DOI: 10.1002/JLB.4LT0122-018RR

JLB JOURNAL OF LEUKOCYTE BIOLOGY

LETTERS TO EDITOR

# Circulating IL-6 but not neutrophil extracellular traps levels can predict anakinra effectiveness in patients with severe COVID-19

Dear Editor,

We read with great interest the very recent study from Belaïd et al. These authors confirm in an Algerian cohort that IL-6 circulating level is a good and simple parameter to predict COVID-19 outcome on severe patients.[1] Other reports already suggested that the most severe cases are linked to a hyperinflammatory status linked to the release of cytokines such as IL-6 or IL-1$\beta$, or neutrophil-derived products such as elastase or neutrophil extracellular traps (NETs). Therefore, various targeted therapies have been rapidly proposed, such as anakinra, a recombinant IL-1 receptor antagonist, previously used to treat auto-inflammatory syndromes involving the inflammasome pathway. In March 2020, our group treated 52 consecutive severe COVID-19 patients with anakinra and found that it reduced both the need for invasive mechanical ventilation and the mortality without serious side effects.[2] They had severe bilateral pneumonia, a confirmed Severe Acute Respiratory Syndrome-Coronavirus-2 infection (RT-PCR or computed tomography [CT] scan), bilateral lung infiltrates and critical pulmonary function (oxygen saturation of 93% or less under 6 L/min of oxygen or more, or oxygen saturation of less than 93% on 3 L/min with a saturation on ambient air decreasing by 3% in the previous 24 h). Exclusion criteria were as follows: refusal to participate, patients bedridden and near the end of life, alternative etiology of respiratory failure, and ICU admission. Several other studies and meta-analyses have now confirmed anakinra's beneficial effect on severe patients. Our group participated in the most recent patient-level meta-analysis that concluded that anakinra was safe and reduced mortality in moderate to severe cases, especially when hyperinflammation was observed.[3] Finally, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (December 2021) now officially recommend the use of anakinra with severe patients. Kyriazopoulou et al. recently pointed out the possibility to guide anakinra treatment with soluble urokinase plasminogen activator receptor circulating levels.[3] We analyzed other potential and simpler biomarkers of anakinra effectiveness in our cohort.[2] We decided to focus on circulating IL-6 and NET levels, known to be closely linked to COVID-19 severity, in particular in Belaïd et al.'s study[1] and another cohort recently published by our group.[4] Blood samples were taken just before (Day 0) and 3–4 days after the beginning of anakinra treatment. IL-6 levels were measured using a commercial Electrochemiluminescence assay test (Elecsys IL-6) on COBAS ROCHE platform. NETs were quantified by measuring serum myeloperoxidase (MPO)–DNA complexes using a previously described in-house capture ELISA.[4,5]

Both biomarkers could be assayed in 49 patients with severe COVID-19 belonging to our cohort[2] (13 females/36 males, mean [SD] age 72.6 years [12.0], mean [SD] C-reactive protein 192.4 mg/L [88.2]). All patients had a severe COVID-19-related bilateral pneumonia on chest X-ray or lung CT scan. Patients were treated with subcutaneous anakinra (100 mg twice a day for 72 h, then 100 mg daily for 7 days) and standard treatments.

At Day 0, all patients had high circulating levels of IL-6 at 75.5 pg/ml [46.3–123.7] (median [Q1–Q3]), since the reference value from the manufacturer is below 7 pg/ml. Similarly, higher circulating levels of MPO–DNA complexes were found at Day 0 in the patients (58.4 arbitrary units [au] [10.9–136.8]) compared to healthy controls (4.2 au [4.2–10.2]) ($p < 0.0001$). Both results are in line with previous reports, which showed increased IL-6[1,3,4] and MPO–DNA complexes[4] in comparable patients. The 28-day probability of survival during anakinra treatment was significantly higher in patients who had IL-6 levels below the median value at Day 0, but not in those with MPO–DNA complex levels below the median value at Day 0 (Figure 1A,B).

In a subgroup of 32 patients, a significant decrease in IL-6 levels was observed at Day 3 or 4 (23.2 pg/ml [7.5–45.1], $p = 0.012$ using paired *t*-test) as compared to Day 0 values (64.3 pg/ml [45.7–107.8]) (Figure 2A). MPO–DNA complex levels were not significantly modified during these 3–4 days (47.1 au [29.0–86.5] at Day 3–4 vs. 51.1 au [8.4–140.6] at Day 0, $p = 0.31$) (Figure 2B).

