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SHORT COMMUNICATION

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High sera levels of SARS-CoV-2 N antigen are associated with death in hospitalized COVID-19 patients

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Abstract

The presence of free severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid-antigen in sera (N-antigenemia) has been shown in COVID-19 patients. However, the link between the quantitative levels of N-antigenemia and COVID-19 disease severity is not entirely understood. To assess the dynamics and clinical association of N-antigen sera levels with disease severity in COVID-19 patients, we analyzed data from patients included in the French COVID cohort, with at least one sera sample between January and September 2020. We assessed N-antigenemia levels and anti-N IgG titers, and patient outcomes was classified in two groups, survival or death. In samples collected within 8 days since symptom onset, we observed that deceased patients had a higher positivity rate (93% vs. 81%; $p < 0.001$) and higher median levels of predicted N-antigenemia (2500 vs. 1200 pg/mL; $p < 0.001$) than surviving patients. Predicted time to N-antigen clearance in sera was prolonged in deceased patients compared to survivors (23.3 vs 19.3 days; $p < 0.0001$). In a subset of patients with both sera and nasopharyngeal (NP) swabs, predicted time to N-antigen clearance in sera was prolonged in deceased patients

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($p < 0.001$), whereas NP viral load clearance did not differ between the groups ($p = 0.07$). Our results demonstrate a strong relationship between N-antigenemia levels and COVID-19 severity on a prospective cohort.

KEYWORDS
antigenemia, COVID-19, hospitalized, serological marker, severity

1 | INTRODUCTION

Diagnostic of COVID-19 infection relies on several assays detecting either severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens or viral RNA, with the gold standard being the reverse-transcriptase polymerase chain reaction (RT-PCR) performed on nasopharyngeal (NP) swabs or other respiratory samples.[1] RT-PCR techniques are sensitive and allow an estimation of the viral load with the cycle threshold (CT) value. Several works have demonstrated a link between NP viral load and disease severity.[2] However this association has been questioned by other studies.[3,4] Indeed, many biases can interfere in the quantitative evaluation.

Direct detection of SARS-CoV-2 by the RT-PCR has also been performed on blood samples and it seems to be associated with the severity of the disease, although discrepancies exist.[5,6] In addition, other works have evaluated the detection of N-antigen in blood. The detection of this circulating antigen in the serum appears to provide a good sensitivity for clinical diagnosis[7,8] even in patients with negative NP RT-PCR, suggesting it might reflect viral replication in the lung.[7] Recently, several works suggested an association between N-antigen sera levels and disease severity.[9–12]

Thus, N-antigen detection and quantification could provide an easy-to-use, scalable, and inexpensive diagnostic tool that would also provide a prognostic assessment. However, the kinetic of this marker has not been evaluated and compared to other markers such as NP viral loads. In this work, we used samples from hospitalized patients presenting various degrees of disease severity to assess the SARS-CoV-2 N-antigen dynamics and association with disease severity.

2 | METHODS

2.1 | Study population

We selected 320 patients that were included in the French COVID cohort (clinicaltrials.gov NCT04262921) between January 25 and September 2, 2020, when the historical strain of SARS-CoV-2 was the dominant variant in Europe. We had at least one serum per patient collected within 60 days since symptoms onset (SSO). Patients were classified according to their clinical outcome that is, (I) Survival for recovery (with or without ICU transfer) or (II) Death (for patients who died within 60 days).

2.2 | N-antigen and viral load assays

N antigenemia levels were determined with a certified in-vitro diagnostic (CE-IVD) ELISA assay, COV-Quanto® (AAZ, Boulogne-Billancourt, France), as previously described.[7] The use of standards made of recombinant N antigen allowed the quantification of N-antigenemia levels. The range of linearity was between 2.89 and 150 pg/mL and the limit of quantification was 2.97 pg/mL.

2.3 | Anti-SARS-CoV-2 IgG titers

Anti-N IgG titers were assessed using the multiplex immunoassay V-PLEX panel on the Meso Scale Discovery (MSD) platform, according to manufacturer recommendations. Standards were ranging from 0.004 to 200 units/mL.

