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Antibody and viral RNA kinetics in SARS-CoV2 infected patients admitted to a Romanian University Hospital of Infectious Diseases

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ABSTRACT

Objectives: To assess the antibody and viral kinetics in asymptomatic/mild confirmed SARS-CoV-2 infections compared to more severe patients.
Material and methods: Retrospective analysis of data obtained from adult patients with a confirmed SARS-CoV2 infection having at least one SARS-CoV-2 pair of specific IgM/IgG tests, admitted in The University Hospital of Infectious Diseases Cluj-Napoca, Romania (28 February to 31 August 2020). The database also included: demographic, clinical, chest X-ray and/or CT scan results, RT-PCR SARS-CoV-2, and dexamethasone treatment. A total of 469 patients were evaluated as "asymptomatic/mild" and "moderate/severe/critical" cases.
Results: The median time since confirmation to SARS-CoV-2 PCR negativity was 15 days [95% CI: 13–18] in asymptomatic/mild cases and 17 days [95% CI: 16–21] in moderate/severe ones. The median time to seroconversion for both IgM and IgG was 13 days [95% CI: 13–14] in asymptomatic/mild cases and 11 days [95% CI: 10–13] in moderate/severe ones. For both antibody types, the highest reactivity was significantly associated with more severe presentation (IgM: OR = 10.30, IgG: OR = 7.97).
Conclusion: Asymptomatic/mild COVID-19 cases had a faster RT-PCR negativity rate compared to moderate/severe/critical patients. IgG and IgM dynamics were almost simultaneous, more robust for IgG in more severe cases, and at one month after confirmation, almost all patients had detectable antibody titers.

Introduction

As of 31 August 2020, the coronavirus disease 2019 (COVID-19) pandemic has affected almost 25 million people worldwide (World Health Organization (WHO), 2020a; INSP, 2020). The laboratory tests for diagnosis include detecting SARS-CoV-2 RNA by real-time- polymerase chain reaction (RT-PCR) from nasopharyngeal/oropharyngeal swabs and antibody detection as markers of recent or previous infection and a tool for seroprevalence estimates of SARS-CoV-2 infections. Antibodies specific to SARS-CoV-2 antigens have been detected in asymptomatic and symptomatic patients with better sensitivity for pair testing (Seow et al., 2020, Atyeo et al., 2020; Ripperger et al., 2020; Wang et al., 2020; Watson et al., 2020).

Immunoglobulin (Ig) M is produced as an early immune response; the test's (enzyme-linked immunosorbent assays, electrochemiluminescence immunoassays, lateral flow assays) sensitivity in days 15–21 is 75.4% (64.3–83.8%) and specificity is 98.7% (97.4–99.3%). IgG are developed simultaneously; the test's sensitivity is 88.2% (83.5–91.4) and specificity is 99.1% (98.3–99.6) in days 15–21 (Deeks et al., 2020). In mild cases, antibody development can take ≥4 weeks, and in some cases, antibodies are not detected (Wellinghausen et al., 2020; Wajnberg et al., 2020; Dan et al., 2021).

In Romania, from the beginning of the pandemic until 23 June, hospitalization was mandatory even in asymptomatic and mild infections, and two negative, consecutive RT-PCR SARS-CoV-2 tests were required before discharge. Later, hospitalization was mandatory for at least ten days. Therefore, many of the study subjects had a prolonged hospitalization allowing us to perform repeated molecular and antibody testing (Ministry of Health, 2020).

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Our study aimed to compare the viral kinetics and antibody response in asymptomatic/mild and moderate/severe/critical SARS-CoV-2 patients.

Methods

We retrospectively evaluated the antibody profiles and RT-PCR SARS-CoV-2 kinetics in asymptomatic/mild confirmed SARS-CoV-2 infections compared to more severe patients.

