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ORIGINAL ARTICLE

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Antibody levels remain high to one-year's follow-up after moderate and severe COVID-19, but not after mild cases

Anne Kallaste[a,b], Kalle Kisand[c], Agnes Aart[b], Kai Kisand[d], Pärt Peterson[d] and Margus Lember[a,c]

[a]Department of Internal Medicine, Tartu University Hospital, Tartu, Estonia; [b]South-Estonian Hospital, Võru Vald, Estonia; [c]Department of Internal Medicine, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia; [d]Molecular Pathology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

ABSTRACT

Background: Understanding the longevity of antibodies against SARS-CoV-2 infection is of utmost importance in predicting the further course of the pandemic and to plan vaccination strategies. Here we report a cohort of COVID-19 patients with different disease severities whose antibody dynamics we evaluated during one-year of follow-up.

Methods: We conducted a longitudinal study of 123 COVID-19 patients and 45 SARS CoV-2 negative outpatients with upper respiratory tract infection. We analyzed the demographic and clinical features of the patients with COVID-19 in relation to different disease severities according to the WHO classification. The antibody response was evaluated by a Luciferase Immunoprecipitation System (LIPS) assay at 3, 6, and 12 months after the acute infection.

Results: Amongst the enrolled COVID-19 patients, 15 (12%) had mild, 42 (34%) had moderate, 39 (32%) had severe and 27 (22%) had critical disease courses; 79% of the patients were hospitalized. During follow-up, all patients had anti-SARS RBD-IgG levels above the cut-off value on all visits, but the antibody levels varied significantly between the different disease severity groups. Between the six- and 12-month follow-up visits, 41% of patients were vaccinated, which enhanced their antibody levels significantly.

Conclusion: Our data demonstrate sustained antibody levels at one-year after moderate and severe COVID-19 infection. Vaccination of patients with the mild disease is important to raise the antibody levels to a protective level.

KEYWORDS
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CONTACT
Anne Kallaste
anne.kallaste@kliinikum.ee
Department of Internal Medicine, Tartu University Hospital, L. Puusepa 8, 51014 Tartu, Estonia

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Introduction

COVID-19 has caused a global pandemic that as of 30 May 2021, had resulted in more than 169 million confirmed cases and 3.5 million deaths worldwide [1]. In South Estonia, the Tartu University Hospital catchment area, where the study took place, had a population in 2018 of ~347,000 [2], and during the first year of the pandemic there had been about 16,000 laboratory-confirmed COVID-19 cases in the area [3].

It is well-recognized that COVID-19 is a multifaceted disease. In those who become symptomatic, 40% develop mild, 40% moderate, 15% severe, and 5% critical disease [4]. Even amongst hospitalized patients, the clinical course may vary, ranging from patients without the need for oxygen support to patients with acute respiratory distress syndrome requiring mechanical ventilation.

It is not clear which factors determine the severity of the disease in different individuals. Several currently used therapeutic approaches are targeted against hyperinflammation, so one hypothesis is that the strength of the immune response contributes to disease severity [5]. Therefore, it is of great interest to assess the clinical presentation of the severity of the disease in relation to an antibody response.

Data are still inconclusive with regard to the duration of the humoral immune response to SARS CoV-2. Among seasonal human coronaviruses, the humoral immune responses are short-lived and reinfection is common [6]. Now, more than a year since the beginning of the pandemic, early reinfection with SARS CoV-2 is rare [7–9], yet some studies have demonstrated declining levels of antibodies, especially when the disease had been mild [10,11]. To date, there have been few studies published that evaluate the anti-SARS CoV-2 antibody levels one year after the acute infection [12,13], and the data are still too scarce to make conclusions about long-term immunity. Understanding the kinetics of antibodies against SARS CoV-2 is important to evaluate the duration of immunity and to plan vaccination strategies.

The aim of this study was to analyze the longevity of SARS-CoV-2 IgG antibodies in relation to the severity of the COVID-19 in a one-year follow-up period in vaccinated and unvaccinated cases and to describe the clinical features in the acute phase of the infection.

Patients and methods

Study design and subjects

We conducted a longitudinal cohort study of patients with laboratory-confirmed positive SARS CoV-2 real-time RT-PCR test (reverse transcriptase-polymerase chain reaction) from nasal swab, who was hospitalized in Tartu University Hospital or seen by the Southern Estonian Hospital emergency medicine department. The control group consisted of SARS CoV-2 negative outpatients with upper respiratory symptoms adjusted for the sex and age of the SARS CoV-2 positive patients.

A total of 168 subjects were enrolled in the study, which consisted of (1) patients, who were hospitalized in Tartu University Hospital from the middle of March to the end of May 2020 ($n = 34$); (2) symptomatic SARS CoV-2 positive outpatients, who were managed by the Southern Estonian Hospital emergency medicine department from the beginning of April to the end of May 2020 ($n = 26$); (3) patients, who were hospitalized in Tartu University Hospital from the beginning of August 2020 to the end of April 2021 ($n = 63$). In general, consecutive patients were recruited, but extreme workload pressures in autumn 2020 meant that a small number of patients could not be included; and (4) a control group of SARS CoV-2 negative outpatients with upper respiratory symptoms ($n = 45$) who were seen by the Southern Estonian Hospital emergency medicine department from April to the end of May 2020.

We excluded children, patients with cognitive impairment, and patients who declined to participate. A total of eight patients have withdrawn from the study. Three patients withdrew after their enrolment, three after the three-month visit, and two after the six-month visit.

We collected the key clinical information during hospitalization of inpatients and during the first visit of ambulatory COVID-19 and non-COVID-19 patients. A standardized questionnaire was used in all patients. Height and weight were measured in all patients. Reduced exercise capacity was determined by interviewing the patients, it was not objectivized by any test.

