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Impaired systemic nucleocapsid antigen clearance in severe COVID-19

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Abstract

Objectives: Circulating nucleocapsid (NCP) antigen of SARS-CoV-2 is increased in severely ill COVID-19 patients. However, clinical deterioration of COVID-19 often happens about one week after benign initial presentation. The role of NCP antigenemia as a biomarker in those cases remains unclear. We investigated NCP clearance kinetics in hospitalized patients as a risk assessment tool for predicting necessity of intensive care treatment of COVID-19 patients. **Methods:** Serum NCP was quantified using a commercial NCP-specific ELISA in hospitalized COVID-19 patients (n=63) during their hospital stay. Results were correlated to COVID-19 disease severity, inflammation parameters, antibody response and results of SARS-CoV-2 PCR from nasopharyngeal swabs. **Results:** We demonstrate that NCP antigen levels in serum remained elevated in 45.6% of patients requiring treatment on intensive care units (ICU) after >8 days post positive SARS-CoV-2 PCR, compared to complete clearance in all non-ICU patients. This was in contrast to mucosal clearance of virus as measured by PCR. Antigen clearance was associated with higher IgG against S1 but not NCP. **Conclusions:** Detection of NCP antigenemia after 8 days post COVID-19 diagnosis identifies patients who will require intensive care. Lack of NCP clearance after one week can thus help to assess the risk to develop severe COVID-19.

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Running head: NCP antigenemia in severe COVID-19

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Methods

Serum NCP was quantified using a commercial NCP-specific ELISA in hospitalized COVID-19 patients (n=63) during their hospital stay. Results were correlated to COVID-19 disease severity, inflammation parameters, antibody response and results of SARS-CoV-2 PCR from nasopharyngeal swabs.

Results

We demonstrate that NCP antigen levels in serum remained elevated in 45.6% of patients requiring treatment on intensive care units (ICU) after >8 days post positive SARS-CoV-2 PCR, compared to complete clearance in all non-ICU patients. This was in contrast to mucosal clearance of virus as measured by PCR. Antigen clearance was associated with higher IgG against S1 but not NCP.

Conclusions

Detection of NCP antigenemia after 8 days post COVID-19 diagnosis identifies patients who will require intensive care. Lack of NCP clearance after one week can thus help to assess the risk to develop severe COVID-19.

Key words

COVID-19, nucleocapsid antigen, ELISA, SARS-CoV-2, biomarker

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Introduction

Circulation of viral antigen in blood is a mainstay of diagnostic testing in systemic viral infections, including HBV, HCV or HIV. However, in respiratory infections such as SARS-CoV-2, antigenemia in serum is harder to interpret [1, 2]. Systemic presence of nucleocapsid (NCP) as well as spike (S) protein has been described [3]. A high level of serum NCP antigen was consistently found to be associated with serious illness [4-7]. It was suggested that the serum NCP antigen level may serve as an early biomarker for predicting the maximum disease grade of severe COVID-19, using NCP antigenemia as a tool for allocation of ICU options on admission [8]. However, some patients progress to more severe disease not before the second week of Covid-19; for those patients, no biomarker has been established yet. Here, we demonstrate a delay of NCP antigen clearance in patients who required intensive care during hospitalization.

Patients and Methods

Study Cohort

Patients (n=63) diagnosed with SARS-CoV-2 infection by Real-Time PCR from respiratory specimens (Altona Diagnostics, Hamburg, Germany) were enrolled into the study at Marburg University Hospital (Germany) between March and November 2020. The baseline characteristics of the study population are shown in Table S1.

Antibody and antigen testing

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Serum IgG antibodies against S1 and NCP antigens of SARS-CoV-2 were measured by commercial ELISAs (Anti-SARS-CoV-2-ELISA [IgG] and Anti-SARS-CoV-2-NCP-ELISA [IgG]; Euroimmun, Lübeck, Germany), according to the manufacturer's instructions. Upon manual dilution, sera were processed in a BEPIII analyzer (Siemens, Eschborn, Germany). Results are indicated as Ratio (cutoff: <0.8; borderline: [?]0.8, <1.1; positive: >1.1). Circulating NCP antigen in serum was quantified using the ScheBo(r) SARS-CoV-2^{TM} Antigen ELISA (ScheBo Biotech, Giessen, Germany). Values [?]2.97 pg/mL were considered positive.

Statistical analysis

Continuous variables are presented as means +- SD or min/max as indicated. Unpaired t tests and linear correlations were performed by GraphPad PRISM 7.0e. A two-sided p value of <0.05 was considered significant.

