

SARS-CoV-2 nucleocapsid urine antigen in hospitalized patients with Covid-19

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Summary of the article's main point:

Mechanisms leading to severe forms of Covid-19 remain poorly understood. Herein, we demonstrate that the SARS-CoV-2 nucleocapsid protein is present in high concentrations in the urine of Covid-19 hospitalized patients. The nucleocapsid level in urine is associated with Covid-19 severity.

Abstract

Background: SARS-CoV-2 nucleocapsid antigen (N-Ag) can be detected in the blood of patients with Covid-19. We used a highly sensitive and specific assay to explore the presence of N-Ag in urine during the course of Covid-19, and explore its relationship with the severity of the disease.

Methods: We studied urine and blood N-Ag using highly sensitive immunoassay in 82 patients with a SARS-CoV-2 infection proven by PCR.

Results: In the first and second weeks of Covid-19, hospitalized patients tested positive for urinary N-Ag (81.25% and 71.79%, respectively), and blood N-Ag (93.75% and 94.87%, respectively). High urinary N-Ag levels were associated with the absence of SARS-CoV-2 nucleocapsid antibodies, admission in intensive care units, high C-reactive protein levels, lymphopenia, eosinopenia, and high lactate dehydrogenase. A higher accuracy was observed for urine N-Ag as a predictor of severe Covid-19 compared to blood N-Ag.

Conclusions: Our study demonstrate that N-Ag is present in the urine of patients hospitalized in the early phase of Covid-19. As a direct marker of SARS-CoV-2, urinary N-Ag reflects the dissemination of viral compounds in the body. Urine N-Ag may be a useful marker for disease severity of SARS-CoV-2 infections.

Keywords: Covid-19 ; SARS-CoV-2 ; Nucleocapsid; Urine ; Severity

Introduction

The SARS-CoV-2 pandemic has upset the world and challenged medical knowledge about viral respiratory infections. One of the major characteristics of SARS-CoV-2 infection is the diversity of clinical expression, with a wide range of symptoms reported in infected persons [1]. While severe forms constitute only a small share of Covid-19 cases, they put major pressure on health systems and are responsible for the direct excess mortality associated with the pandemic.

Although knowledge on the natural history of Covid-19 has improved considerably over the last 18 months, the exact mechanisms of impairment in the innate and adaptive immune system leading to severe forms of Covid-19 remain to be elucidated. According to our current understanding of the pathophysiology, different phases of virus and host interactions characterize SARS-CoV-2 infection [2] [3]. Following the incubation period, a high viral replication triggers the innate immune response. A strong inflammatory syndrome and the onset of the adaptive immune response characterize the second phase of Covid-19 before recovery or worsening. Severe forms of Covid-19 are associated with an excessive release of cytokines, known as a “cytokine storm”, occurring generally during the second phase of the disease [4]. The administration of corticosteroids reduces death and time to recovery [5,6], and interleukin-6 antagonist Tocilizumab also have obtained encouraging results [7–9].

On the virus side of the host-virus interplay, the contribution to disease severity of a high SARS-CoV-2 load measured in respiratory samples remains uncertain. The kinetics of SARS-CoV-2 RNA in the upper respiratory tract during the course of Covid-19 is well established, with high concentrations observed during the initial phase, followed by a rapid decrease in the second week after the onset of symptoms, and a low or undetectable level of RNA later [7]. The viral loads of asymptomatic or mild forms of SARS-CoV-2 infections are similar to severe forms [10]. Studies suggest that SARS-CoV-2 RNA decreases faster in mild/asymptomatic infections and in young subjects compared to severe forms and elderly subjects [11,12]. The presence of SARS-CoV-2 components also has been reported outside the respiratory tract, such as in blood, stool and saliva. When detected, the plasma SARS-CoV-2 RNA level is generally low. The detection of SARS-CoV-2 is also found in extrapulmonary organs of immunocompromised patients, including heart, kidney, liver, and spleen [13]. However, no case of SARS-CoV-2 transmission via transfusion has been described [14]. On the other hand, the presence of the SARS-CoV-2 nucleocapsid has been reported in the blood of Covid-

19 patients. Circulating antigen (Ag) is detectable in almost all hospitalized patients in the early phase of the disease [15,16]. A high level of nucleocapsid antigenemia (N-Ag) could be associated with severe forms of Covid-19 and the presence of circulating SARS-CoV-2 RNA [17,18].

In this study, we used a highly sensitive and specific nucleocapsid-Ag assay to: i) explore the presence of N-Ag in the urine of hospitalized patients, ii) study the kinetics of N-Ag concentration in urine and blood during infection, iii) assess the relationship between N-Ag concentrations in urine and blood, and iv) compare urine and blood N-Ag levels in moderate versus severe forms of Covid-19.

Materials and Methods

Design of the study. Plasma, urine and nasopharyngeal samples were collected from 82 SARS-CoV-2 infected patients admitted in Montpellier University hospitals between March 2020 and May 2021 and consenting to be included in a cohort of patients with confirmed SARS-CoV-2 (COVIDotheque cohort). A total of 82 plasma and 82 urine paired samples were taken the same day or within a maximum of 48 hours apart. In addition, series of urine and blood paired samples were collected in seven patients for a follow-up (28 paired samples). Patient characteristics are detailed in Table 1. Controls consisted of consenting patients who were not suspected of Covid-19 and tested negative for SARS-CoV-2 RNA in nasopharyngeal samples. The cohort study received an institutional ethics committee approval (CPP Ile de France III, n°2020-A00935-34; ClinicalTrials.gov Identifier: [NCT04347850](https://clinicaltrials.gov/ct2/show/study/NCT04347850)).

