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LETTER

Long-term follow-up of neutrophil activation after severe-to-critical SARS-CoV-2 infection: A longitudinal study

To the Editor,

The risk factors and characteristics of SARS-CoV-2 infection have been extensively described in the literature including respiratory *sequelae*. The release of neutrophil extracellular trap (NET) activation is associated with the early response and the severity of pulmonary damage and other multi-visceral manifestations of SARS-CoV-2 infection.[1,2] Neutrophil elastase (NE), myeloperoxidase–DNA (MPO–DNA) and histone–DNA complexes are increased at the early stage of the infection.[2,3] NE and histone–DNA complex are independent predictors of COVID-19 lung damage and the number of affected organs. The increased blood concentrations of NE and NETs were related to exacerbation of neutrophil stimulation through IL-8 and CXCR2 axis.[2,3] However, the implication of NETs in post COVID-19 respiratory *sequelae* is unknown. This study evaluated the evolution of biomarkers of NETosis over the first 6 months after severe SARS-CoV-2 infection in a translational study performed in the Regional University Hospital of Nancy (agreement of local ethics committee, Registration No. 2020PI087). Consent was obtained from all participants.

We included 98 survivors of severe SARS-CoV-2 infection (*i.e.*, hospitalized in intensive care unit or in ward for at least 7 days) who had a follow-up visit at 3 months after hospital discharge. In case of respiratory *sequelae*, follow-up visit at 6 months was planned (Figure 1A). Characteristics of the study population at inclusion is available in Appendix S1. Briefly, median age of the 98 patients was 65.5 [56–73] years, 72 (73.5%) were male, with a median BMI of 27.8 [24.6–31.0] kg/m^2, 41 patients (41.8%) were smokers or ex-smokers. The control groups included healthy subjects prior to SARS-CoV-2 pandemic or with no reported contact with infected cases and negative for COVID-19 screening.

Blood levels of NE, MPO–DNA and histone–DNA complexes as well as cell-free DNA were significantly higher than those of healthy controls at 3 and 6 months after SARS-CoV-2 infection ($p < .0001$). Additionally, blood levels of DNAse 3 and 6 months were dramatically lower in patients than in healthy controls (Figure 1B). The changes of these biomarkers between 3 and 6 months are described in Figure 2A and Figure S1. Cell-free DNA and blood levels of CXCR2 showed a significant decrease in their levels between 3 and 6 months, while other biomarkers remain unchanged. Significant correlations between components of NETs and clinical or biological features involved in the severity of SARS-CoV-2 infection were showed. Three-month NE blood level was significantly correlated with initial BMI ($p = .0162$), leukocytes ($p = .0023$) and neutrophils ($p = .0036$), MPO–DNA with lymphocytes ($p = .0072$), and histone–DNA complexes with arterial oxyhemoglobin saturation ($p = .0167$) (Figure S2). The upper quartile of blood levels of CXCR2, a marker of neutrophil activation, was associated with respiratory *sequelae* at 3 months (Figure 2B). In addition, we also observed a significant correlation between CXCR2 and IL8, a macrophage cytokine involved in neutrophil activation. A similar correlation was found between NE and IL8.

Vascular neutrophil recruitment and evidence for NETosis in the acute infection with SARS-CoV-2 leading to severe COVID-19 is now well documented in the literature.[2] The NETosis and increased serum level of CXCR2 at 3 and 6 months after COVID-19 suggested that the activation of neutrophils was maintained in absence of virus particle. Macrophages can be activated by components of NETs. This could explain the increased production of IL-8, which in turn will produce a loop by activating neutrophils and the release of components of NETs through the CXCR2/IL8 pathway (Figure 2C).[4] Moreover, the use of curative anticoagulation during the acute phase of SARS-CoV-2 infection did not affect the levels of any markers of NETs, neither cell-free DNA nor DNase levels.

In conclusion, our study showed that neutrophil activation remained dramatically high 3 and 6 months after severe-to-critical SARS-CoV-2 infection. More studies are needed to assess whether CXCR2 and NETosis influence the development of respiratory sequelae. Whether the long-term post COVID-19 elevation of NETs results from pattern recognition receptor (PRR)-triggered NETosis and other innate immunity mechanisms[5] or adaptative immunity or both should also deserve further attention.