We analyzed data from all adult patients with confirmed SARS-CoV-2 infection and at least one pair of antibody tests (IgM and IgG) treated in the University Hospital of Infectious Diseases Cluj-Napoca, Romania (27 February to 31 August 2020). Data retrieved from our hospital's electronic database also included: demographics, clinical data, chest X-ray, and CT scan results. All patients were confirmed according to the WHO Clinical Progression Scale for SARS-CoV-2 infection (WHO, 2020b, 2020c; Vafea et al., 2020). Symptomatic patients received hydroxychloroquine, lopinavir/ritonavir, low molecular weight heparin, and for severe/critical patients, dexamethasone, remdesivir, tocilizumab, and convalescent plasma were administered (Iwasaki and Yang, 2020; The RECOVERY Collaborative Group, 2021; Ministry of Health, 2020).

Out of 1349 admitted cases, 469 adults fulfilled the inclusion criteria. Mild cases were defined by the presence of some symptoms and normal chest imaging, while moderate/severe/critical cases by the evidence of pneumonia, pneumonia and respiratory failure, pneumonia and mechanical ventilation or septic shock or multiple organ dysfunction syndrome, respectively (World Health Organization (WHO), 2020b; WHO, 2020c).

Laboratory methods

SARS CoV-2 RT-PCR tests used the nucleocapsid (N), envelope (E), RNA dependent RNA polymerase (RdRp), and open reading frame (ORF) genes of the SARS CoV-2 genome. Before 23 April 2020, RNA was extracted with the QIAsymphony RNA Kit (Qiagen), then amplified and detected on the Rotor-Gene Q RT-PCR system (Qiagen) using first Quantabio qScript XLT1-Step RT-qPCR ToughMix and then ViroReal® Kit SARS-CoV-2 (Ingenetix) or EliGene® COVID19 (Elisabeth Pharmacon) (Corman et al., 2020; WHO, 2020b). After 23 April, automatic RT-PCR SARS CoV-2 detection was done on NeuMoDx 96 and 288 molecular system devices, ensuring a higher testing capacity (NeuMoDx™ Molecular, Qiagen) (Mostafa et al., 2020). Molecular testing was usually performed on day ten and repeated at different intervals at the physician's discretion.

IgM and IgG anti-SARS CoV-2 antibodies index were measured with the eCL8000 electrochemiluminescence system (Lifotronic, Shenzhen, China) with kits based on the enzymatic sandwich principle, the diluted sample being reacted with biotinylated nucleocapsid (N) proteins, the receptor-binding domain (RBD) of SARS-CoV-2 spike protein (S) and mouse IgM antibodies marked with a ruthenium complex and Streptavidin-coated particles (Sethuraman et al., 2020). For both antibody types, the thresholds for detectable and positive measurements were 0.8 (IgM) and 1.2 (IgG), respectively. Additionally, we classified patients into high or low reactivity based on IgM and IgG values at 75% quartile (IQR 75%) with thresholds of 2.607 for IgM and 2.906 for IgG.

Statistical methods

Besides the descriptive analyses of clinical and laboratory tests, log2-transformed data and geometric mean ± standard deviation were used for antibody index assessment. Hypothesis testing was performed using odds ratio with 95% confidence intervals and p-value from Fisher tests or Mann-Whitney test, as appropriate, followed by logistic regression for higher IgM/IgG reactivity by age, severity, and lung area abnormalities. Cumulative distribution plot, hazard ratio, and a log-rank test were used for the RT-PCR test results by time and severity. A probit regression model was used to estimate the average proportion of patients with detectable antibodies or negative RT-PCR results at individual time-points after confirmation. Statistical analysis was performed by R 4.0 (R Core Team, 2020). Statistical significance was considered for p-values of <0.05.

Results

The dataset included 469 patients, 208 with asymptomatic/mild infections and 261 with moderate/severe/critical disease. Patient characteristics are presented in Table 1. Higher male proportion, older age, and longer hospitalization were observed in more severe cases ($p < 0.01$).