2.4 | Viral dynamic model

We reconstructed N-antigen kinetics by considering this biomarker as a plasmatic proxy for NP viral load. We used a target-cell limited model described previously[2,13] to which we added a transfer rate to the plasmatic/extracellular compartment, yielding to a N-antigenemia. This transfer rate also allows for consideration of the extra-pulmonary infection producing N-Antigen. The model is given by the following equations:

$$\frac{dAg}{dt} = R(V_i + V_{ni}) - E_1 Ag - E_{\max}\frac{IgG(t)}{IgG(t) + IgG_{50}}Ag$$

$$\{dlgG\}\{dt\} = a \times \exp\text{^}\{(-\exp\text{^}\{(b - c \times t)\})\}$$

Where Ag is N-antigenemia, R the transfer rate, V_i the infectious viral particles, V_{ni} the noninfectious viral particles, E_1 the elimination rate of N-antigen in absence of antibodies, E_{max} the maximal elimination rate of N-antigen mediated by IgG, and IgG_{50} the antibody titer required to achieve 50% of E_{max}. Kinetics of IgG were described using a Gompertz sigmoid function, with a being the maximal concentration of IgG, b and c dimensionless parameters. For a comprehensive understanding of this model, additional details can be found in the supplementary methods.

2.5 | Anti-cytokine auto-antibodies detection

Serum samples were screened for auto-antibodies against 18 targets in a multiplex particle-based flow cytometry assay, in which magnetic beads with differential fluorescence were covalently coupled to recombinant human proteins. Patients with a fluorescence intensity (FI) of >1500 for IFN-α2 or >1000 for IFN-ω were tested for blocking activity, as were patients positive for another cytokine.

ELISA was performed as previously described.[14] In brief, ELISA plates were coated with recombinant human interferon-α (rhIFN-α) or interferon- ω (rhIFN-ω) and incubated with 1:50 dilutions of plasma samples from the patients or controls.

2.6 | Statistical analysis

Continuous variables are presented as median interquartile range (IQR) and compared by the Wilcoxon rank-sum test if not normally distributed. Categorical variables are presented as n (percent) and compared using Fisher's exact test, as implemented in R statistical software (version 4.2.1).

3 | RESULTS

3.1 | Population

A total of 1053 samples from 320 patients collected during the first two waves in France were analyzed. Patients were mainly male (66%) and were 63 years old in median [IQR: 52–71]. 238 patients were discharged alive, including 94 patients that had been admitted to ICU, while 82 patients died within 2 months postinfection. Median delay between symptom onset and hospitalization was slightly different between surviving and deceased patients: 8 days [IQR: 6–10] and 7 days [IQR: 4–10], respectively (Table 1).

3.2 | N-antigenemia kinetics

We used a target-cell limited model with a transfer rate to the plasmatic compartment to reconstruct the viral dynamics of N-antigen concentrations with a nonlinear and saturable effect of anti-N IgG on its clearance.

We estimated the basic reproduction number R_0 to 13.6 and the loss rate of infected cells to 0.78 day^{-1}. Viral particles (estimated at 3.7×10^5 particles/day)[2] produced were transferred to the plasma compartment at a rate R equal to 10^{-4} day^{-1}. Elimination rate of N-Antigenemia in the absence of IgG was estimated at $E_1 = 0.13$ day^{-1}, whereas the maximal elimination rate mediated by IgG was estimated at $E_{max} = 0.7$ day^{-1}. This caused the half-life of N-antigen in the plasmatic compartment to decrease from 5 days to at most 0.8 days due to IgG-mediated elimination. We estimated IgG_{50} (the concentration of IgG required to achieve 50% of this effect) at 2.54 A.U/mL, and the maximal predicted concentration of IgG was estimated at 5.4 A.U/mL.

The entire N-antigen course was reconstructed for patients until clearance. Our model predicted N-antigenemia to peak 2.4 days after symptom onset [IQR: 1.1–6.9 days] and a time to N-antigenemia clearance of 19.8 days SSO [IQR: 17.3–23.9 days] (Figure 1A,B).

3.3 | N-antigenemia levels across severity groups

Overall, ninety percent of patients tested positive for N-antigenemia, with a median concentration of 2.4 log_{10} pg/mL [IQR: 1.96–3.43] on their first positive sample.

