The long-term dynamics of serum antibodies against SARS-CoV-2

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Objective: To analyze the long-term dynamics of antibodies against SARS-CoV-2 and understand the impact of age, gender, and viral load on patients' immunological response.

Methods: Serum samples were obtained from 231 COVID-19 positive patients from Macaé, in Rio de Janeiro state, in Brazil, from June 2020 until January 2021. The production of IgA, IgM, IgG, and IgE against S glycoprotein was analyzed using the S-UFRJ assay, taking into account the age, gender, and viral load.

Results: Analysis of antibody production over seven months revealed that IgA positivity gradually decreased after the first month. Additionally, the highest percentage of IgM positivity occurred in the first month (97% of patients), and declined after this period, while IgG positivity remained homogeneous for all seven months. The same analysis for IgE revealed that almost all samples were negative. The comparison of antibody production between genders showed no significant difference. Regarding the age factor and antibody production, patients aged ≥60 years produced almost twice more IgA than younger ones (17–39 years old). Finally, a relationship between viral load and antibody production was observed only for older patients.

Conclusions: Our work provides an overview of long-term production of antibodies against SARS-CoV-2, suggesting prolonged production of IgA and IgM antibodies for three months and continued IgG production for over seven months. In addition, it identified a correlation between viral load and IgM titers in the older group and, finally, different IgA production between the age groups.

1   **TITLE**

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35   **ABSTRACT**

36   Objective: To analyze the long-term dynamics of antibodies against SARS-CoV-2 and

37   understand the impact of age, gender, and viral load on patients' immunological

38   response.

39   Methods: Serum samples were obtained from 231 COVID-19 positive patients from

40   Macaé, in Rio de Janeiro state, in Brazil, from June 2020 until January 2021. The

41   production of IgA, IgM, IgG, and IgE against S glycoprotein was analyzed using the

42   S-UFRJ assay, taking into account the age, gender, and viral load.

43   Results: Analysis of antibody production over seven months revealed that IgA

44   positivity gradually decreased after the first month. Additionally, the highest

45   percentage of IgM positivity occurred in the first month (97% of patients), and declined

46   after this period, while IgG positivity remained homogeneous for all seven months. The

47   same analysis for IgE revealed that almost all samples were negative. The comparison

48   of antibody production between genders showed no significant difference. Regarding

49   the age factor and antibody production, patients aged ≥60 years produced almost twice

50   more IgA than younger ones (17–39 years old). Finally, a relationship between viral

51   load and antibody production was observed only for older patients.

52   Conclusions: Our work provides an overview of long-term production of antibodies

53   against SARS-CoV-2, suggesting prolonged production of IgA and IgM antibodies for

54   three months and continued IgG production for over seven months. In addition, it

55   identified a correlation between viral load and IgM titers in the older group and, finally,

56   different IgA production between the age groups.

57   **KEYWORDS**

58   S-UFRJ; COVID-19 patients; SARS-CoV-2-specific antibodies; spike glycoprotein

59

60   **INTRODUCTION**

61   COVID-19 is caused by severe acute respiratory syndrome coronavirus 2

62   (SARS-CoV-2), whose first cases were reported in Wuhan, in Hubei Province, in

63   China, in December 2019, subsequently spreading all over the world. The COVID-19

64   pandemic caused considerable mortality worldwide, with moderate and severe cases.

65   However, approximately 80% of all cases reported are from mildly symptomatic

66   patients[1]. The death rate in Brazil was very high, at 119.9 per 100 thousand individuals,

67   and the mean age of non-survivors was over 70[2]. However, some municipalities, such

68   Macaé, had the one of lower mortality indices of the northeastern of Rio de Janeiro in

69   Brazil in which, the first COVID-19 case was recorded on March 24th, 2020[3].

70   SARS-CoV-2 is a positive-sense, single-stranded RNA virus with four structural

71   proteins: spike (S) glycoprotein, envelope (E) protein, membrane (M) protein

72   nucleocapsid (N) protein and it also has 16 nonstructural proteins (nsp1−16)[4].

73   Nonetheless, the S glycoprotein is critical in the process of SARS-CoV-2 invading host

74   cells, binding to them via the angiotensin-converting-enzyme-2 (ACE-2) receptor and

75   mediating membrane fusion and virus entry[5]. The S glycoprotein also plays a crucial

76   role in elucidating the immune response during disease progression, and is known to

77   represent a major target for neutralizing antibodies, thus making it a key antigen for the

78   development of specific and sensitive tests to evaluate the antibody response to SARS-

79   CoV-2[6,7]. Previous study demonstrated the importance of S glycoprotein as a target of

80   immunological response, in which the IgG produced is directed to the SARS-CoV-2

81   receptor-binding domain (RBD) accounts for half of S protein-induced antibody

82   responses[8]. Many types of enzyme-linked immunosorbent assays (ELISAs) based on S

83   glycoprotein have been previously developed, showing minimal cross-reactivity with

84   sera against circulating "common cold" coronaviruses (but with some cross-reactivity

85   with SARS and MERS-COV antisera)[7]. These serological methods have been used to

86   investigate the poorly understood immunological response of the host and have been

87   used to better comprehend how the virus generates an inflammatory stage, and how it

88   induces serological antibody production[9].

89   However, despite having many studies on the production of antibodies, few are

90   carried out analyzing the long-term dynamic of antibody production against SARS-

91   CoV-2, some of them, have revealed that, high levels of antibodies could be associated

92   with the abnormal inflammatory response, disease severity and with the presence of

93   comorbidities. It is known that the outcome of severe SARS-CoV-2 infection varies

94   widely and, the age factor is important since the majority of younger patients

95   experience mild disease, while older patients display severe cases[10]. Some studies have

96   presented evidence that antibody response may be different in children, adolescents,

97   and adults, which potentially influences the clinical manifestations[11]. However, the

98   causes of differential severity between ages remain unclear, presumably due to

99   differences in the elicited immune responses[12]. Furthermore, gender is an important

100   factor to be analyzed. Males are over-represented among patients with severe

101   disease[1,12]. Finally, studies focusing on viral kinetics and antibody responses during the

102 period of infection, as well as on the monitoring of COVID-19 patients for longer

103 periods, are limited in the literature[6,13].Therefore, to better understand the

104 immunological response to SARS-CoV-2, numerous studies have been performing, and

105 strategies have been establishing aiming to prevent the further spread of COVID-19

106 and also could contribute to the development of new therapeutic approaches [14,15,16].

107 Then, in order to gain a better understanding of the host immune response, the

108 IgA, IgM, IgG, and IgE production was monitored in the serum for seven months in

109 mildly symptomatic patients, using the S-UFRJ assay against S glycoprotein. The

110 patients were randomly selected based in a polymerase chain reaction (PCR)-confirmed

111 COVID-19 infection and were grouped corresponding to how long after the first

112 COVID-19 symptoms the serum was obtained (from first to seventh month).

113 Additionally, the samples were analyzed taking account of the age and separated in

114 three groups (younger, adults and older), gender (men and women), and the viral load

115 correlating this measurement with semi-quantitative levels of IgA, IgM, IgG and IgE.

116 **METHODOLOGY**

117 ***Inclusion and exclusion criteria and sample collection***

118 Serum samples were obtained from 231 positive volunteers (RT-qPCR) to

119 COVID-19 which, were mildly symptomatic, then, the samples were grouped taking

120 into account how long after the first COVID-19 symptoms the serum was obtained. The

121 231 serum samples covered approximately seven months, from June 2020 to January

122 2021 (n=34;17;45;22;16;72;25, respectively to first until seventh month).   The

123 inclusion criteria were a RT-qPCR positive diagnosis of COVID-19 and acceptance of

124   a term of consent received written to use the information informed from the study

125   participants. The exclusion criteria were a negative COVID-19 test, lack of willingness

126   or ability to provide the informed consent, lack of an appropriate legal guardian or

127   representative, or other medical contraindications for donating blood and

128   nasopharyngeal samples. Pregnant women, children, and adolescents under 17 years

129   old were also excluded.