A total of 1199 RT-PCR and 571 paired IgM & IgG measurements were included (on average 2.21 tests/person). Half of the cases in asymptomatic/mild vs. moderate to critical ones had the first undetectable RT-PCR result at 15 days [95% CI:13–18] and 17 days [95% CI:16–21], respectively ($p < 0.01$) (Figure 1A). At 28 days after confirmation, the cumulative proportion of cases with at least one negative RT-PCR test was 93.1% in asymptomatic/mild cases and 75.8% in more severe ones ($p < 0.01$) (Supplementary Table 1).

All patients were tested for IgM/IgG at least once after confirmation, and half of them had the first detectable result for either IgM or IgG at 13 days [95% CI:13–14] in asymptomatic/mild cases and at 11 days [95% CI: 10–13] in more severe ones ($p = 0.001$) (Figure 1B, C). At 28 days after confirmation, the cumulative proportion of cases who had at least one detectable IgM test was 85.0% in asymptomatic/mild cases and 94.0% in moderate to critical cases ($p < 0.01$), and for at least one detectable IgG test was 96.7% in asymptomatic/mild cases and 94.2% in moderate to critical cases ($p < 0.01$) (Supplementary Table 1).

RT-PCR positivity rate decreased faster among patients with a milder presentation to 60% at 14 days and 25% at one month compared to more severe cases which were 70% at 14 days and 50% at one month (Figure 2A).

Table 1
Patients' characteristics by clinical presentation.

		Moderate to critical 208 (%)	Asymptomatic/mild 261 (%)	Total 469 (%)	
Age	Median	54.5	32	43	**MW: $p < 0.001$**
Sex	M	116 (55.8)	110 (42.1)	226 (48.2)	**OR = 1.73 [1.20, 2.50]**
	F	92 (44.2)	151 (57.9)	243 (51.8)	**p = 0.004**
Hospitalization (days)	Median (range)	14 (1–101)	10 (1–44)	12 (1:101)	**MW: $p < 0.001$**
ICU admission		27 (13.1)	1 (0.4)*	28 (6.0)	NA
CT: >30% lung abnormalities		68 (36.2)	0	68 (15.2)	NA

MW = Mann–Whitney Test; OR = odds-ratio [95% CI], p value Fisher test, * patient admitted in ICU for a coincident severe condition.

