J Bras Patol Med Lab. 2021; 57: 1-7.

10.5935/1676-2444.20210021

Timeline analysis of IgA and IgG levels in Covid-19 hospitalized patients according to the clinical outcome

Análise temporal das concentrações de IgA e IgG em pacientes hospitalizados com Covid-19 de acordo com o desfecho clínico

Thalia Medeiros[1]; Fabiana R. Carvalho[1]; Renan Faustino[1]; Gabriel M. C. Guimarães[1]; Lilian S. Alves[1]; Any Caroline Oliveira[1]; Elzinandes L. Azeredo[2]; Jorge R. Almeida[1]; Luzia Maria O. Pinto[2]; Andrea Alice Silva[1]

1. Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil. 2. Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Rio de Janeiro, Brazil.

ABSTRACT

Introduction: Studies suggest the association between antibody production and the severity of coronavirus disease 2019 (Covid-19). **Objectives:** To evaluate the concentrations of immunoglobulins class A (IgA) and class G (IgG) during the hospitalization period of Covid-19 patients according to the outcome (survival vs death). **Materials and methods:** Patients with severe acute respiratory syndrome of coronavirus 2 (Sars-CoV-2) infection confirmed by reverse transcriptase reaction followed by polymerase chain reaction (RT-PCR) were included in this prospective study. Samples were obtained weekly during the follow-up of individuals, considering symptom onset. Titers of anti-Sars-CoV-2 IgA and IgG were measured using a commercial immunoassay. Correlations between IgA/IgG and cycle threshold (Ct) values for *N1* and *N2* target genes were also assessed. **Results:** We studied 55 Covid-19 patients (59.7±16.2 years, 63.6% male), of which 28 (50.9%) died. We observed IgA and IgG positivity (IgA+ and IgG+) in 90.9% and 80% of patients, respectively. The highest IgA+ frequency was observed at weeks 2 and 3 and the highest IgG+ at weeks 3 and 4. It is important to note that patients who died presented lower IgA titers in the first two weeks ($p < 0.05$); however, a significant increase in IgA levels was observed in the subsequent weeks. Lastly, we identified that significant correlations between Ct values and immunoglobulins levels, both IgA and IgG were correlated with Ct *N2* in patients who died. **Conclusion:** Our results suggest that lower IgA titers in early Covid-19, which is associated with lower Ct values, may indicate patients at higher risk for death.

Key words: antibodies; Covid-19; mortality.

RESUMO

Introdução: *Estudos sugerem a associação entre a produção de anticorpos e a gravidade da coronavirus disease 2019 (Covid-19).* ***Objetivos:*** *Avaliar as concentrações de imunoglobulinas da classe A (IgA) e da classe G (IgG) durante a internação de pacientes com Covid-19 de acordo com o desfecho (sobrevida* vs *óbito).* ***Materiais e métodos:*** *Pacientes com infecção pela síndrome respiratória aguda grave do coronavírus 2 (Sars-CoV-2) confirmada por reação da transcriptase reversa seguida de reação em cadeia da polimerase (RT-PCR) foram incluídos neste estudo prospectivo. As amostras foram obtidas semanalmente durante o acompanhamento dos indivíduos, considerando o início dos sintomas. Os títulos de IgA e IgG anti-Sars-CoV-2 foram mensurados por meio de um imunoensaio comercial. Correlações entre IgA/IgG e valores de limiar de detecção [*cycle thresholds *(Ct)] para os genes alvos* N1 *e* N2 *também foram avaliadas.* ***Resultados:*** *Estudamos 55 pacientes com Covid-19 (59,7 ± 6,2 anos; 63,6% do sexo masculino); destes, 28 (50,9%) morreram. Observamos positividade para IgA e IgG (IgA+/IgG+) em 90,9% e 80% dos pacientes, respectivamente. A maior frequência de IgA+ foi verificada nas semanas 2 e 3, e a maior frequência de IgG+, nas semanas 3 e 4. É importante observar que os pacientes que morreram apresentaram títulos de IgA mais baixos nas primeiras duas semanas (*$p < 0,05$*); no entanto, um aumento significativo na concentração de IgA foi observado nas semanas subsequentes.*

First submission on 03/15/21; last submission on 03/17/21; accepted for publication on 03/17/21; published on 03/20/21