130   Nasopharyngeal swabs (infused in 2 mL of Hanks' balanced salt solution) and

131   blood samples were collected in the Trial Center for COVID-19 patients (CTC) and the

132   real-time polymerase chain reaction (RT-qPCR)[3] and ELISA test with AC-purified S

133   protein (S-UFRJ ELISA)[8] were performed at the NUPEM-UFRJ Institute and the

134   acceptance of a term of consent for the use of information. The study was approved by

135   the Comitê de Ética em Pesquisa (Research Ethics Committee, Brazilian Ministry of

136   Health: approval number 32868720.4.0000.5699). All research was performed in

137   accordance with the relevant guidelines and regulations.

138   ***RT-qPCR and viral load estimation***

139   For viral RNA extraction, magnetic beads (Magmax Magnetic Kit −

140   Thermofisher) were used following the manufacturer's instructions. RT-qPCR

141   reactions for the identification of SARS-CoV-2 positive samples were performed using

142   a TaqMan™ approach, as previously described by Corman[17] or the CDC (Prevention

143   CfDCa) protocols. RT-qPCR tests were considered positive when two regions of

144   SARS-CoV-2 genomes were amplified. All RT-qPCR assays were performed in a

145   QuantStudio™ 3 Real-Time PCR System (Applied Biosystems).

146   Positivity for SARS-CoV-2 infection was determined by RT-qPCR of

147   nasopharyngeal swabs using the Allplex™ 2019-nCoV Assay kit (Seegene Inc.), with

148   modifications (supplementary material). Viral loads were estimated based on a

149   standard curve with serially-diluted plasmid-carrying cloned viral genes, as described

150   by Chung et al.[18], with modifications, including normalization with endogenous

151   RNAse P RNA (Supplementary Figure 1A, B).

152   ***S-UFRJ ELISA for anti-S IgM, IgG, IgE, and IgA detection***

153   The S-UFRJ ELISA was performed using the trimeric spike protein of SARS-

154   COV-2 produced in stable recombinant HEK293 cells as described in Alvim et

155   al.(2022) [7] and it was provided by the Cell Culture Engineering Laboratory - LECC

156   from the Universidade Federal do Rio de Janeiro – UFRJ. The secondary antibodies

157   concentration used was 1:200 goat anti-human IgM (Fc) HRP-labeled antibody (Sigma,

158   #MFCD00162459), anti-human IgG (Fc) HRP-labeled antibody (Sigma,

159   #SAB3701282), anti-human IgE (Fc) HRP-labeled antibody (Invitrogen, #A18793),

160   and anti-human IgA (Fc) (Invitrogen, #PA1-74495), incubated for 1 hour at room

161   temperature. The reaction was read at 450 nm in a Thermo Scientific Multiskan Sky

162   microplate reader. The results were expressed either as optical density units (OD) or as

163   the ratio of sample OD/cut-off value. The pre pandemic healthy donors (blood samples

164   before 2019) were selected as negative controls. Then, the cut-off was made by means

165   of O.D. of the samples plus 3 times the O.D. standard deviation [7].

166   ***Statistical analysis***

167   The entire data analysis was performed with the GraphPad Prism 8 (GraphPad
168   Software Inc., USA) software. The data were expressed as mean ± standard error of the
169   mean. The normal distribution was verified by the Kolmogorov-Smirnov test. The
170   Mann-Whitney test was used for comparison between two groups and the Kruskal-
171   Wallis test was used for comparison of more than two groups, followed by Dunn's post
172   hoc test. To assess the relationship between viral load and antibody production by linear
173   regression, the Spearman's test was used. Variance in the groups were analyzed by
174   coefficient of variation (CV). Differences were considered to be significant when P <
175   0.05.

176

177   **RESULTS**

178   ***Overview of features***

179   The median age of the SARS-CoV-2 patients was 52.5 years old, and 54.9%
180   were women while 45.1% were men. The chronic diseases characterizing them were as
181   follows: 1.3% asthmatic bronchitis, 18.6% chronic cardiac disease, 4.8% diabetes, and
182   32.5% obesity (Table 1).

183   ***Serological analysis of IgA, IgM, IgG, and IgE against SARS-CoV-2***

184   To understand the long-term response of antibodies against the entire spike
185   glycoprotein, serological tests were performed with the serum of mildly symptomatic
186   patients in the post disease period and analyzed semi-quantitatively for IgA, IgM, IgG,
187   and IgE positivity. Antibody production was monitored every month for the different
188   patients, grouped taking into account the exact period of the time after the first

189   symptoms that the serum sample was taken, covering a total of seven months. The

190   samples with OD values under the cut-off were considered as not producing

191   immunoglobulins.

192   Almost all the patients were IgA positive for SARS-CoV-2, but a small fraction,

193   though positive in the RT-qPCR, were IgA negative (11.4%) in the first month. The

194   IgA positivity among the patients decreased in the following months, showing a

195   percentage of 64.7% in the second month, and gradually declining until reaching 53.2%

196   in   the   seventh   month   (1.38nm/0.40;0.72nm/0.50;0.68nm/0.65;0.77nm/0.55;

197   0.53nm/0.44;0.68nm/0.50;0.73nm/0.52, respectively to first until seventh month;

198   mean/coefficient of variance). The highest OD values were observed in the samples

199   from the first month and a decrease in these values was observed until the seventh

200   month after the first onset of symptoms. However, minor group of patients are positive

201   to IgA among the months. (Figure 1A).

202   Analyzing the IgM production profile, the majority of samples were IgM positive

203   and only one sample was IgM negative in the first month. As expected, IgM positivity

204   declined in the following months (0.67nm/0.42; 0.45nm/0.60; 0.44nm/0.52;

205   0.34nm/0.53 0.24nm/0.58; 0.23nm/0.50 and 0.26nm/0.45, respectively to first until

206   seventh month; mean/coefficient of variance), and the percentage of IgM positivity

207   decreased gradually from 97% in the first month to 25% in the seventh month(Figure

208   1B).

209   IgG positivity remained homogeneous for all seven months. However, the mean

210   OD, representing the semi-quantitative IgG values, was very high in the first month of

211   analysis   and   perceptibly   decreased   over   subsequent   months   (2.31nm/0.18;

212   2.13nm/0.31; 2.13nm/0.21; 2.13nm/0.19; 1.77nm/0.28; 1.61nm/0.32 and 2.01nm/0.24,

213   respectively to first until seventh month; mean/coefficient of variance), with a 1.35

214   times reduction in the fifth month and a 1.45 times reduction in the sixth month.

215   Interestingly, the average of semi-quantitative IgG values increased again in the

216   seventh month. It is worth noting that some patients were not IgG positive in the first,

217   second, and sixth months (Figure 1C).

218   IgE production was under the cut-off values for almost all the samples analyzed

219   during the study. However, interestingly, of the only six IgE positive samples, two were

220   positive in the first month, one in the third, and, finally, two in the seventh month

221   (0.16nm/0.31;0.13nm/0.19;0.13nm/0.24;0.14nm/0.16;0.14nm/0.23;0.13nm/0.15;0.14

222   nm/0.24, respectively to first until seventh month; mean/coefficient of variance).

223   Notably, the OD values for these six IgE positive patients were remarkably low when

224   compared to the values obtained for IgM, IgG, and IgA, and only two samples, one of

225   them in the first and the other in the third month, showed higher OD values compared

226   to all the IgE positive samples (Figure 1D).