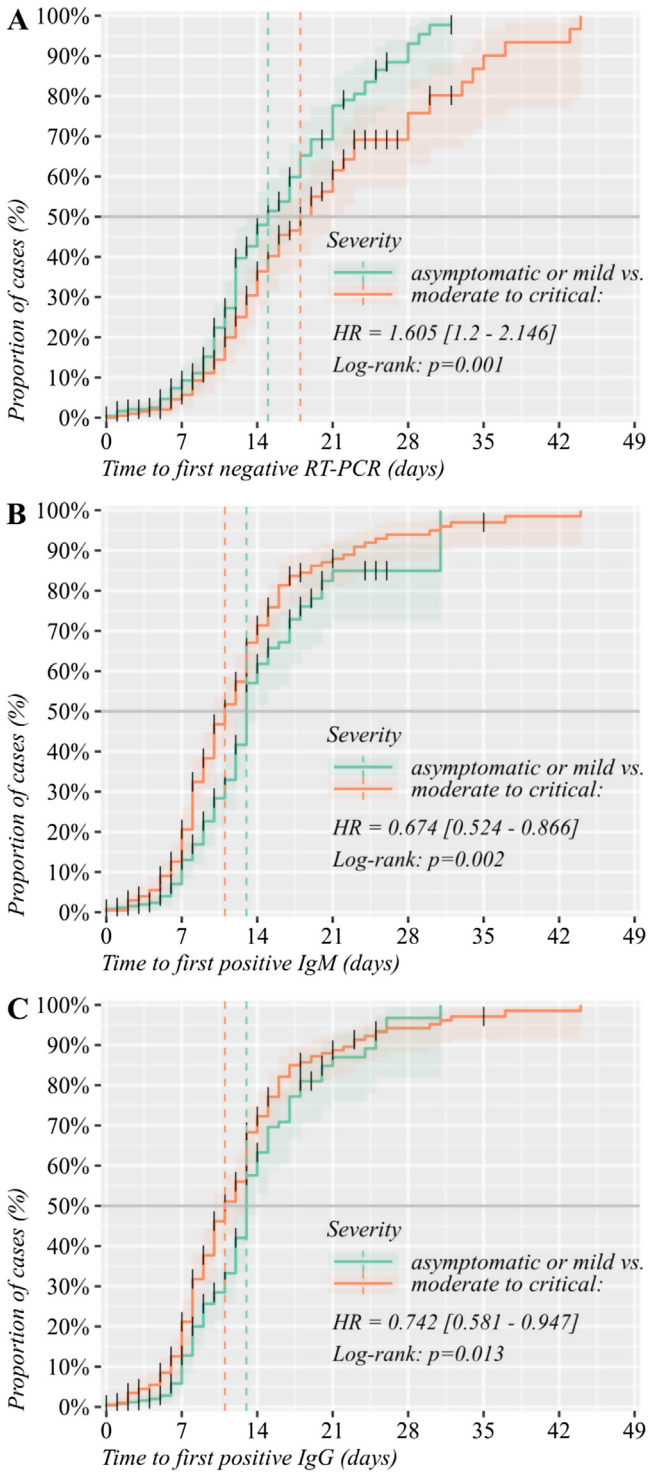

Figure 1. Cumulative distribution plot, RT-PCR negativity rate (A), IgM (B), IgG seroconversion (C) by time since confirmation and severity. Dates were calculated to the first event (negative PCR, detectable antibody). Inverse hazard-ratio (HR) comparing more severe presentations to asymptomatic/mild, 95% CI and log-rank test.

Both antibody types had higher variability in patients with more severe clinical pictures (Fig. 2B, C).

The geometric mean (± geometric standard deviation), median and interquartile range [M (IQR)] for paired IgM and IgG tests and the RT-PCR tests are presented in Table 2. Patients with higher IgM and IgG reactivity were men (albeit not significant), older, with a more severe presentation, >30% lung area abnormalities, higher fatality rate, longer hospitalization, and ICU admission ($p < 0.01$). In the multivariate logistic regression, higher IgM and IgG reactivity was significantly associated with severity and >30% lung area abnormalities. Also, higher IgG reactivity was more often present in patients who received dexamethasone treatment, while IgM reactivity showed a similar pattern but without statistical significance (Table 3).

Both antibody types showed an upwards trend in positivity rate across time; IgG became positive after one month in almost all patients, regardless of severity (Figure 2B and C).

Discussion

In Romania, 87540 cases were confirmed, and 3621 deaths (fatality rate 4.13%) from the end of February until 31 August 2020. The University Hospital of Infectious Diseases Cluj-Napoca is the primary referral center for COVID-19 patients in Cluj County, where 1349 cases were hospitalized, and 28 cases died (fatality rate 2.07%) up to 31 August. Due to the legal context during the first four months of the epidemic, asymptomatic and patients with a mild condition (56%) were hospitalized along with those in a more severe condition. At the beginning of the COVID-19 epidemic in Romania, a lower proportion of very old subjects were affected compared to other European countries (Italy, Spain, UK, Belgium) during the same period and the fatality rate was lower, probably due to early lockdown (INSP, 2020; Kontis et al., 2020).

We evaluated IgM, IgG, and RT-PCR SARS-CoV-2 dynamics during a relatively prolonged hospitalization in all cases. We found that 50% of the patients became RT-PCR SARS-CoV-2 negative after two weeks since confirmation, faster in asymptomatic/mild cases (15 days vs. 17 days, $p = 0.001$) and at one month almost 75% asymptomatic/mild cases and 50% of the more severe ones became RT-PCR-negative. Viral clearance occurred later, and viral shedding was prolonged in patients with COVID-19 pneumonia and severe presentation. Lee et al. found a similar duration of viral shedding in asymptomatic and symptomatic patients (17 and 19.5 days, respectively, $p = 0.07$) (Lee et al., 2020). Long et al. found the same proportion of positivity at 20 days, but contrary to our study, prolonged duration until RT-PCR negative testing was observed in asymptomatic SARS-CoV2 infections (Long et al., 2020).

