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Research note

Impact of dexamethasone on SARS-CoV-2 concentration kinetics and antibody response in hospitalized COVID-19 patients: results from a prospective observational study

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ABSTRACT

Objectives: Dexamethasone has become the standard of care for severe coronavirus disease 2019 (COVID-19), but its virological impact is poorly understood. The objectives of this work were to characterize the kinetics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) concentration in the upper respiratory tract (URT) and the antibody response in patients with (D^+) and without (D^-) dexamethasone treatment.
Methods: Data and biosamples from hospitalized patients with severe COVID-19, enrolled between 4th March and 11th December 2020 in a prospective observational study, were analysed. SARS-CoV-2 virus concentration in serial URT samples was measured using RT-PCR. SARS-CoV-2-specific immunoglobulins A and G (IgA and IgG) were measured in serum samples using S1-ELISA.
Results: We compared 101 immunocompetent patients who received dexamethasone (according to the inclusion criteria and dosage determined in the RECOVERY trial) to 93 immunocompetent patients with comparable disease severity from the first months of the pandemic, who had not been treated with dexamethasone or other glucocorticoids. We found no inter-group differences in virus concentration kinetics, duration of presence of viral loads $>10^6$ viral copies/mL (D^+ median 17 days (IQR 13–24), D^- 19 days (IQR 13–29)), or time from symptom onset until seroconversion (IgA: D^+ median 11.5 days (IQR 11–12), D^- 14 days (IQR 11.5–15.75); IgG: D^+ 13 days (IQR 12–14.5), D^- 12 days (IQR 11–15)).
Conclusion: Dexamethasone does not appear to lead to a change in virus clearance or a delay in antibody response in immunocompetent patients hospitalized with severe COVID-19. **Barbara Mühlemann, Clin Microbiol Infect 2021;27:1520.e7–1520.e10**

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Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection has a clinical spectrum ranging from asymptomatic to severe disease [1]. Inflammation-mediated lung injury plays a crucial role in progression to severe coronavirus disease 2019 (COVID-19). Glucocorticoids are widely used for treatment of infectious and non-infectious diseases involving a dysregulated immune response. In severe COVID-19, dexamethasone showed beneficial effects when administered >7 days after symptom onset [2] and became the standard of care [3]. However, the impact of dexamethasone on viral concentration and the adaptive immune response has not been well studied [4]. We analysed the impact of dexamethasone on SARS-CoV-2 RNA kinetics and antibody response in a prospective observational cohort of hospitalized immunocompetent COVID-19 patients.

Materials and methods

Data collection was performed within the Pa-COVID-19 study, a prospective observational cohort study at Charité—Universitätsmedizin Berlin [5,6], approved by the local ethics committee (EA2/066/20), conducted according to the Declaration of Helsinki and Good Clinical Practice principles (ICH 1996), and registered in the German and WHO international clinical trials registry (DRKS00021688).

Patients were treated according to national guidelines. Treatment with 6 mg dexamethasone orally or intravenously was introduced as the standard of care shortly after the press release of the RECOVERY trial on 16th June 2020 in patients fulfilling the criteria of ≥7 days symptom duration and requiring at least supplemental oxygen therapy (i.e. score ≥4 on the WHO scale for clinical improvement [7]). This analysis includes patients enrolled between 4th March and 11th December 2020 who fulfilled the above-mentioned criteria in the absence of specific contraindications [2]. We excluded patients receiving non-standard doses of dexamethasone or other glucocorticoids and with immunosuppression (at least one of the following: immunodeficiency, immunosuppressive or cancer therapy within the previous 3 months, history of organ transplant, HIV infection, lymphoma, or leukaemia).

SARS-CoV-2 RNA was measured by real-time (RT)-PCR in swabs from the upper respiratory tract (URT) [8] and is given here as $\log_{10}$ RNA copies per 1 mL sampling buffer. In routine testing, a weekly RT-PCR was performed, with test frequency sometimes increasing towards the end of the isolation period. All patients with at least one positive RT-PCR test result were included in the analysis. RNA measurements taken up to 40 days after admission were included for statistical analysis.

