Clinical significance of early IgA anti-SARS-CoV-2 antibody detection in patients from a Romanian referral COVID-19 hospital

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Abstract. Controlling the spread of coronavirus disease 2019 (COVID-19) includes institute isolation, quarantine measures and appropriate clinical management, which all require effective screening, diagnostic and prognostic tools. The present study aimed to analyze severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immunoglobulin (Ig)A detection and determine the potential association with the clinical course of COVID-19 and the levels of inflammation. In the present study, the presence of IgA and IgG SARS-CoV-2 antibodies in 75 consecutive patients with confirmed COVID-19 infection was investigated. No significant differences were found between the IgA positive and negative groups, regarding the presence of symptoms, haematological and inflammatory variables, or the presence of pneumonia. In the majority of cases, antibody detection was comparable, for example, 79.7% of patients in the IgA positive group exhibited both types of antibodies, while 80.9% of patients in the IgA negative group were also IgG negative. A total of four patients in the IgA negative group presented with anti-SARS-CoV-2 IgG antibodies. Early detection of IgA was more frequent in patients who later developed severe forms of the disease. In addition, the IgG SARS-CoV-2 antibody response was higher in patients with the severe form of the disease.

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Introduction

Although it has previously been established that coronaviruses infect humans and generate mild respiratory infections (1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the newly identified member of the betacoronaviruses, is responsible for the most significant pandemic since the Spanish flu pandemic of 1918-1920 (2).

SARS-CoV-2 has a large RNA genome and a complex antigenic profile (3). Due to its genetic profile, the large number of infected patients and the immunological pressure, the virus exhibits the ability to mutate and novel variants can elude the natural or vaccine-generated antibody response (3,4). The onset of the current pandemic occurred with great speed; since 2019, >363 million cases have been confirmed worldwide, with ~2 million in Romania. Despite the fact the lethality of the disease is currently <2% worldwide and 2.87% in Romania (5), in specific patient categories (6), for example patients within an ageing population or with comorbidities, patients with an absence of previous immunization, or those in overwhelmed public health systems, this figure may be much higher. In addition, patients who recover from this disease may present with long-term complications [long coronavirus disease 2019 (COVID-19)] (7). Notably, despite quarantine mechanisms in place, the virus remains and continues to pose a serious threat to public health.

COVID-19 exhibits a wide array of clinical manifestations. Originating in China as a predominantly respiratory disease (8), at present it is regarded a disease involving almost any organ or system. Symptoms vary according to individual factors, including age, genetic background, comorbidities and the viral subtype involved (9-11). The evolution of certain diseases is often difficult to predict. However, numerous early risk factors for the development of a severe form of disease had been proposed (10) and may help to promote the correct management of the patient.

Despite ethical barriers for investigation in human subjects (12), research has been dedicated to the development of safe vaccines (13,14) and effective antiviral therapies to target this disease (15,16).

The interactions between SARS-CoV-2 and the immune system remain a key focus of current research, to improve

understanding of the pathogenesis and effect that COVID-19 may have on patients. Antibody responses serve a primary role in protecting individuals against SARS-CoV-2, particularly by the activity of neutralizing antibodies that can block viral infection (17). Specific immune response to viral antigens involves the action of lymphocytes, including B cells and T cells, chemokines, cytokines and antigen-neutralizing immunoglobulin (Ig)A (18). The potency of IgA is highlighted in various sites of the body, including the saliva, blood or bronchoalveolar lavage in the first stage of COVID-19 disease. Thus, the presence of SARS-CoV-2 neutralization in the first weeks following symptom onset is more closely associated with IgA, than IgM or IgG antibodies (19). Elevated titers of IgG antibodies are essential for developing immune memory in order to prevent reinfection. The specific type of IgG against SARS-CoV-2 following infection remains undetermined; however, results of a previous study highlighted a decrease in IgG antibodies in week 5-7 after infection, that only continued for 1-2 weeks (20).