Seow et al. found that antibodies to SARS-CoV-2 can be detected in most infected individuals 10–15 days after the onset of COVID-19 symptoms (Seow et al., 2020). Van Elslande et al. found a similar timing of seroconversion for IgG, detected between five and 14 days after symptom onset and not significantly shorter for IgM and IgA (Van Elslande et al., 2020). Similar to other studies, we found that half of the patients developed antibodies in the first 14 days, and within one month, almost all patients had detectable antibodies, with similar dynamics for IgM and IgG, significantly faster in more severe cases (Long et al., 2020; Laing et al., 2020). In asymptomatic patients, the distribution of IgM and IgG was more homogenous compared to the scattered plot area for IgM and IgG in more severe cases, suggesting that there is a subset of highly immune reactive patients among those who developed severe disease. A robust immune response observed in more severe cases is probably associated with a higher viral load, or conversely, antibodies may have a role in disease severity (Seow et al., 2020; Wang et al., 2020; Iwasaki and Yang, 2020). Liu et al. found results close to ours regarding the dynamics of RT-PCR detection and the IgM/IgG positivity rate in moderate to critical cases, concluding that antibody tests might be complementary to false-negative nucleic acid tests for SARS-CoV-2 (Liu et al., 2020). In a detailed COVID-immunophenotyping study, Laing et al. evaluated a heterogeneous cohort of patients finding a clear trend towards higher index values of RBD-specific and N-specific IgG antibodies in severe patients (Laing et al., 2020).