The COVID-19 patients were allocated into four severity groups according to the WHO guidelines [4]. The definitions were as follows: mild—symptomatic patients without evidence of pneumonia; moderate—evidence of pneumonia, but no signs of severe pneumonia ($SpO_2 \geq 90\%$ in room air); severe—pneumonia plus one of the following—respiratory rate ≥ 30 breaths/min or $SpO_2 < 90\%$ (in room air); and critical—patients with ARDS (Berlin definition), sepsis or septic shock.

Ethics approval and consent to participate

The study was approved by the Ethics Committee for Human Research of the University of Tartu (protocol

318/T-1 from 2020) and written informed consent was obtained from all subjects. The procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

Follow-up of participants

The follow-up of the COVID-19 patients was at 3, 6, and 12 months after the acute infection. The control group was re-examined once—at three months after the acute infection. During follow-up visits, we collected blood into BD Vacutainer® Serum Tubes. The tubes were centrifuged for 10 min at 1800 rpm and aliquoted serum samples were stored at −75 °C until analysis.

SARS CoV-2 RT-PCR and SARS-CoV2 antibody tests

SARS-CoV2 infection was diagnosed in the United Laboratories of Tartu University Hospital. Three methods for initial detection of the virus were used (during different waves): (1) Alinity m SARS-CoV-2 AMP Kit (Abbott, 09N78-095); (2) Allplex™ SARS-CoV-2 Assay (Seegene, RV10248X); and (3) Xpert Xpress SARS-CoV-2 (GeneXpert, XPRSARS-COV2-10).

To assess the serum antibody response, we used a Luciferase Immunoprecipitation System (LIPS) assay to analyze IgG antibody responses to RBD protein.

SARS-CoV-2 S RBD (aa 329–538) fragment was cloned into a pNanoLuc vector, and LIPS was performed as reported [14,15]. The transfected HEK293 cell supernatants containing NanoLuc-fusion protein (10^6 luminescence units; LU) were incubated with serum samples (in triplicate) and Protein G Sepharose beads (Creative BioMart) to capture antibodies. After washing, the substrate was added (Nano-Glo™ Luciferase Substrate, Promega), and luminescence was measured in VICTOR X Reader (PerkinElmer Life Sciences). Results are expressed in Arbitrary Units (AU) which are their percentage of the positive control LU signal (a serum sample from a convalescent person) with a threshold of 0.6%. The threshold was set according to the values from 120 pre-pandemic serum samples as mean plus three standard deviations.

Data management

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Tartu. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for data integration and interoperability with external sources (www.project-redcap.org).

Statistical analysis

Statistical analysis was performed using SPSS Statistics. Continuous variables with normally distributed data are reported as means and standard deviations (*SD*), and non-normally distributed data as medians and interquartile ranges (IQR). Categorical variables are reported as frequency and percentages. Fisher's exact test was used to compare categorical variables. For normally distributed data of continuous variables, the independent samples *t*-test for two groups and one-way ANOVA test for more than two groups were used. For non-normally-distributed data of continuous variables, we used the Mann–Whitney *U* test for two study groups and the Kruskal-Wallis test for more than two groups with subsequent Dunn's multiple comparisons, with significance values adjusted by the Bonferroni correction for multiple tests. The Friedman test was used for repeated measures. For all statistical analyses, *p*-values $<.05$ (two-tailed) were considered statistically significant.

Results

Demographics and clinical features

A total of 123 COVID-19 patients were included for analysis, 59% ($n = 73$) of whom were male.

The main clinical characteristics and results of statistical analysis of hospitalized and non-hospitalized COVID-19 patients, as well as those of the non-COVID-19 patients, are displayed in Table 1.

The mean age of COVID-19 patients was 57.5 years, but COVID-19 outpatients were significantly younger than COVID-19 inpatients (mean age difference of 9.9 years). Hospitalized COVID-19 patients were more frequently previously diagnosed with hypertension than non-hospitalized COVID-19 patients. They had a significantly higher temperature and more frequently had dyspnoea, tachypnoea and cough compared with the non-hospitalized COVID-19 patients, who more frequently described reduced exercise capacity, rhinorrhoea, anosmia, and ageusia or dysgeusia.

Table 1. Demographic and clinical characteristics of inpatients and outpatients with COVID-19 and non-COVID-19 patients with respiratory infection.