The present study aimed to evaluate the presence of IgA and IgG SARS-CoV-2 antibodies in patients with COVID-19 that had been confirmed using reverse transcription-quantitative (RT-q) PCR. The main purpose of the present study was to analyse SARS-CoV-2-specific IgA detection and associate it to the clinical course of COVID-19 and the inflammatory response of patients.

Materials and methods

Study population. A cross-sectional study involving 75 consecutive patients was conducted. A total of 40 (53.33%) males and 35 (46.67%) females that were hospitalized during October 2020 in Sfanta Parascheva Infectious Diseases Hospital of Iași, Romania were included. Demographic data, including age, sex, occupation and residential region of each participant, were collected. All patients were confirmed positive for COVID-19 via SARS-CoV-2 detection in nasopharyngeal swabs using RT-qPCR.

Detection of SARS-CoV-2 antibodies. After obtaining written informed consent from all patients, serum samples were collected (between 7 and 28 days post-onset of symptoms) and ELISA kits were used for the detection of anti-SARS-CoV-2 (cat. nos. EI 2606-9601 A and EI 2606-9601 G; EUROIMMUN AG), in order to provide semi-quantitative *in vitro* determination of IgA and IgG against SARS-CoV-2. Each kit contained microplate strip wells coated with a recombinant structural protein of SARS-CoV-2: S1 domain of spike protein expressed in the human embryonic kidney cell line (293 cells). Semi-quantitative evaluation of the results was calculated using the following formula: Optical density (OD) ratio of the extinction of the control or patient sample over the extinction of the calibrator. The result was interpreted as recommended by the manufacturer: <0.8 for negative samples; ≥0.8 to <1.0 as borderline and >1.1 for positive samples. Diagnostic sensitivity of the ELISA kit to anti-SARS-CoV-2 IgA in samples taken between day 10 and 20 following symptom onset was determined by the manufacturer and amounted to 91.7%. In samples taken after >20 days, the sensitivity of the ELISA kit amounted to 100%. The specificity of the ELISA kit to

anti-SARS-CoV-2 IgA amounted to 88.2 and 82.4% during the aforementioned timeframes, respectively. In samples obtained between day 10 and 20 after symptom onset, the sensitivity of the ELISA kit to anti-SARS-CoV-2 IgG declared by the manufacturer amounted to 75 and 99% for specificity.

Following serological testing, the patients were split into two groups; namely, group 1: Positive for IgA anti-SARS-CoV-2 (n=54) and group 2: negative for IgA anti-SARS-CoV-2 (n=21). Blood collection was performed between the 7 and 14th day following disease onset, in order to evaluate the presence of IgA anti SARS-CoV-2. Results revealed 77.8% in group 1, compared with 85.7% in group 2 (Table I).

Other laboratory/imagistic tests. All patients included in the study were also examined using an imaging system, such as computerized tomography scans or chest radiography and evaluated for blood inflammatory markers, liver transaminase levels (RX Imola analyzer) and hematological parameters (fully automated bidirectionally analyzer fluorescence & flow cytometry-Sysmex xn550). These included the mean leukocyte count, percentage of neutrophils, mean neutrophil count and mean platelet count.

Statistical analysis. Statistical analysis was performed using Analyse-it Add-on for Microsoft Excel (Analyse-it Software, Ltd.). Descriptive data are presented as absolute values, percentages and means. Differences between groups were tested for statistical significance using unpaired T-Student and χ^2 square tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic and clinical characteristics of the patients. The comparative demographic and clinical characteristics of the patients from the two groups are displayed in Table II.