TABLE 1 Epidemiological and clinical description of the 320 patients with SARS-CoV-2 included.

Characteristics	Overall (*n* = 320)	Survival group (*n* = 238)	Death group (*n* = 82)	*p* Value
Male gender	236 (66.1%)	146 (61.1%)	69 (81.4%)	****
Age ≥ 65 years old	162 (45.4%)	89 (37.3%)	46 (56.1%)	**
Hypertension	134 (42.9%)	83 (34.8%)	35 (42.6%)	ns
Chronic cardiac disease	68 (20.9%)	38 (15.9%)	20 (24.3%)	ns
Chronic pulmonary disease	46 (13.8%)	28 (11.7%)	14 (17.1%)	ns
Asthma	33 (10.2%)	26 (10.9%)	7 (8.5%)	ns
Chronic renal disease	33 (9.9%)	17 (7.1%)	13 (15.8%)	*
Plasma samples per patient median [IQR]	3 [2–4]	3 [2–4]	2 [2–3]	ns
Delay between SO[a] and hospitalization median [IQR]	8 [5–10]	8 [6–10]	7 [4–10]	ns
Delay between hospitalization and PS[b] median [IQR]	2 [1–3.25]	2 [1–3]	2.5 [1.5–4]	ns
First positive N antigenemia[c] log_{10} pg/mL—median [IQR]	2.4 [1.96–3.43]	2.2 [1.67–3.04]	3.2 [2.50–3.97]	***

Note: Mann–Whitney and Fisher's tests were performed, with: ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ and ns: $p > 0.05$.

[a]Symptom Onset.

[b]Plasma sample collection.

[c]Within the first 8 days of hospitalization.