Ethics statement

The study protocol was reviewed and approved by the Ethics Commission of the Medical Faculty of Philipps University Marburg (vote 57/20). Informed consent was obtained from all study participants.

Results

NCP antigenemia and SARS-CoV-2-specific antibody responses

In sera of hospitalized COVID-19 patients without detectable antibodies, NCP antigen was detected in high concentrations >100 pg/mL (Fig. S1A,B). As expected, a subset of patients with detectable antibodies against S1 or NCP had NCP antigen levels <2.97 pg/mL, consistent with successful clearance of the infection. We assumed that the appearance of NCP-specific IgG would show a strong association with the reduction of NCP antigenemia. Instead, we found that only 33.0% of anti-NCP-positive, but 40.6% of anti-S1-positive sera had an NCP antigen concentration below the cutoff. Simultaneous detection of antibodies and NCP antigen was also found (29.3% of S1 IgG-positive sera, 38.3% of NCP IgG-positive sera, Fig. S1A,B). Accordingly, NCP antigen was lower in anti-S1-positive compared to anti-NCP-positive sera (Fig. S1C), suggesting that S1-specific IgG is more relevant than NCP-specific IgG to reduce NCP antigenemia.

Inflammatory response and NCP antigenemia in ICU and non-ICU patients

Patients were grouped into those requiring intensive care (ICU patients) versus those without need for ventilatory support (non-ICU patients). Higher levels of CRP and IL-6 were found in ICU compared to non-ICU patients (Fig. S2A,B). Based on this differentiation, we found that NCP antigenemia correlated positively with both CRP and IL-6 in ICU, but not in non-ICU patients (Fig. S2C,D). NCP antigenemia was significantly higher in patients [?]75 years of age (Table S1). Sex was not associated with increased antigenemia.

Time course of systemic NCP antigenemia in COVID-19 patients

Next, we compared NCP serum concentrations between non-ICU and ICU patients. During the first week, NCP antigen was detected in 36/43 samples from ICU patients (83.7%) and 23/25 samples from non-ICU patients (92.0%, Fig. 1A) with similar peak values (Fig. 1B). After day 8 post COVID-19 PCR diagnosis, non-ICU patients had invariably cleared NCP (14/14, 100%). In contrast, NCP was still elevated in 26/57 (45.6%) samples from ICU patients obtained during the late phase of illness (>8 days, Fig. 1B). Mean NCP antigen concentrations remained significantly higher in ICU compared to non-ICU patients after the 8th day of illness (Fig. 1B). Importantly, the Ct values in swab PCRs from ICU patients above vs. below the NCP cutoff did not differ significantly (Fig. S3A-C), suggesting that NCP antigenemia is independent of mucosal clearance. Thus, while high NCP antigenemia was a phenomenon in all hospitalized COVID-19 patients during the first week of illness, prolonged systemic circulation of NCP beyond the first week was detected only in ICU patients.

Antibody responses in NCP-positive ICU patients

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To understand the possible defect in ICU patients that have prolonged NCP antigenemia (after 8 days of COVID-19), ICU patients were grouped into those with NCP levels above and those below the cutoff, and the S1- and NCP-specific antibody responses were analyzed. ICU patients without detectable antigenemia had antibodies against S1 (Fig. 2A) and NCP (Fig. 2B) with ratios similar to non-ICU patients, suggesting normal antibody responses. In contrast, ICU patients with antigenemia after day 8 of COVID-19 had significantly lower S1-specific antibodies compared to ICU patients without antigenemia. This difference was restricted to S1-specific antibodies, while no difference was found for IgG against NCP (Fig. 2B). Thus, the prolonged NCP antigenemia in COVID-19 patients on ICU was associated with decreased antibody responses against S1, but not NCP.

Discussion

The present study investigated the role of circulating SARS-CoV-2 NCP in COVID-19 with relation to antibody status, disease severity, time post diagnosis and inflammation markers. We show that high systemic concentrations of NCP antigen are a consistent feature of the early phase of hospitalized COVID-19 patients, independent of disease severity. Remarkably, NCP antigen clearance to levels below the detection threshold was impaired after the 8^{th} day of disease in COVID-19 patients needing intensive care compared to non-ICU patients, suggesting a reduced systemic antigen clearance in severe disease.