ELISA for the detection of immunoglobulins G and A (IgG and IgA) to the S1 domain of the SARS-CoV-2 spike protein (S1-ELISA) was performed once per week during hospital stay, as described elsewhere [9]; a median of four measurements were performed per patient. All patients with at least one reactive S1-ELISA test (optical density (OD) ratio >1.1) were included in the analysis ($n = 174$). S1-ELISA OD ratios were capped at a value of 5. Outliers—defined as patients who seroconverted or reached a threshold OD ratio of 5 after more than 30 days—were excluded ($n = 5$). Distribution of continuous variables is shown as median and interquartile range (IQR). Differences of continuous variables between groups were examined by Mann–Whitney *U* test and of categorical variables using χ^2 tests. Local regression (LOWESS) was used for the comparison of SARS-CoV-2 RNA kinetics. Analyses were conducted with R (V3.6.1), JMP (V14.2.0), and statsmodels (V0.12.0) in Python (V3.7.9).

Results

We identified 216 patients in our cohort with an indication for dexamethasone treatment according to current national guidelines; of these patients 202 were not immunosuppressed and were included in the analysis. One hundred and one patients received dexamethasone treatment (D^+) of 6 mg/day orally or intravenously for a median duration of 10 days (IQR 8.5–11), initiated at a median of 7 days (IQR 5–10) after symptom onset or 1 day (IQR 0–2) after hospital admission; 93 patients, mostly hospitalized before June 2020, did not receive dexamethasone or other glucocorticoids (D^-, Supplementary Material Fig. S1). Eight patients received other glucocorticoids or different daily doses of dexamethasone and were excluded. We found no statistically significant differences between clinical baseline characteristics, including duration of symptoms until hospital admission, age, gender, presence of comorbidities, or level of respiratory support at admission in the groups (Supplementary Material Tables S1 and S2).

Virus concentration

SARS-CoV-2 RNA concentration kinetics in the URT did not differ between patient groups (Fig. 1A), even though D^- patients had lower RNA concentrations throughout the study than D^+ patients (Supplementary Material Fig. S2). Similarly, no difference was seen when excluding patients ($n = 18$) undergoing antiviral treatment (Supplementary Material Table S1). We also found no evidence for differences in the time from symptom onset to the last test with a viral load $>10^6$ viral copies/mL between patient groups (D^+ median 17 days (IQR 13–24), D^- median 19 days (IQR 13–29), p 0.37) (Fig. 1B).

Antibody response

Analysis of time to seroconversion was feasible for 50 patients showing a non-reactive S1-ELISA result upon inclusion into the study. There was no difference in time from symptom onset to first reactive IgA or IgG S1-ELISA test (i.e. seroconversion) between D^+ and D^- patients (median days to IgA seroconversion 11.5 (IQR 11–12) versus 14 (IQR 11.5–15.75), respectively, p 0.47; median days to IgG seroconversion 13 (IQR 12–14.5) versus 12 (IQR 11–15), respectively, p 0.70. Figs. 2A,B). Seventy-nine percent of patients reached IgA and IgG OD ratios ≥5 within 20 days after symptom onset. There was no difference between D^+ and D^- patients in time from symptom onset to the first test with an OD ratio ≥5 (median days from symptom onset to first IgA test with OD ratio ≥5, 13 (IQR 10–16) versus 13 (IQR 11–15.5), respectively, p 0.76; median days from onset to first IgG test with OD ratio ≥5, 15 (IQR 13–18) versus 15 (IQR 12–16), respectively, p 0.20) (Figs. 2C,D).

Discussion

The introduction of dexamethasone for the treatment of severe COVID-19 raised concerns regarding possible subsequent delay in virus clearance, based on observational studies [10] and data on glucocorticoid therapy in other respiratory virus infections, including SARS-CoV, MERS-CoV [11], and influenza A [12]. Accurate analysis of the impact of dexamethasone on virus clearance and adaptive immune response in COVID-19 would require randomized controlled trials. As collection of virological and laboratory data was not included in the published RECOVERY trial protocol [2], this approach seems beyond reach. Most studies available to date are based on heterogenous medication regimens and show no influence of corticosteroids on virus clearance and antibody response in COVID-19 [3,13,14].