227

228   ***Gender-related analyses of IgA, IgM, IgG, and IgE production against SARS-CoV-***

229   ***2***

230   Subsequently, the data were analyzed comparing antibody production between

231   genders over seven months. The IgA positivity profile for the comparison of women

232   and men patients was very similar for every month analyzed, and the previously noted

233   decrease in IgA production was observed throughout the seven months for both

234   genders. Nonetheless, it is interesting to note the dispersion in OD in the first month

235   (1.39nm/0.39 and 1.38nm/0.43 respectively, to women and men; mean/coefficient of

236   variance), when women showed an elevated variation in IgA OD values in comparison

237   to the values registered for men, which were higher and less dispersive (Figure 2A).

238   IgM positivity did not show significant differences between the genders for most

239   of the months. However, when comparing women and men from the fourth month after

240   the onset of symptoms (0.26nm/0.30 and 0.45nm/0.51 respectively, to women and men;

241   mean/coefficient of variance) there was a difference, and lower production was

242   observed for women. Regarding the intersample variation, a difference between

243   genders was noted, with female patients presenting more disperse and lower OD values

244   than male patients (Figure 2B).

245   The most homogeneous antibody production over the study period was observed

246   for IgG, and no discrepant differences between men and women were observed at all,

247   both for absolute production and for the individual dispersion in the semi-quantitative

248   analysis (Figure 2C).

249   Comparing IgE production between genders, it is possible to note that of the six

250   IgE positive samples, four samples were from women and two were from men (Figure

251   2D).

252   ***Age-related analyses of IgA, IgM, IgG, and IgE production against SARS-CoV-2***

253   To analyze antibody production among the different age groups, the samples

254   were divided into three groups (17-39, 40-59, and >60 years old), and the analysis were

255   performed using only the data from the first month, because all the antibodies tested

256   (IgA, IgM, IgG, and IgE) were produced at high levels mainly in the first month. IgA

257   was produced after the onset of symptoms in all age groups (1.07nm/0.48; 1.41nm/0.31

258   and 1.86nm/0.23, respectively, to 17-39, 40-59 and >60 years old mean/coefficient of

259   variance). Interestingly, most samples that tested negative for IgG were from the young

260   age group, while all the patients from the older group were IgA positive (Figure 3).

261   Notably, the average OD value was significantly different between the groups. These

262   data indicate an increase in anti-SARS-CoV-2 IgA production with age, as the group

263   containing older individuals produced almost 50% more IgA than the group containing

264   the younger patients (Figure 3A).

265   Semi-quantitative production of both IgM and IgG presented a homogeneous

266   distribution among the age groups. A subtle, non-significant increase in IgM production

267   was observed in the older age group. The only IgM negative sample belonged to the

268   40-59 age group; while the only IgG negative patient was in the 17-39 age group

269   (Figure 3B, C).

270   The IgE analysis showed positivity in only one sample from the 40-59 age group

271   and one sample from the ≥60 age group, as shown in the Figure 3D.

272   ***Analysis of SARS-CoV-2 viral load and IgA, IgM, IgG, and IgE production***

273   In order to investigate a possible correlation between viral load and antibody

274   production, the samples from the first month were subjected to the non-parametric

275   Spearman's rank correlation analysis. The Spearman's coefficient (ρ) indicates a

276   relationship between the production of each immunoglobulin and the viral load of the

277   respective patient. This analysis revealed that there was a low or inexistent positive

278   correlation between viral loads and IgA, IgG, and IgE levels (ρ−values: 0.0508; 0.1398;

279   0.1782, for IgG, IgA, and IgE, respectively). Although not robust, a slightly increased

280   positive correlation was observed for IgM production ($\rho = 0.2202$) (Figure 4A, B, C,

281   D).

282   Next, an analysis was performed considering the genders (men and women) and

283   viral load. Once again, the data revealed that there were no differences between men

284   and women in the relationship between IgA, IgG, and IgE and viral load (Figure S2A,

285   C, D). The ρ coefficients considering the IgM levels once again presented a moderate

286   correlation (ρ= 0.3261) for women. Men patients didn't show correlation (ρ= -0.0090)

287   between viral load and IgM, possibly reflecting the fact that higher viral loads induce

288   higher IgM production only in women (Figure S2B).

289   Finally, the correlation analysis was performed taking into account the age

290   groups of the COVID-19 patients. Again, no correlation was evident between the viral

291   loads and levels of IgG, IgA, and IgE (Figure S3). Interestingly, the ρ coefficients

292   considering the IgM levels once again presented a statistical correlation (Figure S3B).

293   **DISCUSSION**

294   The current study followed the long-term dynamics of semi-quantitative serum

295   levels of IgA, IgM, IgG, and IgE against S-glycoprotein from patients presenting mild

296   COVID-19 symptoms over seven months after infection. It is widely known that the

297   antibody response is very important to eliminate viruses and to prevent new

298   infections[19]. Previous studies have reported high IgA and IgM production in the first

299   month after the onset of COVID-19 symptoms, as well as a marked production decrease

mainly in IgM and IgA after the first month, accompanied by continued production of IgG[20,21,22]. This study presents a different scenario, which IgA production persists in more than 60% of patients during the second and third months after the onset of symptoms for patients with mild symptoms. Regarding IgG, its prolonged production was observed here as in previous studies that have shown the involvement of this antibody class to prevent reinfection. Indeed, one previous study revealed that detectable IgG levels persisted for more than two years in SARS-CoV and MERS-CoV infected patients[23]. The present study showed continued IgG production over the seven months, and a slight decrease was observed for the last months indicating that from fifth month after infection, the IgG production are not produced for long periods in high quantities. In this study, some positive samples of IgM was observed after the first month, generally, in a clinical diagnosis, a high level of IgM antibody is considered as an indicator of acute infection or recent reinfection. However, in this study, the patients did not report symptoms of COVID-19 or did not perform a RT-qPCR more the once time during the seventh months analyzed, indicating that they may not had a reinfection. However, this hypothesis cannot be discarded, because some patients are asymptomatic[24]. Another two hypothesis which could explain positive samples of IgM after first month is concerning a persistent SARS-CoV-2 infection in some patients, culminating in a persistent positive production of IgM antibodies as reported by some authors[25]. Finally, Fanfan et al., 2021 reported, in patients infected once time by SARS-CoV-2, who were analyzed IgM titers and followed up for one year, a persistent positive IgM production (12.80%)[26]. Then, it is not reliable and proper to assist clinical diagnosis of infection only by IgM detecting results. Moreover, the reason underlying

323   such a long duration of SARS-CoV-2 IgM in some patients still unclear and needs

324   additional study.

325   Taking gender into account, previous research identified that there were more

326   women with high levels of IgG relative to men with a severe COVID-19 status. Here,

327   in this study analyzing mild cases, no difference between genders was observed for IgG

328   and, only a subtle difference was observed for IgM in the fourth month, indicating that

329   severity could to influence in the dynamic of the humoral response among genders;

330   however, with respect to mild cases gender may not have an influence[27].

331   Interestingly, in this study, taking account of viral load, IgM, and gender,

332   revealed that only women produced IgM according to their viral load, even though,

333   men did not show correlation between IgM and viral load, they presented the high IgM

334   production comparing to women. This fact could be explained by a lower viral load

335   that women were exposure during this study. In another study performed in Macae,

336   which analyzed only viral load and gender, and did not consider IgM titers, it was

337   observed that, there were a higher percentage of men at working efforts compared to

338   women that showed high viral loads. Men make up the main workforce in the essential

339   activities of offshore companies from Macaé involved in oil exploration of the Brazilian

340   coast, which were not interrupted during the quarantine. Taken together, these results

341   suggest that actively working men were more exposed to virus, showing a high

342   production of IgM than women.