Por fim, identificamos correlações significativas entre os valores de Ct e imunoglobulinas; tanto IgA quanto IgG foram correlacionadas com Ct N2 *em pacientes que morreram.* ***Conclusão:*** *Nossos resultados sugerem que títulos mais baixos de IgA no início da Covid-19 – que estão associados a valores mais baixos de Ct – podem indicar pacientes com risco elevado de evoluir para óbito.*

Unitermos: *anticorpos; Covid-19; mortalidade.*

RESUMEN

Introducción: *Los estudios sugieren una asociación entre la producción de anticuerpos y la gravedad de la enfermedad por coronavirus 2019 (Covid-19).* ***Objetivos:*** *Evaluar las concentraciones de inmunoglobulinas clase A (IgA) y clase G (IgG) durante la hospitalización de pacientes con Covid-19 según el desenlace (supervivencia vs muerte).* ***Materiales y métodos:*** *Se incluyeron en este estudio prospectivo pacientes con síndrome respiratorio agudo severo de infección por coronavirus 2 (Sars-CoV-2) confirmado por la reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR). Las muestras se obtuvieron semanalmente durante el seguimiento de los individuos, considerando la aparición de los síntomas. Los títulos de IgA e IgG anti-Sars-CoV-2 se midieron usando un inmunoensayo comercial. También se evaluaron las correlaciones entre IgA/IgG y los valores de los umbrales de ciclo [*cycle threshold *(Ct)] para los genes* N1 *y* N2*.* ***Resultados:*** *Se estudiaron 55 pacientes Covid-19 (59,7 ± 16,2 años, 63,6% varones), de los cuales 28 (50,9%) fallecieron. Observamos positividad de IgA e IgG (IgA+ e IgG+) en el 90,9% y el 80% de los pacientes, respectivamente. La frecuencia más alta de IgA+ se observó en las semanas dos y tres y la IgG + más alta en las semanas tres y cuatro. Es importante señalar que los pacientes que fallecieron presentaron títulos de IgA más bajos en las dos primeras semanas (*p *< 0,05); sin embargo, se observó un aumento significativo en los niveles de IgA en las semanas siguientes.* ***Conclusión:*** *Identificamos correlaciones significativas entre los valores de Ct y los niveles de Ig, tanto IgA como IgG se correlacionaron con Ct* N2 *en los pacientes que fallecieron. Nuestros resultados sugieren que los títulos de IgA más bajos en Covid-19 temprano, que se asocia con valores de Ct más bajos, pueden indicar que los pacientes tienen un mayor riesgo de muerte.*

Palabras clave: *anticuepos; Covid-19; mortalidad.*

INTRODUCTION

The current pandemic caused by the coronavirus disease-2019 (Covid-19) reached Brazil in February 2020[1], leading to more than 150,000 deaths in just eight months after the virus started circulating in the country[2]. Regarding Covid-19 severity, previous comorbidities and coagulation disorders appear to compromise viral clearance and are associated with poor outcomes[3, 4]. Besides, an exacerbated immune response often referred as cytokine storm, was also associated with disease severity[5, 6].

The humoral response against the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) is important for viral control with the production of neutralizing antibodies[7, 8]. Concerning the kinetics of antibody production, immunoglobulin class M (IgM) and class G (IgG) titers seem to maintain the same profile during the acute infection[9]; however, immunoglobulin class A (IgA) has been described as an early marker of acute respiratory infection by Sars-CoV-2, also presenting a high capacity for viral neutralization[10]. Moreover, some studies have been suggesting an association between immunoglobulin production and disease severity[11-13]. To support these results, we evaluated IgA and IgG levels produced against the S1 subunit of the spike protein from Sars-CoV-2 during the hospitalization period of Covid-19 patients with moderate to severe disease according to the clinical outcome (survival vs death). Using parameters obtained from the from the reverse transcriptase reaction followed by polymerase chain reaction (RT-PCR), we also aimed to evaluate the correlation between IgA and IgG levels with cycle threshold (Ct) values.

MATERIALS AND METHODS

Study design and sample collection

We conducted a prospective observational longitudinal study performed with hospitalized Covid-19 patients. All patients were hospitalized from April to July 2020 at the Hospital Universitário Antônio Pedro (HUAP), located in Niterói, Rio de Janeiro, Brazil, presenting moderate to severe disease. This study was approved by the Research Ethics Committee of the Universidade Federal Fluminense (CAAE: 30623520.5.0000.5243). Demographic and clinical data were obtained from patient's medical records.

