, 10^9/L	3.5±1.9 (2.2−4.2)	
Platelet count, 10^9/L	219.2±79.9 (165.5−258.8)	
SARS-CoV-2 RT-PCR cycle	29.6±6.1 (25.1−34.3)	
IP-10pg/mL	163.1±195.6 (45.7−197.0)	
IP-10 plasma levels and clinical correlations	**Pearson correlation coefficient**	***P* value**
IP-10 and CRP	0.809	0.005[a]
IP-10 and LDH	0.341	0.005[a]
IP-10 and lymphocyte count	-0.292	0.013[a]
IP-10 and neutrophil count	0.520	0.001
IP-10 and Charlson score	0.491	0.001[a]
IP-10 and COVID-19 RT-PCR cycle	-0.059	0.656

[a] Correlation is significant at the 0.05 level (2-tailed)
CRP: C-reactive protein; CXR: chest X-ray; ICU: intensive care unit; IP-10: interferon gamma inducible protein-10; IQR: interquartile range; LDH: lactate dehydrogenase; RT-PCR: reverse transcriptase–polymerase chain reaction; SD: standard deviation

P<0.01), high CRP (>50mg/L, *P*<0.05), and high IP-10 level (≥200pg/mL, *P*<0.05). A logistic regression model using the laboratory data with significant univariate association with ICU admission (CRP, thrombocytosis and high IP-10 level) was found to have an area under the ROC curve of 0.9546, implying a strong predictive performance.

SARS-COV-2 is a zoonotic RNA betacoronavirus. Infection with SARS-CoV can trigger an exaggerated immune response with excessive production of chemokines including IP-10, leading to inflammation and destruction of lung tissue through the infiltration of neutrophils, alveolar macrophages and Th1 lymphocytes.[5] This may also be the case with SARS-Cov-2 as elevated IP-10 levels have been reported to be associated with prolonged fever, increased hypoxia and disease severity in COVID-19 patients.[6-8] A recent observational cohort study of 52 hospitalised patients from Israel found that IP-10 levels were higher in patients with severe disease and those requiring ICU admission, findings in keeping with those of our study.[9]

The limitation of our cross-sectional study is that levels of IP-10 were performed an average of 7 days after symptom onset, thus the utility of IP-10 for early risk stratification is not fully known.

The addition of blood IP-10 levels to the risk factor profile may hold promise for early identification of individuals with oncoming severe COVID-19 lung disease, facilitating the institution of therapy that could potentially avert intensive care and prevent mortality, Larger, adequately powered longitudinal studies will be required for this to be determined.

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