Letter to the Editor

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The early antibody response to SARS-CoV-2 infection

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To the Editor,

In the early stage of covid-19 disease, the use of serological tests has recently been proposed but its role in the diagnostic process is not yet clear [1]. There is some indirect evidence of a protective role of specific antibodies, such as the administration of plasma immune from healed subjects to sick patients [2]. This represents a very interesting challenge but definitive evidence is still lacking.

In order to assess whether the antibodies can help the early diagnosis of COVID-19, we evaluated a series of samples who presented at the Ospedale dell'Angelo (Mestre, Venice, Italy) from March 4 to March 29, 2020 with symptoms suggestive of SARS-CoV-2 infection, subsequently diagnosed definitively with clinical and laboratory criteria. In three of these cases the disease was diagnosed along with history, images and other clinical data, despite the negative RT-PCR nose-pharyngeal swab. From all these cases we excluded patients whose symptom onset time was not clearly reported. Therefore, 46 patients (38 males and 8 females, median age 66 years, minimum 36, maximum 89) were included, of which 30 with one blood sample for serological tests, 7 with two withdrawals in the following days, 5 with 3 withdrawals, 2 with 4 withdrawals and 2 with 5 withdrawals, for a total of 77 assessed samples. Moreover, 35 healthy controls with negative swab and no symptoms were included (20 female, 15 males, age 24–65). Despite the limited number of cases evaluated in the control group, the absence of the detection of false positive results is encouraging about the specificity of these tests. The samples were obtained with polyethylene tubes (BD Vacutainer®; Becton Dickinson, CA, USA) containing clot activator and gel separator. Eighteen samples were also collected in polyethylene tubes containing K$_2$EDTA for the comparison of blood matrices. The presence of specific IgM and IgG antibodies was assessed by a chemiluminescence immunoassays (MAGLUMI 2019-nCoV IgM and MAGLUMI 2019-nCoV IgG, respectively), that according to the manufacturer is able to detect both spike and nucleocapside proteins antibodies, the main immunogens proteins of this coronavirus [3]. This type of antibody seems to correlates with neutralizing antibodies responses [4]. The assays were carried out on MAGLUMI 800 platform (Snibe, Shenzen, PRC) according the manufacturer’s instruction.

The results were interpreted following the indications of the manufacturer: (i) IgG considered reactive if >1.1 AU/mL; (ii) not reactive if <0.9 and (iii) doubt between 0.9 and 1.1. A single cut off limit of 1.0 for IgM was proposed.

Five aliquots of three sera (S1, S2 and S3) were stored and analyzed in triplicate in five different analytical sessions over seven days to verify the intra-series and total imprecision according to the CLSI EP-15-A2 protocol [5].

Linearity was verified by dilution test (four dilutions 1/2–1/10) mixing two samples, one with concentrations of 11.8 and 4.1 AU/mL, the second of 0.12 and 0.1 AU/mL for IgM and IgG respectively.

All investigations have been conducted by following the tenets of the Declaration of Helsinki and has been complied with institutional policies. Statistical analysis were performed with MedCalc© Software, Version 19.2.1 (MedCalc Software, Mariakerke, Belgium).

The data of imprecision study for IgG and IgM antibodies are shown in Table 1. The verification of the precision performances in no case is higher than the manufacturer’s claim (overall CVs <6% within-laboratory). However, the IgM assay showed imprecision data a little higher but still acceptable. In fact the within-laboratory precision estimate were less than the upper verification value calculated as CLSI guideline [5]. Dilution test showed good linearity for IgM (recovery

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For IgG a deviation from linearity (recovery 74.4%) was found at concentration <0.8 AU/mL.

We compared the results of EDTA matrix vs. serum with the Passing-Bablock regression: a good correlation was found as suggested by the manufacturer, both for IgG (range 0.17 – 88.1 U/mL) and IgM (range 0.11 – 12.9 U/mL). The slope of EDTA vs. serum for IgG was 1.005 (95% CI 0.97/1.03) and the intercept of 0.0003 (95% CI −1.09/+0.57). The slope of EDTA vs. serum for IgM was 0.95 (95% CI 0.91/0.99) and the intercept of 0.016 (95% CI −0.06/+0.06).