Nasopharyngeal samples were collected by the medical team on admission and subsequently transported to the Multiuser Laboratory for Research Support in Nephrology and Medical Science [Laboratório Multiusuário de Apoio à Pesquisa em Nefrologia e Ciências Médicas (LAMAP)] for Covid-19 diagnosis.

Serum samples from Covid-19 hospitalized patients were collected for routine laboratory analysis at the Clinical Pathology Service (HUAP). We retrieved 116 samples from 55 patients, which were carefully checked for the timepoint of disease progression post-symptom onset (PSO). We evaluated the following timepoints considering the time elapsed between the onset of symptoms and blood sampling: i) week 1 (< 7 days PSO; $n = 26$); ii) week 2 (7-14 days PSO; $n = 40$); iii) week 3 (15-21 days PSO; $n = 34$); and iv) week 4 (> 21 days; $n = 16$). Serum samples were stored at -80°C until analysis.

Diagnosis of Sars-CoV-2 infection

Ribonucleic acid (RNA) extraction was performed using the QIAamp Viral RNA kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. RT-PCR was performed in three separate reactions per specimen for each target – *N1*, *N2* and the internal control, human RNaseP (RP) – using the 2019-nCOV RUO Kit (catalog no. 10006770, Integrated DNA Technologies, Inc – IDT, Iowa, USA] and the GoTaq® Probe 1-Step RT-qPCR (Catalog no. A6121, Promega Corporation, Wisconsin, USA), also following the manufacturer's specifications and protocols. Ct values for *N1*, *N2*, and RP were analyzed. It is important to note that all tests for Sars-CoV-2 detection were performed within the first week after the onset of symptoms.

Serological tests

Serum levels of IgA and IgG produced against the S1 subunit of the Sars-CoV-2 Spike protein were measured in all samples using the Anti-Sars-CoV-2 Elisa IgG and IgA commercial kit (Euroimmun, Lüebeck, Germany). Optical densities (ODs) were obtained according to the manufacturer's instructions and the results were calculated in a semi-quantitative fashion using a ratio of samples ODs (or controls) divided by the calibrator's OD. When the result was ≥ 1.1, it was interpreted as positive.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Comparisons between two groups were analyzed using the Student's *t*-test/Mann-Whitney according to variables distribution. For three or more groups, differences were calculated using the analysis of variance (Anova)/Kruskal-Wallis with Bonferroni/Dunn's post-tests, also according to variables distribution. Correlations between serum levels of immunoglobulins and other variables such as Ct and days since symptom onset were calculated, and Spearman's coefficients were assessed. *p*-values < 0.05 were considered statistically significant. All statistical and graphical analyses were performed using the GraphPad Prism 8.0 software (San Diego, California, USA).

RESULTS

Patients' characteristics

We studied 55 hospitalized Covid-19 patients admitted at HUAP from April to July 2020. The demographic and clinical characteristics of patients according to the clinical outcome are described in **Table 1**. In general, patients presented a mean age ± SD of 59.7 ± 16.2 years, and 63.6% ($n = 35$) were male. All hospitalized patients presented moderate to severe Covid-19, the symptoms most frequently observed were fever (67.3%), cough (61.8%), dyspnea (47.3%), and hypoxia (41.8%); which were identified in association

TABLE 1 – Demographic and clinical characteristics of Covid-19 hospitalized patients

