To the editor,

Serology may offer valuable information during COVID-19 pandemic however published papers mainly reported the results of symptomatic patients having positive RT-PCR on upper respiratory tract specimens [1]. More studies are needed to address whether asymptomatic patients, or patients with chest imaging compatible with COVID-19 but negative RT-PCR, have different antibody response that could influence assays performances. We wanted to share our data from Turkey where **4.323.596** COVID- 19 cases were detected out of **44.087.628** PCR tests by April, 20, 2021 but there are only a couple of published studies about serodiagnosis of the infection.

According to the interim guidance of WHO (Diagnostic testing for SARS-CoV-2), interpretations of serology should be made by an expert and are dependent on several factors including the timing of the disease, clinical morbidity, the epidemiology and prevalence within the setting, the type of test used, the validation method, and the reliability of the results . IDSA (Infectious Diseases of South America) guideline suggest not to use any serological test during the first 2 weeks following infection and to use IgG antibody to provide evidence of COVID-19 infection in symptomatic patients with a high clinical suspicion and repeatedly negative PCR results [2]. Commercially available serological assays for SARS-CoV-2 like ELISAs and lateral flow assays are high throughput, relatively inexpensive and use readily available instrumentation. These assays are performed with recombinant antigens, such as the spike protein (the main surface glycoprotein that is used to attach and enter cells) of SARS-CoV-2; the receptor-binding domain (RBD), which is part of the spike protein; or the viral nucleoprotein and can be handled at biosafety level 2. However neutralization assays with replication-competent SARS-CoV-2 have to be performed in biosafety level 3 facilities, which limits their application. IgM titer may be detectable 10 to 12 days after the first manifestation of the symptoms and IgG is measurable subsequently to IgM, after 12–14 days from the infection [3]. The maximum viremia levels are measured during the initial period of the disease. From the 10–14th day forward, the concentration decreases as a consequence of the immune response that justify the therapeutic use of convalescence plasma at an early stage of severe COVID–19 [4]. Positive serology can suggest an early, active, or late phase of the disease. After the 15th day, the sensitivity of RT–PCR is 45, 5% and serological tests above 90% [5]. Due to the high sensitivity of serological tests after the 10th day from the onset of the disease, it is recommended to utilize qualitative and quantitative anti–SARS–CoV2 IgM and IgG detection in the advanced stage of the infection, particularly, in patients with negative RT–PCR results

[6]. The nature of the virus-specific IgA response against SARS-CoV-2 infection in humans remains poorly understood. In a recent study assessing the prevalence of IgG, IgA, and IgM antibodies recognizing the SARS-CoV-2 from 132 infected patients, neutralization was more closely correlated with IgA than IgM or IgG in the first weeks after symptom onset although this response was not associated with COVID-19 severity [7].

We have analyzed serum samples of 245 patients among PCR positive patients (n: 154) who are eligible for convalescent plasma donation and PCR negative patients (n: 91) with high clinical and/or radiological suspicion of COVID-19. Our study was approved by the ethics committee of the Marmara University Training and Research Hospital (approval number: 09.2020.740). Viral RNA was extracted from nasopharyngeal swab samples by using Bio-speedy® viral nucleic acid buffer (Bioexen LTD, Turkey) and RT-PCR was performed with Bio-speedy® COVID-19 qPCR detection kit, Version 2 (Bioexen LTD) using primers and probes targeting the RNA-dependent RNA polymerase (RdRp) gene fragment in a LightCycler® 96 System (Roche, Switzerland). The Weimi Diagnostic COVID-19 IgG cassette lateral flow assay was performed with 10 µl of serum/plasma and the result was read at 10 min visually. Serum samples were kept in -20 ºC until antibody assays were available. The Abbott SARS-CoV-2 ELISA assay was performed on an i2000 Abbott Architect (Abbott Diagnostics, USA) for detecting IgG antibodies against the viral nucleocapsid protein. The Euroimmun SARS-CoV-2 ELISA assay was performed on a Euroimmun Analyser I (Euroimmun Diagnostics, Germany) for detecting anti-SARS-CoV-2 IgG and IgA directed against the S1 domain of viral spike protein. Percent agreement of ELISA tests was calculated using Cohen's Kappa test. Chest CT exams were performed within 1-3 days of PCR assay. Image analysis was performed using PACS (Picture Archiving and Communication System) workstation (INFINITT Healthcare Co., Ltd).

