BRIEF REPORT

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Interleukin-6 in Critical Coronavirus Disease 2019, a Driver of Lung Inflammation of Systemic Origin?

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OBJECTIVES: To examine whether interleukin-6 in critical coronavirus disease 2019 is higher in arterial than in central venous blood, as a sign of predominantly local pulmonal rather than systemic interleukin-6 production.

DESIGN: Prospective cohort pilot study with repeated weekly measurements of interleukin-6 in arterial and central venous blood. Respiratory function, assessed with Pao_2/Fio_2 ratio, was measured at the time of blood sampling.

SETTING: ICU at a university hospital.

SUBJECTS: Nine adult patients with critical coronavirus disease 2019, actively treated and receiving mechanical ventilation.

MEASUREMENTS AND MAIN RESULTS: No difference between arterial and central venous interleukin-6 was found. There was a significant negative relationship between interleukin-6 concentration and P/F ratio in both arterial ($p = 0.04$) and central venous ($p = 0.03$) blood.

CONCLUSIONS: The absence of an arteriovenous interleukin-6 difference implies that interleukin-6 in critical coronavirus disease 2019 is mainly produced outside the lungs as part of a systemic inflammatory response syndrome and act as a driver of local inflammation and damage in the lungs.

KEY WORDS: coronavirus disease 2019; interleukin-6; interleukin-6/analysis; interleukin-6/antagonists and inhibitors; respiratory distress syndrome; systemic inflammatory response syndrome

The cytokine interleukin-6 (IL-6) is produced by immune cells in the human body and has numerous functions in infectious and autoinflammatory diseases (1). Previous studies have found a negative correlation between IL-6 and respiratory function in patients with coronavirus disease 2019 (COVID-19) (2), and IL-6 antagonist tocilizumab is shown to increase survival in COVID-19 patients with severe or critical disease (3, 4). These findings suggest that IL-6 is not only a marker of respiratory failure but also contributes to pulmonary inflammation and injury in this disease.

Higher concentrations of IL-6 in arterial than central venous blood have previously been found in critically ill patients with sepsis-related acute respiratory distress syndrome (ARDS) and respiratory failure of other etiology (5, 6), suggesting local production of IL-6 in the lungs. Because COVID-19 is primarily a respiratory disease, it could be assumed that IL-6 is mainly produced in the lungs in this condition. It should then be possible to detect arteriovenous differences in IL-6. On the other hand, it has been speculated that inhibition of IL-6 in COVID-19 may only be beneficial if IL-6 is produced outside the lungs, being a systemic driver of lung inflammation (7). If IL-6 is produced systemically, no arteriovenous IL-6 difference would be expected.

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The aim of this study was to investigate whether IL-6 in critical COVID-19 is higher in arterial than that in central venous blood, which would suggest predominantly pulmonal rather than systemic IL-6 production.

MATERIALS AND METHODS

Subjects

IL-6 was measured in nine critically ill adult patients with COVID-19, hospitalized in an ICU at a university hospital, actively treated, and receiving noninvasive or invasive mechanical ventilation. All patients were included in a prospective cohort study (Norwegian SARS-CoV2 study; NCT04381819) (2).

Timing of Blood Sampling

Blood was preferably sampled once per week while the patient was fulfilling inclusion criteria. Due to practical challenges during the COVID-19 pandemic, blood samples were not obtained from three patients during the first week at the ICU, and one of these patients was transferred to a neighboring hospital after 2 weeks and was not observed in our study after this. Another patient was admitted to ICU several weeks before the study was initiated. For the remaining five patients, all blood samples were obtained as projected.