Characteristics	Total ($n = 55$)	Survival ($n = 27$)	Death ($n = 28$)	*p*-value
Age (years, mean ± SD)	59.7 ± 16.2	52.6 ± 17.3	66.5 ± 11.8	**0.0004**
Male, *n* (%)	35 (63.6)	16 (59.2)	19 (67.8)	0.6
Comorbidities, *n* (%)				
Diabetes	22 (40)	8 (29.6)	14 (50)	0.2
CVD	36 (65.4)	16 (59.2)	20 (71.4)	0.4
CKD	9 (16.3)	4 (14.8)	5 (17.8)	1.0
COPD	11 (20)	6 (22.2)	5 (17.8)	0.7
Immunosuppression	12 (21.8)	5 (18.5)	7 (25)	0.7
Cancer	25 (45.4)	11 (40.7)	14 (50)	0.6
Obesity	12 (21.8)	5 (18.5)	7 (25)	0.7
Clinical symptoms, *n* (%)				
Fever	37 (67.3)	20 (74.1)	17 (60.7)	0.4
Cough	34 (61.8)	17 (62.9)	17 (60.7)	1
Dyspnea	26 (47.3)	12 (44.4)	14 (50)	0.8
O_2 saturation < 95%	23 (41.8)	10 (37)	13 (46.4)	0.6
Fatigue	26 (29.1)	9 (30)	7 (25)	0.6
Myalgia	6 (10.9)	4 (14.8)	2 (7.1)	0.4
Anosmia/Ageusia	8 (14.5)	4 (14.8)	4 (14.3)	1
Headache	6 (10.9)	2 (7.4)	4 (14.3)	0.4
Diarrhea	6 (10.9)	3 (11.1)	3 (10.7)	1
Complications during hospitalization, *n* (%)				
Sars	32 (58.2)	9 (30)	23 (82.1)	**0.0003**
Admission at the ICU	32 (58.2)	8 (29.6)	24 (85.7)	**< 0.0001**
Mechanical ventilation	33 (50)	8 (29.6)	25 (89.3)	**< 0.0001**
Hospitalization time (days, mean ± SD)	26 ± 17.6	33.8 ± 22.6	19.4 ± 7.2	**0.001**

Data are expressed as mean ± SD or n *(%). p-values were calculated using the Mann-Whitney test or chi-square test and were considered statiscally significant when < 0.05 (in bold). Covid-19: coronavirus disease 2019; SD: standard deviation; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease; ICU: intensive care unit; Sars: severe acute respiratory syndrome.*

with the presence of pulmonary involvement [e.g. ground-glass pattern on chest computed tomography (CT)]. Patients presented various comorbidities, such as diabetes (40%), cardiovascular disease (65.4%), cancer (23.6%), and obesity (21.8%).

It is important to note that, as expected, patients who died due to Covid-19 ($n = 28$) were older (52.6 ± 17.3 vs 66.5 ± 11.8 years-old for patients who survived and died, respectively; $p = 0.0004$) and presented a higher frequency of complications during hospitalization, such as development of Sars ($p = 0.0003$) and requirement of intensive care ($p < 0.0001$), including invasive mechanical ventilation support ($p < 0.0001$). Moreover, the average length of stay ± SD was 26 ± 17.6 days, and this was also significantly different between groups (33.8 ± 22.6 vs 19.4 ± 7.2 days for patients who survived and died, respectively; $p = 0.001$).

Analysis of IgA and IgG during the hospitalization of Covid-19 patients

Overall, we observed IgA and IgG positivity in 90.9% and 80% of hospitalized patients ($n = 55$), respectively. Moreover, we observed a significant correlation between serum levels of immunoglobulins and days since the onset of symptoms in patients with moderate to severe Covid-19 ($p < 0.0001$), as shown in **Figure 1**. Next, we evaluated the frequency of IgA and IgG positivity (IgA+ and IgG+) at each timepoint. At week 1, only four patients (30.9%) presented IgA+. However, this rate progressively increased to 85% and 94.4% by the second and third weeks of hospitalization, respectively ($p < 0.0001$ for week 1 vs week 2 and week 1 vs week 3). At week 4, serum levels of IgA started to decrease, but this was not statistically significant (IgA+ of 94.4% to 88.9%, $p = 0.3$). For IgG, the positivity was 23.1% in the first week and increased to 60% by the second week ($p = 0.005$). After week 2, IgG+ was observed in 88.9% of patients, and this rate was sustained until the end of follow-up ($p < 0.0001$ for week 1 vs week 3, and week 1 vs week 4).

Interestingly, some differences regarding the IgA and IgG positivity were observed between groups (survival vs death). In the first week, both IgA and IgG positivity was observed in 41.7% of the patients who survived and 21.4% of the patients who died, but this was not statistically significant. For IgG+, we identified that this difference was more evident in the second week (83.3% vs 50%, $p = 0.05$, survival vs death); meanwhile, IgA+ rates were similar between groups in subsequent weeks.