The median age (IQR) was 43(35-52.5) for PCR positive patients (70.6% of them were male) and 51(38-68.2) for PCR negative patients (47.8% of them were male). For PCR positive patients, Abbott IgG was positive in 92,9% , Euroimmune IgG was positive in 91.6%, Euroimmune IgA was positive in 91.6% , Weimi Diagnostic Lateral flow assay was positive in 86.6% whereas positivity was detected for the given methods in 39.6%, 39.6%, 43.9% and 40.6% in PCR negative patients. The highest positivity rate was detected in samples taken 21-29 days after the PCR request. (Table) . There was a lineer increase of Kappa values (percent agreement between assays) which was 0.56, 0.79 and 0.88 between Abbott IgG and Euroimmun

IgG tests and 0.48, 0.54. and 0.57 between Abbott IgG and Euroimmun IgA tests for samples have been taken 14-20 days, 21-29 days and ≥30 days, respectively for PCR positive patients. Kappa values were 1 between Abbott IgG and Euroimmun IgG tests and 0.80, 0.90. and 1 between Abbott IgG and Euroimmun IgA tests for samples have been taken 14-20 days, 21-29 days and ≥30 days, respectively for PCR negative patients. In 91 patients PCR was negative despite there were COVID-19 related changes in chest CT and 77% of them were treated empirically. When antibody tests are available positivity was detected in about 40% of the samples that might support the diagnosis.

Mei et al., [8] compared the utility of Roche, Abbott, and Euroimmun SARS-CoV-2 assays for correlation with neutralizing antibodies and a modest correlation, but poor concordance was reported for all assays. Authors suggested that patients infected with SARS-CoV-2 develop a broad-based antibody repertoire against multiple proteins and epitopes, but only some of these antibodies have neutralizing properties. Patients who were intubated, had cardiac injury, or acute kidney injury from COVID-19 infection had higher neutralizing titers relative to those with mild symptoms. In a meta review, 57 publications reporting on a total of 54 study cohorts with 15,976 samples, of which 8526 were from cases of SARS-CoV-2 infection were evaluated [9]. The sensitivity of antibody tests is too low in the first week since symptom onset to have a primary role for the diagnosis of COVID-19, but they may still have a role complementing other testing in individuals presenting later, when RT-PCR tests are negative, or are not done. Beavis et al [10] have studied 86 samples from SARS-CoV-2 PCR-negative patients, and 82 samples from SARS-CoV-2 PCR-positive patients, and reported that Euroimmun Anti-SARS-CoV-2 ELISA assay demonstrated good sensitivity for detection of IgA and excellent sensitivity for detection of IgG antibodies from samples collected ≥4 days, good specificity for IgA and excellent specificity for IgG.

Limiting factors of our study that single serum samples for each patients were tested and since consecutive samples are not available we have no information about the duration of the seropositivity. Moreover, there is a debate on the possibility of waning immunity, and research on kinetics of antibody responses to SARS-CoV-2 is therefore needed to assess added value of serology in diagnosing COVID-19 in the future [11].

Table . Antibody positivity according to the days between PCR and serology tests

ELISA Test	PCR positive patients			PCR negative patients		
	14-20 days (n=45)	21-29 days (n=47)	>=30 days (n=62)	0-20 (n=58)	21-29 days (n=23)	>=30 days (n=10)
Euroimmun IgG, positive, n (%)	38 (84.4)	45 (95.7)	58 (93.5)	15 (25.9)	16(69.6)	5(50.0)
Abbott IgG, positive, n (%)	42 (93.3)	44 (93.6)	57 (91.9)	15(25.9)	16(69.6)	5(50.0)
Euroimmun IgA, positive, n (%)	41 (91.1)	46 (97.9)	54 (87.1)	20(34.5)	15(65.2)	5(50.0)

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