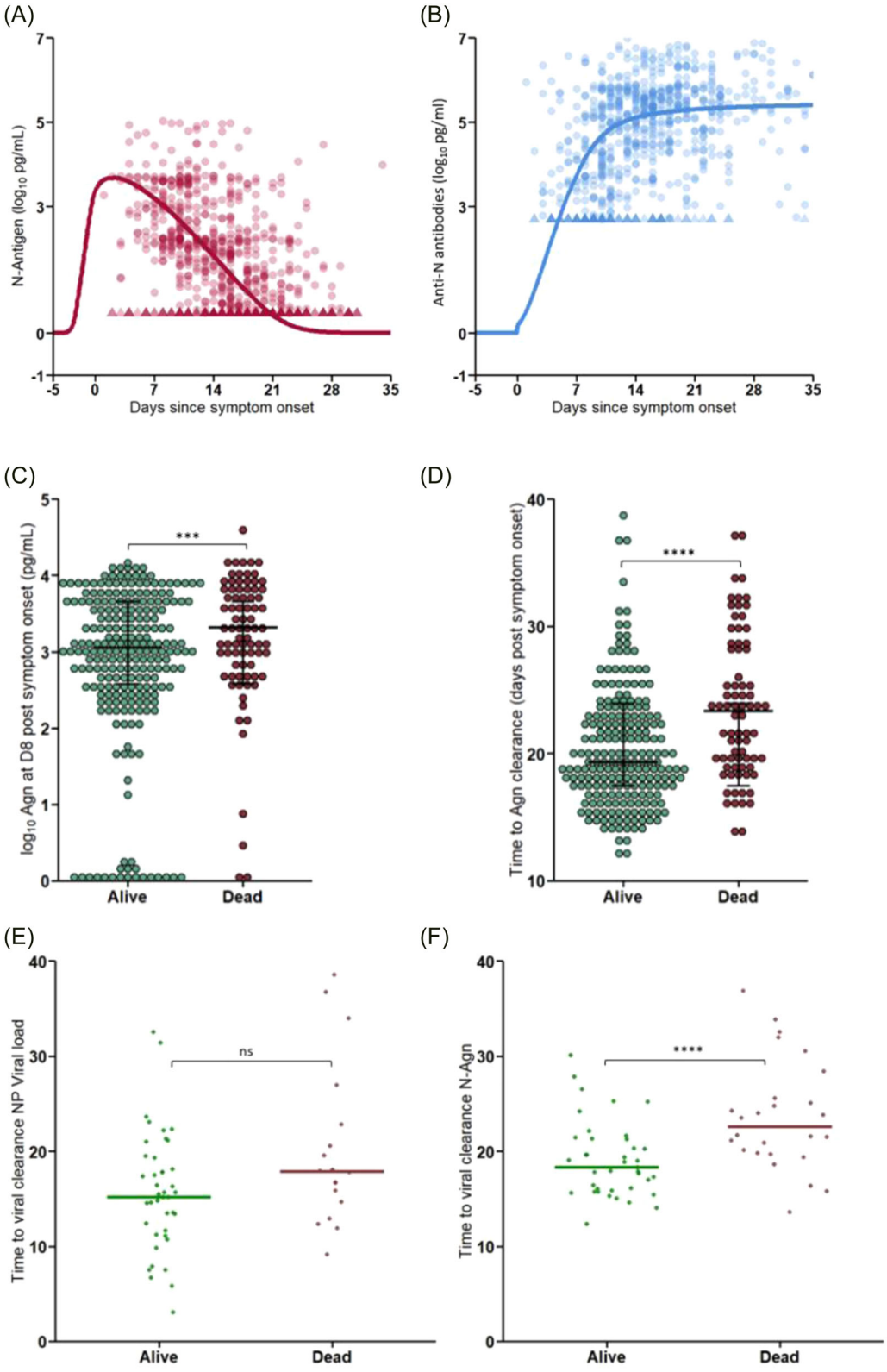

FIGURE 1 Evolution and dynamic of sera N-antigen levels according to patient's outcome. (A, B) Evolution of sera N-antigen levels (A, red) and anti-N antibodies (IgG) titers (B, blue) according to the delay since symptom onset (SSO). Triangles represent data under the Limit of Quantification (LoQ) (2.97 pg/mL for N-Antigen and 500 pg/mL for anti-N IgG). (C) Predicted sera N-antigen levels (log_{10} pg/mL) at day 8 SSO according to survival status. (D) Predicted time to N-antigen clearance in sera according to survival status. (E, F) Predicted time to viral clearance in nasopharynx (E) and in sera (F) according to survival status, in the subset of patients with both sera and nasopharyngeal samples. In panels C to F, bars indicate median, patients discharged alive are shown in green, and deceased patients are shown in the dark red. Wilcoxon–Mann–Whitney tests were performed, with: $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ and ns: $p > 0.05$.

In a subset of patients with early samples (collected within 8 days SSO), N-antigenemia was positive in 84% (126/148) of patients, with different rates across severity groups: 93% (41/44) and 81% (85/104) for Death and Survival groups, respectively ($p < 0.001$). In these early samples, a significant gradient was found in the first positive N-antigenemia levels according to disease severity, with a median concentration of 3.2 $\log_{10}$ pg/mL [IQR: 2.50–3.97] and 2.2 $\log_{10}$ pg/mL [IQR: 1.67-3.04] in Death and Survival groups, respectively ($p < 0.001$) (Table 1). This gradient was also found for all patients at Day 8 SSO using the prediction model, with significant differences in predicted N-antigenemia levels according to disease severity, with a median concentration of 2500 versus 1200 pg/mL in Death and Survival groups, respectively ($p < 0.001$) (Figure 1C). Predicted time to viral clearance was significantly delayed in deceased patients compared to survivors (23.3 vs. 19.3 days SSO, respectively; $p < 0.0001$) (Figure 1D). To note, no significant differences were observed in predicted N-antigenemia levels ($p = 0.39$) (Supporting Information S1: Figure 1A) OR predicted time to viral clearance ($p = 0.24$) (Supporting Information S1: Figure 1B) between patients who recovered from their SARS-CoV-2 infection after an ICU stay and patients who were never transferred to ICU.

3.4 | Comparison with NP viral load

A subset of patients was common between our analysis and a previous work on NP viral load data.[2] We compared between deceased patients and survivors the delay to viral clearance in both compartments: nasopharynx (Figure 1E) and sera (Figure 1F). In this subset of patients, clearance of viral load in nasopharynx did not differ ($p = 0.07$) (Figure 1E), while the clearance of viral N-antigen was delayed in sera of deceased patients ($p < 0.0001$) (Figure 1F).