343   The viral load analysis comparing age and IgA, IgM, IgG, and IgE titers showed a

344   positive correlation only between IgM production and viral load in the older age group

345   (>60 years old). IgM normally occurs in high quantities during the first days of an

acute infection, playing an important role in initiating the host humoral defense mechanism. Due to its early role in the humoral immune response, it is conceivable that IgM levels exhibit a positive correlation with viral loads, as occurs with the older COVID-19 patients in this study[28]. In contrast, the younger patients do not exhibit this correlation, suggesting that initial humoral immune responses to COVID-19 differ depending on the age, but not on the gender of the patient, and, it may be that an immune cellular response could be more activated being more effective than a humoral response to eliminate the SARS-CoV-2 virus in the younger group. Nonetheless, it is important to understand the pathophysiologic basis underlying the different disease manifestations and outcomes of SARS-CoV-2 infection in patients from all age groups.

Our findings suggest that the differences in the clinical manifestations of COVID-19 in different age groups might be partly due to age-related immune responses. Here, we observed significantly increased titers not for IgG but for IgA levels, when comparing the age groups. Thus, the older (>60 years old) patients showed a higher level of IgA than the younger ones. Similarly to our results, comparing IgG titers between ages revealed the same pattern of antibody production[28].

Nevertheless, the exact mechanisms inherent in the different SARS-CoV-2 immune responses based on age remain unclear. It may be that adolescents and young adults have attenuated immune responses that culminate in more tolerance to the virus. On the other hand, some other studies have suggested a more active innate immune response in adolescents and young adults than in older adults, which culminates in efficient virus elimination and low levels of antibodies[29,30,31].

In addition, some research has proposed that trained immunity may develop a function and that innate immune memory generated by other vaccines may confer a nonspecific protective effect against SARS-CoV-2[32]. Interestingly, our data revealed that IgA and IgM production does not present a substantial correlation to viral load, indicating that viral load does not induce a high humoral response to eliminate the virus and that the innate response could perhaps develop a key role in SARS-CoV-2 infection. Thus, these data indicate that more investigations are needed to better understand the age dynamic of the cellular and humoral immune response.

Some other previous studies exploring serological assays directed at SARS-CoV-2, but analyzing only the IgG and total antibody titers, observed higher levels in the serum of severe patients than in non-severe ones in the older age group, which was associated with the establishment of a critical inflammatory process and hospitalization[28]. These data correlate an elevated humoral response with the presence of previous chronic diseases in older patients.

Zervou et al., 2021 conducted an study analyzing IgA to SARS-CoV-2 and concluded that IgA levels were highest in patients with a severe and critical illness. Here, the elevated IgA production was observed in a mild symptomatic older age group and could be associated with an increase in comorbidities, such as obesity, diabetes, and chronic cardiac diseases, among others[33]. These comorbidities, especially obesity, were described to have a high baseline of pro-inflammatory cytokines and SARS-CoV-2 infection could have a stimulatory effect on humoral response, which culminates here with the observation of high IgA production in these patients[34,35]. It may be that the humoral responses in the older group could be more activated because

392 some comorbidities such as diabetes and chronic cardiac diseases are prevalent in this

393 group, which are known for their influences on the cellular immune responses,

394 including impairing phagocytic cell capacities and inducing high levels of ACE2

395 receptors[36].

396 Analyzing this hypothesis, the comorbidities data in the age groups revealed that

397 the percentage of obesity is high in all age groups (19.6% in adolescents and young

398 adults, 45.5% in adults, and 31.2% in seniors) and this probably was not the main

399 factor that influenced the high IgA production. However, comparing chronic cardiac

400 diseases and diabetes and the age groups, it was possible to note that these diseases

401 were more prevalent in the older group than in other age groups and may contribute to

402 the high levels of IgA observed here. Lastly, more investigation is currently needed to

403 better understand the age-related dynamics of the cellular and humoral immune

404 responses elicited against SARS-CoV-2.

405 Regarding IgE, the most common responses to viruses are mediated by IgM,

406 IgG, and IgA. Non expected, type-2 immunity, common for anti-helminth defense

407 through IgE, was observed in severe COVID-19 patients, who showed increased levels

408 of IgE during the first 10 days of disease[37]. Here, IgE production was also analyzed

409 during the seven months after the onset of symptoms in mild COVID-19 patients. Our

410 data revealed that only six patients were IgE positive, showing slight production of

411 these molecules. Indicating that more researches are needed to disclose the function of

412 the IgE in the dynamic of antibodies production of the COVID-19 disease.

413 We recognize that there are some limitations to this study. We used 231 serum

414 samples from RT-qPCR-confirmed COVID-19 patients; however, serum samples were

415   not available every month for each patient during the seven months. Nevertheless, we

416   believe this study provides valuable information regarding the serological analysis of

417   COVID-19 considering the parameters of gender, age, viral load, comorbidities, and

418   duration of responses for IgM, IgG, IgA, and IgE antibodies in mildly symptomatic

419   patients.

420   In summary, our study provides additional evidence to help guide the use of

421   serologic testing in the diagnosis and management of COVID-19 infection.

422   Additionally, the data presented here can help in a correlation between s-antibodies to

423   SARS-Cov-2 and the emerging knowledge for the development of immunotherapeutics

424   and vaccines. However, further studies of antibody persistence in SARS-CoV-2,

425   reinfection and long COVID are warranted.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interests.

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REFERENCES

[1] Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA 2020;323:1239–42. https://doi.org/10.1001/jama.2020.2648.

459   [2] Sanchez MN, Moura EC de, Moreira JR, Lima RT de S, Barreto IC de HC, Pereira

460   CC de A, et al. Mortalidade por COVID-19 no Brasil : uma análise do Registro Civil

461   de óbitos de janeiro de 2020 a fevereiro de 2021. Mortality from COVID-19 in Brazil :

462   analysis of death's civil registry from 2020 January to 2021 February 2021.

463   https://doi.org/10.1590/SciELOPreprints.2012.

464   [3] Prefeitura Municipal de Macaé n.d. https://macae.rj.gov.br/saude (accessed April

465   21, 2022).

466   [4]   Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication,

467   and pathogenesis. J Med Virol 2020;92:418–23. https://doi.org/10.1002/JMV.25681.

468   [5] Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike

469   glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with

470   SARS-CoV. Nat Commun 2020;11. https://doi.org/10.1038/s41467-020-15562-9.

471   [6]   To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal

472   profiles of viral load in posterior oropharyngeal saliva samples and serum antibody

473   responses during infection by SARS-CoV-2: an observational cohort study. Lancet

474   Infect Dis 2020;20:565–74. https://doi.org/10.1016/S1473-3099(20)30196-1.

475   [7] Alvim RGF, Lima TM, Rodrigues DAS, Marsili FF, Bozza VBT, Higa LM, et al.

476   Development and large-scale validation of a highly accurate SARS-COV-2

477   serological test using regular test strips for autonomous and affordable finger-prick

478   sample collection, transportation, and storage 2021:2020.07.13.20152884.

479   https://doi.org/10.1101/2020.07.13.20152884.

480   [8] Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an

481   inactivated vaccine candidate for SARS-CoV-2. Science 2020:eabc1932.

482   https://doi.org/10.1126/science.abc1932.

483   [9] Taefehshokr N, Taefehshokr S, Hemmat N, Heit B. Covid-19: Perspectives on

484   Innate Immune Evasion. Frontiers in Immunology

485   2020;11.https://doi.org/10.3389/fimmu.2020.580641.

486   [10]] Brodin P. Why is COVID-19 so mild in children? Acta Paediatr Int J Paediatr

487   2020;109:1082–3. https://doi.org/10.1111/apa.15271.