It is important to highlight that we performed a separate analysis of IgA and IgG positivity in immunocompromised ($n = 12$) – one kidney transplant patient, three patients with autoimmune disease, submitted to corticotherapy, and eight patients undergoing chemotherapy. We observed that only one patient was not positive for both IgA and IgG. Moreover, six (50%) immunocompromised patients did not show positive IgG until the end of the follow-up.

We also evaluated IgA and IgG levels weekly in a longitudinal fashion aiming to assess the kinetics of antibody production during the hospitalization period in addition to identify any differences of antibody positivity according to the outcome (survival vs death) (**Figure 2**). We observed that patients who died presented lower levels of IgA at week 1, and significantly lower levels of IgA at week 2 ($p < 0.05$) when compared to patients who survived. Moreover, a significant increase in IgA levels after 14 days of follow-up was only observed for patients who died (week 1 vs week 3, $p < 0.01$, and week 1 vs week 4, $p < 0.05$). The analysis of IgG between timepoints showed that both groups presented a significant increase in IgG levels also after the second week; however, no differences between groups were observed when timepoints were analyzed separately, although the mean IgG levels from patients who survived were slightly increased in all timepoints.

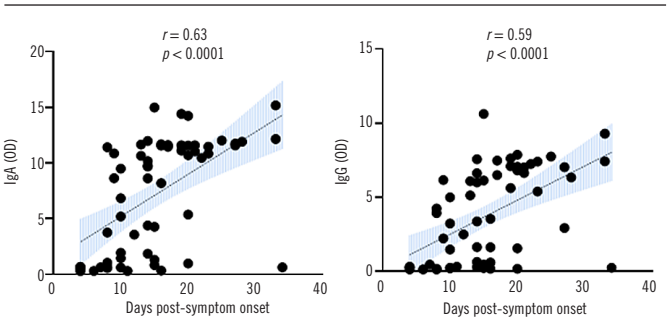

FIGURE 1 – *Correlation between serum levels of IgA and IgG and days after the onset of symptoms. Spearman's coefficients (r) were analyzed*

IgA: immunoglobulin class A; IgG: immunoglobulin class G; OD: optical density.

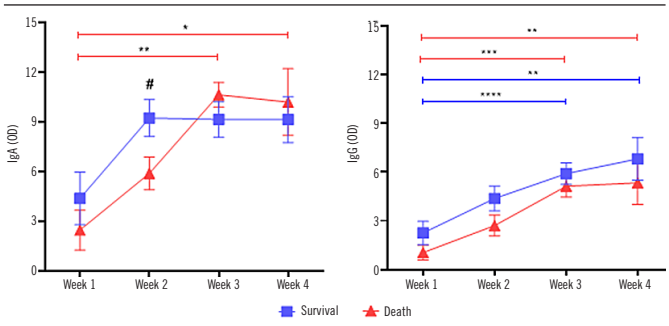

FIGURE 2 – *Weekly assessment of IgA and IgG levels according to the outcome (survival vs death). Data are shown as mean ± SEM*

**p < 0.05; **p < 0.01; ***p < 0.00; ****p < 0.0001 indicates comparison between timepoints for each group (Kruskal-Wallis test with Dunn's post-test; survival – blue lines vs death – red lines); #p < 0.05 indicates the comparison between survival vs death in at week 2 (Mann-Whitney test).*

IgA: immunoglobulin class A; IgG: immunoglobulin class G; SEM: standard error of the mean.

Associations between immunoglobulins and Ct values

Lastly, to identify possible associations between serum levels of immunoglobulins and viral load, we evaluated IgA and IgG in association with Ct values obtained by the RT-PCR, which are representative of viral load[14]. When considering all patients, we observed a significant correlation between the Ct values for *N1* and *N2* and serum levels of IgA ($p = 0.03$ and $p = 0.007$, respectively). No significant correlations were observed for IgG. Interestingly, when evaluating patients according to the outcome, we only observed correlations between immunoglobulins and Ct *N2* ($r = 0.757, p = 0.01$ for IgA vs *N2*; and $r = 0.745, p = 0.01$ for IgG vs Ct *N2*) in non-surviving patients. No correlations were observed between immunoglobulins levels and Ct values in patients who survived. These results are shown in **Table 2**.