None of the 35 control samples resulted positive for IgM and IgG. Forty-seven samples (61%) were positive for IgM, while 66 samples (85.7%) were positive and two in gray zone for IgG.

The correlation of the results with respect to the days since the onset of the symptoms is shown in Figure 1. We have distinguished the samples analyzed before 15 days of illness (Group 1) from those analyzed 15 or more days later (group 2). Analyzing data of IgG in Group 1 the positivity rate was 71.1% (27 samples out of 38) but we found 100% positivity rate in the remaining 39 samples collected more than 15 days after the symptoms (Group 2).

The median concentration of IgG was 24.4 U/mL (25°–75° perc: 0.92–50.6) in Group 1 and 60.6 U/mL (25°–75° perc: 29.9–66.5) in Group 2 (Mann–Whitney test p = 0.0003). Among the 10 patients with negative results, we were able to collect more than one sample for five patients. We also observed that all of them showed a rapid seroconversion.

The trend was similar for the IgM but less significant (Mann–Whitney test p = 0.026). The positivity rate in Group 1 was 44.7%, with a median concentration of 0.83 U/mL (25°–75° perc: 0.39–2.36) and in Group 2 the positivity

### Table 1: Evaluation of tests imprecision in three serum samples at different concentrations. The verification value represents the imprecision limit according to the CLSI document [5].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean AU/mL</th>
<th>Intra-assay CV%</th>
<th>Within-laboratory CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obtained</td>
<td>Upper verification value</td>
<td>Obtained</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG S1</td>
<td>0.98</td>
<td>3.8</td>
<td>7.2</td>
</tr>
<tr>
<td>S2</td>
<td>16.9</td>
<td>4.3</td>
<td>7.2</td>
</tr>
<tr>
<td>S3</td>
<td>46.3</td>
<td>2.8</td>
<td>7.1</td>
</tr>
<tr>
<td>IgM S1</td>
<td>&lt;0.1</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>S2</td>
<td>1.67</td>
<td>5.7</td>
<td>7.7</td>
</tr>
<tr>
<td>S3</td>
<td>1.93</td>
<td>4.68</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Figure 1: Distribution of IgM (circles) and IgG (square) in relation to the days since the onset of symptoms in 77 cases of 46 patients. Data from control group of normal subjects are not represented. The horizontal solid line represents the limit of a reactive test for IgM (1.0 AU/mL) and the dotted line the limit for IgG (1.1 AU/mL).
rate was 76.9%, with a median of 1.96 U/mL (25°–75° perc: 1.2–2.82).

Considering COVID-19 symptomatic patients, the true positive results were 60.8% for IgM, with mainly low concentrations, and 85.1% for IgG at the first test. In particular, all the 39 samples evaluated more than 15 days after the onset of symptoms showed positive IgG results. It is moreover interesting to note that there are numerous cases with high IgG even after the first days of symptoms. It must be emphasized that the incubation time of the disease varies from two to a maximum of 14 days [6], and therefore it is also conceivable that cases with early high IgG may be related to patients with longer incubation times. However, in our case series the onset of the appearance of the serological response remains rather short, especially in consideration of the limited presence of IgM, which seems to have a parallel trend but with much lower concentrations than IgG.

Our findings are in agreement with recent studies, with an almost complete seroconversion within two weeks after disease onset [7–10]. In particular, in a recent report by Guo et al. the evidence of positive RT-PCR and the appearance of specific antibodies is very similar to that shown in our study [7]. Their conclusions report a high sensitivity for the swab in the first 5.5 days of symptoms and a high sensitivity for serology in the following days. For this reason, Guo proposes the early study of suspected patients with both diagnostic methods. Our data also seems to confirm this approach.

Finally, we observed that all the positive IgM cases except one also showed IgG positivity, so that the IgM value seems to provide a small contribution to the assessment of the immunological response in COVID-19 patients.

Competing interests: Authors state no conflict of interest.
Ethical approval: The local Institutional Review Board deemed the study exempt from review.

References