488   [11] Weisberg SP, Connors TJ, Zhu Y, Baldwin MR, Lin WH, Wontakal S, et al.

489   Distinct antibody responses to SARS-CoV-2 in children and adults across the

490   COVID-19 clinical spectrum. Nat Immunol 2020 221 2020;22:25–31.

491   https://doi.org/10.1038/s41590-020-00826-9.

492   [12] Feitosa NM, da Costa Rodrigues B, Petry AC, Nocchi KJCV, de Moraes

493   Brindeiro R, Zilberberg C, et al. Molecular testing and analysis of disease spreading

494   during the emergence of COVID-19 in Macaé, the Brazilian National Capital of Oil.

495   Sci Rep 2021;11:1–13. https://doi.org/10.1038/s41598-021-99475-7.

496   [13] Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex

497   differences in immune responses that underlie COVID-19 disease outcomes. Nat

498   2020 58878372020;588:315–20. https://doi.org/10.1038/s41586-020-2700-3.

499   [14] Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody

500   responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020b;26:845–8.

501   https://doi.org/10.1038/s41591-020-0897-1.

502   [15] Okba NMA, Müller MA, Li W, Wang C, Geurtsvankessel CH, Corman VM, et

503   al. Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody Responses

504   in Coronavirus Disease Patients. Emerg Infect Dis 2020;26:1478.

505   https://doi.org/10.3201/EID2607.200841.

506   [16] Yang HS, Costa V, Racine-Brzostek SE, Acker KP, Yee J, Chen Z, et al.

507   Association of Age with SARS-CoV-2 Antibody Response. JAMA Netw Open

508   2021;4:1−12. https://doi.org/10.1001/jamanetworkopen.2021.4302.

509   [17] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al.

510   Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro

511   Surveill 2020;25:2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

512   [18] Chung YS, Lee NJ, Woo SH, Kim JM, Kim HM, Jo HJ, Park YE, Han MG.

513   Validation of real-time RT-PCR for detection of SARS-CoV-2 in the early stages of

514   the COVID-19 outbreak in the Republic of Korea. Sci Rep. 2021;11(1):14817.

515   https://doi.org/10.1038/s41598-021-94196-3.

516   [19] Chvatal-Medina M, Mendez-Cortina Y, Patiño PJ, Velilla PA, Rugeles MT.

517   Antibody Responses in COVID-19: A Review. Front Immunol 2021;12:633184.

518   https://doi.org/10.3389/fimmu.2021.633184.

519   [20] Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. Diagnostic value and dynamic

520   variance of serum antibody in coronavirus disease 2019. Int J Infect Dis 2020;94:49−

521   52. https://doi.org/10.1016/j.ijid.2020.03.065.

522   [21] Aita A, Basso D, Cattelan AM, Fioretto P, Navaglia F, Barbaro F, et al. SARS-

523   CoV-2 identification and IgA antibodies in saliva: One sample two tests approach for

524   diagnosis. Clin Chim Acta 2020;510:717–22.

525   https://doi.org/10.1016/j.cca.2020.09.018.

526   [22] Sterlin D, Mathian A, Miraya M, Mohr, Audrey et al. IgA dominates the early

527   neutralizing antibody response to SARS-CoV-2 virus. Sci Transl Med.

528   2021;13(577):1–10. https://doi.org/10.1126/scitranslmed.abd2223.

529   [23] Zhou W, Xu X, Chang Z, Wang H, Zhong X, Tong X, et al. The dynamic

530   changes of serum IgM and IgG against SARS-CoV-2 in patients with COVID-19. J

531   Med Virol 2021;93:924–33. https://doi.org/10.1002/jmv.26353.

532   [24] Pozzobon APB, Petry AC, Zilberberg C, De Barros CM, Da silva JLN, Feitosa

533   NM, et al. Schools reopening and the COVID-19 pandemic: a case study from Macaé,

534   Rio de Janeiro, Brazil. An Acad Bras Cienc. 2022; 94(Suppl. 3): e20211361.

535   https://doi.org/10.1590/0001-3765202220211361.

536   [25] Choudhary MC, Crain CR, Qiu X, Hanage W, Li JZ. Severe Acute Respiratory

537   Syndrome Coronavirus 2 (SARS-CoV-2) Sequence Characteristics of Coronavirus

538   Disease 2019 (COVID-19) Persistence and Reinfection. Clin Infect Dis. 2022 Jan

539   29;74(2):237-245. https://doi.org/10.1093/cid/ciab380.

540   [26] Fanfan Z; Mengjun W; Jinbiao W; Jianyu L; Guoyun H; Lin W. Over 1-year

541   duration and age difference of SARS-CoV-2 antibodies in convalescent COVID-19

542   patients. J Med Virol. 2021; 93(12): 6506-6511. https://doi.org/10.1002/jmv.27152

543   [27] Zeng F, Dai C, Cai P, Wang J, Xu L, Li J, et al. A comparison study of

544   SARS-CoV-2 IgG antibody between male and female COVID-19 patients: A possible

545   reason underlying different outcome between sexes. J Med Virol

546   2020:10.1002/jmv.25989. https://doi.org/10.1002/jmv.25989.

547   [28] Yang HS, Costa V, Racine-Brzostek SE, Acker KP, Yee J, Chen Z, et al.

548   Association of Age with SARS-CoV-2 Antibody Response. JAMA Netw Open

549   2021;4:1–12. https://doi.org/10.1001/jamanetworkopen.2021.4302.

550   [29] Elahi S. Neonatal and Children's Immune System and COVID-19: Biased

551   Immune Tolerance versus Resistance Strategy. J Immunol 2020;205:1990–7.

552   https://doi.org/10.4049/jimmunol.2000710.

553   [30] Zimmermann P, Curtis N. Coronavirus infections in children including COVID-

554   19: An overview of the epidemiology, clinical features, diagnosis, treatment and

555   prevention options in children. Pediatr Infect Dis J 2020;39:355–68.

556   https://doi.org/10.1097/INF.0000000000002660.

557   [31] Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, et al. Severe

558   Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and

559   adolescents: A systematic review. JAMA Pediatr 2020;174:882–9.

560   https://doi.org/10.1001/jamapediatrics.2020.1467.

561   [32] Fidel PL, Noverr MC. Could an unrelated live attenuated vaccine serve as a

562   preventive measure to dampen septic inflammation associated with covid-19

563   infection? MBio 2020;11:1–4. https://doi.org/10.1128/mBio.00907-20.

564   [33] Zervou FN, Louie P, Stachel A, Zacharioudakis IM, Ortiz-Mendez Y, Thomas K,

565   Aguero-Rosenfeld ME. SARS-CoV-2 antibodies: IgA correlates with severity of

566   disease in early COVID-19 infection. J Med Virol. 2021; 93:5409–5415.

567   https://doi.org/10.1002/jmv.27058

568   [34] Ejaz H, Alsrhani A, Zafar A, Javed H, Junaid K, Abdalla AE, et al. COVID-19

569   and comorbidities: Deleterious impact on infected patients. J Infect Public Health

570   2020;13:1833–9. https://doi.org/10.1016/j.jiph.2020.07.014.

571   [35] Kulcsar KA, Coleman CM, Beck SE, Frieman MB. Comorbid diabetes results in

572   immune dysregulation and enhanced disease severity following MERS-CoV

573   infection. JCI Insight 2019;4. https://doi.org/10.1172/jci.insight.131774.

574   [36] Rao S, Lau A, So HC. Exploring Diseases/Traits and Blood Proteins Causally

575   Related to Expression of ACE2, the Putative Receptor of SARS-CoV-2: A Mendelian

576   Randomization Analysis Highlights Tentative Relevance of Diabetes-Related Traits.

577   Diabetes Care 2020;43:1416–26. https://doi.org/10.2337/dc20-0643.

578   [37] Lucas C, Wong P, Klein J, Castro TBR, Silva J, Ellingson MK, et al.