Circulating NCP antigen is detected at higher concentrations in severely ill COVID19 patients [7, 9-11]. Our study identified a moderate but significant correlation between NCP antigenemia and CRP / IL-6 in ICU patients. Earlier investigations had observed an association of higher NCP antigenemia with CRP [6], IL-10, IP-10 and RAGE [10]. As a prognostic marker, NCP concentrations of >1000 pg/mL upon hospitalization were associated with later ICU admission and delayed hospital discharge [10, 12]. Our study now adds that clearance of NCP antigenemia is delayed in >40% of severely ill COVID19 patients who required intensive care. Thus, if NCP is detectable after 1 week of illness, a potentially severe course of disease is likely, emphasizing its role as a prognostic biomarker.

The biological basis of NCP antigenemia is still ill-defined. Plasma NCP antigenemia was found not to correlate with mucosal Ct values [8]. However, it is unresolved whether NCP antigen in plasma reflects ongoing systemic viral replication [12], or rather dissemination from the respiratory tract [10]. Our study is the first to show that prolonged antigenemia in ICU patients is associated with lower S1- but not NCP-specific IgG after 8 days post diagnosis. Reduced S1-specific antibodies could thus result in less efficient neutralization and permit systemic dissemination of virions.

Of note, the present study found that PCR results from nasopharyngeal swabs did not differ significantly between ICU and non-ICU patients, as also described by Yilmaz et al. [13]. In contrast, systemic NCP antigenemia could differentiate both groups, suggesting independent regulation of viral clearance from the mucosa and systemic clearance of viral antigen. Consequently, detection of systemic NCP antigen after 8 days post COVID-19 diagnosis identifies patients that progress to clinical deterioration requiring intensive care, while those who clear antigenemia after this time are likely to recover. Thus, prolonged antigenemia can serve as a novel biomarker to recognize severe courses of COVID-19 infection.

Conflict of interest

CB reports funding by the European Fund of Regional Development (EFRE). CK reports funding by the European Fund of Regional Development (EFRE) and the Pandemic Network of the State of Hessen as well as lecture honorarium from Roche (unrelated to present study).

Funding

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Contribution

CB: investigation, formal analysis, writing – original draft. EM: conceptualization, patient recruitment. VH: investigation, writing-review and editing. AF: investigation. KV: investigation. CS: conceptualization, patient recruitment, writing-review and editing. AN: patient recruitment, writing-review and editing. TG: writing-review and editing, supervision. SB: writing-review and editing, supervision. CK: conceptualization, investigation, formal analysis, writing – original draft. All authors read and approved the final manuscript.

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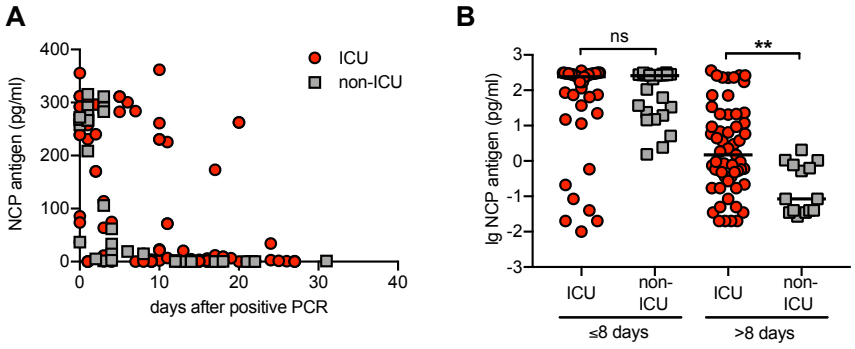
Figure legend

Fig. 1. The clearance of NCP antigen is delayed in ICU patients

Antigen concentration of systemic NCP in patient sera was measured by ELISA (see Supplementary Methods). Samples were grouped based on ICU admission. (A) NCP concentration at indicated days after positive SARS-CoV-2 PCR. (B) Samples were grouped into those collected before or after 8 days since COVID-19 diagnosis. ICU and non-ICU patients exhibited high NCP levels in the early phase of COVID-19 infection. NCP antigenemia remained significantly higher in ICU compared to non-ICU patients after 8 days since diagnosis. Means +- SD. ** $p<0.01$; ns=not significant by t test.

Fig. 2. ICU patients with impaired NCP antigen clearance show lower antibody concentrations against S1 but not NCP

(A) S1 IgG concentrations of individuals after day 8 post first positive PCR, from ICU (red) and non-ICU patients (grey). The ICU patients were split into the participants with NCP antigenemia above and below the test threshold defined by the manufacturer (2.97 pg/ml). S1-specific antibody concentrations (n=20, 24 and 10, respectively). (B) NCP-specific antibody concentrations (n=19, 24 and 11, respectively). Means +- SD. ** $p<0.01$; ns=not significant by t test.



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