	All COVID-19 patients ($n = 123$)	COVID-19 inpatients ($n = 97$)	COVID-19 outpatients ($n = 26$)	Non-COVID-19 outpatients ($n = 45$)	p-Value* between COVID-19 inpatients and outpatients	p-Value* between COVID-19 and non-COVID-19 outpatients
Age, mean (*SD*) (years)	57.5 (13.5)	59.6 (13.1)	49.5 (12.3)	53.8 (13.9)	.0021	ns
Male gender, *n* (%)	73 (59.3)	62 (63.9)	11 (42.3)	22 (48.9)	ns	ns
BMI, mean (*SD*)	30.4 (5.5)	30.9 (5.7)	28.4 (4.5)	29.6 (6.4)	ns	ns
Chronic diseases and comorbidity, *n* (%)						
Any comorbidity	85 (69.1)	71 (73.2)	12 (46.2)	32 (71.1)	ns	ns
Hypertension	62 (50.4)	60 (61.9)	2 (7.7)	16 (35.6)	.0000012	.033
COPD	6 (4.9)	6 (6.2)	0	1 (2.2)	ns	ns
Asthma	12 (9.8)	12 (12.4)	0	2 (4.4)	ns	ns
Diabetes	14 (11.4)	13 (13.4)	1 (3.8)	3 (6.7)	ns	ns
Coronary artery disease	11 (8.9)	10 (10.3)	1 (3.8)	3 (6.7)	ns	ns
Cerebrovascular disease	3 (2.4)	3 (3.1)	0	1 (2.2)	ns	ns
Tumour	6 (4.9)	6 (6.2)	0	1 (2.2)	ns	ns
Signs and symptoms, *n* (%)						
Temperature, mean (*SD*)	38.7 (0.8)	38.8 (0.8)	38.1 (0.8)	37.8 (0.9)	.00021	ns
1–4 symptoms	34 (27.6)	28 (28.9)	6 (23.1)	17 (37.8)	ns	ns
5–9 symptoms	64 (52.0)	52 (53.6)	12 (46.2)	20 (44.4)	ns	ns
>10 symptoms	25 (20.3)	17 (17.5)	8 (30.8)	8 (17.8)	ns	ns
Fatigue	107 (87)	84 (86.6)	23 (88.5)	30 (66.7)	ns	ns
Chills	45 (36.6)	34 (35.1)	11 (42.3)	17 (37.8)	ns	ns
Dyspnoea	44 (35.8)	42 (43.3)	2 (7.7)	12 (26.7)	.0015	ns
Tachypnoea	37 (30.1)	34 (35.1)	3 (11.5)	9 (20)	ns	ns
Reduced exercise capacity	70 (56.9)	49 (50.5)	21 (80.8)	28 (62.2)	.021	ns
Cough	89 (72.4)	76 (78.4)	13 (50)	19 (42.2)	.018	ns
Rhinorrhoea	20 (16.3)	11 (11.3)	9 (34.6)	17 (37.8)	.039	ns
Sore throat	25 (20.3)	19 (19.6)	6 (23.1)	17 (37.8)	ns	ns
Myalgia	45 (36.6)	35 (36.1)	10 (38.5)	22 (49.9)	ns	ns
Arthralgia	34 (27.6)	28 (28.9)	6 (23.1)	20 (44.4)	ns	ns
Chest pain	31 (25.2)	22 (22.7)	9 (34.6)	11 (24.4)	ns	ns
Headache	42 (34.1)	26 (26.8)	16 (61.5)	16 (35.6)	.006	ns
Abdominal pain	8 (6.5)	6 (6.2)	2 (7.7)	4 (8.9)	ns	ns
Nausea	32 (26)	27 (27.8)	5 (19.2)	7 (15.6)	ns	ns
Vomiting	18 (14.6)	16 (16.5)	2 (7.7)	2 (4.4)	ns	ns
Anorexia	68 (55.3)	55 (56.7)	13 (50)	11 (24.4)	ns	ns
Anosmia	33 (26.8)	21 (21.6)	12 (46.2)	6 (13.3)	ns	.012
Ageusia/dysgeusia	39 (31.7)	25 (25.8)	14 (53.8)	4 (8.9)	.027	.00015

BMI: body mass index; COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019; IQR: interquartile range; ns: non-significant; SD: standard deviation.

Fisher's exact test for categorical variables, independent samples *t*-test for normally distributed and Mann–Whitney *U* Test for non-normally distributed continuous variables were used.

*All *p*-values are adjusted for multiple comparisons. The significance level is *p*-value <.05.

We found that the age, sex, and BMI of COVID-19 outpatients and non-COVID-19 outpatients did not differ significantly. Fewer non-hospitalized COVID-19 patients had been previously diagnosed with hypertension, compared to non-COVID-19 outpatients (7.7 *vs.* 35.6%, respectively).

COVID-19 patients with different disease severity

The demographics and clinical features of the groups with different disease severity as evaluated by the WHO guidelines are displayed in Table 2. We found that the critical and severe disease groups did not differ statistically in demographics, comorbidity, and symptoms. Previously diagnosed hypertension was more frequent among the severe and critical disease groups.

Laboratory biomarkers at hospitalization of COVID-19 patients with different disease severity is presented in Table 3. The neutrophil-to-lymphocyte ratio (NLR) at admission was significantly higher in the critical and severe disease groups compared with the moderate disease group. Moreover, patients in the critical disease group had significantly higher C-reactive protein (CRP), procalcitonin, alanine aminotransferase (ALT), creatinine, and ferritin levels at baseline, compared with the moderate disease group. Also, patients with severe disease had significantly higher procalcitonin, ferritin, and alanine aminotransferase compared with the moderate disease group.

Table 2. Demographic and clinical characteristics of COVID-19 patients with different disease severity as graded by the WHO guidelines.