AUTHOR CONTRIBUTIONS

Simon Valentin: Conceived and designed the analysis, Collected the data, Contributed analysis tool, Performed the analysis, Wrote the paper, Final approval of the manuscript; Veronique Regnault: Contributed analysis tool, Performed the analysis, Wrote the paper, Final approval of the manuscript; Jean-Louis

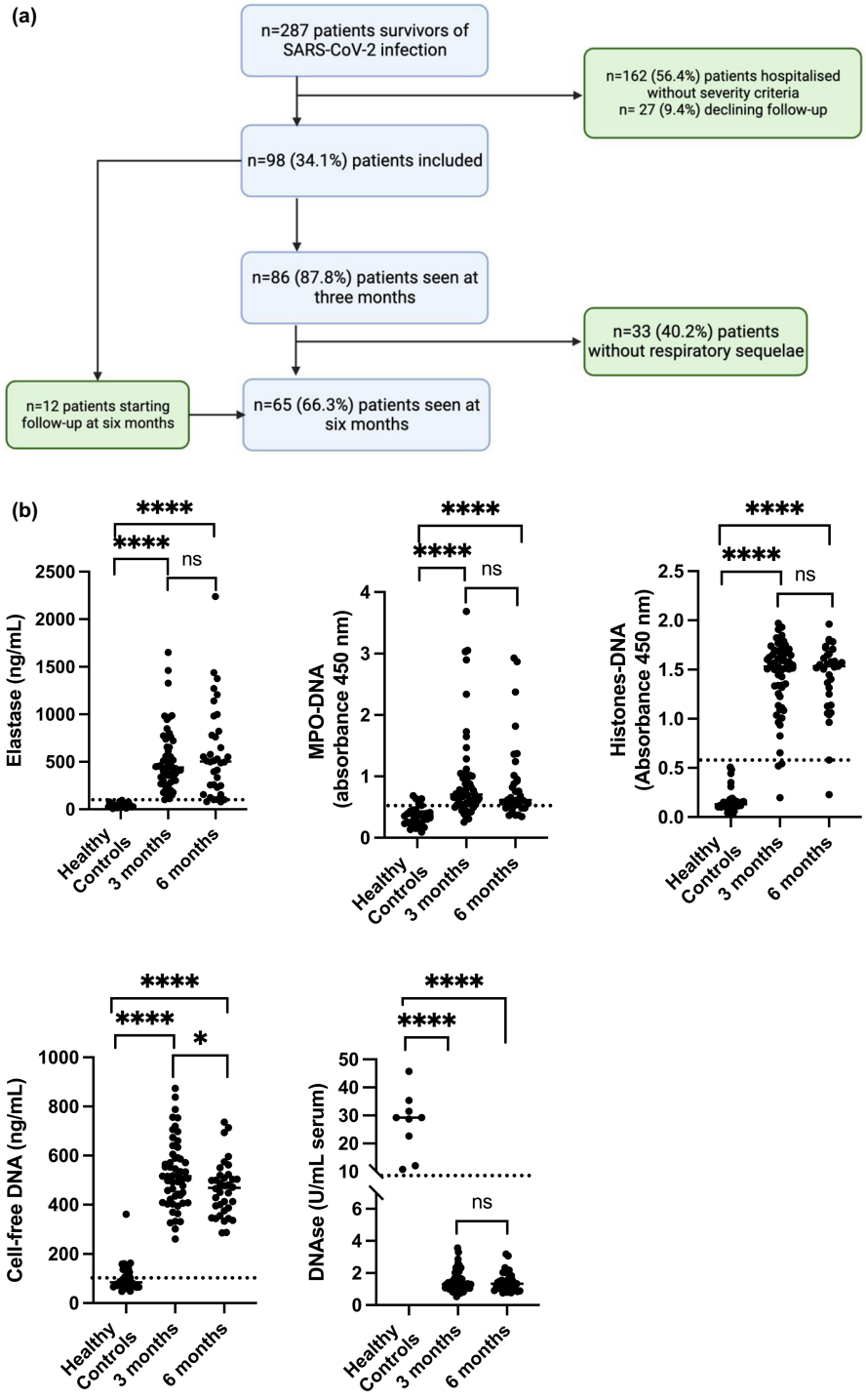

FIGURE 1 (A) Diagram of the inclusion of patients. (B) Blood levels of components of neutrophil extracellular traps (NETs) in healthy controls and survivors at 3 and 6 months after severe-to-critical SARS-CoV-2 infection. The dashed lines represent the cutoffs defined by mean + 2 standard deviation of concentrations determined in controls. Data were compared with Mann–Whitney test. ****p-value <.0001; ***p-value <.001; *p-value <.05.

