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

POSTER #197

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BACKGROUND

- The presence of free SARS-CoV-2 nucleocapsid-antigen circulating in patients' sera (N-antigenemia) has been recently demonstrated by several studies and suggested as a potential diagnostic marker [1]
- A few studies also suggested a link between the quantitative levels of N-antigenemia and COVID-19 disease severity [2], [3]
- **In the current study, we assessed the N-antigemia levels and modelized the kinetic of this marker among COVID-19 hospitalized patients according to disease severity**

METHODS

- We retrospectively included patients with at least one sera sample from the prospective French COVID cohort, that is part of the ORCHESTRA project, between January 25, 2020 and September 2, 2020
- All patients included within French COVID cohort were hospitalized with a positive SARS-CoV-2 RT-PCR
- N-antigenemia levels were determined with the COV-Quanto® assay (AAZ), a commercial ELISA 96-wells microplates assays with a limit of detection at 2.98 pg/mL, according to manufacturer recommendations.
- IgG antibodies levels were assessed using the V-PLEX® SARS-CoV-2 Panel 1 Kit (MesoScale Discovery), a multiplex serology assay allowing the quantification of anti-N, anti-S and anti-RBD
- Patient's outcome was classified within three groups: recovery without ICU transfer (Hospital group), recovery with ICU transfer (ICU group) and patients dying during hospitalization (Death group)
- Statistical analysis and graphical representations were performed using R.4.0.4.
- All patients have provided written informed consent, 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)

Variable	Overall (n=320)	Hospital group (n=142)	ICU group (n=96)	Death group (n=82)
Male gender	**236 (66.1%)**	79 (55.6%)	67 (69.7%)	69 (81.4%)
Age ≥ 65 years old	**162 (45.4%)**	45 (31.6%)	38 (39.5%)	46 (56.1%)
Hypertension	**134 (42.9%)**	43 (30.2%)	40 (41.6%)	35 (42.6%)
Chronic cardiac disease	**68 (20.9%)**	23 (16.1%)	15 (15.6%)	20 (24.3%)
Chronic pulmonary disease	**46 (13.8%)**	15 (10.5%)	13 (13.5%)	14 (17.1%)
Asthma	**33 (10.2%)**	16 (11.2%)	10 (10.4%)	7 (8.5%)
Chronic renal disease	**33 (9.9%)**	11 (7.7%)	6 (6.2%)	13 (15.8%)
Delay SSO[a] and hospitalization – *median [IQR]*	**8 [5-10]**	8 [6-11]	7 [5-10]	7 [4-10]
First N antigenemia[b] - *log_{10}pg/mL*	**2.2 [1.60-3.27]**	1.9 [0.80-2.43]	2.4 [1.68-3.43]	3.3[2.09-3.97]

a: Since Symptom Onset
b: within the first 5 days of hospitalization

***Table 1* : Epidemiological and clinical description of the 320 SARS-CoV-2 included patients.**

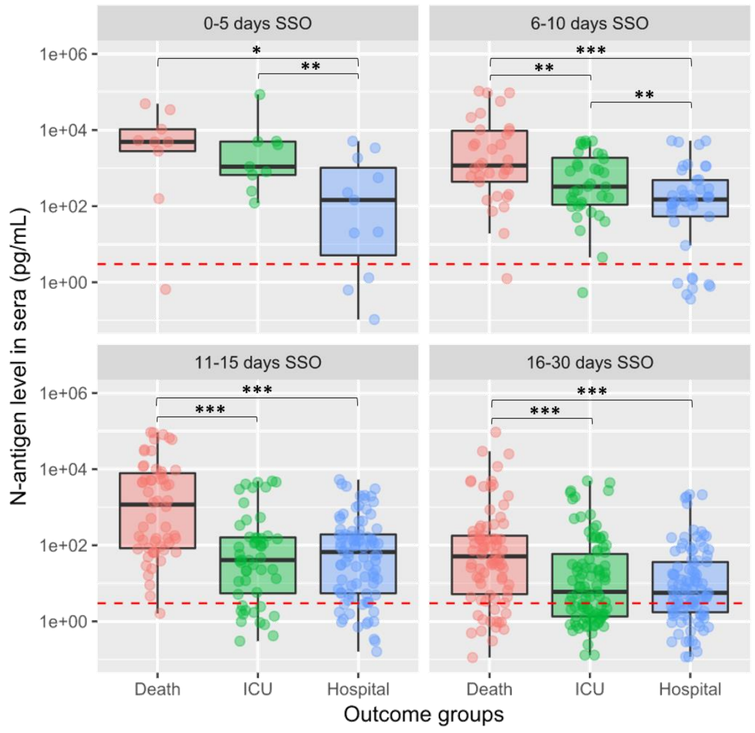


***Figure 1 :* Sera N-antigen levels according to severity groups (i.e. Death, ICU and Hospital groups) and time since symptoms onset (SSO).**
The red dashed lines indicate Nantigen positivity threshold (2.98 pg/mL). Statistical significance between groups is indicated by asterisks (* p<0.05; ** p<0.025; *** p<0.001).