	All COVID-19 patients ($n = 123$)	Mild COVID-19 patients ($n = 15$)	Moderate COVID-19 patients ($n = 42$)	Severe COVID-19 patients ($n = 39$)	Critical COVID-19 ($n = 27$)	p-Value*
Age, mean (*SD*) (years)	57.5 (13.5)	51.3 (12.7)	53.3 (12.8)	61.2 (15.2)	62.2 (9.3)	.004[a]
Male gender, *n* (%)	73 (59.3)	7 (46.7)	20 (47.6)	25 (64.1)	21 (77.8)	ns
BMI, mean (*SD*)	30.4 (5.5)	27.3 (3.6)	30.6 (6.8)	30.9 (4.8)	31.3 (4.7)	.028[b]
Chronic diseases and comorbidity, *n* (%)						
Any comorbidity	85 (69.1)	7 (46.7)	26 (61.9)	30 (76.9)	20 (74.1)	ns
Hypertension	62 (50.4)	2 (13.3)	16 (38.1)	26 (66.7)	18 (66.7)	.0005[c]
COPD	6 (4.9)	0	0	3 (7.7)	3 (11.1)	ns
Asthma	12 (9.8)	0	4 (9.5)	4 (10.3)	4 (14.8)	ns
Diabetes	14 (11.4)	0	4 (9.5)	4 (10.3)	6 (22.2)	ns
Coronary artery disease	11 (8.9)	2 (13.3)	3 (7.1)	3 (7.7)	3 (11.1)	ns
Cerebrovascular disease	3 (2.4)	0	1 (2.4)	2 (5.1)	0	ns
Tumour	6 (4.9)	0	1 (2.4)	2 (5.1)	3 (11.1)	ns
Signs and symptoms, *n* (%)						
Temperature, mean (*SD*)	38.7 (0.8)	37.7 (0.8)	38.6 (0.7)	38.9 (0.8)	38.9 (0.7)	.00004[d]
1–4 symptoms	34 (27.6)	5 (33.3)	8 (19.0)	11 (28.2)	10 (37.0)	ns
5–9 symptoms	64 (52.0)	9 (60)	22 (52.4)	21 (53.8)	12 (44.4)	ns
>10 symptoms	25 (20.3)	1 (6.7)	12 (28.6)	7 (17.9)	5 (18.5)	ns
Fatigue	107 (87)	11 (73.3)	40 (95.2)	34 (87.2)	22 (81.5)	ns
Chills	45 (36.6)	5 (33.3)	17 (40.5)	12 (30.8)	11 (40.7)	ns
Dyspnoea	44 (35.8)	2 (13.3)	10 (23.8)	19 (48.7)	13 (48.1)	.014
Tachypnoea	37 (30.1)	2 (13.3)	13 (31.0)	12 (30.8)	10 (37.0)	ns
Reduced exercise capacity	70 (56.9)	8 (53.3)	24 (57.1)	25 (64.1)	13 (48.1)	ns
Cough	89 (72.4)	5 (33.3)	33 (78.6)	32 (82.1)	19 (70.4)	.005[e]
Rhinorrhoea	20 (16.3)	4 (26.7)	9 (21.4)	6 (15.4)	1 (3.7)	ns
Sore throat	25 (20.3)	5 (33.3)	8 (19.0)	10 (25.6)	2 (7.4)	ns
Myalgia	45 (36.6)	4 (26.7)	21 (50.0)	10 (25.6)	10 (37.0)	ns
Arthralgia	34 (27.6)	1 (6.7)	18 (42.9)	7 (17.9)	8 (29.6)	.018
Chest pain	31 (25.2)	4 (26.7)	14 (33.3)	8 (20.5)	5 (18.5)	ns
Headache	42 (34.1)	6 (40)	20 (47.6)	8 (20.5)	8 (29.6)	ns
Abdominal pain	8 (6.5)	0	3 (7.1)	2 (5.1)	3 (11.1)	ns
Nausea	32 (26)	1 (6.7)	12 (28.6)	13 (33.3)	6 (22.2)	ns
Vomiting	18 (14.6)	0	8 (19.0)	6 (15.4)	4 (14.8)	ns
Anorexia	68 (55.3)	5 (33.3)	25 (59.5)	22 (56.4)	16 (59.3)	ns
Anosmia	33 (26.8)	5 (33.3)	16 (38.1)	9 (23.1)	3 (11.1)	ns
Ageusia/dysgeusia	39 (31.7)	6 (40.0)	14 (33.3)	1 (35.9)	5 (18.5)	ns

BMI: body mass index; COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019; ns: non-significant; SD: standard deviation.
*p-Values of the investigated severity groups that are shown in the Table were calculated for normally distributed continuous data with a one-way ANOVA test, non-normally distributed continuous data with the Kruskal–Wallis test and with Fisher's exact test for categorical variables. Statistically significant pairwise comparison (adjusted p-value corrected for six hypotheses) of the groups are shown in footnotes a-e. The significance level is p-value <.05.
[a]COVID-19 patients with a critical disease course were older than patients with moderate ($p = .039$) disease courses.
[b]BMI of patients with a severe disease course was greater compared with patients with a mild disease course (adjusted $p = .031$).
[c]There was less hypertension among patients with a mild disease course compared with severe ($p = .006$) and critical ($p = .006$) disease courses.
[d]Patients with a mild disease course had lower maximum temperature than in patients with severe ($p = .00004$) and critical ($p = .00024$) disease courses.
[e]Patients with a mild disease course had less cough than patients with moderate ($p = .018$) and severe ($p = .006$) disease courses.

In-hospital treatment of COVID-19 patients is presented in Table 4. Of all the enrolled COVID-19 patients, 79% (97 of 123) were hospitalized, including 66% of the moderate and all patients in the severe and critical disease groups. There was no difference between the median number of days from the first symptom to the hospitalization in the moderate, severe, and critical disease groups. However, the patients with more severe diseases had a longer hospital stay.

Anti-SARS CoV-2 antibody dynamics of COVID-19 patients

The mean intervals (days ± SD) between symptom onset and the follow-up serum collections were 97.8 (±6.0, $n = 59$), 190.7 (±5.5, $n = 57$), and 371 (±5.3, $n = 51$), respectively.

As displayed in Figure 1, patients with severe COVID-19 had significantly higher anti-SARS RBD-IgG levels than patients with mild and moderate disease severity at the three-month and six-month follow-up visits. At one-year follow-up, patients with a severe disease course had higher anti-SARS RBD-IgG levels compared with patients with a mild disease course. Notably, relatively large variability in antibody levels was observed between COVID-19 severity groups.

The dynamics of anti-SARS RBD-IgG levels across the different follow-up visits are presented in Figure 2. Among all COVID-19 patients, antibody levels at month six were significantly higher than at month three. We

Table 3. Laboratory biomarkers at hospitalization of COVID-19 patients with different disease severity as graded by the WHO guidelines.