TABLE 2 – Correlations between IgA and IgG levels and Ct for *N1* and *N2* Sars-CoV-2 gene targets values at week 1 according to the outcome

	IgA		IgG	
	Correlation with Ct values		Correlation with Ct values	
	N1	*N2*	*N1*	*N2*
All patients	**$r = 0.491$**	**$r = 0.6$**	$r = 0.386$	$r = 0.445$
($n = 55$)	**$p = 0.03$**	**$p = 0.007$**	$p = 0.1$	$p = 0.06$
Survival	$r = 0.4$	$r = 0.45$	$r = -0.083$	$r = 0.067$
($n = 27$)	$p = 0.3$	$p = 0.2$	$p = 0.8$	$p = 0.8$
Death	$r = 0.474$	**$r = 0.757$**	$r = -0.596$	**$r = 0.745$**
($n = 28$)	$p = 0.1$	**$p = 0.01$**	$p = 0.07$	**$p = 0.01$**

Spearman's coefficients (r) were analyzed. p-*values were considered statiscally significant when < 0.05 (in bold).*
IgA: immunoglobulin class A; IgG: immunoglobulin class G; Ct: cycle threshold; Sars-CoV-2: severe acute respiratory syndrome coronavirus 2.

DISCUSSION

This study was focused on the assessment of anti-Sars-CoV-2 IgA and IgG titers during the hospitalization period of Covid-19 patients. In general, we observed a higher frequency of IgA positivity at weeks 2 and 3, which for IgG it was observed at weeks 3 and 4. Several studies have been published to report data about antibody detection in Covid-19. Our data are in agreement with studies demonstrating peaks of IgA production at 10-14 days after the onset of symptoms[12, 13, 15, 16] concomitantly with IgG levels starting to increase[13, 16-18]; however, different results can be found in the literature concerning antibody kinetics during Covid-19 progression. For example, Beavis *et al*. (2020)[19] also compared IgA and IgG produced against the S1 subunit of the Sars-CoV-2 Spike protein in 82 positive samples for Sars-CoV-2. They observed 100% positivity for IgG in the first week after diagnosis, but it was not clear when the diagnosis was performed considering the onset of symptoms.

Overall, we identified IgA and IgG positivity in 90.9% and 80% of hospitalized patients, respectively. These results are in accordance with other studies showing IgA positivity higher than 90% in the firsts four weeks of follow-up, with a slightly lower frequency of IgG positivity[15, 16, 20]. Despite the high number of studies in the field, the data concerning antibody production in Covid-19 is still dispersed, and we need to consider the possibility of different kinetics of antibodies produced against different targets and the use of several types of commercial kits with different sensitivities, besides patient's characteristics, such as immunosuppression and other comorbidities. In our cohort, the high prevalence of cancer patients (25/55; 45.4%), especially those undergoing chemotherapy (8/25; 32%), may influence seroconversion rates; however, the recent study by Marra *et al*. (2020)[17] showed that seroconversion in cancer patients infected with Sars-CoV-2 is similar to IgG antibody response in subjects with no comorbidities[17].

Of note, we observed a slight decrease in IgA positivity rate after 21 days of follow-up. In our cohort, the number of patients who reached the "week 4" timepoint was reduced ($n = 16$). This was probably due to the high number of patients who died before completing 21 days of hospitalization (18/28; 64.3%). Indeed, the average length of stay for patients who died was significantly shorter. Nevertheless, other studies also demonstrate the decrease in IgA levels after 3-4 weeks PSO[13, 15, 20, 21]. This is especially important for the application of IgA in the diagnosis of acute Covid-19. In this regard, Guo *et al*. (2020)[16] observed that the combination of anti-Sars-CoV-2 serology and viral RNA detection by RT-PCR significantly improves the performance of Covid-19 diagnose, especially for those cases presenting negative RT-PCR with typical clinical presentation. Together, these data suggest that IgA can be used for Covid-19 diagnosis with high sensitivity from seven to 21 days PSO if it is not possible to perform molecular tests for inconclusive cases.