Data Collection and Blood Sample Processing

Blood samples were drawn successively from arterial and central venous cannulas, and the order of the blood sampling was randomized. Patients were observed in the study for 1–3 weeks, and one (n = 2), two (n = 5), or three (n = 2) pairs of EDTA blood samples were obtained from each patient. Within 6 minutes after sampling, blood was centrifuged at 200 × g in room temperature for 10 minutes, and then, at 4,000 × g in 4°C for 15 minutes. Platelet-poor plasma was stored at –80°C until analysis. After thawing, plasma was centrifuged at 10,000 × g in 4°C for 10 minutes, the supernatant was diluted (1:4), and cell debris and the upper lipid layer was removed. IL-6 was then quantified using a Luminex IS 200 instrument and a Human Cytokine 8-plex assay (Bio-Rad, Hercules, CA). Pao_2/Fio_2 ratio (P/F ratio) was registered at the time of blood sampling. C-reactive protein (CRP) concentrations, Sequential Organ Failure Assessment (SOFA) score, body mass index (BMI), and other patient data were collected from the patient record.

Statistics

Concentrations of IL-6 were log-transformed to achieve normal distribution, and data were analyzed with the paired sample t test or mixed models using R (R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS Statistics (IBM Corp., Armonk, NY). p values of less than 0.05 were considered statistically significant. Continuous variables are expressed with median value (range). Illustrations were made with Graph Pad Prism 8 (GraphPad Software, San Diego, CA).

Ethics

Informed consents were obtained from all patients or next of kin if patients were incapacitated of giving consent. The study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (reference number: 106624).

RESULTS

Nine patients (seven men and two women) with median age 61 years (46–70 yr) were included. Five patients (56%) had a fatal outcome. Baseline characteristics for the patients are presented in **Table 1**. Time from disease onset, hospital admission, and ICU admission to first blood sample is presented in **Table 2**. In the seven patients where repeated pairs of blood samples were obtained, blood was sampled with intervals of 6 days (4–9 d).

No difference between arterial and central venous IL-6 was found (**Fig. 1*A***). There was a significant negative relationship between IL-6 and P/F ratio (**Fig. 1*B***) in both arterial blood (r = –0.52; p = 0.04) and central venous blood (r = –0.54; p = 0.03).

Post hoc analyses with a selection of blood sample pairs were performed. We analyzed the first pair of blood samples from all patients and the first pair of blood samples from the five patients where blood was sampled the first week at the ICU. In another analysis, only the pair of blood samples with the highest IL-6 concentration from every patient was included. These analyses showed no arteriovenous IL-6 differences.

TABLE 1.
Baseline Characteristics for Patients

Variable	Median (Range)
Charlson comorbidity index	1 (0–4)
Clinical frailty score	3 (2–4)
Body mass index	28.1 (21.3–34.6) kg/m^2
Sequential Organ Failure Assessment score at admission	4 (2–6)
CRP at admission	70 (32–483) mg/L
CRP at day of interleukin-6 blood sampling	107 (8–435) mg/L

CRP = C-reactive protein.

TABLE 2.
Timing of Blood Sampling

Number of Days to First Blood Sample	Median (Range)
From disease onset	16 (11–42)
From hospital admission	10 (3–35)
From ICU admission	6 (1–35)
From disease onset in subgroup[a]	14 (11–18)
From hospital admission in subgroup[a]	8 (3–11)
From ICU admission in subgroup[a]	3 (1–6)

[a]Subgroup of patients (n = 5) where blood was sampled the first week in the ICU.
Number of days from disease onset, hospital admission, and ICU admission to first blood sample.

DISCUSSION

The absence of an arteriovenous IL-6 difference disputes the hypothesis of predominantly local IL-6 production in inflamed lungs in critical COVID-19. This suggests that IL-6 is of systemic origin in this condition. A higher IL-6 concentration in arterial than central venous blood has previously been observed in sepsis-related ARDS (5), and if the lungs were the main source of IL-6 in critical COVID-19, we would expect that an arteriovenous difference would be detectable.

The observed relationship between IL-6 and respiratory failure is in accordance with previous studies (2). The beneficial effect of blocking IL-6 in critical COVID-19 has been established (3, 4), and it seems evident that IL-6 contributes to pulmonary inflammation and injury in this condition. It has been proposed that antagonizing IL-6 in COVID-19 may only be beneficial if IL-6 is mainly produced outside the lungs (7). Critical COVID-19 often follows a prolonged course resembling ARDS in sepsis. In this condition, there may be a biological rationale for blocking systemic IL-6, as has recently been demonstrated in a rat sepsis model (8).