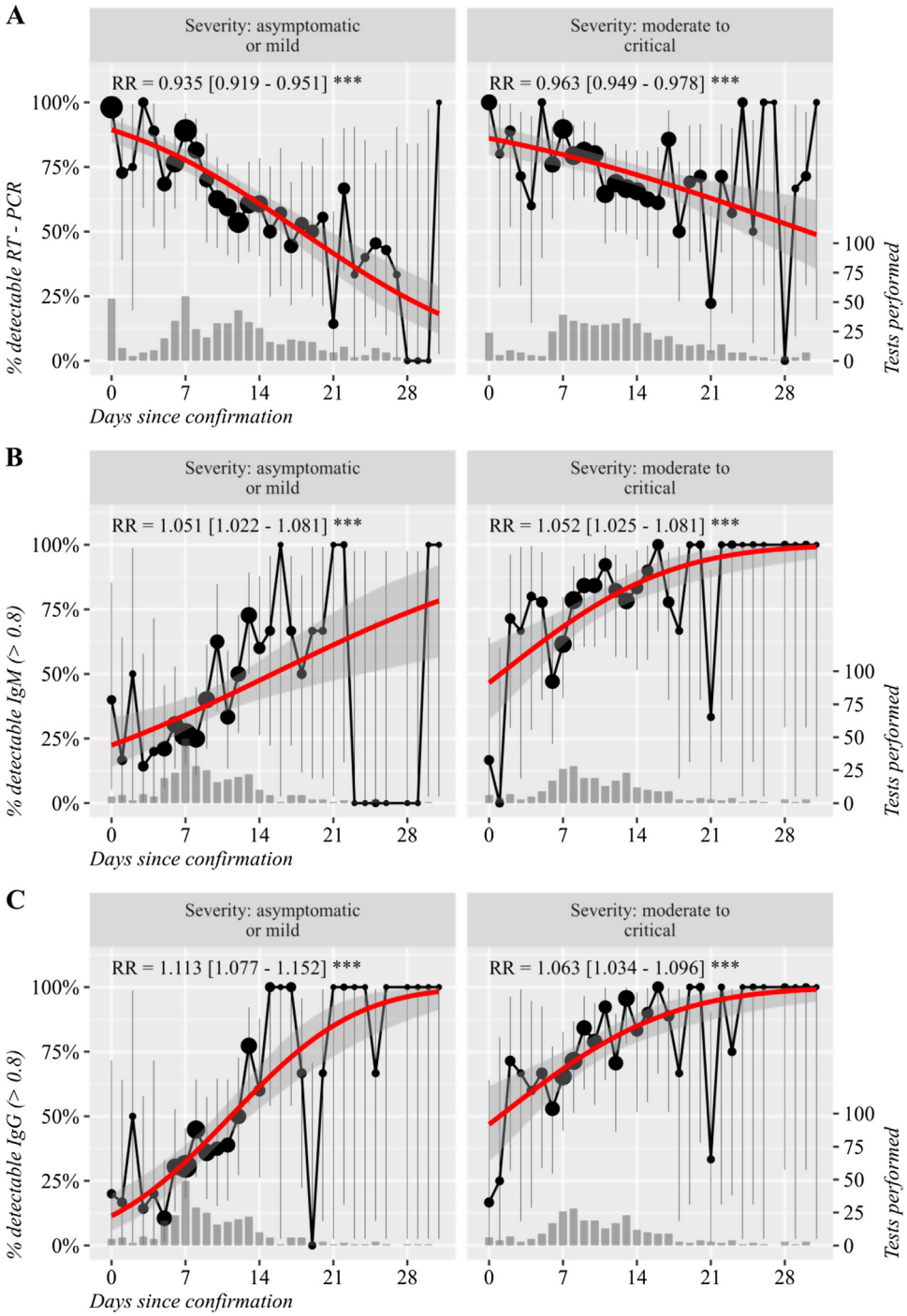

Figure 2. The proportion of patients with detectable RT-PCR (A), IgM (B) and IgG (C) results, by time since confirmation (within one month) and clinical severity (asymptomatic/mild and moderate to critical). Also shown: weighted probit regression line, risk-ratio change per day, number of tests performed (dot area and bottom histogram with right y-axis) and binomial 95% confidence interval around average proportion.

Within 14–21 days after symptoms onset, SARS-CoV-2 specific IgM and IgG are developed against the nucleocapsid (N) and spike (S) proteins (Long et al., 2020; Atyeo et al., 2020; Watson et al., 2020; Deeks et al., 2020). Our electrochemiluminescence kits consisted of biotinylated N and RBD-specific SARS-CoV-2 spike proteins; therefore, we have a fair degree of certainty that antibodies were well ascertained. However, besides humoral immunity, response to SARS-CoV-2 has been demonstrated to be more complex, involving CD4+ and CD8+ T lymphocytes, IFN-γ production by CD4+ T cells, NK cells, or their complements (Atyeo et al., 2020; Laing et al., 2020; Dan et al., 2021). We found similar results regarding the timing of seroconversion and virus shedding as other authors, demonstrating that in different populations, viral kinetics and immunological features are the same (Long et al., 2020; Ng et al., 2020; Stephens and McElrath, 2020; Wölfel et al., 2020).

Chest imaging is an essential tool in assessing the diagnosis and severity of SARS-CoV-2 infection. A Cochrane review of chest CT scan diagnostic accuracy in confirmed COVID-19 patients showed a high pooled sensitivity (93.1%, 95%CI: 90.2–95.0%) (Islam et al., 2020). Noteworthy, in our study, the relationship between disease severity ascertained by chest CT scan (lung area damage >30%) and

Table 2
RT-PCR kinetics (positive, number and %), IgM and IgG index (geometric mean ± standard deviation, median, interquartile range) by time since confirmation, in patients under follow-up.

