Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Research Note

Detection of SARS-CoV-2 N-antigen in blood during acute COVID-19 provides a sensitive new marker and new testing alternatives

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ABSTRACT

Objectives: Molecular assays on nasopharyngeal swabs remain the cornerstone of COVID-19 diagnostics. The high technicalities of nasopharyngeal sampling and molecular assays, as well as scarce resources of reagents, limit our testing capabilities. Several strategies failed, to date, to fully alleviate this testing process (e.g. saliva sampling or antigen testing on nasopharyngeal samples). We assessed the clinical performances of SARS-CoV-2 nucleocapsid antigen (N-antigen) ELISA detection in serum or plasma using the COVID-19 Quantigene® (AAZ, France) assay.
Methods: Performances were determined on 63 serum samples from 63 non-COVID patients and 227 serum samples (165 patients) from the French COVID and CoV-CONTACT cohorts with RT-PCR-confirmed SARS-CoV-2 infection, including 142 serum samples (114 patients) obtained within 14 days after symptom onset.
Results: Specificity was 98.4% (95% CI 95.3–100). Sensitivity was 79.3% overall (180/227, 95% CI, 74.0–84.6) and 93.0% (132/142, 95% CI, 88.7–97.2) within 14 days after symptom onset. Ninety-one of the included patients had serum samples and nasopharyngeal swabs collected in the same 24 hr. Among those with high nasopharyngeal viral loads, i.e. Ct value below 30 and 33, only 1/50 and 4/67 tested negative for N-antigenaemia, respectively. Among those with a negative nasopharyngeal RT-PCR, 8/12 presented positive N-antigenaemia; the lower respiratory tract was explored for six of these eight patients, showing positive RT-PCR in five cases.
Discussion: This is the first evaluation of a commercially available serum N-antigen detection assay. It presents a robust specificity and sensitivity within the first 14 days after symptoms onset. This approach provides a valuable new option for COVID-19 diagnosis, only requiring a blood draw and easily scalable in all clinical laboratories. **Quentin Le Hingrat, Clin Microbiol Infect 2021;27:789.e1–789.e5**

Introduction

Molecular assays on nasopharyngeal swabs remain the cornerstone of COVID-19 diagnostics. Despite massive efforts, the high technicalities of nasopharyngeal sampling and molecular assays, as

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well as scarce resources of reagents, limit our testing capabilities. Several strategies have failed, to date, to fully alleviate this testing process, e.g. saliva sampling [1,2] or antigen testing on nasopharyngeal samples [3,4]. Nucleocapsid-antigen (N-antigen) has been detected in the serum of SARS-CoV-infected patients and, recently, it has been demonstrated in a single study of SARS-CoV-2-infected patients to have a global sensitivity of 41/64 patients [5,6].

In this work, we assessed the performances of N-antigen sera detection in a large patients' population using the first commercially available assay, the COVID-19 Quantigene® (AAZ France) providing a low limit of detection at 2.98 pg/mL.

Materials and methods

Patients and ethics

Negative samples comprised 50 pre-pandemic samples (collected between 2 December 2019 and 13 January 2020) and 13 pandemic samples from SARS-CoV-2 non-infected patients that tested positive for other microbial antigens (i.e. NS1 antigen, HBs antigen, HIV-1 p24 antigen, HKU1 coronavirus or malaria antigens). Positive samples were collected between 25 January 2020 and 2 September 2020 from study participants included in the French COVID (clinicaltrials.gov NCT04262921) and CoV-CONTACT cohorts (clinicaltrials.gov NCT04259892). We selected the first serum samples available after COVID-19 diagnosis (cf. Fig. S2). The following serum samples of those patients, when collected at the physician's discretion, were also included. They provided written informed consent for the use of their samples for research. Ethics approval 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).

For COVID-19 patients, available serum samples were classified into different categories according to the delay since symptom onset: serum collected $\leq$14 days post-symptom onset (142 serum samples from 114 patients), serum samples collected >14 days post-symptom onset (81 serum samples from 72 patients), serum samples collected from asymptomatic patients (three serum samples from three patients) and patient without date of symptom onset (one serum sample from one patient). Distribution of serum samples according to date of sampling and hospitalization status is detailed in Fig. S2.