The patients in our study were comparable to the general intensive care population with COVID-19 in our hospital with regard to age, sex, BMI, comorbidity,

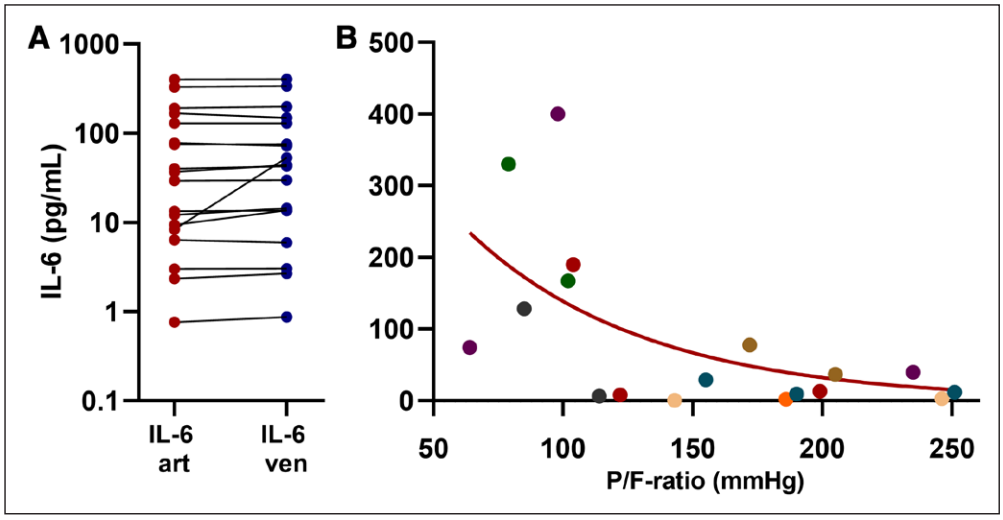

Figure 1. Interleukin-6 in arterial and central venous blood. **A**, Pairs of arterial (art) and central venous (ven) IL-6 blood successively drawn, on a logarithmic scale. No difference in arterial and central venous IL-6 was found. **B**, Association between P/F ratio and arterial IL-6, with nonlinear best fit. Values from the same patient are indicated with the same color. IL = interleukin, P/F ratio = Pao_2/Fio_2 ratio.

and frailty. SOFA score and CRP upon admission were also similar (9). However, the patients in our study had much higher mortality (56% vs 9%) and, thus, represented the most critically ill group of COVID-19 patients in our hospital.

One previous study measuring IL-6 repeatedly in patients with infectious or traumatic pulmonary inflammation detected arteriovenous IL-6 differences only when the blood samples with the highest IL-6 concentrations were analyzed (6). When performing a similar post hoc analysis on our data, we still did not find any arteriovenous IL-6 difference.

Limitations of this study are the small number of patients and the fact that patients with less severe or early COVID-19 were not included for comparison. Measurements of arteriovenous IL-6 concentrations in mild or moderate disease would have required arterial and central venous cannulations where this was not otherwise necessary, and such investigations were beyond the scope of this small study. It could be speculated that local pulmonary IL-6 production is prominent in early COVID-19 but disappears as the disease progresses and becomes predominantly systemic. Post hoc analyses including only the first pairs of blood samples did not reveal any trend toward an arteriovenous IL-6 difference early in the ICU stay. However, there was often a prominent delay from disease onset, hospital admission, and ICU admission to blood sampling, and from our data, it cannot be ruled out that an arteriovenous IL-6 difference is detectable in the earlier phases of COVID-19.

CONCLUSIONS

We found no arteriovenous difference in IL-6 and, hence, postulate that IL-6 in critical COVID-19 is mainly produced outside the lungs as part of a systemic inflammatory response syndrome and act as a driver of local inflammation and damage in the lungs.

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