We also compared patients who had no autoantibodies directed against IFNα2 and anti-IFNω to those who had at least one anti-type 1 IFN auto-antibody. We did not find any difference in N-antigenemia at Day 8 SSO between these two groups ($p = 0.66$) (Supporting Information S1: Figure 2).

4 | DISCUSSION

SARS-CoV-2 N-antigen has been described as a potential diagnostic tool,[7] but its link with disease severity has been scarcely explored.[11,12,15,16]

In this work, we observed a strong link between N-antigenemia levels and COVID-19 disease severity. Deceased patients had higher N-antigen levels than surviving patients. This difference in the N-antigen levels also translated into a delayed clearance of the viral antigen values in deceased patients. This paves the way for the use of this antigen to detect patients at higher risk of death.

Circulating N-antigen levels may reflect the viral replication in both the upper and lower respiratory tracts.[17] Therefore, it might be an interesting prognostic marker and could be used for diagnosis in late-presenting patients in which lower respiratory tract samples are not feasible. The longer time required for clearance of N-antigenemia in deceased patients might indicate a less effective immunological control of the infection and a contribution of viral replication to disease progression. Its use in patients receiving antivirals (nirmatrelvir and remdesivir) could also be evaluated. In addition, measurement of N-antigen levels immediately after admission to hospital might improve risk stratification.

Anti-type 1 IFN auto-antibodies have been associated with disease severity in COVID-19 patients.[18] One could hypothesize that the impaired innate immune response in these patients could lead to increased viral replication and potentially higher circulating N-antigen levels. As this has not been studied, to the best of our knowledge, we compared the N-antigen levels in sera of patients with or without auto-antibodies directed against type 1 interferon. No difference was noted, suggesting that a defect in the innate immune response was not responsible for the higher plasma N-antigen levels in patients with more severe disease in our cohort. However, it should be noted that all patients with auto-antibodies against interferon type 1 had detectable N-antigen levels.

Our study has limitations, including the fact that our patients were included during the first two COVID-19 waves, and results might differ with the later SARS-CoV-2 variants. Furthermore, the immune status of patients could influence the dynamics of N-antigen, leading to reduced delays to viral clearance during the latter SARS-CoV-2 waves. In our study, patients were included before the unfolding of SARS-CoV-2 vaccines and specific preventive therapies.

To note, our model could underestimate N-antigen if endothelial cells are significantly involved in N-antigen production. However, recent studies using endothelial cell lines or primary endothelial cells from various human organs have demonstrated that endothelial cells are resistant to SARS-CoV-2 infection due to lower or absence of ACE2 expression,[19] suggesting nonproductive replication of SARS-CoV-2 in human pulmonary endothelial cells (HPEC). Nevertheless, HPECs may play an active and important role in regulating cytokine storm, leukocyte recruitment, and contributing to vascular injury through epithelial-endothelial cell interactions and direct infection of circulating leukocytes.[20]

Our model provides insight into the clinical importance of circulating SARS-CoV-2 N-antigen. This new diagnostic tool might help to evaluate the prognostic of patients. Such markers could also be evaluated in other respiratory viral infections.

AUTHOR CONTRIBUTIONS

Charlotte Charpentier, Samuel Lebourgeois and Houssem Redha Chenane contributed to conception and design of the study. Reyene Menidjel, Cédric Laouenan, and Sarah Tubiana performed the experiments and organized the database. Jérémie Guedj and Guillaume Lingas performed viral kinetics modelization and statistical analyses Laurent Abel performed anti-cytokine auto-antibodies analyses. Houssem Redha Chenane, Benoit Visseaux, and Guillaume Lingas wrote the first draft of the manuscript. Diane Descamps, Quentin Le Hingrat, Surbi Malhotra, Samir kumar-Singh, and Jade Ghosn contributed to manuscript revision. All authors read and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethics approval for the French COVID cohort was given by the French Ethics Committee CPP-Ile-de-France 6 (ID RCB: 2020-A00256-33 and ID RCB: 2020-A00280-39) and the French National Data Protection Commission (approval #920102).

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SUPPORTING INFORMATION

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

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