N-antigen level assessment

Prior to analysis, serum samples were stored at −80°C. N-antigenaemia levels were determined with a CE-IVD ELISA microplate assay, COVID-Quantigene® (AAZ), according to manufacturer's recommendations. Briefly, in each well of 96-well microplates previously coated with anti-SARS-CoV-2 N-antibodies, 50 μL of a solution containing biotinylated anti-SARS-CoV-2 N-antibodies and 50 μL of serum were added. After incubation at 37°C for 60 min, plates were washed five times with a phosphate buffer solution. Then, 100 μL of a solution containing a Horseradish peroxidase-conjugated streptavidin was added, followed by incubation for 30 min at 37°C. Plates were washed five times with the phosphate buffer solution, then 50 μL of a solution containing the substrate (3,3′,5,5′-tetramethylbenzidine (TMB)) and 50 μL of a second solution containing urea were added. After 15 min at 37°C, the colorimetric reaction was stopped by adding 50 μL of H_2SO_4. Absorbance values were measured at 450 nm, with the reference set at 630 nm. In each plate, standards made of recombinant N-antigens were tested, to quantify the N-antigenaemia levels for each patient's sample. As the purpose of this study was to assess the sensitivity of this new assay, samples with titres above 180 pg/mL were not diluted for precise quantification.

RT-PCR assays

For all patients included in this study, diagnosis of SARS-CoV-2 infection was performed in the virology department of Bichat-Claude Bernard University Hospital by RT-PCR on nasopharyngeal swabs, as recommended. Different techniques were performed throughout the study period for nasopharyngeal samples, due to frequent shortages issues and requirements for fast turnaround time: RealStar® SARS-CoV-2 (Altona, Hamburg, Germany), Cobas® SARS-CoV-2 (Roche Diagnostics, Branchburg, NJ, USA), Simplexa® COVID-19 Direct (DiaSorin, Gerenzano, Italy), BioFire® SARS-CoV-2 (BioMerieux, Salt Lake City, UT, USA), QIAstat-Dx® Respiratory SARS-CoV-2 (Qiagen, Hilden, Germany) and NeumoDX® (QIAgen, Hilden, Germany) using IP2 Institute Pasteur and WHO E gene primers [7]. E gene cycle threshold (Ct) values, available for all techniques except Simplexa® COVID-19 Direct and BioFire® SARS-COV-2, were used as a proxy for viral load for 104 samples from 91 patients with paired nasopharyngeal swabs and sera (i.e. collected in the same 24 hr).

For a subset of 146 samples, corresponding to 89 patients included in the French COVID-19 cohort, paired sera and plasma samples were available, allowing one to determine the presence of viral RNA in plasma. Briefly, viral nucleic acids were extracted from 200 μL of plasma with the MagNA Pure LC Total Nucleic Acid Isolation Kit − Large Volume (Roche Diagnostics, Branchburg, NJ, USA) and eluted in 50 μL. RT-PCR was performed on 10 μL of eluate using the RealStar® SARS-CoV-2 assay (Altona, Germany), according to the manufacturer's recommendations. Samples with RT-PCR cycle threshold values above 40 were considered negative.

Detection of anti-SARS-CoV-2 nucleocapsid IgG

For a subset of 85 serum samples, corresponding to 80 patients (ICU patients: $n = 21$, ward patients: $n = 36$ and outpatients: $n = 23$), we performed a chemiluminescent microparticle immunoassay detecting anti-N immunoglobulin G (Architect SARS-CoV-2 IG Assay, Abbott). Results were reported as a signal to cut-off (S/Co) value. The positivity threshold was set to 1.4, as recommended by the manufacturer.

Data availability

A file compiling all data used in this article is available on Mendeley Data public repository (https://data.mendeley.com/datasets/fjz6zbkxvm/1).

Results

Specificity of the COVID-19 Quantigene® was 98.4% (95% confidence interval (CI) 95.3–100), as N-antigenaemia was negative for 62 samples out of 63 non-COVID-19 patients.

N-antigenaemia sensitivity was determined on 227 serum samples, obtained from 165 patients included in the French COVID and CoV-CONTACT cohorts with RT-PCR confirmed SARS-CoV-2 infection. Among them, 180/227 serum samples tested positive, leading to a sensitivity of 79.3% (95% CI 74.0–84.6). When restricting sensitivity analysis to samples collected in the first 2 weeks after symptom onset, 132 out of 142 samples tested positive for N-antigenaemia, leading to a sensitivity of 93.0% (95% CI 88.7–97.2) (Fig. 1A,B). Patients with positive RNAaemia (viraemic patients) exhibited higher N-antigen sera levels (Fig. 1C). In serum samples collected more than 14 days after symptom onset, N-