The age of IgA anti-SARS-CoV-2 positive patients ranged from 40-88 years, with a median age of 63 years. Among the patients with detectable antibodies, 42.6% were >65 years old. Group 2 (negative for IgA anti SARS-CoV-2) included hospitalized patients aged between 23-101 years old, with a median age of 67 years. Among the patients who did not present with SARS-CoV-2 detectable antibodies, 66.7% were >65 years of age. Analysis of sex distribution indicated that males were dominant over females in group 2 [male (M)/female (F), 1.08], compared with a higher number of female patients in group 1 (M/F, 0.75), but the difference was not statistically significant. In addition, almost no differences were noted regarding the frequency of the main clinical manifestations of the disease; namely, fever, cough, anosmia or ageusia between the two groups (Table II). Radiologically detectable pneumonia was present in most patients in both groups (85.2 vs. 90.3%) and the hospitalization period of both groups of patients was comparable (11 vs. 10 days).

In order to further evaluate the presence of IgA anti-SARS-CoV-2, all patients included in the study were evaluated for existing comorbidities (Table III). Infections are often associated with comorbidities that increase the risk of certain medical conditions; thus, leading to a higher severity of the disease.

Table I. Evaluation of the presence of IgA anti SARS-CoV-2 and the stage of the infection.

	Between 7-14 day of evolution			After the 14 days of evolution		
Group	No. of patients	%	Mean	No. of patients	%	Mean
Group 1 (anti SARS-CoV-2 IgA positive)	42	77.8	10	12	22.2	16
Group 2 (anti SARS-CoV-2 IgA negative)	18	85.7	9	3	14.3	15

IgA, immunoglobulin A; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table II. Patient clinical and demographic characteristics.

Characteristic	Group 1 (positive for IgA anti SARS-CoV-2)	Group 2 (negative for IgA anti SARS-CoV-2)	P-value
Median age (years)	63	67	0.59
Sex ratio (M/F)	0.75	1.08	0.66
Hospitalization period (days)	11.1	10.5	0.49
Fever (>38˚C, %)	81.1	83.4	0.72
Cough (%)	90.5	93.4	0.61
Anosmia/Ageusia (%)	33.3	38.4	0.51
Pneumonia (%)	85.2	90.3	0.46

IgA, immunoglobulin A; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table III. Comorbidities in patients with COVID-19.

	Group 1 (positive for anti-SARS-CoV-2 IgA)		Group 2 (negative for anti-SARS-CoV-2 IgA)		
Characteristic	No. of patients	%	No. of patients	%	P-value
Without comorbidities	11	20.4	2	9.5	P=0.26
With comorbidities	43	79.6	19	90.5	

COVID-19, coronavirus 19; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgA, immunoglobulin A.

Hematological and biochemical characteristics of the patients. Disturbance of the immune system in patients with COVID-19 has been considered as one of the distinctive features of SARS-CoV-2 infection, particularly lymphopenia (21). Results of a previous study demonstrated that SARS-CoV-2 infection is not only a pulmonary disease, but also a systemic inflammatory illness (7). Key laboratory parameters of the patients included in the present study are detailed in Table IV.

IgA, IgG anti SARS-COV-2 antibodies and clinical severity. The illness severity among symptomatic infections varied widely, from mild cases to critical ones with respiratory failure, or dysfunction of multiple other organ systems. In the present study, the association between the presence of IgA anti-SARS-CoV-2 antibodies and disease severity revealed significant differences between the two groups (Table V).

No significant differences were determined between the ODs for IgA anti SARS-CoV-2 of the patient samples and disease severity. Notably, mild forms were established at 5.45, compared with medium forms at 6.94 and severe forms at 6.97 (P=0.98; Fig. 1).

Patients who developed moderate to severe disease exhibited significantly higher ODs for anti-SARS-CoV-2 IgG antibodies than those with mild disease (1.27 vs. 5.49 vs. 6.73; Fig. 2).

Antibody detection was comparable in most cases; in the IgA positive group, 79.7% of patients presented with both types of antibodies and in the IgA negative group, 80.9% of patients were also IgG negative (Table VI).