Reverse transcription polymerase chain reaction (RT-PCR). The Allplex 2019-nCoV Assay (Seegene) kit was used to confirm SARS-CoV-2 infection.

SARS-CoV-2 N IgG and IgA assays on plasma and urine. SARS-CoV- N IgG and IgA detection was performed on plasma samples by indirect semi-quantitative ELISA ID Screen® ID.Vet [19]. According to manufacturer's instructions, a signal ratio « Sample/Positive control% » (S/P%) $\geq 40\%$ was considered positive, $30\% < S/P\% < 40\%$ was considered suspicious and $S/P\% \leq 30$ was considered negative.

N-antigen detection. N-SARS-CoV-2 antigen levels in urine and plasma were determined with a CE-IVD ELISA microplate assay, COV-QUANTO® (AAZ-LMB, Boulogne-Billancourt, France). The cut-off value was defined by the manufacturer's instructions: samples with antigen N concentration ≥ 2.97 pg/mL were considered positive. Assay reproducibility was established by the manufacturer using three batches: CV is 9.01% at 6.49pg/mL, and 4.8% at 168.02 pg/mL.

Software and statistical analyses. Mann-Whitney U test was used to compare the different quantitative variables with non-normal distribution. Exact binomial's test was used to compare the proportion of patients with urine N-Ag and/or plasma N-Ag. Correlations were calculated by Spearman's coefficient. The median and interquartile range (IQR) were used to describe cohort characteristics. Data were analyzed using Excel 2016 (Microsoft Corp, Redmond, Washington) and GraphPad Prism 9.1.1.0 (Microsoft Corp, Redmond, Washington).

Results

N-Ag is present in the urine of patients hospitalized with SARS-CoV-2 infection

We tested the presence of N-Ag in urine samples of 82 patients hospitalized for a SARS-CoV-2 infection confirmed by RT-PCR. N-Ag was present in the urine of 41/55 patients (74.55%) tested during the first two weeks after the onset of symptoms, (Se: 81.25% and 71.79%, respectively) with concentrations ranging from 1.00 to 9,613.03 pg/mL (Figure 1.A). All urine samples from 30 control subjects tested negative for N-Ag (Sp: 100%; data not shown). In blood-paired samples, N-Ag was detected in 53/55 (94.64%) patients tested during the first two weeks after the onset of symptoms (Se: 93.75% and 94.87%, respectively) with concentrations ranging from 2.97 to 16,019.30 pg/mL (Figure 1.B). Urine N-Ag levels correlated with blood concentrations ($r=0.74$) (Supplementary Figure 1).

Urinary N-Ag concentrations decrease over time but remain detectable during the second week after the onset of symptoms, and after nucleocapsid IgG (N-IgG) seroconversion

N-Ag levels in urine was inversely correlated with the number of days after the onset of symptoms (Figure 1.C), ($r = -0.43$; $p < 0.0001$). Urinary N-Ag levels were high in samples collected during the first and second week after the onset of symptoms and decreased sharply during the third week

(Supplementary Figure 2.A). Blood N-Ag correlated well with the number of days after the onset of symptoms (Figure 1.D) ($r = -0.55$; $p < 0.0001$), and also decreased after the first two weeks (Supplementary Figure 2.B). The follow-up of seven SARS-CoV-2 infected patients confirmed the gradual decrease of urine and blood N-Ag levels over time (Figure 2).

The analysis of blood N-Ag according to serological status against SARS-CoV-2 nucleocapsid showed lower concentrations in N-IgG positive patients compared to N-IgG negative patients. However, most patients (81.36%) who tested positive for SARS-CoV-2 nucleocapsid IgG remained positive for blood N-Ag (Figure 3.B). The level of N-Ag in the urine was also significantly lower in patients who tested positive for circulating anti-N IgG compared to N IgG negative patients, but we observed a less pronounced difference between the two groups than in blood and both differ by about 1 log₁₀ (Figure 3.A). Cycle threshold (CT) results of SARS-CoV-2 RNA PCR in nasopharyngeal swabs were not correlated with urine N-Ag level ($r = -0.24$, $p = 0.1077$; Figure 3.C), and weakly correlated with blood N-Ag ($r = -0.35$, $p = 0.0162$; Figure 3.D).

Urine samples also were assessed for antibodies against SARS-CoV-2 nucleocapsid (Figure 4). All patients tested negative for nucleocapsid antibodies in plasma tested also negative for both N-IgA and N-IgG in urine (data not shown). Signal ratios for N-IgA and N-IgG were higher in urine samples collected after seroconversion has occurred ($p < 0.0001$). Using the same threshold as for plasma, N-IgA were detected in 17/82 patients (20.7%), and N-IgG in 6/82 patients (7.3%). All urine samples from SARS-CoV-2 negative controls tested negative for N-IgG and N-IgA (data not shown).