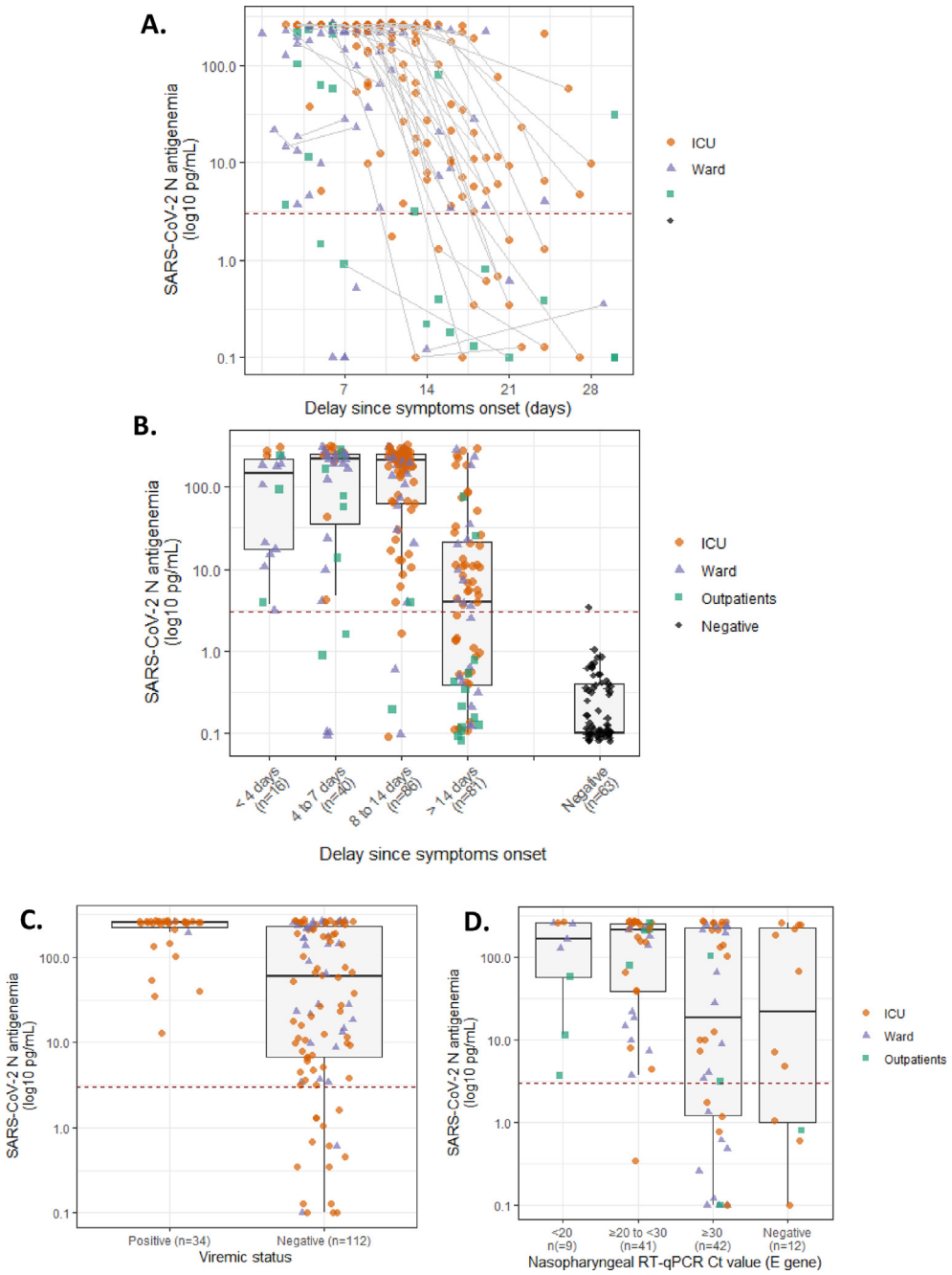

Fig. 1. (A) Evolution of N-antigen sera levels in SARS-CoV-2-infected patients according to hospitalization status ($n = 227$ serum samples from 165 patients); sequential samples are connected with a grey line, while the positivity threshold value for N-antigen (2.97 pg/mL) is indicated with a dashed red line. (B) N-antigenaemia levels according to delay since symptoms onset. (C) N-antigen sera levels according to positive and negative RNAaemia status ($n = 146$ sera, $n = 89$ patients). (D) N-antigen sera levels according to E-gene cycle threshold value of 104 nasopharyngeal swabs collected within 24 hr ($n = 91$ patients).

antigenaemia frequently declined and was undetectable in 84.6% (11/13), 42.1% (8/19) and 32.7% (16/49) samples of outpatients, ward and ICU patients, respectively. The lower detection in late-stage samples appears linked with the apparition of anti-N IgG (Fig. 1A and supplementary material).