Discussion

The clinical manifestations following SARS-CoV-2 infection vary in severity, from asymptomatic, to mild, moderate or

Table IV. Results of blood tests obtained from all evaluated patients.

Variable	Group 1 (positive for anti-SARS-CoV-2 IgA)	Group 2 (negative for anti-SARS-CoV-2 IgA)	P-value
Mean leukocyte count (/mm³)	6,390	6,050	0.7
Percent of neutrophils	72.4	73.5	0.76
Mean neutrophils count (/mm³)	4726.8	4848.0	0.87
Mean platelet count (/mm³)	216,157.6	184,907.1	0.22
Mean ALT (UI/l)	37.6	39.8	0.79
Mean CRP (mg/l)	68.2	55.2	0.48

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgA, immunoglobulin A; CRP, C-reactive protein; ALT, alanine aminotransferase.

Table V. Clinical evaluation of patients with COVID-19 and the presence of IgA anti-SARS-CoV-2.

Disease severity	Group 1 (positive for anti-SARS-CoV-2 IgA)		Group 2 (negative for anti-SARS-CoV-2 IgA)		P-value
	No. of patients	%	No. of patients	%	
Mild	7	12.9	0	0	0.037
Moderate	30	55.6	18	85.7	
Severe	17	31.5	3	14.3	

COVID-19, coronavirus 19; IgA, immunoglobulin A; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
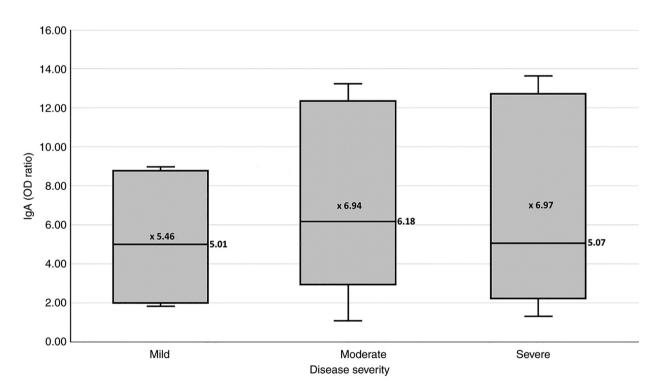

Figure 1. Average OD values of IgA positive samples obtained from patients with mild and moderate forms compared with severe forms of COVID-19. OD, optical density; COVID-19, coronavirus 19; IgA, immunoglobulin A.

severe respiratory disease and multi-organ failure requiring intensive care. The course of infection depends mainly on the individual immune responses and is therefore difficult to predict. The main objective of the present study was to evaluate the association between IgA anti SARS-Cov-2 antibodies and the severity of disease in early COVID-19 infection.
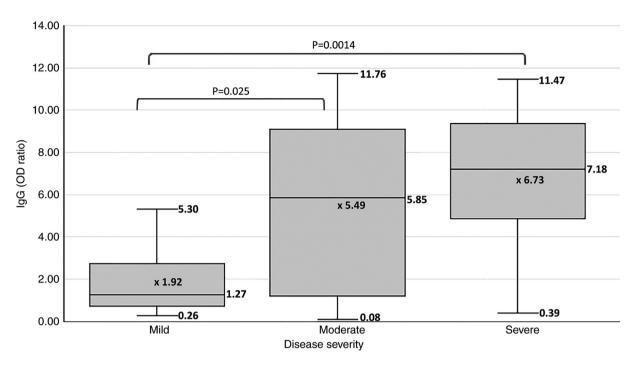
Figure 2. Average OD values of IgG positive samples obtained from patients with mild and moderate forms compared with severe forms of SARS-CoV-2 infection. OD, optical density; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

In addition, the association of age, sex, laboratory variables and duration of symptoms with the presence of IgA antibodies were investigated.