579   Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature

580   2020;584:463–9. https://doi.org/10.1038/s41586-020-2588-y.Longitudinal.

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583   **LEGENDS**

584   **Figure 1: The long-term quantification of IgA (A), IgM (B), IgG (C), and IgE (D)**

585   **by S-UFRJ-ELISA assays.** The serum from 231 RT-qPCR-positive mildly

586   symptomatic SARS-CoV-2 patients was measured for 7 months. Each dot represents

587   an individual sample (n=34;17;45;22;16;72;25, respectively to first until seventh

588   month).   The results were analyzed using the Kruskall-Wallis test followed by Dunn's

589   post hoc test. The representative difference between: the first and subsequent months

590   (*); the second and subsequent months (#); the third and subsequent months (o); the

591   fourth and subsequent months (□), and the fifth and subsequent months (●). The

592   symbols indicating statistical significance were [*,#,o,●] $p<0.05$; [**,##,□□] $p<0.01$;[***]

593      $p<0.001$; [****,oooo] $p<0.0001$.

594   **Figure 2: The gender comparative analysis of IgA (A), IgM (B), IgG (C), and IgE**

595   **(D) by S-UFRJ-ELISA assays.** The serum 231 RT-qPCR-positive mildly

596   symptomatic SARS-CoV-2 patients was measured for 7 months. Each dot represents

597   an individual sample (first month n = 22 and 12; second month: n = 9 and 8; third

598   moth: n = 29 and 16; fourth month: n = 14 and 8; fifth month: n = 8 and 8; sixth month:

599   n = 35 and 37 and seventh month: n = 14 and 11, for women and men, respectively).

600   The results were analyzed using the Mann-Whitney test. The representative difference

601   between: the first and subsequent months (*); the second and subsequent months (#);

602   the third and subsequent months (o); the fourth and subsequent months (□); for women

603   and men on month fourth (ø), and the sixth and subsequent months (●). The

604   significances were [*,#,o,□,ø,●] $p<0.05$; [**,□□] $p<0.01$;[***,ooo] $p<0.001$;[****,oooo] $p<0.0001$.

605 **Figure 3: The age groups comparative analysis of IgA (A), IgM (B), IgG (C), and**

606 **IgE (D) by S-UFRJ-ELISA assays.** Three age groups were separated into young

607 adults, adults, and older adults. The serum of 34 RT-qPCR-positive mildly

608 symptomatic SARS-CoV-2 patients from the first month was analyzed. Each dot

609 represents an individual sample (17-39: n = 14; 40-59: n = 12; >60: n = 9, for age

610 group). The results were analyzed using the Kruskall-Wallis test followed by Dunn's

611 post hoc test. The representative difference between the age groups (*). The

612 significance was *** p<0.001.

613 **Figure 4: Profiles of IgA (A), IgM (B), IgG (C), and IgE (D) and viral load.** A

614 panel of 34 swab samples from PCR-positive mildly symptomatic SARS-CoV-2

615 patients was used to quantify viral load, which was presented as log(10) and calculated

616 according to standard curve and numbers of copies/mL. The results were analyzed

617 using Spearman's test.

618

Figure 1

Figure 1: The long-term quantification of IgA (A), IgM (B), IgG (C), and IgE (D) by S-UFRJ-ELISA assays.

The serum of 231 RT-qPCR-positive SARS-CoV-2 patients was measured for 7 months. The results were analyzed using the Kruskall-Wallis test followed by Dunn's post hoc test. The representative difference between: the first and subsequent months (*); the second and subsequent months (#); the third and subsequent months (o); the fourth and subsequent months (□), and the fifth and subsequent months (●). The symbols indicating statistical significance were $^{*,\#,●}$ p<0.05; $^{**,\#\#,□□}$ p<0.01; ^{***,ooo} p<0.001; ^{****,oooo} p<0.0001.
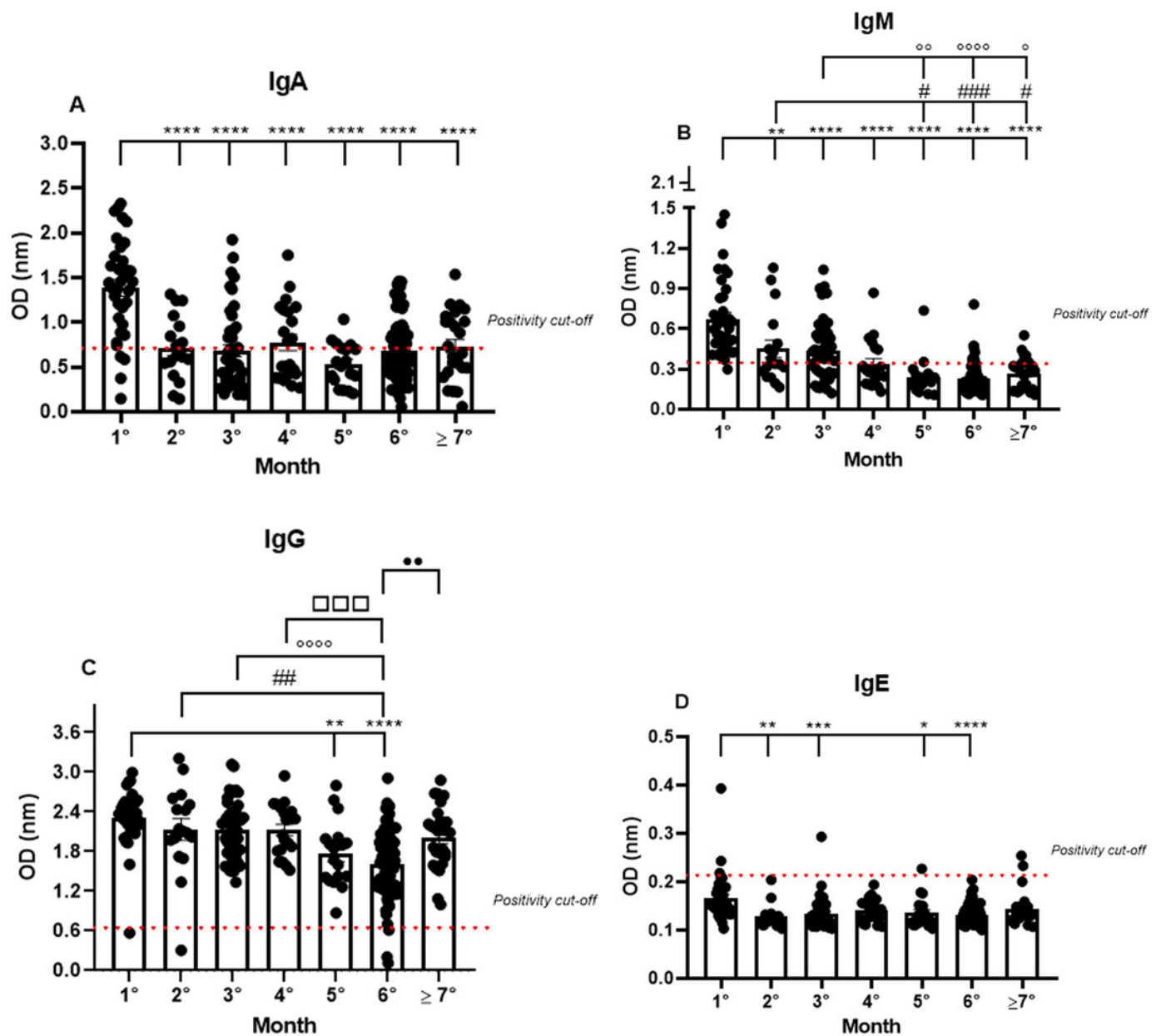
Figure 2

Figure 2: The gender comparative analysis of IgA (A), IgM (B), IgG (C), and IgE (D) by S-UFRJ-ELISA assays.