Our next step was to assess immunoglobulins levels in a longitudinal approach according to the clinical outcome. We found that patients who died presented lower IgA titers at weeks 1 and 2 compared to patients who survived; however, this promptly changed in the third week of follow-up, when IgA levels continued to increase in patients who died and, for patients who survived, IgA reached a plateau. We observed a similar trend for IgA when comparing critically ill patients with patients who did not develop severe acute respiratory syndrome nor required invasive O_2 support (data not shown). Similar to our results, Fourati *et al*. (2020)[11] also observed that patients who died presented significantly lower immunoglobulins titers on admission at intensive care unit (ICU), and higher IgA was considered as a protective factor.

Considering these results, we suggest that decreased serum levels of anti-Sars-CoV-2 immunoglobulins, especially IgA, at an early timepoint during the hospitalization period may be indicative of poor prognosis. This may reflect a delayed immune response, leading to an insufficient production of antibodies, which could be essential for combating Sars-CoV-2 infection[9]. Nevertheless, further studies are necessary to confirm the neutralizing potential of these antibodies.

In support of this, we evaluated correlations between immunoglobulins titers and Ct values. Previous studies have demonstrated strong significant associations between Sars-CoV-2 viral load and disease severity[14, 22]. Since Ct values obtained from the RT-PCR are representative of viral load, we aimed to evaluate whether serum levels of IgA and IgG could be associated with this promising marker of Covid-19 severity. We observed that, in patients who died, both IgA and IgG were significantly and directly correlated with Ct values for *N2* target gene. In other words, patients who present lower Ct values at admission (high viral load) also present lower IgA and IgG titers. Similarly to our data, a recent study carried out with severe Covid-19 patients also found significant correlations between IgA and IgG levels with Ct values[11]. It is important to mention that Ct values at admission have been shown as strong independent predictive factors associated with death by Covid-19[22]. Thus, considering the importance of the humoral response for combating Sars-CoV-2[7, 8, 23], one may notice that lower levels of immunoglobulins at the first two weeks of acute infection are associated with high viral load and would directly impact the response against the virus.

Later in Covid-19 progression, some reports demonstrate that higher levels of anti-Sars-CoV-2 IgA, as well as total IgA, are increased in critically ill patients[12, 13, 15, 24]. These observations follow our results, in which the patients who died presented a significant increase in IgA levels in the third and four weeks of hospitalization. Higher levels of IgA were also associated with hypoxia and extensive pulmonary lesions[12]. Zhang *et al*. (2020) [21] demonstrated an association between increased risk of death and high IgA levels, besides other laboratory abnormalities, such as thrombocytopenia and increased levels of C-reactive protein. Moreover, higher IgG titers and faster seroconversion were also observed in critically ill patients[25], and this may be the reflection of the exacerbated immune response observed during severe Covid-19 progression.

Our study has some limitations. Despite the small cohort (n = 55), we were unable to obtain serum samples from all patients at all timepoints. As mentioned above, a significant parcel of our patients died of Covid-19 (50.9%), most of them before the fourth week of follow-up, which hampered our paired analysis. In our study, the average hospital stay was 26 days; therefore, it was not possible to carry out a long-term follow-up. It is important to note that we did not evaluate IgM titers during the hospitalization period of Covid-19 patients; however, some studies report greater sensitivity to IgA during the acute phase[10].

CONCLUSION

Our results suggest that IgA can be used for Covid-19 diagnosis in the early stages of hospitalization (7-21 days after the onset of symptoms). We also observed a high frequency of IgG positivity after 14 days of follow-up. The associations observed between IgA levels and Ct values may indicate that patients at higher risk for Covid-19 severity and death also present lower IgA titers in the first two weeks of hospitalization. Thus, the high viral load associated with lower levels of immunoglobulins at an early stage during acute infection may directly impact the response against the virus and increase the mortality risk, even for immunocompetent patients. Further studies are necessary to confirm these findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Thalia Medeiros and Fabiana R. Carvalho equally contributed to this study.

FUNDING

We thank the financial support by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, #001), Prefeitura de Niterói (#37, #306), Universidade Federal Fluminense and Funding Authority for Studies and Projects (FINEP, REDE COVID-19 #27968*6).

REFERENCES

1. Rodriguez-Morales AJ, Gallego V, Escalera-Antezana JP, et al. COVID-19 in Latin America: the implications of the first confirmed case in Brazil. Travel Med Infect Dis. 2020; 35: 101613. doi:10.1016/j.tmaid.2020.101613.