A growing body of evidence suggests a pathogenic role of IL-1$\beta$ in the COVID-19- related inflammatory cascade since high levels are found in the circulation, and patients' monocytes or neutrophils exhibit an hyperactivation of IL-1$\beta$ pathway.[3,4] Moreover, anakinra treatment was also associated with an increased survival rate in a recent mouse model of SARS-CoV-2-induced pneumonia.[6] Our results suggest that anakinra-induced IL-1 inhibition participates in a more global blocking effect upon the proinflammatory cytokine cascade as we found a decrease in IL-6 circulating levels after 3–4 days of anakinra. Therefore, monitoring IL-6 levels during anakinra treatment seems to be a simple and useful way to assess anakinra effectiveness. Conversely, we found no decrease in MPO-DNA soluble complex levels, a hallmark of NETs, after 3–4 days of anakinra.

  

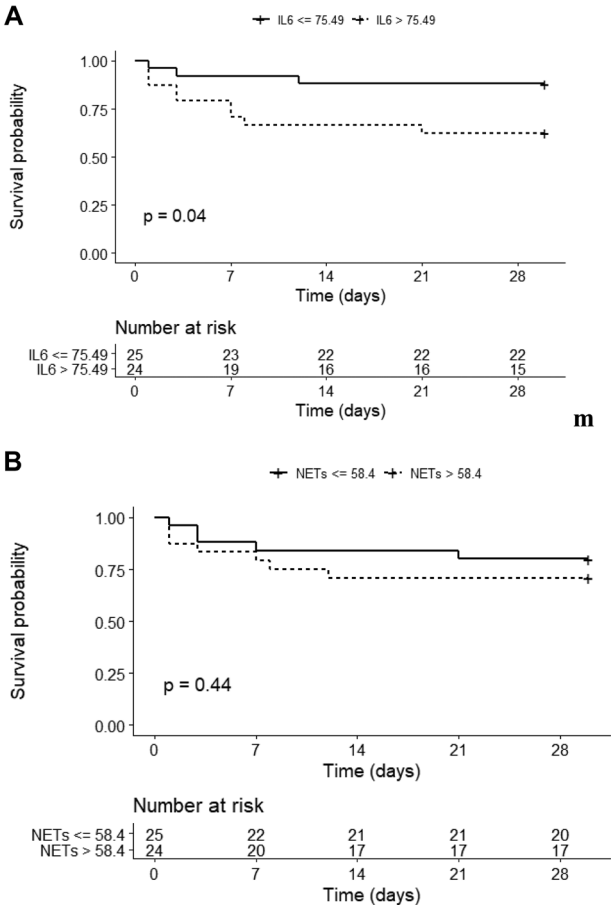


**FIGURE 1** Kaplan–Meier cumulative estimation of probability of survival according to IL-6 (**A**) or NET (**B**) levels at Day 0 and log-rank test. The median values of IL-6 levels (75.49 pg/ml) and NET levels (58.39 au) are used for the analysis