The serum of 231 RT-qPCR-positive SARS-CoV-2 patients was measured for 7 months. The results were analyzed using the Mann-Whitney test. The representative difference between: the first and subsequent months (*); the second and subsequent months (#); the third and subsequent months (o); the fourth and subsequent months (□); and for women and men on month four (ø). The significances were $^{*,\#,o,\Box,\o}$ p<0.05; ^{**,oo} p<0.01; *** p<0.001; ^{****,oooo} p<0.0001.
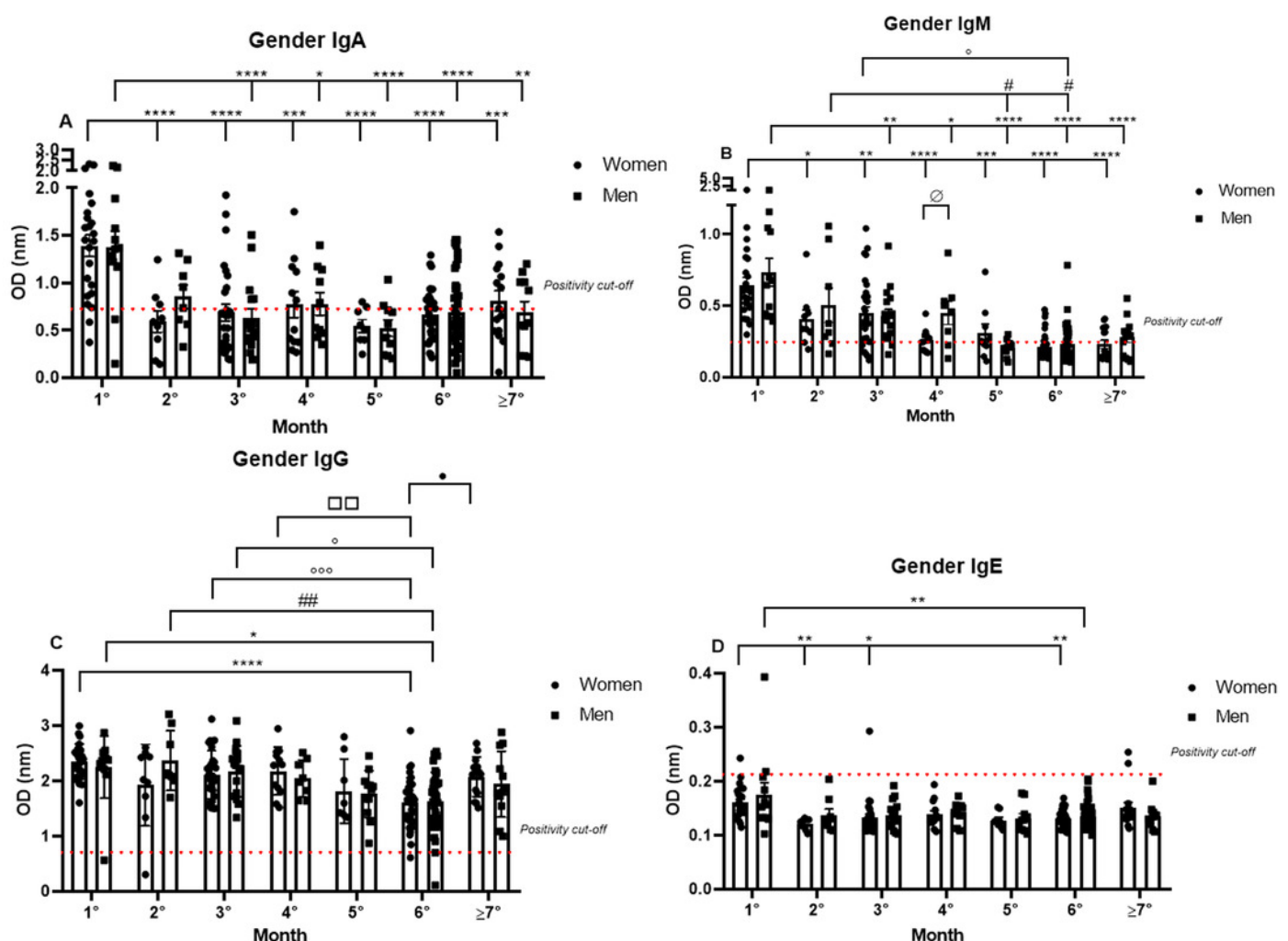
Figure 3

Figure 3: The age groups comparative analysis of IgA (A), IgM (B), IgG (C), and IgE (D) by S-UFRJ-ELISA assays.

Three age groups were separated into young adults, adults, and older adults. The serum of 34 RT-qPCR-positive mildly symptomatic SARS-CoV-2 patients from the first month was analyzed. Each dot represents an individual sample (17-39: n = 14; 40-59: n = 12; >60: n = 9, for age group). The results were analyzed using the Kruskall-Wallis test followed by Dunn's post hoc test. The representative difference between the age groups (*). The significance was [***]p<0.001.
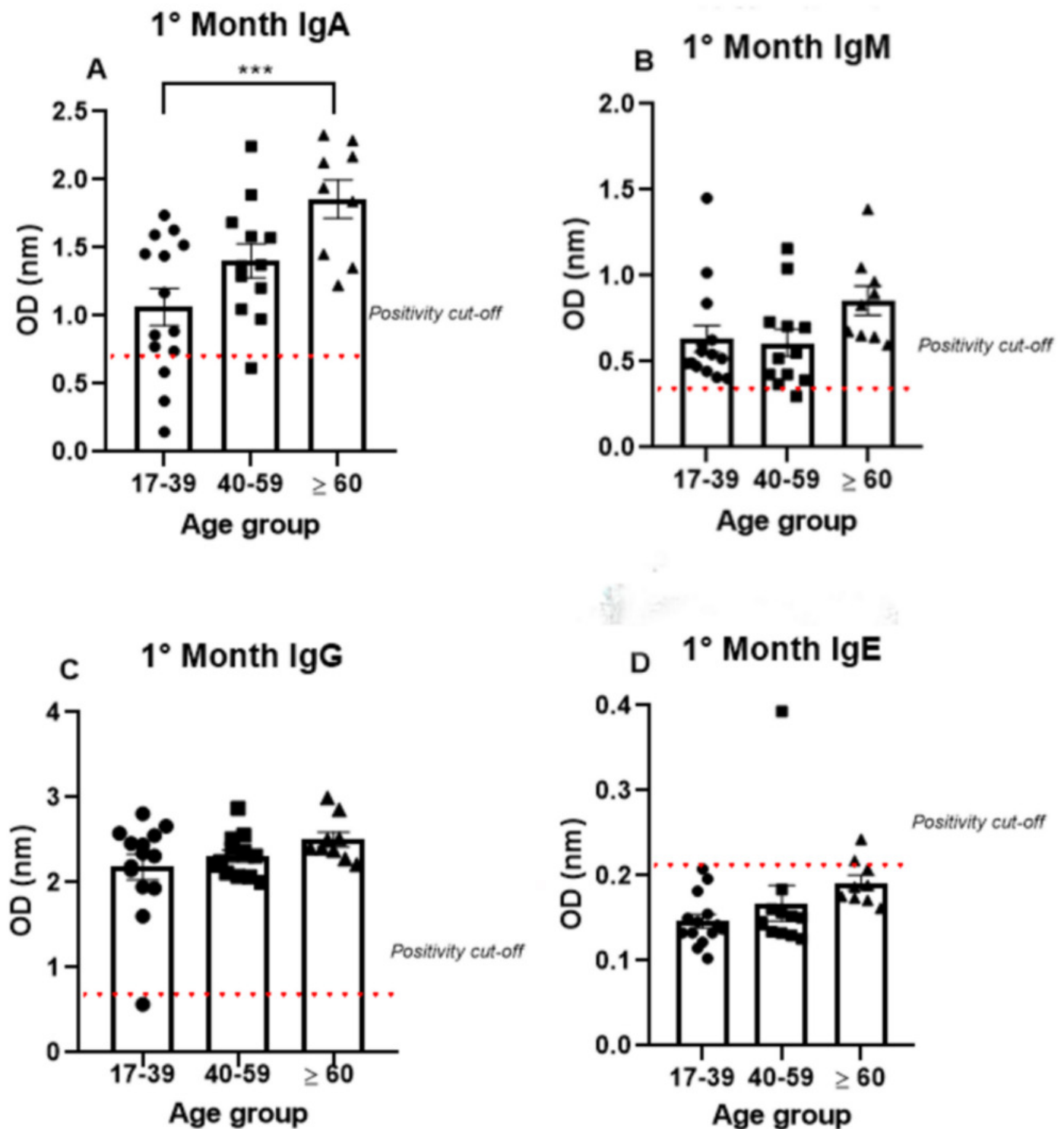
Figure 4