High urinary N-Ag levels are associated with admission in intensive care units

Patients had higher urinary N-Ag levels when hospitalized in intensive care units (ICU) compared to medical wards ($p = 0.0077$). The levels were higher in ICU seronegative hospitalized patients ($p = 0.0112$), and a trend toward a higher level of N-Ag was observed in ICU seropositive patients ($p = 0.0559$) (Figure 5.A). Blood N-Ag levels were also higher in patients hospitalized in ICU compared to medical wards ($p = 0.0250$) (Figure 5.B). The ROC curves show that both high urine and blood N-Ag levels were predictive of a condition requiring intensive care (Figure 5.C, D). A higher accuracy was observed for urine N-Ag as a predictor of severe Covid-19 compared to blood N-Ag, especially on seronegative patients (AUC: 0.8300 vs 0.7115, respectively).

Relationship of urine and blood N-Ag levels with biological markers of Covid-19 severity

Levels of urine and plasma N-Ag were analyzed according to abnormalities in biological markers associated with Covid-19 severity (Figure 6, supplementary Figure 3). Higher urine and blood N-Ag levels were measured among patients with a C-reactive protein (CRP) over 100 mg/L. The differences remained significant when N-Ag levels in urine and blood were analyzed according to N-IgG serological status. Higher urine and blood N-Ag levels also were measured among patients with lymphopenia and low eosinophil counts. Urine N-Ag levels were higher when the Lactate dehydrogenase (LDH) level was elevated. We did not observe an association between N-Ag levels and low platelet counts ($<200/\mu\text{L}$), abnormal troponin levels ($>60\text{ mg/mL}$), high D-dimer levels ($>1200\text{ mg/mL}$), or the Glomerular Filtration Rate (GFR) based on the Modification of Diet in Renal Disease (MDRD) study equation ($<60\text{ ml/min/1.73m}^2$). Urine N-Ag levels were lower in patients with elevated alanine aminotransferase (ALT) concentrations.

Discussion

In this study, we assess N-Ag in urine of patients hospitalized for a Covid-19 infection confirmed by PCR. Our results demonstrate that N-Ag is present in urine of patients hospitalized for a SARS-CoV-2 infection. Urinary N-Ag concentrations decreased progressively after the onset of symptoms following a low decay during the first and second weeks, and a sharp decrease during the third week. The presence of circulating antibodies against nucleocapsid was associated with a lower level of urine N-Ag, but the SARS-CoV-2 nucleocapsid remained detectable in the urine samples of most of the patients seropositive for N-IgG.

Levels of SARS-CoV-2 RNA in the upper respiratory track do not appear to be a reliable marker of Covid-19 severity since high concentrations of virus in nasopharyngeal and saliva specimens are observed in asymptomatic, mild and severe forms of SARS-CoV-2 infections [6]. By contrast, blood SARS-CoV-2 RNA is more frequently detectable and found at higher levels in severe forms of Covid-19 [8,9]. SARS-CoV-2 viremia is associated with disease severity, patient outcome and inflammatory biomarkers [20]. Systemic clinical manifestations suggest that SARS-CoV-2 also can infect different organs through the bloodstream, such as endothelial cells [21], gastrointestinal cells and angiotensin

converting enzyme 2 receptor (ACE2) positive distal tubule cells [22]. Furthermore, the administration of convalescent plasma therapy and monoclonal antibodies (mAb) against the Spike protein help improve Covid-19 recovery, although studies shown conflicting results [23–28]. These observations suggest that SARS-CoV-2 replication and plasma viremia may contribute to the severity of Covid-19.

The first reports on Covid-19 infrequently detected circulating SARS-CoV-2 RNA [29]. Recent studies have inconsistently detected SARS-CoV-2 RNA, and generally with a low viral load [30,31]. In contrast, in the study of Le Hingrat *et al.*, N-antigenemia appeared to be a sensitive marker of SARS-CoV-2 infection in hospitalized patients, able to provide a surrogate test to molecular approaches [15]. Dandan S. *et al.* confirmed this observation using a digital enzyme-linked immunosorbent method [17]. In line with these studies, we observed that the N-Ag levels in our population of hospitalized patients most of the time were over 100 pg/mL during the first week after the onset of symptoms, and remained largely over the lower limit of quantification of the assay during the second week. Given the much better analytical sensitivity of PCR methods compared to antigen immunoassays, these findings are surprising. SARS-CoV-2 nucleocapsid antigen may be released in the bloodstream or circulate after destruction of the virus particle or produced in excess.

Detection of SARS-CoV-2 RNA in urine specimens has been reported in less than 5% of confirmed Covid-19 cases [32]. Using mass spectrometry, Mishra C *et al.* have reported detection of nucleocapsid-derived peptides in urine of a third of Covid-19 patients [33]. Our results using a highly sensitive immunoassay demonstrate the presence of N-antigen in urine in most of Covid-19 hospitalized patients during the first two weeks after onset of the symptoms. The kidney is among the most frequently affected extrapulmonary organs during SARS-CoV-2 infection, and varying degrees of renal damage have been reported in Covid-19 patients [34,35]. Acute kidney disease was observed in a quarter of the patients included in our study. We did not observed any association between urine N-Ag levels and altered renal function. N-Ag in urine may originate from the blood and be excreted by the kidney, as suggested by the correlation between N-Ag concentrations in blood and urine. The SARS-CoV-2 nucleocapsid is a 46 kd protein. The glomerular permeability and filtration probably permit the excretion of this small-size protein. However, after seroconversion against the nucleocapsid, immune complexes also form, with a size that makes them unable to be filtered. Of note, although Covid-19 associated glomerular disease has been reported, this type of kidney injury

seems infrequent among acute kidney diseases associated to SARS-CoV-2 infection [36]. A local production of SARS-CoV-2 nucleocapsid may be another possible origin of the antigen detected in urine. Alongside hypoxia, circulating disorder and inflammation, SARS-CoV-2 infection may directly contribute to kidney injury. Cells expressing ACE2 are present in the tubules, and studies have shown that SARS-CoV-2 RNA, nucleocapsid and spike protein accumulate in tubules [22,37].