Coronaviruses are recognized as having the largest RNA genomes, which are transcribed by 14 different lengths of open reading frames (1). This aspect increases the chance of mutagenesis and the efficacy of viral replication and decreases the possibility of being eliminated by the immune system (22,23). The spike glycoprotein is a fusion protein responsible for initiating SARS-CoV-2 entry into susceptible cells by binding to cell receptors. The spike comprises two functional subunits that enable viral attachment to the surface of host cells (S1 subunit) and the fusion of the viral envelope and cellular membranes (S2 subunit). Once inside the cell, the virus replicates its RNA genome using the replicase gene (24). Coronavirus recombination serves an important role in viral evolution, favoring the appearance of novel strains with unpredictable consequences for animals and humans. These viruses have an extensive range of natural origins and can cause respiratory, hepatic, enteric and neurologic diseases (25).

IgA antibody constitutes 15-20% of the total immunoglobulins circulating in human serum. IgA is present in blood and mucous secretions. The essential biological function is to protect the body against molecular antigens that could be absorbed, mainly by endocytosis. This immunoglobulin constitutes the first line of defense against infection by blocking viral adhesion to epithelial cell receptors (26). IgA is also involved in pathogen or antigen elimination through an IgA-mediated excretory pathway, characterized by the development of a poly-immunoglobulin receptor-mediated transport of immune complexes (27). The specific humoral responses against SARS-CoV-2 spike-1 receptor-binding domain (RBD) and nucleocapsid proteins indicate that in a significant number of patients, neutralizing IgG and IgA antibodies were detected within 2-3 weeks from the initial onset of symptoms. Subsequently, the levels of neutralizing anti-RBD IgG increased until the fourth week following symptom onset after a plateau, whereas IgA levels decreased by day 28 (28).

Antibodies targeting various virus-encoded proteins are central players in conveying protective immunity against viral infections such as SARS-CoV-2. Antibody detectability has broadly been associated with COVID-19 severity (29); thus, IgA and IgG antibody detection following seroconversion provides data for further understanding the dynamics of the immune response to infection. Accurate interpretation of serology tests depends on antigen specificity. In the present study, anti-spike antibody assays were used, demonstrating high-fidelity performance characteristics. These data can be used to guide epidemiology and seroprevalence studies; however, the specific length of time for which these antibodies stay detectable and in what specific populations remains to be fully elucidated. Although the diagnostic uses of serological testing in the acute phase of illness is limited, it may be useful for identifying symptomatic patients suspected of suffering with long COVID-19. In addition, detection of IgA and IgG antibodies against SARS-CoV-2 spike protein may be a useful tool for evaluating SARS-CoV-2 infection in patients with PCR-positive COVID-19, mainly in asymptomatic cases and in patients with a low viral load (30). In addition, immunoassays are useful in post-infection immunity evaluation and also in analyzing the efficacy of vaccines. In patients with PCR-confirmed SARS-CoV-2 infection in the present study, 28% presented as IgA negative at the time of sampling (>7 days after the onset of symptoms) and these patients presented with an increased age and an increased number of comorbidities. Yu et al (31) detected IgA seroconversion on day 2 and IgM/IgG on day 5 following the onset of symptoms.

Table VI. Summary of SARS CoV-2 RNA-positive hospitalized patients (n=75) blood test results and clinical forms of the disease.

A, SARS CoV-2 IgA-positive patients (n=54)

Parameter	No. of patients
Disease severity	
Mild disease	7
Moderate disease	30
Severe disease	17
SARS CoV-2 IgG status	
IgG-positive	43
IgG-negative	11

B, SARS CoV-2 IgA-negative patients (n=21)

Parameter	No. of patients
Disease severity	
Mild disease	0
Moderate disease	18
Severe disease	3
SARS CoV-2 IgG status	
IgG-positive	4
IgG-negative	17

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ig, immunoglobulin.

Results of a previous study demonstrated that sensitivity and specificity of the commercially available SARS-CoV-2 immunoassays is adequate, ranging between 80-90%, highlighting a suitable diagnostic performance (32).