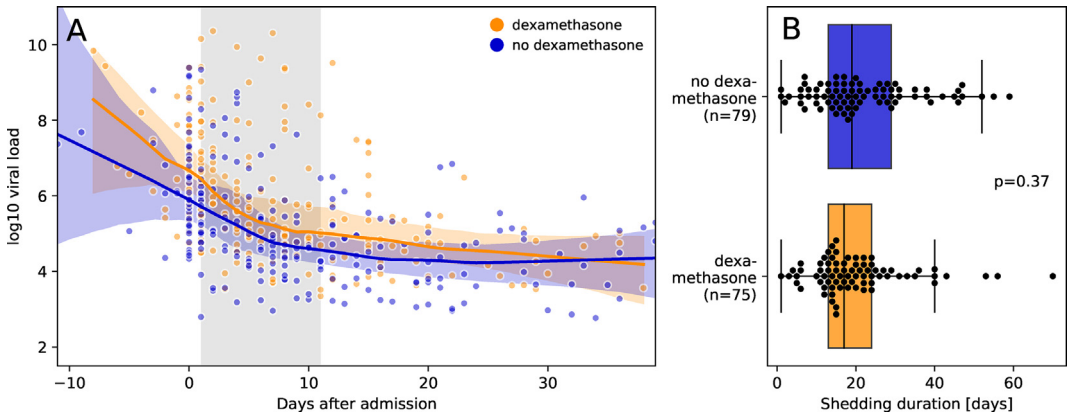

Fig. 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) concentration kinetics and shedding. (A) Virus concentration over time in upper respiratory tract (URT) samples in two groups of hospitalized COVID-19 patients with comparable disease severity (WHO score ≥ 4), D^+ patients in orange and D^- in blue. Bold lines and shaded areas indicate the LOWESS fit (smoothing parameter 0.5) and 95% confidence intervals (calculated from 1000 bootstrap replicates of 90 samples, corresponding to ~90% of the full dataset) for the two groups. The grey area indicates the typical time span during which dexamethasone was administered (a median of 1–11 days after admission). (B) Duration of shedding was measured as the time from symptom onset to the last PCR test with a viral load $>10^6$ viral copies/mL for D^+ and D^- patients. Median shedding duration: D^+ median 17 days (IQR 13–24); D^- median 19 days (IQR 13–29), p 0.37 (Mann–Whitney *U* test). During their time in hospital, D^+ patients had a median of three tests (IQR 2–6), a median of 4 days (IQR 2–7) apart, and D^- patients had a median of five tests (IQR 3–8), a median of 4 days (IQR 2–7) apart.