Both blood and urine N-Ag levels may reflect SARS-CoV-2 disseminated infection. We observed a moderate correlation between N-Ag in blood and urine, perhaps better if the couple of blood and urine were collected in the same time but the kinetic of this marker may be different in these two compartments [13]. The development of anti-nucleocapsid humoral response may induce the formation of immune complexes that interfere with N-Ag quantification in blood. Hence, after seroconversion N-Ag and N-IgG levels may represent only the unbound fraction available to be measured by the immunoassays. In urine, antibodies directed against nucleocapsid were only detected in one quarter of the patients who tested positive for circulating N-IgG, limiting the risk of underestimation of N-Ag levels by the immunoassay. In other words, N-Ag levels in urine may be more accurate since nucleocapsid Ag quantitation in urine is probably less impacted by the formation of immune Ag-Abs complexes compared to blood. N-Ag in urine may better reflect disseminated infection than nucleocapsid antigenemia, especially after seroconversion against SARS-CoV-2 nucleoprotein. Besides interfering with assay measurement, the presence of circulating antibody-antigen complexes may bind FC receptors activating monocytes/macrophages, and fuel the hyper inflammation observed in the second phase of Covid-19 [38].

Risk factors related to age and comorbidities, alongside inflammation and cytopenia, are associated with the development of severe forms of Covid-19 requiring hospitalisation and intensive care. At present, however, the progression to a severe form of Covid-19 remains unpredictable. We observed higher concentrations of urine N-Ag in samples collected in patients hospitalised in ICU compared to medical wards. This result is in line with the study of Caceres P. *et al.*, reporting that SARS-CoV-2 viral load in urine sediments was associated with higher mortality in hospitalized patients [6]. Furthermore, in our study, urine and plasma N-Ag levels were associated with several early markers of Covid-19 severity, such as lymphopenia, low eosinophil count, and CRP levels. N-Ag level should be evaluated in a combination of markers to predict the risk of severe form of Covid-19. We observed lower levels of urine N-Ag in patients with abnormal ALT, which may be because liver injury is

delayed after the first week in the course of prolonged Covid-19 while the decay of N-Ag is already underway [39].

Our study has several limitations. The population is not representative of SARS-CoV-2 infected individuals. All of the subjects had moderate or severe forms of Covid-19 and required oxygen, whereas a majority of SARS-CoV-2 infected individuals do not require hospitalization. In addition, patients requiring critical care are over-represented because their urine samples were frequently collected in routine care. We did not assess the value of N-Ag as a predictive marker of adverse evolution but only as a marker associated to severe Covid-19. Finally, N-Ag levels were analysed on a single urine sample while results on urine samples collected taken over a 24-hour period would be more accurate.

In conclusion, these results demonstrate that N-Ag is present in urine of patients hospitalized for Covid-19. A broad SARS-CoV-2 systemic dissemination may be the hallmark of severe forms of Covid-19. Herein, we demonstrate that the SARS-CoV-2 nucleocapsid protein is present in high concentrations in the urine of Covid-19 hospitalized patients. The nucleocapsid level in urine is associated with admission in intensive care units and markers of Covid-19 severity.

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Acknowledgments

The author would like to thank Grace Delobel for English language editing and review services.

Funding statement

This work was funded by the Montpellier University Hospital, Muse I-SITE Program Grant, University of Montpellier.

Conflict of Interest Statement

The authors declare that there are no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Patient characteristics

Clinical and biological characteristics†	Confirmed COVID-19			<i>P</i> value	Controls (n=32)
	Total (n=82)	Medical ward patients (n=24)	ICU patients (n=58)		
M/F	58/24	15/9	43/15	0.29 ^u	15/17
Median age	63.0 (53.0 - 74.25)	66.50 (49.00 - 79.25)	62.50 (54.75 - 73.25)	0.82 [#]	55.0 (31.5 - 68.75)
Age ≥ 65 (%)	39 (47.6)	13 (54.2)	26 (44.8)	0.44 ^u	13 (40.6)
Median time since onset of symptoms (days)	12 (9 - 16)	11.5 (7.5 - 17.0)	12.0 (9.0 - 16.0)	0.75 [#]	-
SARS-CoV-2 RNA PCR cycle threshold (CT)	27.8 (22.6 - 32.8)	29.5 (21.1 - 36.3)	26.9 (22.7 - 31.5)	0.54	-
SARS-CoV-2 nucleocapsid IgG positive (%)	48 (58.5)	13 (54.2)	35 (60.3)	0.61	-
Platelet count (10 ³ /mm ³)	236.0 (167.0 - 334.5)	256.5 (135.3 - 391.8)	232.0 (185.0 - 301.5)	0.68 [#]	-
Lymphocyte count (10 ³ /mm ³)	0.93 (0.55.0 - 1.29)	1.11 (0.93 - 1.41)	0.82 (0.49 - 1.16)	0.01[#]	-
Neutrophil count (10 ³ /mm ³)	5.91 (3.70 - 9.19)	4.62 (3.09 - 6.89)	6.42 (3.91 - 9.57)	0.11 [#]	-
Eosinophil count (10 ³ /mm ³)	0.06 (0.03 - 0.11)	0.06 (0.03 - 0.11)	0.06 (0.03 - 0.12)	0.67 [#]	-
C-reactive protein (mg/L)	118.4 (49.2 - 211.6)	82.6 (20.7 - 131.3)	146.2 (55.7 - 241.2)	0.0058[#]	-
Glomerular Filtration Rate (mL/min)	81.5 (39.5-101.5)	86.5 (67.8-99.3)	79.5 (37.3-102.8)	0.41 [#]	-
D-dimer (ng/mL)	1110 (594 - 2024)	985 (529 - 1392)	1211 (594 - 2221)	0.37 [#]	-
Alanine aminotransferase (ALT) (IU/mL)	33 (22.0-53.8)	30 (18-51.3)	33 (23-64.3)	0.2382 [#]	-
Lactate dehydrogenase (LDH) (U/L)	386 (293-451.3)	327 (201.3-420.5)	398 (307.5-495.5)	0.0192[#]	-
Troponin (pg/mL)	12.9 (8.8-29.6)	12.3 (6.0-17.3)	13.1 (9.85-39.6)	0.0970 [#]	-