Laboratory biomarkers, median (IQR)*	All COVID-19 patients ($n = 123$)	Mild COVID-19 patients	Moderate COVID-19 patients ($n = 28$)	Severe COVID-19 patients ($n = 39$)	Critical COVID-19 ($n = 27$)	p-Value**
Neutrophils (E9/L)	3.8 (2.8–5.9)	–	3.2 (2.4–4.6)	3.8 (3.0–6.4)	4.2 (3.1–6.8)	.018[a]
Lymphocytes (E9/L)	1.01 (0.7–1.4)	–	1.2 (0.9–1.6)	0.9 (0.65–1.5)	0.84 (0.6–1.2)	.022[b]
NLR	3.9 (2.5–6.2)	–	2.8 (1.6–3.9)	4.3 (2.7–6.6)	5.8 (3.3–10.8)	.0004[c]
CRP (mg/L)	56 (26–104)	–	48 (17–70)	63 (25–153)	72 (37–181)	.029[d]
Procalcitonin (μg/L)	0.11 (0.07–0.23)	–	0.08 (0.07–0.10)	0.14 (0.07–0.22)	0.28 (0.12–0.44)	.00002[e]
LDH (U/L)	494 (398–611)	–	447 (382–554)	528 (415–622)	536 (419–640)	ns
ALT (U/L)	31 (20–48)	–	28 (17–37)	28 (21–42)	47 (31–65)	.008[f]
Creatinine (μmol/L)	80 (63–97)	–	69 (58–86)	81 (63–99)	92 (68–114)	.005[g]
NT-proBNP (ng/L)	114 (43–332)	–	77 (35–196)	143 (48–413)	151 (75–934)	ns
Ferritin (μg/L)	586 (380–1128)	–	441 (334–570)	781 (380–1309)	852 (633–1485)	.001[h]
D-dimers (mg/L)	0.87 (0.59–1.36)	–	0.79 (0.48–1.33)	1.07 (0.63–1.82)	0.85 (0.65–1.17)	ns

ALT: alanine aminotransferase; COVID-19: coronavirus disease 2019; CRP: C-reactive protein; IQR: interquartile range; LDH: lactate dehydrogenase; NLR: neutrophil-to-lymphocyte ratio; ns: non-significant.

*Baseline laboratory biomarkers at admission to hospital. Data are not displayed for the mild disease group as only three such patients were hospitalized.

**p-Values of the investigated severity groups that are shown in the Table were calculated for normally distributed continuous data with a one-way ANOVA test, non-normally distributed continuous data with the Kruskal–Wallis test. Statistically significant pairwise comparison (adjusted p-value for three hypotheses) of the groups are shown in footnotes a–h. The significance level is p-value $<.05$.

[a]Patients with a critical disease course had higher neutrophil count than patients with a moderate disease course ($p = .016$).

[b]Patients with a critical disease course had lower lymphocyte count than patients with a moderate disease course ($p = .02$).

[c]Patients with a moderate disease course had lower neutrophil-to-lymphocyte ratio than patients with severe ($p = .012$) and critical ($p = .00037$) disease courses.

[d]Patients with a critical disease course had higher CRP values than patients with a moderate disease course ($p = .028$).

[e]Patients with a moderate disease course had lower procalcitonin values than patients with severe ($p = .016$) and critical ($p = .000012$) disease courses.

[f]Patients with a critical disease course had higher ALT activity than patients with severe ($p = .037$) and moderate ($p = .010$) disease severity.

[g]Patients with a critical disease course had higher creatinine levels than patients with a moderate disease course ($p = .004$).

[h]Patients with a moderate disease course had lower ferritin levels than patients with severe ($p = .035$) and critical ($p = .001$) disease courses.

Table 4. In hospital treatment[a] of COVID-19 patients.

	All COVID-19 inpatients ($n = 97$)	Mild COVID-19 patients ($n = 3$)	Moderate COVID-19 patients ($n = 28$)	Severe COVID-19 patients ($n = 39$)	Critical COVID-19 ($n = 27$)
Days from the first symptom to hospitalization, median (IQR)	8 (6–10)	3	8 (5–10)	8 (6–10)	8 (7–10)
Length of hospital stay, median (IQR)	9 (5–13)	2	5 (4–7)	9 (6–11)[b]	17 (12–29)[c]
Parenteral antibiotics, *n* (%)	36 (37.1)	0	0	17 (43.6)	19 (70.4)
Remdesivir, *n* (%)	18 (18.8)	0	4 (9.5)	6 (15.4)	8 (29.6)
Hydroxychloroquine, *n* (%)	25 (25.8)	0	2 (4.8)	16 (41)	7 (25.9)
Glucocorticoids, *n* (%)	53 (54.6)	0	15 (35.7)	17 (43.6)	21 (77.8)
Supplemental oxygen, *n* (%)	89 (91.8)	1 (33.3)	22 (78.6)	39 (100)	27 (100)
HFNO, *n* (%)	19 (19.6)	0	0	1 (2.6)	18 (66.7)
NIV, *n* (%)	16 (16.5)	0	0	1 (2.6)	15 (55.6)
Invasive ventilation, *n* (%)	11 (11.3)	0	0	0	11 (40.7)
ICU admission, *n* (%)	18 (18.6)	0	1 (2.4)	2 (5.1)	15 (55.6)
Haemodialysis, *n* (%)	3 (3.1)	0	0	0	3 (11.1)
ECMO, *n* (%)	1 (1.0)	0	0	0	1 (3.7)

BMI: body mass index; COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019; HFNO: high-flow nasal oxygen; ICU: intensive care unit; IQR: interquartile range; ECMO: extracorporeal membrane oxygenation; NIV: non-invasive ventilation; SD: standard deviation.

[a]Treatment recommendations have changed during the study period (COVID-19 wave 1 in Spring 2020 *vs.* wave 2 in autumn and winter 2020/2021).

[b]Patients with a severe disease course had significantly longer hospital stay than patients with a moderate disease course (adjusted $p = .008$).

[c]Patients with a critical disease course had significantly longer hospital stay than patients with a moderate disease course (adjusted $p = .0002$).

found no significant difference between the antibody levels at months 6 and 12 when we analyzed all COVID-19 patients together and excluded vaccinated cases.

Evaluation of the dynamics of the antibody levels in different disease severity groups is displayed in Figure 2.