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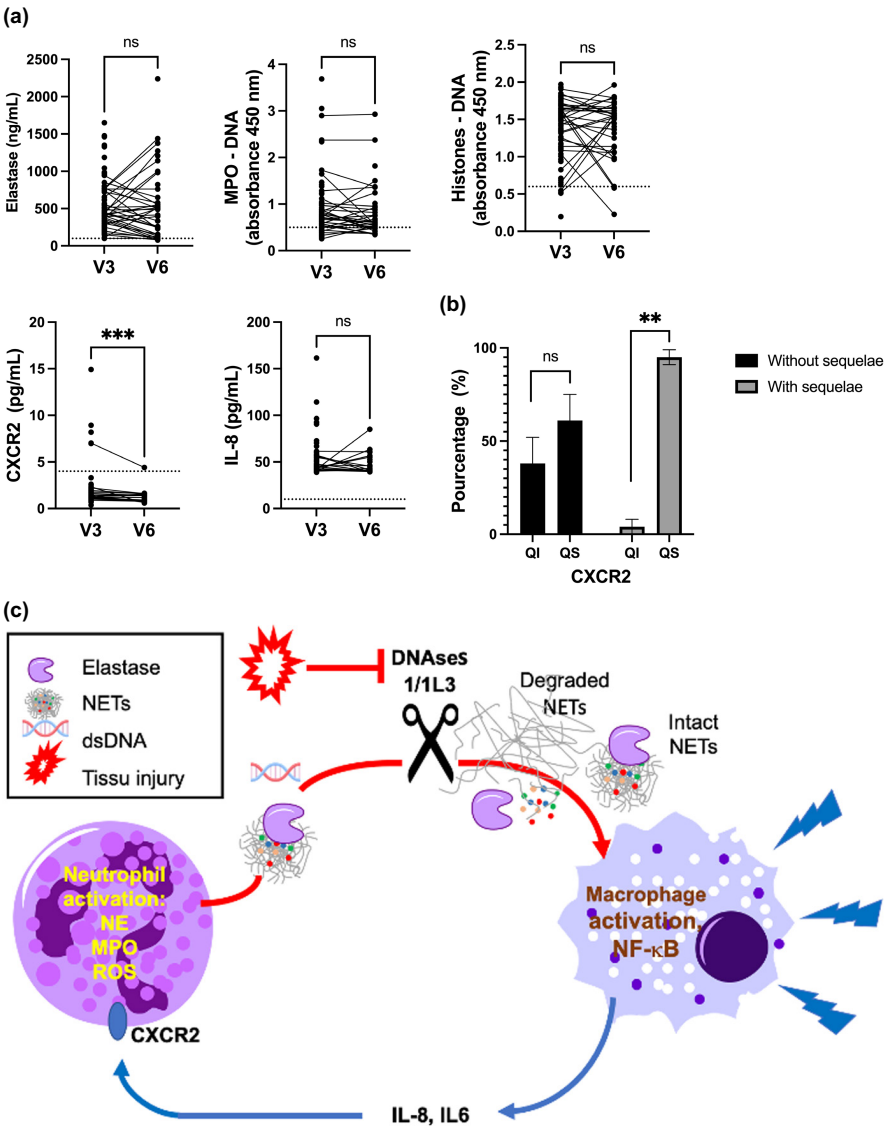

FIGURE 2 (A) Evolution of components of NETs and CXCR2 between three and six months after severe-to-critical SARS-CoV-2 infection. (B) Quartile rate analysis of CXRC2 between patients with and without *sequelae*. (C) The "macrophage/neutrophil loop" hypothesis of long-term NETosis in absence of virus replication. The increased production of IL8 by macrophages activates neutrophils though the IL-8/CXCR2 pathway and in turn, the release of NET components by activated neutrophils activates the macrophage production of IL8. ***p*-value <.01; ****p*-value <.001.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare related to this manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.