Antibodies against type-I Interferon: detection and association with severe clinical outcome in COVID-19 patients

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Conflict of interest: All authors declare no conflict of interest

Funding: This research is being supported by Hospices Civils de Lyon and by Fondation des Hospices Civils de Lyon.
Abstract

Objectives Impairment of type I interferon (IFN-I) immunity has been reported in critically ill COVID-19 patients. This defect can be explained by the presence of circulating autoantibodies against IFN-I. We set out to improve the detection and the quantification of such antibodies (Abs) in a cohort of severe Covid-19 patients, in an effort to better document the prevalence of these Abs as the pandemics evolves and how they correlate with the clinical course of the disease.

Methods Anti-IFN-α Abs was investigated 84 critical COVID-19 patients who were admitted to ICU at the Lyon University Hospital, France with a commercially available kit (Thermo-Fisher).

Results Twenty-one patients out of 84 (25%) had anti-IFNα2 Ab above cut-off (>34ng/mL) in sera. A neutralizing activity against IFN-α2 was evidenced in 15 of them, suggesting that 18% of patients were positive for neutralizing anti-IFN-α and -ω auto-Abs. In addition, in most of patients with neutralizing IFN-I Abs, we noticed an impairment of the IFN-I response. However, we did not find any difference in terms of clinical characteristics or outcome between critical COVID-19 patients “with or without” neutralizing anti-IFN-α2 auto-Abs in these conditions. Finally, we detected anti-type I IFN auto-Abs in COVID-19 patients’ sera were detected throughout the ICU stay.

Conclusions We report that 18% of severe COVID-19 patients were positive for these Anti-Type-I IFN Abs, confirming the detrimental role of these Abs on the antiviral response. Our results further support the use of recombinant type I IFNs not targeted by the auto-Abs (e.g., IFN-β) in COVID-19 patients with an impaired IFN-I response.

Keywords – Type I interferon, COVID-19, SARS-CoV2 virus, Intensive care unit, viral infection, auto-antibodies
Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) infection leads to coronavirus disease 19 (COVID-19), a disease with a wide spectrum of clinical presentations, including severe pneumonia. The anti-SARS-CoV-2 immune response has been extensively studied and defects of antiviral mechanisms have been linked to disease severity. In particular, impairment of type I interferon (IFN-I) immunity has been reported in critically ill COVID-19 patients infected during the first wave of the pandemics. This defect was explained either by inborn genetic defects of the IFN-I pathway or by the presence of circulating autoantibodies against 14 or the 17 individual IFN-I (1-4). These auto-Abs against type I IFNs were also found in a third of patients from a small international cohort who had suffered from severe adverse events following Yellow Fever vaccination (YFV-17D) vaccination (5).

These findings advocate for the development of diagnostic tools in routine laboratories. Here, we set out to improve the detection and the quantification of such antibodies (Abs) in a cohort of severe Covid-19 patients, in an effort to better document the prevalence of these Abs as the pandemics evolves and how they correlate with the clinical course of the disease.
Results

Our cohort comprised 84 critical COVID-19 patients who were admitted to ICU at the Lyon University Hospital, France between September and December 2020. A local ethical committee for biomedical research (Comité de Protection des Personnes HCL, Supplemental material and methods) approved our study. The presence of anti IFN-α2 Abs was investigated with a commercially available kit (Thermo-Fisher). We first sought to determine a positive cut-off value for Ab detection by performing measurements in 76 putative control sera ie from healthy donors retrieved before the Covid19 outbreak. The mean value +3SD of these measurements gave a cut-off value at 34ng/mL. We then assessed the presence of IFN-α2 Abs in putative positive sera, ie sera from patients with autoimmune polyendocrinopathy type 1 syndrome (APS-1), a condition known to be associated with anti-cytokine autoAbs. All 11 patients tested were positive for anti-IFN-α2 autoAbs (>100ng/mL for all of them). We also evaluated the presence of anti-IFN-α2 autoAbs in 10 mild COVID-19 healthcare workers for which no autoAb was found.

We then measured anti-IFNα2 Ab levels in sera from the 84 COVID-19 patients in our ICU cohort, and values above cut-off (>34ng/mL) were found in 21 patients. The neutralizing capacity of patients’ sera against IFN-α and IFN-ω was then evaluated as previously described (5). A neutralizing activity was evidenced in 15 out the 21 sera with IFN-α2 Abs, suggesting that 18% of patients were positive for neutralizing anti-IFN-α and -ω auto-Abs (Figure 1A). Importantly, all sera with anti-IFN-α2 auto-Abs concentrations above 1µg/mL potently neutralized IFN-α and -ω in vitro. In addition, in most patients with neutralizing IFN-I Abs, we noticed an impairment of the IFN-I response, as determined by the measurement of i) plasma IFN-α2 levels using the new digital ELISA technology single-molecule arrays (Simoa) and ii) blood Interferon Stimulating Genes (ISG) expression using the Nanostring nCounter technology in blood samples collected in the first 15 days after symptoms (Figure 1B-C).