Some 41% of the COVID-19 patients (21 of the 51) in our follow-up cohort were vaccinated with the Pfizer-BioNTech or AstraZeneca COVID-19 vaccines between six-months and one year. The mean interval between vaccination and the 12-month visit was 36 days ($SD \pm 28$). The steep rise in antibody levels after vaccination is illustrated in Figure 3. The unvaccinated patients with mild, moderate, and severe disease courses had significantly lower anti-SARS RBD-IgG levels at the 12-month follow-up visits than vaccinated patients from the same disease severity groups, as shown in Figure 4. Three patients had lower antibody levels after vaccination compared with others—two were vaccinated only

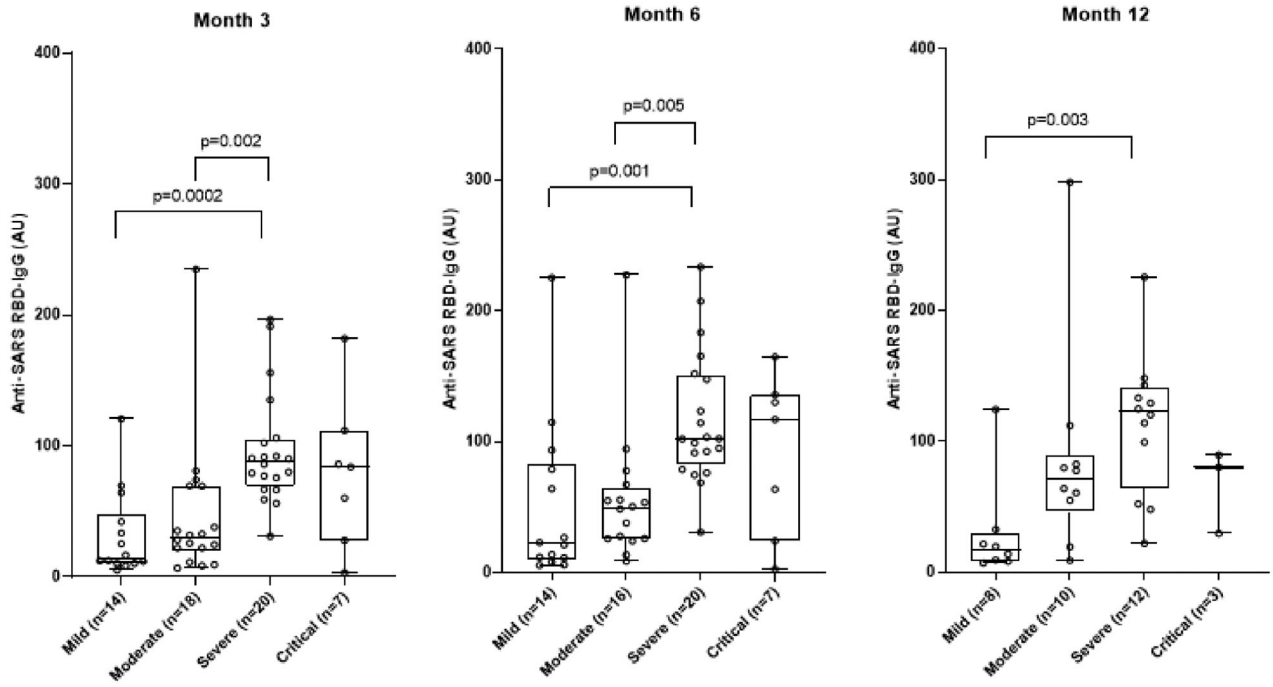

Figure 1. Anti-SARS RBD-IgG in different COVID-19 severity groups during follow-up visits (Dunn's multiple comparison test).

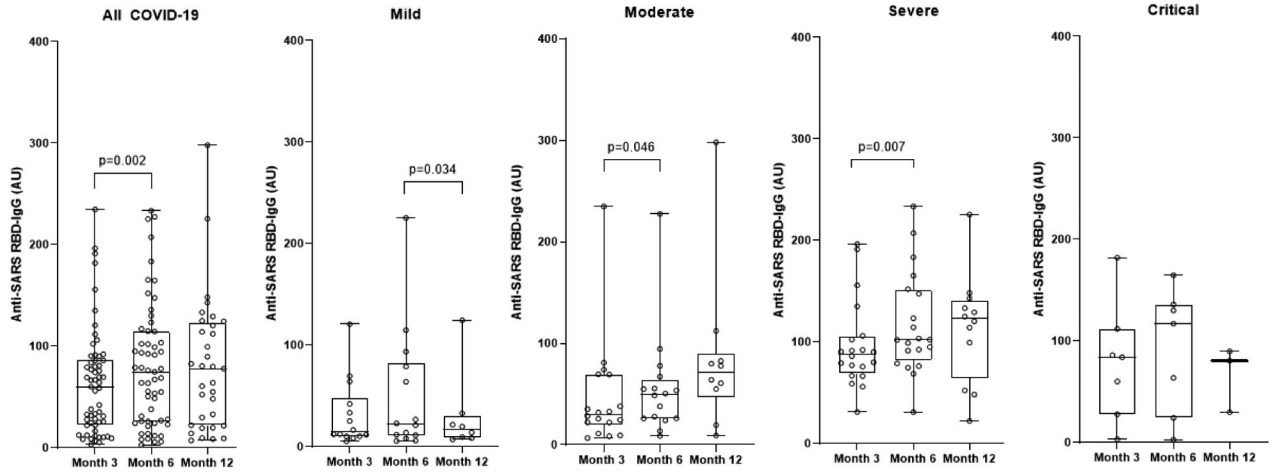

Figure 2. Anti-SARS RBD-IgG levels in different disease severity groups during follow-up visits, vaccinated cases at month 12 excluded (Friedman test).

a few days before the follow-up visits and one patient had been diagnosed with chronic lymphocytic leukaemia. Apart from these exceptions, the differences in anti-SARS RBD-IgG levels seen between COVID-19 severity groups before vaccination were no longer observed after vaccination.