For 91 patients, 104 paired serum samples and nasopharyngeal swabs collected in the same 24 hours were available, allowing us to compare N-antigen detection with E gene Ct values for those patients (Fig. 1D). Among patients with E gene Ct value below 30 and below 33 on their nasopharyngeal swab, only 1/50 and 4/67 tested negative for N-antigenaemia, respectively (Fig. S1B). For patients with positive nasopharyngeal samples with Ct values ≥ 33, only 15/25 (60%) were positive for N-antigenaemia. Interestingly, eight out of 12 patients with a negative nasopharyngeal RT-PCR presented positive N-antigenaemia. The lower respiratory tract was explored for 6 of these 8 patients either the same day or in the 5 following days. RT-PCR on the lower respiratory tract sample was positive in five of these six patients.

Discussion

This is the first evaluation of a commercially available SARS-CoV-2 N-antigen serum or plasma detection. This assay presented a low detection limit at 2.98 pg/mL and a sensitivity above 90% during the acute phase of the disease (i.e. <14 days after symptoms onset in PCR confirmed COVID-19 patients). In the first 2 weeks, N-antigen negativity was associated with anti-N IgG detection (6/10) and/or low nasopharyngeal viral load in the same 24 hr (7/7, Ct value > 30). This sensitivity could allow its use for COVID-19 diagnostic and is in line with RT-PCR on nasopharyngeal samples whose reported sensitivity rates ranged between 71% and 98%, based on negative RT-PCR tests which were positive on repeat testing [8].

Detection of viral antigens in the blood of COVID-19 patients has been recently described by Ogata and collaborators, who detected N and S1 antigens in the blood of 41 out of 64 COVID-19 patients [5]. Antigen circulation in blood is not uncommon in infectious diseases, antigenaemia tests usually target blood-borne pathogens, notably Dengue, CMV, HBV or HIV. In respiratory diseases, antigen circulation into non-respiratory body fluids is usually not considered, even, if likely, because of the focal nature of the infection or possible pre-existence of antibodies. Antigen detection in non-respiratory fluids is still used for two respiratory bacteria: *Streptococcus pneumonia* and *Legionella pneumophila*. Interestingly, it has also been reported in SARS-CoV-1 infection [6]. Whether the circulation of free viral antigens has an impact on disease physiopathology should be assessed in future studies.

N-antigenaemia was also detectable in outpatients but the decrease seems to occur earlier in our study. Detection of viral antigens in this population was not evaluated in the study by Ogata and collaborators. Detection of N-antigenaemia was higher for patients with either high nasopharyngeal viral loads, i.e. Ct below 30, or active replication in the lung, i.e. high Ct values (>30) in nasopharyngeal samples but positive RT-PCR in lower respiratory tract samples.

This innovative marker may also be of help for prognostic evaluation of patients. An association between high N-antigen levels and higher ICU admission rates has been reported by Ogata et al. [5]. In our study, we observed higher N-antigen levels in serum of viraemic patients, in line with the possible association of viraemia, or RNAaemia, with disease severity and/or immunosuppression [9–11].

Our study presents several limitations. We included a very small number of outpatients and this population needs to be explored in larger cohorts, ideally with longitudinal samples of paucisymptomatic and asymptomatic patients. The case–control design is another limitation and the test performances could be slightly deteriorated in real life condition.

In conclusion, sensitive N-antigen detection in serum or plasma provides a valuable new marker for COVID-19 diagnosis, only requiring a blood draw, that is scalable in all clinical laboratories. It allows potential new developments to design rapid antigen blood test or combined ELISA assays, detecting both antigens and antibodies. It also raises new questions about the physiological mechanisms at play explaining blood circulation of this antigen and its potential correlation with disease severity.

Transparency declaration

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Author contributions

Q.L.H., B.V., C.Cha., D.D., N.H.F. conceptualized the study and its methodology. Q.L.H., H.I., F.D., N.B., M.B. performed the experiments. C.L., S.T., C.B., C.Cho., X.D., J.F.T., L.B., J.G., Y.Y. collected data and participated to the validation of the study. Q.L.H., B.V. wrote the first draft. All authors reviewed and edited the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.11.025.

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