†Quantitative results were expressed as median (IQR)

^uPearson's Chi-squared test

[#]Mann-Whitney

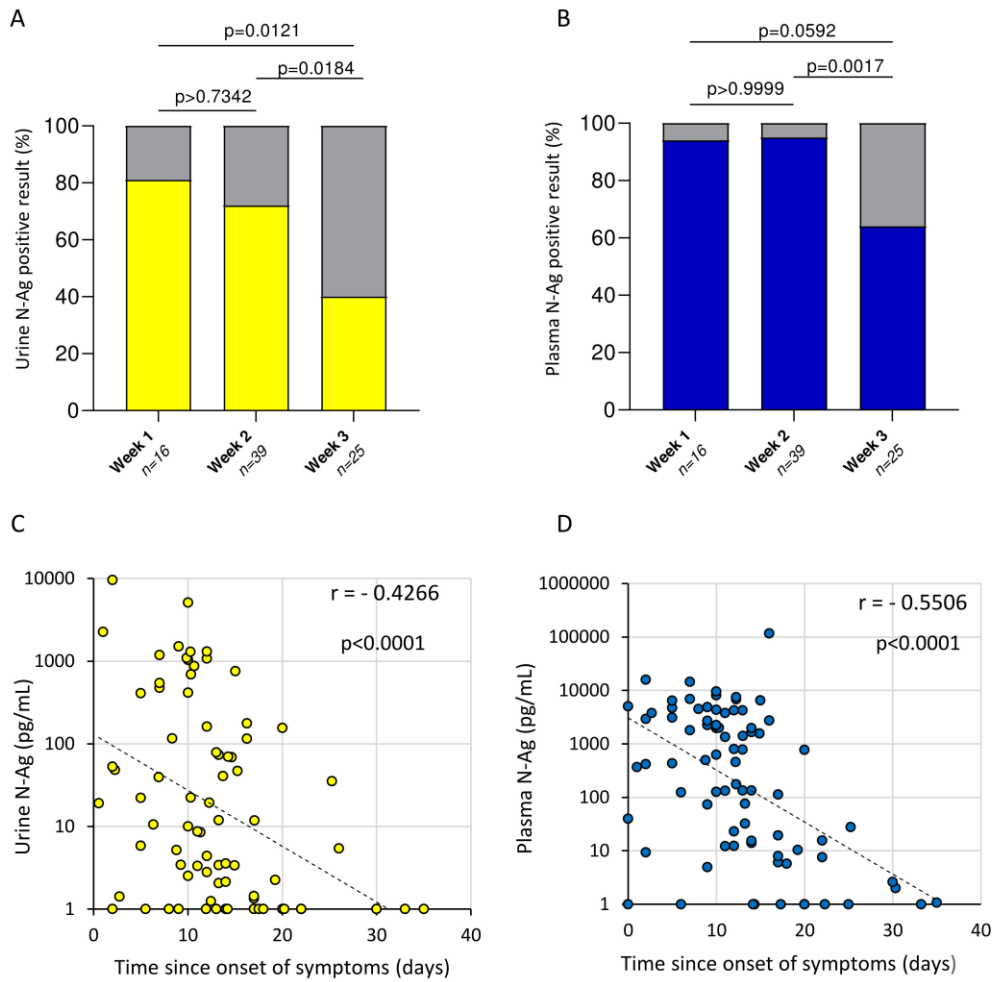


Figure 1. SARS-CoV-2 N-Ag in urine and plasma samples according to the time. A) Detection of urine N-Ag according to the week since the onset of symptoms in SARS-CoV-2-infected patients. Proportion of patients tested positive for N-Ag in urine were represented in yellow. B) Detection of plasma N-Ag according to the week since the onset of symptoms in SARS-CoV-2-infected patients. Proportion of patients tested positive for N-Ag in plasma were represented in blue. C) Urine N-Ag levels according to the time since the onset of symptoms, with exponential fits (dotted line). D) Plasma N-Ag levels according to the time since the onset of symptoms, with exponential fits (dotted line).