Moreover, we found no difference in clinical parameters (age, sex ratio, co-morbidity) or outcome (death, O2 support) between critical COVID-19 patients “with or without” neutralizing anti-IFN-α2
auto-Abs in these conditions (Table 1). Finally, we detected anti-type I IFN auto-Ab in COVID-19 patients’ sera throughout the ICU stay, as shown in Figure 1D.

Conclusions

A previous study reported that IFN-I auto-Abs were undetectable in 663 individuals with asymptomatic or mild COVID-19 and only detected in 0.33% of healthy individuals (1) whereas we report here that 18% of severe COVID-19 patients were positive for these Abs. This finding further confirms the deleterious role of IFN-I autoAbs in the antiviral immune response, and reciprocally the central importance of the IFN-I pathway. We noticed that only a part of auto-Abs detected were able to neutralize IFN-I in the conditions we used, which confirms previous studies in COVID-19 patients and systemic lupus erythematosus subjects (1, 6). Our results further support the use of recombinant type I IFNs not targeted by the auto-Abs (e.g., IFN-b) or type III IFNs in COVID-19 patients with an impairment of the IFN-I response. First clinical trials evaluating the administration of recombinant IFN-b did not show any beneficial effect in ICU patients (7). Nevertheless, post-hoc analysis have demonstrated the benefit of cytokine therapies (ie IL-1RA or GM-CSF) in a sub cohort of septic shock patients, when initial clinical trials had reported no positive effect (8-11). Moreover, such treatments could be more adapted to selected patients with IFN-α2 autoAb. Yet, inhaled IFNβ was efficient in patients with moderate or severe disease (12) and could be given in ambulatory patients, symptomatic or not, or even in contact cases.

Methods

Ethics

Critical COVID-19 patients

Plasma samples and Paxgene® tubes were collected from COVID-19 patients hospitalized in the university hospital of Lyon (Hospices Civils de Lyon), France. Diagnosis of COVID-19 was confirmed in all patients by RT-PCR.
All critically ill patients, admitted to ICU, were included in the MIR-COVID study. This study was registered to the French National Data Protection Agency under the number 20-097 and was approved by an ethical committee for biomedical research (Comité de Protection des Personnes Hôpitaux) under the number N°20-41. In agreement with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the French data protection law (Law n°78-17 on 06/01/1978 and Décret n°2019-536 on 29/05/2019), we obtained consent from each patient or his next of kin.

Mild COVID-19 patients

Plasma samples and Paxgene® tubes were collected from symptomatic healthcare workers at SARS-CoV-2 diagnosis. Written informed consent was obtained from all participants; ethics approval was obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France; ID RCB 2020-A00932-37), and the study was registered on ClinicalTrials.gov (NCT04341142) (13).

Auto-Abs anti Type I IFN investigation

The presence of anti IFN-α2 Abs was investigated in plasma with a commercially available kit (Thermo-Fisher). The positive cut-off value for Ab detection was 34ng/mL. The neutralization capacity of these antibodies against IFN-α2 and -α was determined as previously described (5).

Plasma protein quantification

Plasma IFN-α concentrations (fg/ml) were determined by single molecule array (Simoa) using a commercial kit for IFN-α2 quantification (Quanterix™, Lexington, MA, USA) on plasma samples of COVID-19 patients. The assay was based on a 3-step protocol using an HD-1 Analyzer (Quanterix).

IFN score assessment

RNA was extracted from whole blood contained in Paxgene® tubes (Kit PreAnalytix, Qiagen©, SW) and quantified by spectrophotometric assay (Nanodrop 2000, Thermo Scientific™, MA, USA). RNA integrity was then evaluated by Agilent RNA microarray (Agilent Technologies©, Santa Clara, CA,
USA. mRNA quantification of 6 ISGs (interferon alpha inducible protein 27 (IFI27), interferon induced protein 44 like (IFI44L), Interferon Induced Protein With Tetratricopeptide Repeats 1 (IFIT1), ISG15 Ubiquitin Like Modifier (ISG15), Radical S-Adenosyl Methionine Domain Containing 2 (RSAD2), Sialic Acid Binding Ig Like Lectin 1 (SIGLEC1) and 3 housekeeping genes (Actin Beta (ACTB), Hypoxanthine Phosphoribosyltransferase 1 (HPRT1), RNA Polymerase II Subunit A (POLR2A)), was performed using nanostring technology (Nanostring Technologies®, WA, USA). Data standardization was obtained using the geometric mean of internal control and housekeeping genes count number. Interferon score was calculated as previously described (14).