Figure 4: Profiles of IgA (A), IgM (B), IgG (C), and IgE (D) and viral load.

A panel of 34 swab samples from PCR-positive mildly symptomatic SARS-CoV-2 patients was used to quantify viral load, which was presented as log(10) and calculated according to standard curve and numbers of copies/mL. The results were analyzed using Spearman's test.
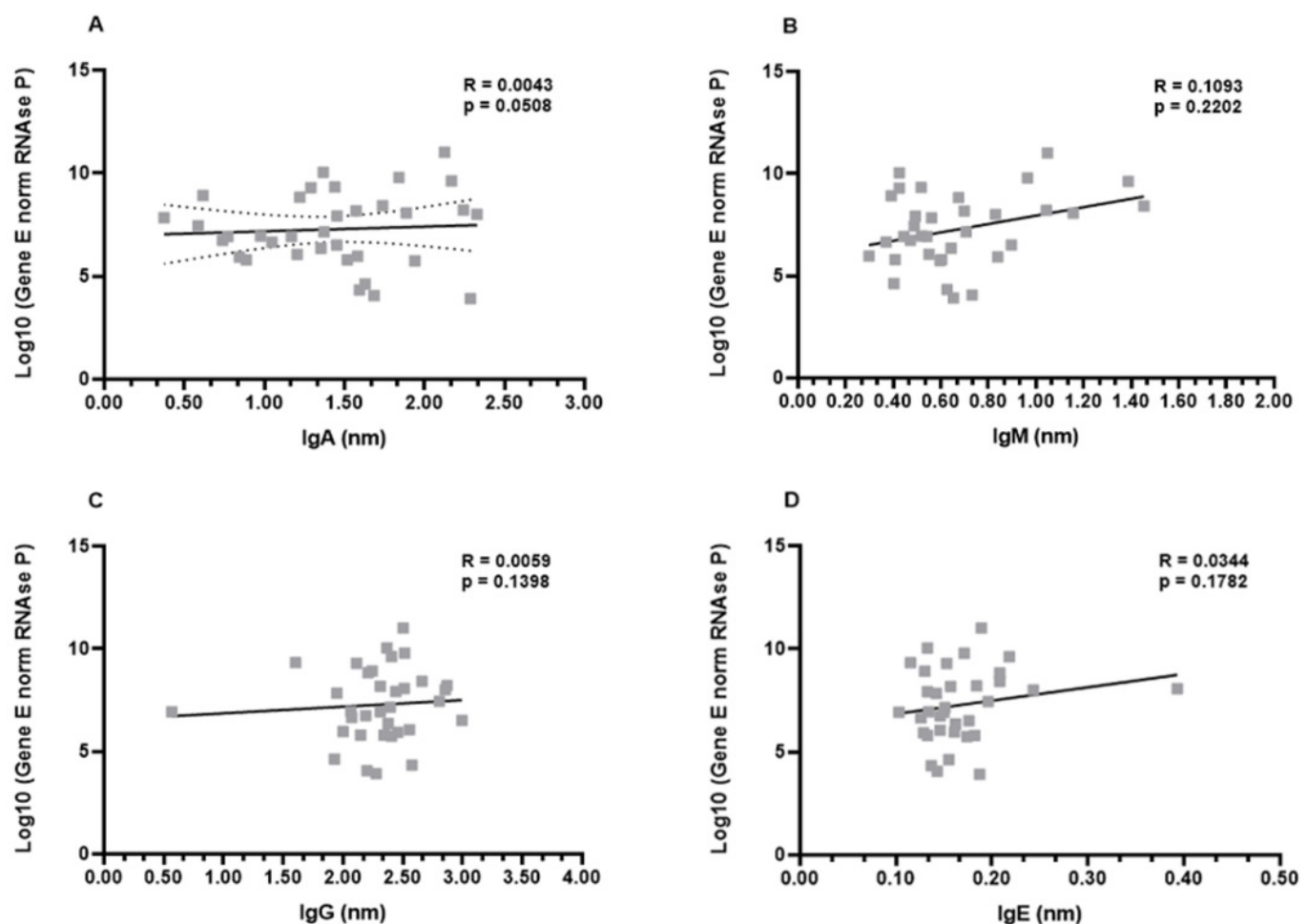
Table 1: Characteristics of mildly symptomatic SARS-CoV-2 patients.

Percentages are shown for each category with respective counts in parentheses. Select COVID-19 risk factors were scored by a clinical infectious disease physician.

1 Table 1: Characteristics of mildly symptomatic SARS-CoV-2 patients. Percentages are shown for

2 each category with respective counts in parentheses. Select COVID-19 risk factors were scored by

3 a clinical infectious disease physician.

Characteristic	Patients, No/Total No.			
	Total (N = 231)	Adolescents and young adults (17-39 years old, n =99)	Adults (40-59 years old, n=100)	Seniors (> 60 years old, n =32)
Sex				
Women	128/231 (54.9%)	60/99 (58.8%)	60/99 (58.8%)	17/32 (53.1%)
Men	105/231 (45.1%)	42/99 (41.2%)	42/99 (41.2%)	15/32 (46.9%)
Age				
Median (range)	52.5 (17-89)	28 (17-39)	49.5 (40-59)	74.5 (60-89)
Comorbidities				
Bronchitis	3/231 (1.3%)	2/99 (1.96%)	1/100 (1.0%)	-
Women	3/3 (100%)	2/99 (1.96%)	1/100 (1.0%)	-
Men	-	-	-	-
Chronic cardiac disease	37/231 (16%)	4/99 (3.9%)	24/100 (24%)	9/32 (28.1%)
Women	21/37 (56.8%)	3/99 (3%)	13/100 (13%)	5/32 (15.2%)
Men	16/37 (43.2%)	1/99 (1%)	11/100 (11%)	4/32 (12.5%)
Diabetes	9/231 (3.9%)	-	6/100 (6%)	3/32 (9.4%)
Women	6/9 (66.7%)	-	4/100 (4%)	2/32 (6.3%)
Men	3/9 (33.3%)	-	2/100 (2%)	1/32 (3.1%)
Obesity	62/231 (26.8%)	18/99 (18.2%)	37/100 (37%)	7/32 (21.9%)
Women	32/62 (51.6%)	10/99 (10%)	17/100 (17%)	5/32 (15.6%)
Men	30/62 (48.4%)	8/99 (8%)	20/100 (20%)	2/32 (6.3%)