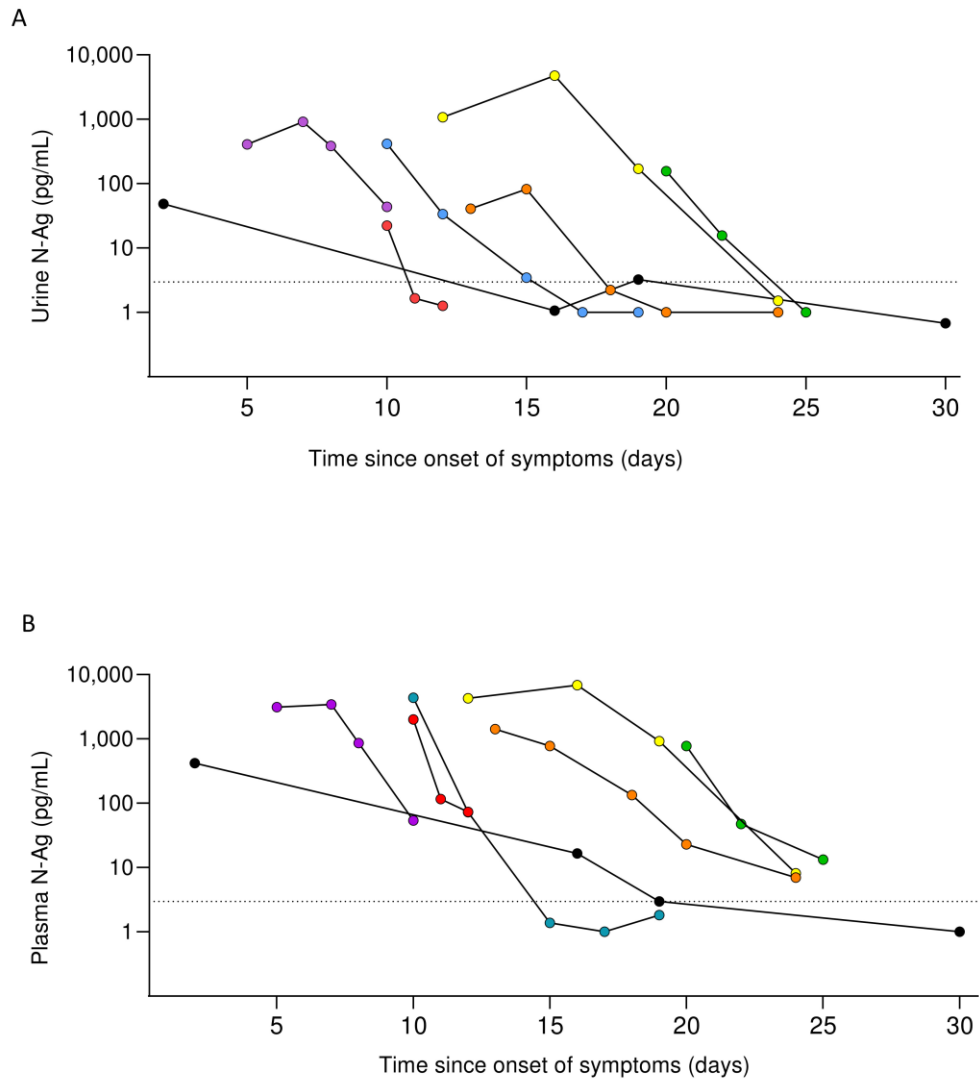


Figure 2. Follow-up of N-Ag in urine and blood samples. A) Longitudinal assessment of N-Ag in urine samples collected in seven patients (dotted line: lower limit of detection). B) Longitudinal assessment of N-Ag in plasma samples collected in seven patients (dotted line: lower limit of detection). Matched urine and plasma samples have the same color of symbol.