Time since confirmation (days)		≤7	8–14	15–21	22–28	≥29
RT - PCR tests		349 (29.1%)	515 (43.0%)	220 (18.3%)	84 (7.0%)	31 (2.6%)
Positive RT - PCR		269 (86.5)	301 (67.6)	119 (56.7)	47 (56.6)	16 (51.6)
Antibodies tests*		194 (55.6)	272 (52.8)	66 (30.0)	23 (27.4)	16 (51.6)
IgM	Gμ ± SD	0.766 ± 3.03	1.46 ± 3.65	2.77 ± 3.79	1.77 ± 2.84	2.82 ± 3.13
	M (IQR)	0.54 (0.35–1.23)	1.14 (0.53–2.6)	2.56 (0.87–5.36)	1.24 (0.71–3.74)	4.03 (0.99-7.92)
Detectable IgM		73 (37.6)	175 (64.3)	51 (77.3)	16 (69.6)	13 (81.2)
IgG	Gμ ± SD	1.11 ± 2.99	1.58 ± 3.23	3.29 ± 4.07	3.75 ± 3.47	12.81 ± 5.65
	M (IQR)	0.71 (0.62–0.96)	0.98 (0.68–2.2)	2 (1.01–8.41)	3.8 (1.15–9.05)	21.46 (2.03–37.66)
Detectable IgG		73 (37.6)	177 (64.8)	55 (83.3)	21 (91.3)	15 (93.8)

Gμ ± SD = Geometric mean ± geometric standard deviation; M (IQR) = Median (interquartile range).
* Total IgM & IgG paired determinations, relative to RT-PCR tests during the same period (both antibody types were measured simultaneously).

Table 3
Patient characteristics by IgM and IgG reactivity.

		IgM reactivity (IQR75%>2.607=high)			IgG reactivity (IQR75%>2.906 = high)		
		High 110 (23.4%)	Univariate analysis	Multivariate analysis	High 112 (23.8%)	Univariate analysis	Multivariate analysis
Age (years)	Median	52.5	MW: p < 0.001	aOR = 1.06 [0.91, 1.23] for every 10 years	55	MW: p < 0.001	aOR = 1.01 [0.89, 1.2] for every 10 years p = 0.850
Sex	M	62 (56.4)	OR = 1.52		61 (54.5)	OR = 1.38	
	F	48 (43.6)	[0.99, 2.33] p = 0.064		51 (45.5)	[0.90, 2.11] p = 0.159	
Severity	moderate to critical	90 (83.3)	OR = 10.30 [5.93, 17.88]	aOR = 4.68 [2.34, 9.61]	88 (80.0%)	OR = 7.97 [4.75, 13.35]	aOR = 4.2 [2.14, 8.36]
	asymptomatic or mild	18 (16.7)	p < 0.001	p < 0.001	22 (20.0)	p < 0.001	p < 0.001
Dexamethasone		46 (42.2)	OR = 8.31 [4.86, 14.22] p < 0.001	aOR = 1.74 [0.84, 3.52] p = 0.128	45 (40.5)	OR = 7.43 [4.36, 12.66] p < 0.001	aOR = 2.02 [0.99, 4.02] p = 0.047
ICU admission		17 (15.6)	OR = 5.36 [2.47, 11.62] p < 0.001		14 (12.6)	OR = 3.30 [1.54, 7.07] p = 0.003	
Hospitalization (days)	μ ±SD	19.63 ± 13.1	13.15 ± 8.75		20.57 ± 14.8	12.82 ± 7.52	
	Median (range)	16(1-101)	MW: p < 0.001		16.5 (1–101)	MW: p < 0.001	
CT>30% lung abnormalities		51 (50.0)	OR = 17.21 [9.41, 31.48] p < 0.001	aOR = 4.83 [2.37, 10.18] p < 0.001	49 (47.6)	OR = 14.00 [7.79, 25.17] p < 0.001	aOR = 4.01 [1.98, 8.29] p < 0.001

μ ± SD = Mean ± standard deviation; MW = Mann–Whitney Test; OR = odds-ratio [95% CI], p value from Fisher test); aOR = adjusted odds-ratio.