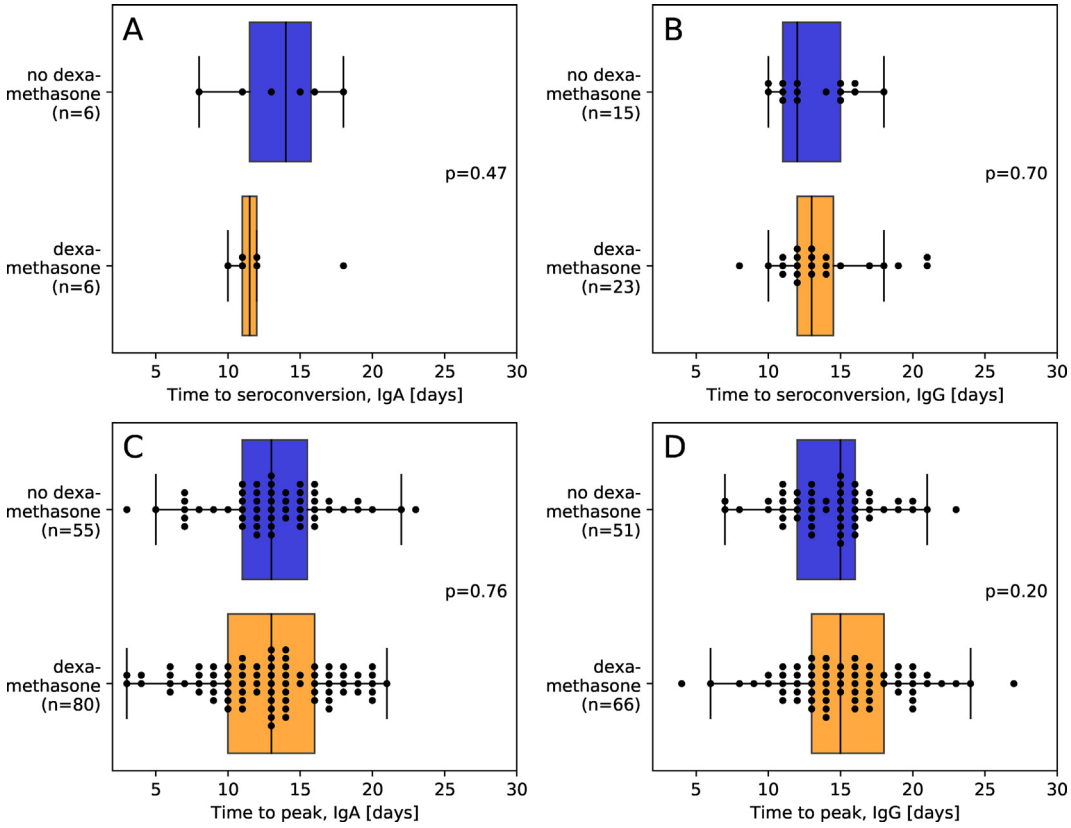

Fig. 2. IgG and IgA S1-ELISA results. (A,B) Time to seroconversion. Time to seroconversion is measured as the time from symptom onset to a reactive S1-ELISA test that is preceded by a non-reactive S1-ELISA test. (A) IgA, median duration to seroconversion: D^+ 11.5 days (IQR 11–12); D^- 14 days (IQR 11.5–15.75). Seroconversion could be observed in six D^+ patients and six D^- patients who were included into the study with a non-reactive IgA S1-ELISA result. (B) IgG, median duration to IgG seroconversion, D^+ 13 days (IQR 12–14.5), D^- 12 days (IQR 11–15). For IgG, 23 D^+ patients and 15 D^- patients were included into the study with a non-reactive IgG S1-ELISA and later seroconverted. (C, D) Time from symptom onset to the first test reaching the threshold OD ratio ≥5. Only patients with a first test taken no more than 20 days after symptom onset were included. (C) IgA, median duration from symptom onset to first IgA S1-ELISA test with OD ratio ≥5, D^+ 13 days (IQR 10–16), D^- 13 days (IQR 11–15.5). (D) IgG, median duration from symptom onset to first IgG S1-ELISA test with OD ratio ≥5, D^+ 15 days (IQR 13–18), D^- 15 days (IQR 12–16). Outliers—defined as patients with >30 days until seroconversion or >30 days to reach the threshold OD ratio of 5—were excluded ($n = 5$).

In this *post hoc* analysis of patients included in a prospective cohort study we found no difference in SARS-CoV-2 RNA kinetics or humoral immune response in two comparable groups of immunocompetent patients hospitalized with COVID-19, treated with or without dexamethasone. We observed a shorter duration of hospital stay and a trend towards reduced progression to

mechanical ventilation and dialysis in the dexamethasone group, yet this observational data may be influenced by factors such as a more strained healthcare system in the second pandemic wave.

A limitation to our study is the relatively small sample size, particularly with regard to biosamples before seroconversion. We could not adjust for demographic and clinical parameters that may influence antibody kinetics [9].

Our results indicate that standard-dose dexamethasone treatment in severe COVID-19 cases—initiated after the peak of SARS-CoV-2 replication during the first week [15]—does not lead to slower virus clearance or antibody response in immunocompetent patients, a finding of importance to clinical management, public health authorities, and the scientific community.

Transparency declaration

VMC and MAM are named with EUROIMMUN on a patent application filed recently regarding antibody diagnostics of SARS-CoV-2. The Pa-COVID 19 Study is supported by grants from the Berlin Institute of Health (BIH). This study was further supported by the German Federal Ministry of Education and Research (BMBF) (NaFoUniMedCovid19—COVIM (01KX2021) and PROVID (01KI20160A)) to LES, MW, CD, FK and VMC. Part of this work was funded by the German Federal Ministry of Education and Research (BMBF) through projects VARIPath (01KI2021) to VMC, the RECOVER project (European Union Horizon 2020 research and innovation programme, GA101003589) to CD, CAPSyS-COVID (01ZX1604B) to MW and NS, and NUM-NAPKON (01KX2021) to MW. TCJ is in part funded through NIAID-NIH CEIRS contract HHSN272201400008C.

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Author contributions

Conceptualization: BM, CT, TCJ, VMC and FK. Methodology: BM, CT, DH, ETH, LJL, PTL, TS, MAM, MW, NS, LES, CD, TCJ, VMC and FK. Formal analysis: BM and CT. Investigation: BM, CT, DH, ETH, LJL, PTL, TS, MAM, MW, NS, LES, CD, TCJ, VMC and FK. Writing (original draft): BM, CT and DH. Writing (review and editing): ETH, LJL, PTL, TS, MAM, MW, NS, LES, CD, TCJ, VMC and FK. Data curation: BM, CT, DH, ETH, LJL and PTL. Visualization: BM, CT, TCJ, VMC and FK. Supervision: MAM, MW, LES, CD, VMC and FK.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.06.008.

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