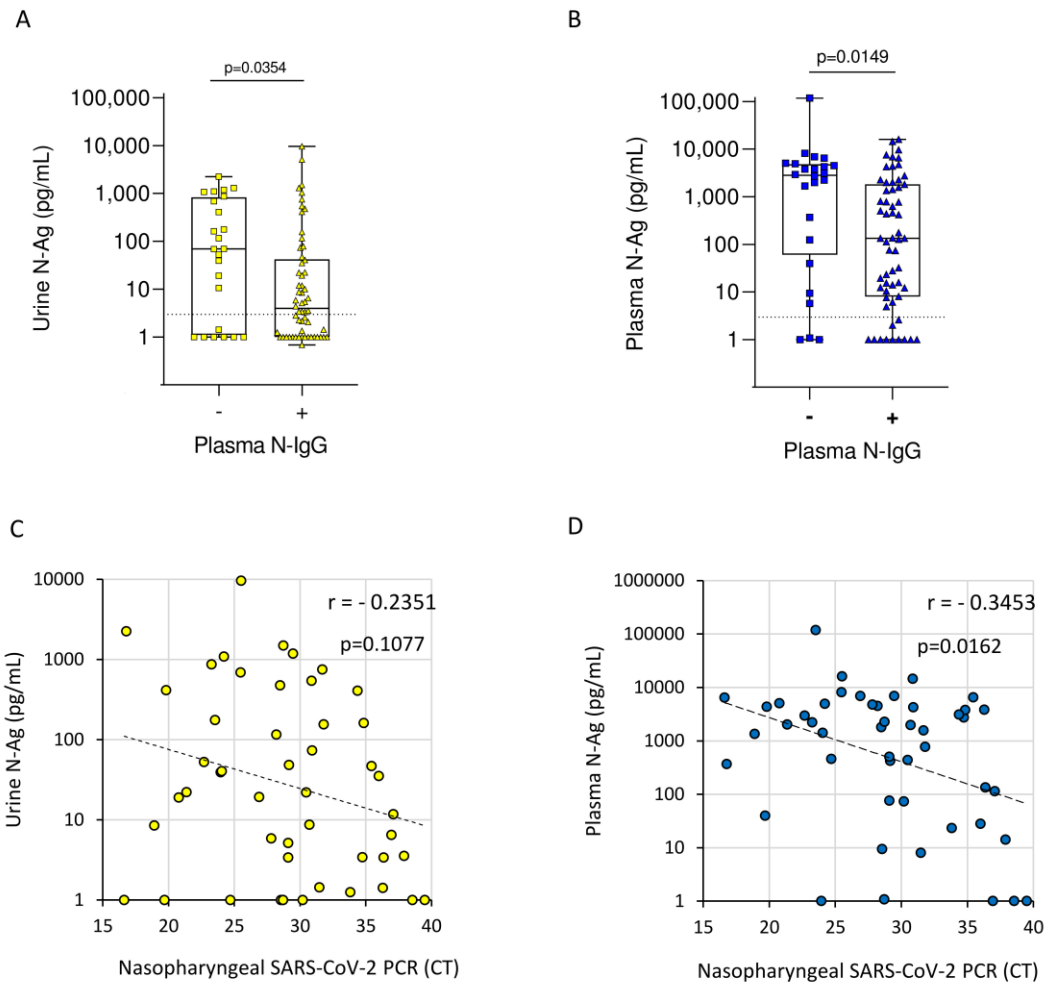


Figure 3. N-Ag according serological status and PCR Ct results A) Urine N-Ag according to serological status for N-IgG. B) Plasma N-Ag according to serological status for N-IgG. C) N-Ag in urine as a function of PCR cycle threshold (CT) in nasopharyngeal samples, with exponential fits (solid lines). D) N-Ag in plasma as a function of PCR CT in nasopharyngeal samples, with exponential fits (solid lines).

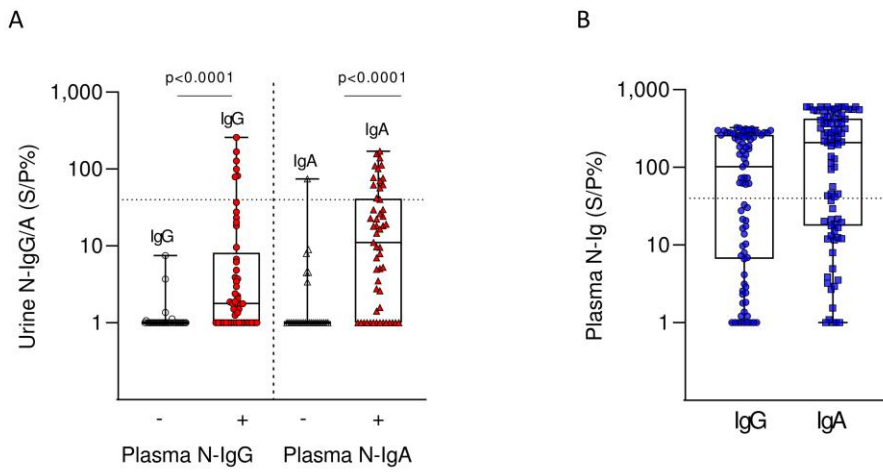


Figure 4. SARS-CoV-2 nucleocapsid-IgG (N-IgG) and -IgA (N-IgA) in plasma and urine samples A) Nucleocapsid levels in urine according to plasma nucleocapsid status; urine N-IgG in plasma N-IgG negative patients (white circles) and plasma N-IgA positive patients (red circles); urine N-IgA in plasma N-IgA negative patients (white triangles) and plasma N-IgA positive patients (red triangles). Nucleocapsid antibody levels were expressed as a percentage sample/positive control ratio (S/P%). B) Nucleocapsid antibody levels in plasma, IgG (blue circles), IgA (blue square), limite of positivity (doted line).