a robust immune response appeared to be bidirectional. Since we found that higher antibody titers were correlated with extended lung area damage, we hypothesize that unknown variables may cause a greater severity as well as higher immunological reactivity or the uncoordinated SARS-CoV-2 antigen-specific response fails to control the disease. These questions were raised by many authors, but we do know that circulating memory B cell, $CD4^+$ T cell, and $CD8^+$ T cell memory for SARS-CoV-2 are of utmost importance for protection against reinfection, as well as for a vaccination strategy (Seow et al., 2020; Iwasaki and Yang, 2020; Rydyznski Moderbacher et al., 2020; Mathew et al., 2020; Dan et al., 2021; Grifoni et al., 2020). Recent studies demonstrated that the previous history of SARS-CoV-2 infection was associated with an 80.5-84% reduction of reinfection, even if antibody titers are lower than those induced by COVID-19 vaccination. This is reasonably and scientifically explained by the fact that the natural immune response involves much more than antibodies targeting spike proteins (Hansen et al., 2021; Hall et al., 2021). More than this, Krammer et al. showed that after one dose of mRNA vaccines (BNT162b2 and mRNA-1273), individuals with previous SARS-CoV-2 infection developed robust antibody titers, ten to 45 times higher than vaccine recipients without previous infection at the same time points after the first vaccine dose, suggesting that a one-dose vaccine strategy might be effective in previously SARS-CoV-2 infected persons (Krammer et al., 2021). Almost all our patients seroconverted at one month and should be less susceptible to reinfection with one-dose vaccination.

Regarding the treatment, corticosteroids represent the cornerstone medication in patients with COVID-19 requiring oxygen supplementation, being associated with lower mortality, no increased risk of bacterial infection nor of delayed viral clearance (WHO 2020b; Yang et al., 2020; Recovery Collaborative Group 2021; Annane, 2021; Bhimraj et al., 2021; Monedero et al., 2021).

Masiá et al. showed that patients on corticosteroids (even in combination with immune-based therapies) do not negatively impact the humoral immune response (Masiá et al., 2021). In univariate analysis, we found that dexamethasone treatment was correlated with higher antibody reactivity (IgM and IgG) and after adjustment for age and severity remained significant for IgG, suggesting that corticosteroids had a minimal effect on antibody response.

The main strength of our study is that viral and antibody kinetics were evaluated in patients with asymptomatic/mild disease for prolonged hospitalization and, in comparison, with more severe cases. Since, during the first four pandemic months, patients' discharge was possible only after two consecutive negative RT-PCR-based tests, we had a fairly good evaluation of

the time when the SARS-CoV-2 was no longer detectable. Also, a parallel serological assessment was done in all cases, allowing us to present a documented antibody dynamics.

The primary limits of our study were that we evaluated only IgM and IgG antibodies without isotyping, and the virus-specific T-cell response was not assessed.

Conclusions

Asymptomatic/mild COVID-19 cases had a faster RT-PCR negativity rate compared to more severe patients, and almost all cleared the virus in one month. RT-PCR tests remained positive in half of all patients for approximately two weeks after confirmation. IgG and IgM dynamics were almost simultaneous, more robust for IgG and more severe cases. At one month after confirmation, almost all patients had detectable antibodies. Both IgM/IgG high reactivity were correlated with disease severity and mirrored by chest CT scan abnormalities.

Author contributions

AR drafted the initial manuscript. AR and ML contributed significantly to the revision of the manuscript and its finalization for submission.

AI, AR were responsible for conception and study design, analysis, and interpretation of data. AI was responsible for data collection, data input, and statistical analysis. AR, ML, and MF were responsible for critical analysis and editorial assistance. All authors have read and approved the final manuscript.

Conflict of interest

None.

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Ethical approval

This study was approved by the University Hospital Ethical Committee, and all patients have signed an Informed consent.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.04.067.

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