Discussion

To understand which factors determine disease course, it is important to classify patients regarding disease severity. In the current study, we used the WHO severity classification [4], which is well-defined and easily used.

The antibody response is also related to the severity of the disease. Despite all the COVID-19 patients in our study having anti-SARS RBD-IgG levels above the diagnostic threshold value throughout the one-year period, antibody levels varied significantly between groups. To analyze IgG antibody responses to RBD protein, we used the LIPS assay, which was among the first methods published at the beginning of the pandemics that permitted the detection of anti-Spike and anti-RBD antibodies. Recently, a good correlation between LIPS and ELISA for

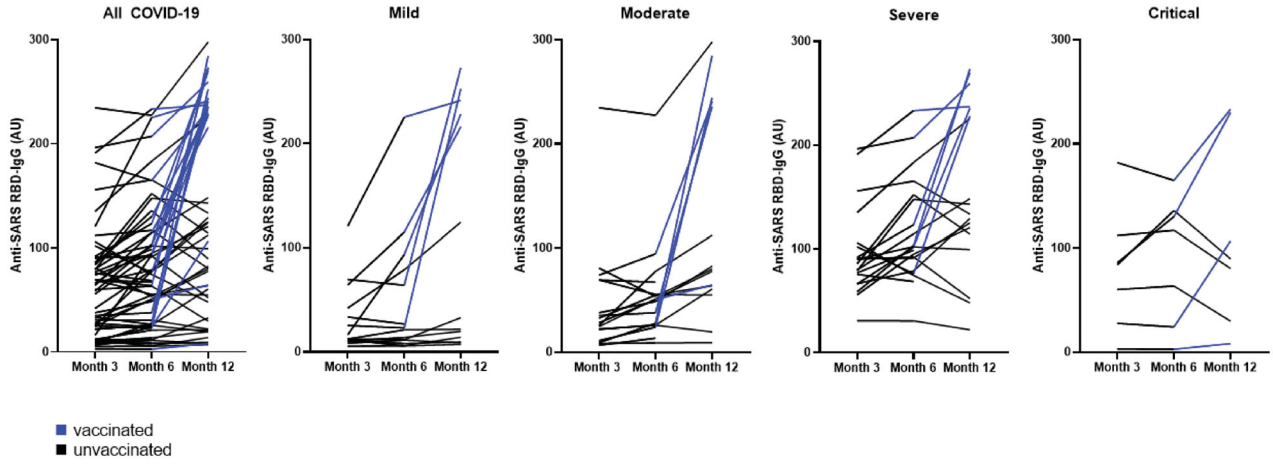

Figure 3. Dynamics of anti-SARS RBD-IgG levels in different disease severity groups during follow-up visits, vaccinated cases at month 12 included (Vaccinated cases between 6 and 12 months in blue).

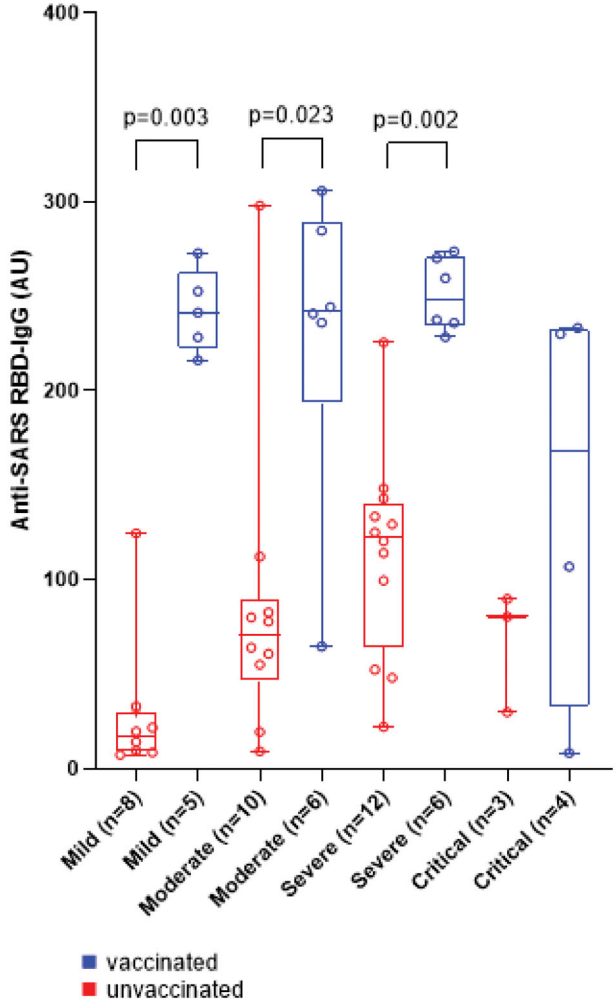

Figure 4. Anti-SARS RBD-IgG in SARS-CoV-2 vaccinated (in blue) and unvaccinated (in red) patients with different COVID-19 severity groups at month 12 (Mann–Whitney *U* test).

anti-RBD detection was shown [16]. LIPS has some advantages over ELISA with a higher dynamic range, and conformation of the antigen is better preserved. Previous studies have revealed that patients with a more severe disease course have higher antibody levels than patients in whom the disease course is milder [17,18]. We could not confirm this in our critical disease group, which may be because of relatively small sample size, but patients in the severe disease group had higher antibody levels compared with the mild and moderate disease groups at months three and six. At month 12, the significantly higher level of antibodies remained in the severe disease group, compared with the mild disease group. Several factors may contribute to the magnitude of the humoral immune responses of different disease severities. Male sex and age [19–21] have been found to correlate with higher antibody levels—both of which are risk factors for a severe disease course. We could not confirm this effect in our study, but there was a tendency towards a higher ratio of males in the severe and critical disease groups. Patients in the critical disease group were also older than patients in the moderate disease groups.