Carnicelli *et al* (33) demonstrated that IgA and IgG antibody responses are connected, although IgA seroconversion begins at an earlier point. Results of this previous study demonstrated that both IgA and IgG responses are detected in patients with severe COVID-19. Results of the present study demonstrated that disease severity led to significant differences between the two groups of patients. Notably, in the positive IgA anti SARS-CoV-2 group, more patients exhibited a severe form of the disease (31.5%), compared with the IgA negative group (14.3%). As IgA detection was associated with disease severity, this may be attributed to higher immune responsiveness of the respiratory system facing a severe respiratory infection.

In contrast with the findings by Korte *et al* (34), no significant differences were found regarding the presence of IgA antibodies between the two sexes.

Ma *et al* (35), Carnicelli *et al* (33) and Zervouz (36) report that patients with severe COVID-19 present high levels of the IgA antibody. These finding could not be confirmed in the present study, where only the IgG antibody levels were increased in severe COVID-19 cases. The stage and severity of COVID-19 infection are important factors associated with the presence of IgA, but the development of immune complexes, the immune system and genetic characteristics of the patient may interfere with the humoral response against SARS-CoV-2.

The evaluation of hematological and other laboratory parameters is essential in understanding how the immune system works following infection with SARS-CoV-2. Neutrophils account for >50% of the total white blood cell count, being the most important white blood cells that fight viral infection (32). The analysis of the blood tests results revealed no significant differences in the number of leukocytes or neutrophils (percentage or absolute) between positive and negative IgA SARS-CoV-2 groups. Platelet count values were increased in patients in group 1, but the difference was not statistically significant. The mean alanine aminotransferase level was above normal in both groups, without significant variation. The mean C-reactive protein values detected in IgA SARS-CoV-2 positive patients was also increased (68.2 vs. 55.2 mg/l).

Analysis of comorbidities and IgA anti-SARS-CoV-2 revealed that more patients (82.7%) that identified with comorbidities were included in the second group of patients (79.6 vs. 90.5%), including those that were positive for SARS-CoV-2 RNA, but negative for IgA anti SARS-CoV-2. Nonetheless, each patient should be considered as a unique model, where COVID-19 clinical evolution depends on other comorbidities.

Results of a previous study noted that SARS-CoV-2 infection elicits strong humoral immune responses, represented by the production of IgA, IgM and IgG virus-specific antibodies (16). A total of 4 patients in the IgA negative group presented with anti-SARS CoV2 IgG antibodies. These patients, 3 males and 1 female, exhibited advanced ages (89, 67, 101 and 67 years) and multiple comorbidities; all of them developed moderate forms of the disease, without identifying with other clinical features or the laboratory variables analyzed.

The present study has some limitations, including the relatively low number of patients included and the large sampling interval (relative to onset of symptoms) for IgA; however, it provides a basis for future sampled research.

In conclusion, results of the present study suggested that the detection of IgA antibodies against SARS-CoV-2 early in the course of the disease may be associated with severe disease development, but further research is required. Their presence may be influenced by several individual factors, such as age, comorbidities or the time of sampling in association with the onset of symptoms. In addition, data obtained during the present study suggested that patients with severe infection may also present a stronger IgG response. Further studies should focus on increasing the sample number and expanding the median age of the patients evaluated.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

AV and AA designed the study. DA and AV performed the experiments and analyzed the data. CDM, FMR, IFM and DA were responsible for the analysis and discussion of the data. AA and AV drafted the manuscript. CML, GS and AV critically revised the manuscript for important intellectual content and made substantial contributions to the interpretation of data. AV and DA confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted with fully adherence to the international norms of medical ethics, as set out in the Helsinki Declaration. The patients gave their informed written consent for enrollment in the study. The study was approved by the Sfanta Parascheva Infectious Diseases Hospital of Iași Ethics Committee (approval no. 14/04.12.2020).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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