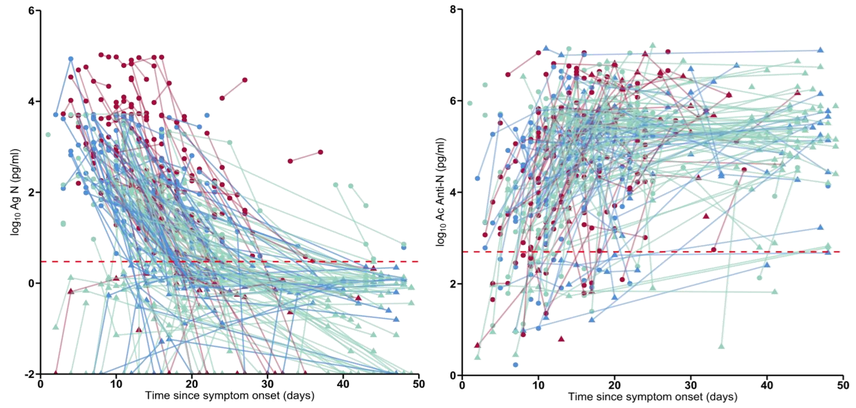


***Figure 2 :* Evolution of sera N-antigen levels (left panel) and anti-N antibodies (right panel) according to delay since symptoms onset (SSO).**
Sequential samples from a single patient are connected by a line. The N-antigen positivity threshold value (2.97 pg/mL) and anti-N (500 RLU) are indicated with a dashed red line. Patients status are indicated by colors: hospital by blue, ICU by green and death by dark red color.

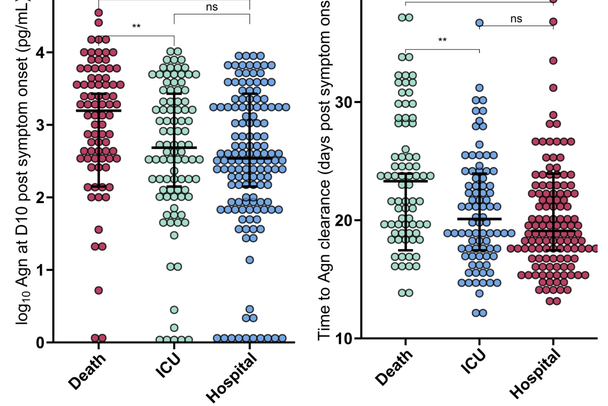


***Figure 3 :* Sera N-antigen dynamic according to death status.**
Sera N-antigen levels (log_{10}) at day 10 since symptoms onset is indicated in the left panel and time to sera N-antigen clearance is indicated in the right panel.
Patients status is indicated by colors: hospital by blue, ICU by green and death by dark red color.

RESULTS

- We included 1052 samples from 320 patients, with 66% of male, with a median age at 63 years [IQR: 52-71], including 162 (45%) patients above 65 years old
- A total of 142, 96 and 82 patients were in the Hospital, ICU and Death groups, respectively
- Main comorbidities are indicated in ***Table 1***

- The overall sensitivity of N-antigenemia was 81% (304/372) within the first 15 days SSO (since symptoms onset) and 61% (190/313) from 16 to 30 days SSO
- Positivity rates were significantly different across severity groups within 15 days SSO: 95% (96/101), 81% (83/105), 78% (117/150) (p<0.001) for Death, ICU and Hospital groups, respectively (cf. ***Figure1***)

- Among positive patients, a significant gradient was found in the levels of N-antigenemia according to disease severity, with median levels at 94, 124 and 254 pg/mL in Hospital, ICU and Death groups, respectively (*p<0.001*)
- When stratifying on the time SSO, this difference was observed for all time strata since symptoms onset, even in the 16-30 days SSO despite being smaller Overall, 95, 80, 43 and 22% of N-antigenemia >10,000, >5,000, >1,000 and <1,000 pg/mL corresponded to patients who died (cf. ***Figure1***)
- IgG antibodies titers were not correlated to severity and the presence of both sera N-antigen and anti-N IgG was observed for 42% (442/1052) samples

- The kinetic of N-antigenemia and N-antibodies according to time since symptoms onset is depicted in ***Figure 2***
- By modeling the N-antigen clearance, the overall N-antigen median time for clearance was 20 days SSO and statistically longer among death patients than surviving patients (23.5 vs 19.6 days) (cf. ***Figure 3***)

CONCLUSION

- In this work conducted on a large prospective cohort of hospitalized patients, we observed a strong link between N-antigenemia levels and COVID-19 disease severity from 0 to 30 days SSO
- The blood free N-antigen levels may reflect the viral replication in both upper and lower respiratory tracts. Thus, it could provide an interesting prognostic markers, more accurate than nasopharyngeal viral loads
- The longer time to N-antigenemia clearance observed among patients form the Death group may suggest a less effective immunological control of the infection and virological part into disease progression
- This is the first time that a free antigen blood circulation is demonstrated in a respiratory viral infection. This could open a new toolbox for COVID-19 and other human coronaviruses infections.

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