Interestingly, when we evaluated antibody dynamics in all cases, we found that antibody levels were higher at month 6 than at the previous month three visit. When we assessed this in relation to disease severity, we found that the elevation of antibody levels was only seen in the moderate and severe disease groups. This cannot readily be explained *via* re-exposure to the virus because most of the six-month follow-up visits occurred in September-October when the disease prevalence in the community was low. However, sustained immune stimulation due to residual antigenic fragments of unpacked viral capsid proteins several months after acute infection could be a possible alternative hypothesis.

We found that antibody levels remained stable over a one-year period in all disease severity groups apart from

the mild disease group. Declining antibody levels in patients with a mild disease course have also been demonstrated in other studies [11]. Sustained levels of antibodies, which we demonstrated in the moderate, severe, and critical disease courses, have also been reported in other studies assessing antibody levels at one-year follow-up [13,22]. Thus, a declining antibody level at one-year follow-up appears a consequence of initially milder courses COVID-19 disease.

Since it is recommended in Estonia to vaccinate convalescent patients with one dose six months after the acute disease, 41% of our patients were vaccinated by the one-year follow-up. Our study demonstrated that in all disease severity groups apart from the critical, vaccinated patients had significantly higher antibody levels than unvaccinated patients. It has previously been demonstrated that all vaccinated convalescent patients have substantially higher antibody levels than unvaccinated convalescent patients [23]. In our study, the critical disease group was too small to show any statistically significant difference, and the group included a patient whose pre-vaccination anti-SARS RBD-IgG levels were just above the threshold value and whose antibody levels had been elevated only slightly after the vaccination. Furthermore, we found no difference in antibody levels between vaccinated patients of different disease severities, which indicates that despite the initial disease course, vaccination of seropositive subjects boosts antibodies to equal levels. This finding is consistent with another study, which also did not find significant differences in antibody responses by disease severity after vaccination [13]. Therefore, the initial variations in antibody levels are not associated with an inability to generate a humoral immune response during the acute phase of the disease. It has been demonstrated that it is reasonable to vaccinate all COVID-19 convalescent patients with one dose [24], but in immunocompromised patients, a more individualized approach may be necessary. Nevertheless, it must be noted that immunity against infection is not only related to antibody levels. SARS-CoV-2 specific $CD4^+$ T cells are known to persist in the majority of convalescent individuals up to 8 months and $CD8^+$ T cells in approximately half of the patients [25]. However, the protection against reinfection seems to be more dependent on antibody responses [26].

An appropriate control group is needed for the correct evaluation of clinical signs of COVID-19. Several studies have compared COVID-19 inpatients with hospitalized influenza patients [27–29]. However, the majority of COVID-19 patients are never hospitalized. Therefore, we formed our control group of outpatients with those with another respiratory tract infection (the common cold). Full data about the aetiology in the non-COVID-19 patients were not available, but viruses that cause upper respiratory diseases in our climate zone are rhinoviruses, respiratory syncytial virus, influenza viruses, parainfluenza viruses, seasonal human coronaviruses, adenoviruses, metapneumoviruses, and others—up to 50% of cases are rhinoviruses [30]. Anosmia and ageusia are well-recognized in COVID-19 and it was not surprising that these symptoms were more frequent in COVID-19 outpatients than non-COVID-19 patients in our study as well. Although the two groups did not differ in age, sex, or BMI, we found that the non-COVID-19 outpatients had more frequently previously been diagnosed with hypertension than COVID-19 outpatients. Hence, this further confirms hypertension as a separate risk factor for severe disease course and hospitalization in COVID-19 patients.

Various laboratory biomarkers have been associated with worse outcomes of COVID-19. In our study, as expected, several of these were expressed differently in the different severity subgroups. Interestingly, although neutrophil and lymphocyte counts did not differ statistically between the moderate and severe disease groups, the NLR was significantly higher in patients with a severe disease course compared with the moderate disease group. Therefore, the NLR may be more sensitive in the early phase, reflecting the imbalance of lymphocytes and neutrophils in a dysregulated immune response.

Only one biomarker significantly differed at admission between the severe and critical disease groups, and interestingly it was ALT, which was slightly higher in the critical disease group. Previously published studies have demonstrated that, although elevation of liver transaminases is generally mild when elevation occurs, it is related to a severe course of the disease [31,32].

There are some limitations regarding our study. First, our study is limited by a relatively small number of subjects enrolled, but our cohort is predominantly of consecutive patients. Secondly, the aetiology of the respiratory tract infection in non-COVID-19 outpatients was not available. Thirdly, we did not obtain serum in the acute phase of the disease, so we were not able to compare antibody dynamics between the acute and convalescent phases. However, the strengths of our study were the standardized approach during hospitalization, as symptoms, medical history, and laboratory biomarkers at admission were collected in a standardized manner from the beginning of the pandemic.

Although we assessed the differences between COVID-19 inpatients and outpatients, we further categorized COVID-19 patients according to the WHO severity classification to comprehensively assess the longevity of antibodies in relation to disease course. We also had a control group of non-COVID-19 outpatients to further evaluate what distinguishes COVID-19 patients from patients with other respiratory tract infections.

In conclusion, anti-SARS RBD-IgG levels remain high in moderate and severe COVID-19 cases, but not in mild cases, to one-year follow-up. To illuminate what determines disease severity and how antibody dynamics are related to disease course, it is important to use standardized classifications of COVID-19 patients, as the clinical course may vary significantly. Although previous studies have revealed that a severe disease course is related to comorbidities, especially to hypertension, we demonstrated this from another perspective, as patients with a mild disease course were healthier compared to individuals with a similar age and sex distribution in the non-COVID-19 control group.

In addition, vaccination in convalescent patients is extremely important as it significantly increases and equalizes antibody levels, of especial value in patients with a mild disease whose spontaneous levels are otherwise low and waning.

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Disclosure statement

The authors have no conflicts of interest to declare.

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