References

1. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2021 Oct 23;370(6515).
2. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science. 2020 Aug 7;369(6504):718-24.
3. Trouillet-Assant S, Viel S, Gaymard A, Pons S, Richard JC, Perret M, et al. Type I IFN immunoprofiling in COVID-19 patients. J Allergy Clin Immunol. 2020 Jul;146(1):206-8.e2.
4. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515).
5. Bastard P, Michailidis E, Hoffmann HH, Chbihi M, Le Voyer T, Rosain J, et al. Auto-antibodies to type I IFNs can underlie adverse reactions to yellow fever live attenuated vaccine. J Exp Med. 2021 Apr 5;218(4).
6. Gupta S, Nakabo S, Chu J, Hasni S, Kaplan M. Association between anti-interferon-alpha autoantibodies and COVID-19 in systemic lupus erythematosus. medrxiv. 2020.
7. Pan H, Peto R, Henao-Restrepo AM, Preziosi MP, Sathiyamoorthy V, Abdool Karim Q, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med. 2021 Feb 11;384(6):497-511.
8. Bo L, Wang F, Zhu J, Li J, Deng X. Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: a meta-analysis. Crit Care. 2011;15(1):R58.
9. Opal SM, Fisher CJ, Jr., Dhainaut JF, Vincent JL, Brase R, Lowry SF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med. 1997 Jul;25(7):1115-24.
Randomised controlled trial of GM-CSF in critically ill patients with impaired neutrophil phagocytosis. Thorax. 2018 Oct;73(10):918-25.

Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. 2016 Feb;44(2):275-81.

Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med. 2021 Feb;9(2):196-206.

Assessment of serological techniques for screening patients for COVID-19 (COVID-SER): a prospective, multicentric study. BMJ Open. 2020 Nov 24;10(11):e041268.

Comparison of RT-qPCR and Nanostring in the measurement of blood interferon response for the diagnosis of type I interferonopathies. Cytokine. 2018 Jan;113:446-52.

Figure 1- Anti-Type I IFN antibodies (Abs) in patients with life-threatening COVID-19

A. Auto-Abs against IFN-α2 concentrations (ng/mL) determined by Thermofisher kit in serum samples collected from COVID-19 patients admitted in ICU (n=84) and COVID-19 patients with mild respiratory symptoms (n=10). Neutral. means auto-Abs with neutralizing capacity against IFN-α and -ω B. C- IFN-α2 concentrations (fg/mL) (B) and ISG score (C) in plasma and whole blood collected from COVID-19 patients in the first 15 days after symptom onset (critical COVID-19 (n=54) and mild COVID-19 (n=10)). D- Longitudinal detection of auto-Abs against IFN-α2 of COVID-19 patients during ICU stay according to time post symptom. Dotted lines represent positive cut-off value (threshold), lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ)
## Table 1 - Clinical characteristics of COVID-19 patients in intensive care unit

Data are median [EIQ] or count (percentage). Mann-Whitney and Fisher tests were used for quantitative and qualitative variables, respectively. ICU - Intensive Care Unit, BMI - Body Mass Index, nAb – neutralizing Auto-Abs against IFN-α.

| Clinical features                              | **Auto-nAb Negative** | **Auto-nAb Positive** | **P-value** |
|------------------------------------------------|-----------------------|-----------------------|-------------|
| **(n=69)**                                      | **(n=15)**            | **(n=15)**            |             |
| Age                                            | 67 [58-72]            | 65 [55-74]            | 0.75        |
| Sex male (%)                                   | 54 (78%)              | 13 (87%)              | 0.72        |
| BMI (kg/m²)                                    | 29 [26-34]            | 29 [25-32]            | 0.49        |
| Auto-immune disease                            | 7 (10%)               | 2 (13%)               | 0.66        |
| Time between 1st symptoms and ICU admission (day) | 9 [7-12]              | 10 [7-11]             | 0.80        |
| Maximal ventilatory support                    |                       |                       |             |
| • Standard oxygen only                         | 4 (6%)                | 0 (0%)                | 0.33        |
| • High flow oxygen only                        | 19 (28%)              | 7 (47%)               |             |
| • Invasive mechanical ventilation              | 46 (67%)              | 8 (53%)               |             |
| ARDS criteria                                  | 44 (64%)              | 8 (53%)               | 0.65        |
| Worst PaO2/FiO2 day 1 in ICU (mm Hg)            | 75 [62-103]           | 89 [62-116]           | 0.40        |
| ECMO                                           | 15 (22%)              | 2 (13%)               | 0.72        |
| SOFA Day 1 in ICU                              | 4 [3-8]               | 3 [2-5]               | 0.11        |
| SAPS2 Day 1 in ICU                             | 40 [31-47]            | 43 [38-48]            | 0.41        |
| Vasopressor requirement in ICU                 | 45 (65%)              | 9 (60%)               | 0.77        |
| Renal replacement therapy in ICU               | 27 (39%)              | 4 (27%)               | 0.56        |
| ICU length of stay (day)                       | 13 [7-35]             | 13 [6-24]             | 0.74        |
| ICU mortality                                  | 29 (42%)              | 5 (33%)               | 0.58        |