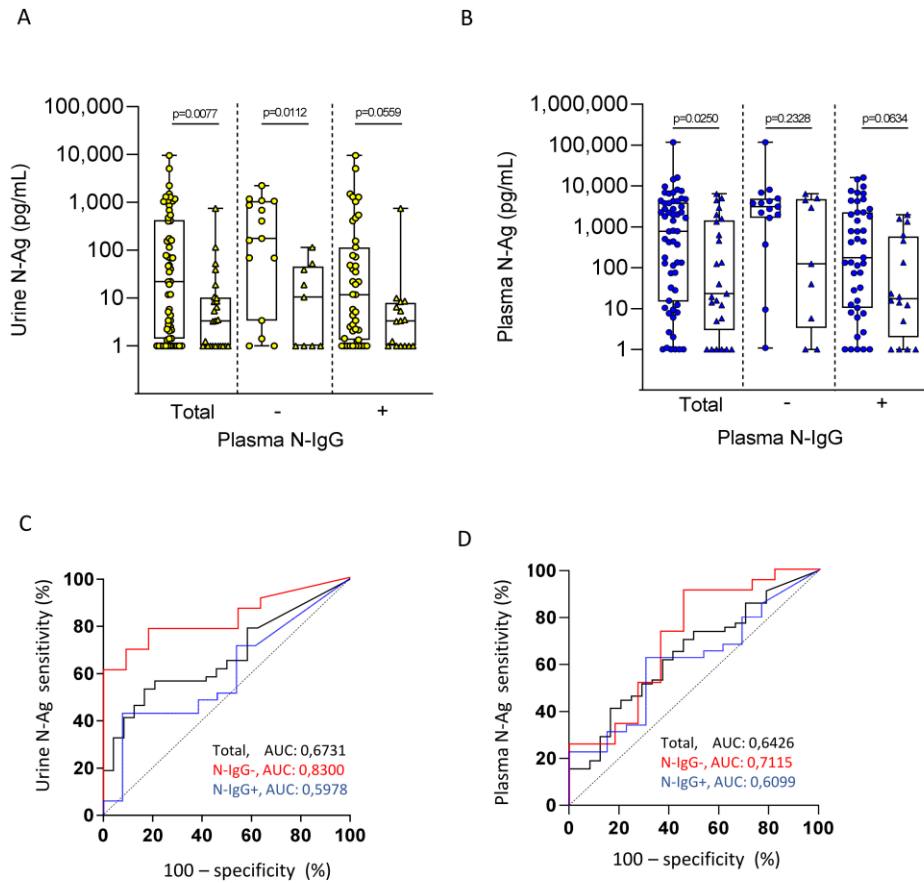


Figure 5. SARS-CoV-2 N-Ag according to Covid-19 severity in hospitalized patients. A) Urine N-Ag levels in patients hospitalized in intensive care units (yellow circles) versus medical wards (yellow triangles) and according to serological status for N-IgG. B) Plasma N-Ag levels in patients hospitalized in intensive care units (blue circles) versus medical wards (blue triangles) and according to serological status for N-IgG. C) Receiver operating characteristic curve (ROC) evaluating the ability of urine N-Ag levels to discriminate patients hospitalized in intensive care units versus medical wards (Black: all patients; red: nucleocapside-IgG seronegative patients; blue: nucleocapsid-IgG seropositive patients). D) ROC evaluating the ability of plasma N-Ag levels to discriminate patients hospitalized in intensive care units versus medical wards (Black: all patients; red: nucleocapside-IgG seronegative patients; blue: nucleocapsid-IgG seropositive patients).

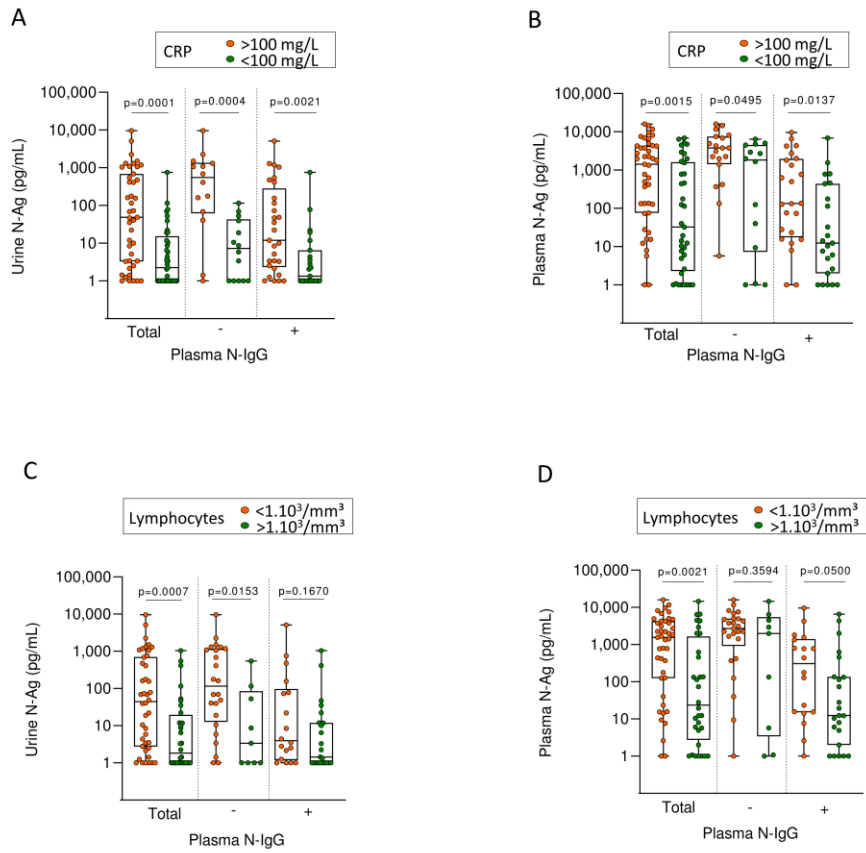


Figure 6. Association of N-Ag levels with biological indicators of Covid-19 severity. A) N-Ag levels in urine according to C-reactive protein (CRP) and N-IgG serological status. B) N-Ag levels in plasma according to CRP and N-IgG serological status. C) N-Ag levels in urine according to lymphocyte count and N-IgG serological status. D) N-Ag levels in plasma according to lymphocyte count and N-IgG serological status.