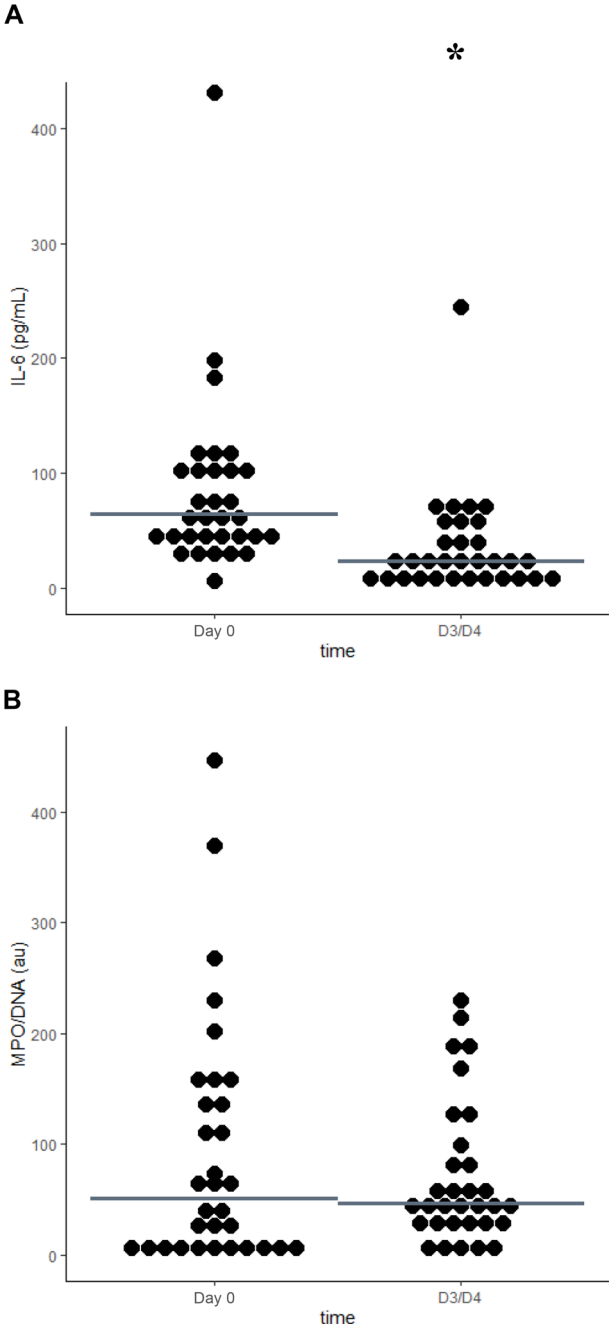


**FIGURE 2** IL-6 circulating levels before (Day 0) and after 3–4 days of anakinra (D3/4). The horizontal bars represent the median values ($n = 32$). *$p = 0.012$ as compared to Day 0 values

In conclusion, in moderate to severe forms of COVID-19, anakinra significantly and rapidly downregulated the release of IL-6 and was more effective in patients with higher baseline IL-6 titers. These results reinforce Belaïd et al.'s study results recently published in the *Journal of Leukocyte Biology*[1] and confirm the importance of monitoring IL-6 circulating levels during severe COVID-19. Due to legal limitations, we could not include a replication cohort. Indeed, the French Agency for Drug Security (ANSM) abruptly stopped the use of anakinra on COVID-19 patients in October 2020 for concerns related to its potential toxicity. The European Medical Agency (EMA) then gave the approval again on December 2021. Even though some patients have been treated since January 2022 in our institution, they are too few to constitute a replicated study at present.

## FUNDING INFORMATION

There was no funding for this work.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

Vanessa Granger[1,6]
Audrey Fels[2]
Thomas Huet[3]
Jean-Louis Laplanche[4]
Sophie Laplanche[5]
Gilles Chatellier[2]
Hélène Beaussier[2]
Sylvie Chollet-Martin[1,6]
Luc de Chaisemartin[1,6]

Gilles Hayem[3]

[1]*Immunology Department, Bichat Hospital, APHP, DMU BIOGéM, Paris, France*

[2]*Clinical Research Department, Paris-Saint Joseph Hospital Group, Paris, France*

[3]*Rheumatology Department, Paris-Saint Joseph Hospital Group, Paris, France*

[4]*Biochemistry & Molecular Biology Department, Saint-Louis Lariboisière and Fernand-Widal Hospital Group, Paris, France*

[5]*Biology Department, Paris-Saint Joseph Hospital Group, Paris, France*

[6]*Inserm, Inflammation, Microbiome and Immunosurveillance, Université Paris-Saclay, Châtenay-Malabry, France*

**Correspondence**

Sylvie Chollet-Martin, Immunology Department, Bichat Hospital, APHP, DMU BIOGéM, Paris 75018, France.

Email: sylvie.chollet-martin@u-psud.fr

## REFERENCES

1. Belaid B, Lamara Mahammad L, Mihi B, et al. T cell counts and IL-6 concentration in blood of North African COVID-19 patients are two independent prognostic factors for severe disease and death. *J Leukoc Biol*. 2022;111:269-281.
2. Huet T, Beaussier H, Voisin O, et al. Anakinra for severe forms of COVID-19: a cohort study. *Lancet Rheumatol*. 2020;2:e393-400.
3. Kyriazopoulou E, Huet T, Cavalli G, et al. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. *Lancet Rheumatol*. 2021;3:e690-e697.
4. Peyneau M, Granger V, Timsit J-F, et al. Innate immune deficiencies are associated with severity and poor prognosis in patients with COVID-19. *Sci Rep*. 2022;12:638.
5. Jönsson F, de Chaisemartin L, Granger V, et al. An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis. *Sci Transl Med*. 2019;11:eaat1479.
6. Xiong S, Zhang L, Richner JM, Class J, Rehman J, Malik AB. Interleukin-1RA mitigates SARS-CoV-2-induced inflammatory lung vascular leakage and mortality in humanized K18-hACE-2 mice. *Arterioscler Thromb Vasc Biol*. 2021;41:2773-2785.