2. Carvalho TA, Boschiero MN, Marson FAL. COVID-19 in Brazil: 150,000 deaths and the Brazilian underreporting. Diagn Microbiol Infect Dis. 2021; 99(3): 115258. doi:10.1016/j.diagmicrobio.2020.115258.

3. Argenziano MG, Bruce SL, Slater CL, et al. Characterization and clinical course of 1000 patients with coronavirus disease 2019 in New York: retrospective case series. BMJ. 2020; 369.

4. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020; 95: 834-47.

5. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2020.

6. Varchetta S, Mele D, Oliviero B, et al. Unique immunological profile in patients with COVID-19. Cell Mol Immunol. 2020; 1-9.

7. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: current state of the science. Immunity. 2020; 52: 910-41.

8. Paces J, Strizova Z, Smrz D, Cerny J. COVID-19 and the Immune System. Physiol Res. 2020; 379-88.

9. Lippi G, Mattiuzzi C. Clinical value anti-SARS-COV-2 serum IgA titration in patients with COVID-19. J Med Virol. 2020.

10. García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. Front Immunol. 2020; 11.

11. Fourati S, Hue S, Pawlotsky JM, Mekontso-Dessap A, de Prost N. SARS-CoV-2 viral loads and serum IgA/IgG immune responses in critically ill COVID-19 patients. Intensive Care Med. 2020; 46: 1781-3.

12. Xue M, Zhang T, Hu H, et al. Predictive effects of IgA and IgG combination to assess pulmonary exudation progression in COVID-19 patients. J Med Virol. 2020.

13. Ma H, Zeng W, He H, et al. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol. 2020; 17: 773-5.

14. Xu T, Wang J, Hu B, et al. Identification of the RNase-binding site of SARS-CoV-2 RNA for anchor primer-PCR detection of viral loading in 306 COVID-19 patients. Brief Bioinform. 2020.

15. Yu H, Sun B, Fang Z, et al. Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. Eur Respir J. 2020; 56: 2001526.

16. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis. 2020; 71: 778-85.

17. Marra A, Generali D, Zagami P, et al. Seroconversion in patients with cancer and oncology healthcare workers infected by SARS-CoV-2. Ann Oncol. 2020; S0923-7534: 42965-5.

18. Tré-Hardy M, Wilmet A, Beukinga I, et al. Analytical and clinical validation of an ELISA for specific SARS-CoV-2 IgG, IgA, and IgM antibodies. J Med Virol. 2020.

19. Beavis KG, Matushek SM, Abeleda APF, et al. Evaluation of the EUROIMMUN Anti-SARS-CoV-2 ELISA Assay for detection of IgA and IgG antibodies. J Clin Virol. 2020; 104468.

20. Dahlke C, Heidepriem J, Kobbe R, et al. Distinct early IgA profile may determine severity of COVID-19 symptoms: an immunological case series [Internet]. Infect Dis (except HIV/AIDS); 2020 Apr [cited 2020 Nov 5]. Available at: http://medrxiv.org/lookup/doi/10.1101/2020.04.14.20059733.

21. Zhang L, Fan T, Yang S, et al. Comparison of clinical characteristics of COVID-19 between elderly patients and young patients: a study based on a 28-day follow-up. Aging. 2020; 12: 19898-910.

22. Rao SN, Manissero D, Steele VR, Pareja J. A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther. 2020; 573-86.

23. Chao YX, Rötzschke O, Tan EK. The role of IgA in COVID-19. Brain Behav Immun. 2020; 87: 182-3.

24. Hasan Ali O, Bomze D, Risch L, et al. Severe COVID-19 is associated with elevated serum IgA and antiphospholipid IgA-antibodies [Internet]. Infect Dis (except HIV/AIDS); 2020 Jul [cited 2020 Nov 5]. Available at: http://medrxiv.org/lookup/doi/10.1101/2020.07.21.20159244.

25. Marklund E, Leach S, Axelsson H, et al. Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders. Walsh SR, editor. PLoS ONE. 2020; 15: e0241104.

CORRESPONDING AUTHOR

Andrea Alice Silva ID 0000-0001-5856-6128
e-mail: aasilva@id.uff.br

CC BY

This is an open-access article distributed under the terms of the Creative Commons Attribution License.