Italian Journal of Emergency Medicine 2021 April;10(1):30-3
DOI: 10.23736/S2532-1285.21.00084-7

COMMENTARY
COVID-19 SECTION

Clinical indications for anti-COVID-19 antibodies use

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In an interesting study conducted during the early days of the COVID-19 pandemic spread, Gabriele Cerini and Emergency Department (ED) COVID-19 Investigators Group from University Hospital Careggi of Florence, have prospectively evaluated the use of titration of immunoglobulins IgM/IgG for SARS-CoV-2 in addition to nasopharyngeal swab for viral nucleic acid detection and real-time reverse-transcription polymerase chain reaction (rRT-PCR) for COVID-19 – recognized as gold-standard essay for diagnosis SARS-CoV-2 infection.[1] In large casuistry, these authors have evaluated serologic and molecular tests in patients observed in ED with at least one risk factor for COVID-19 infection at ED presentation: cough, pharyngitis, temperature >37.5 °C, dyspnea, oxygen saturation ≤94%, respiratory rate ≥20 per minute, need of oxygen implementation or ventilation, close contact with a suspected or confirmed COVID-19 case. Onset of overall signs or symptoms was 2 days, with interval range of 1-4 days. The authors found that the accuracy of physician's evaluation alone (based on clinical features, ECG, EGA, bedside diagnostic imaging tests as lung ultrasound or chest X-ray) was like the accuracy of physician's evaluation plus serology in order to distinguish patients in COVID-19 "likely" or "unlikely;" whereas the sensitivity of nasopharyngeal swab with serology to rule out COVID-19 infection was not significantly different from the sensitivity of nasopharyngeal swab alone. Finally, authors concluded that in early-stage evaluation of clinically suspected COVID-19 infection, the serologic tool was not useful and accurate for diagnosis of acute infection of COVID-19 in ED; therefore, not recommending it to rule out COVID-19 infection in association with physician's gestalt or in association with the first nasopharyngeal swab.

The worldwide impact and rapid spread of the COVID-19 pandemic induced several international research groups to increase diagnostic capacity, especially in ED, in order to accelerate the identification of acute infection cases, early isolation, and identification of patients needing ventilatory support and drug therapy.

Several serological studies conducted around

the world shown that there are two types of antibody tests: binding antibody detection and neutralizing antibody detection, this latter preventing virus infection *in vitro*, and shortly pending authorization to treat acute COVID-19 infection. The former antibody test uses purified proteins of SARS-CoV-2 to determine antibody binding coupled with one of the following platforms: lateral flow immunoassay (LFA), chemiluminescent immunoassay (CIA) and enzyme-linked immunosorbent assay (ELISA). The LFA detects IgM and IgG antibodies to SARS-CoV-2 in serum, plasma, or whole blood specimens, with quick response (15-20 minutes), resulting easily employable. ELISA and CIA tests are qualitative rather than quantitative, with more reliable results and utility to correlate well with neutralizing titers.[2]

Serology tests are currently being developed for the detection of antibodies (in blood plasma or serum) to two main antigenic targets, the N nucleocapsid protein and S spike protein, including subunits S1 and S2 as well as the receptor-binding domain (RBD) of SARS-CoV-2. A meta-analysis was recently conducted to investigate the performance of all available antibody tests and found that tests using the S antigen are more sensitive than N antigen-based tests, whereas some researchers found that antibodies to N protein are more sensitive than to S protein during early phases of infection (using immunoassay called luciferase immunoprecipitation systems).[3] A combined IgG/IgM test displayed better sensitivity than measuring either antibody type alone with ELISA tests being more sensitive than LFA.[4] Serological tests have varying levels of specificity and sensitivity and can detect past infections in those who are RT-PCR negative. On another hand, false positives can result from cross-reactivity with pre-existing antibodies from previous infections such as other coronaviruses (*i.e.* SARS-CoV or MERS-CoV); whereas negative results may be due to antibodies that have not yet formed during the early stages of infections. Recent study evaluated different serological commercial assays for the detection of SARS-CoV-2 specific IgG, IgM and IgA antibodies. They show a very good analytical performance, in terms of sensitivity and specificity, of IgG respect to IgM (low sensitivity and high number of false negative) and IgA (high number of indeterminate results) antibodies that seems to be a useful tool for epidemiological serosurveys and retrospective diagnosis of SARS-CoV-2 infection.[5]

Regarding evaluation of seroconversion response in suspected COVID-19 acute infection, Cassaniti *et al*.[6] have recently performed an interesting study conducted in ED of a tertiary hospital in Northern Italy, evaluating both molecular test (RT-PCR) from nasal swab and COVID-19 IgM/IgG rapid test (LFA). These authors tested 110 subjects: 30 healthy subjects RT-PCR COVID-19 negative, 30 patients RT-PCR COVID-19 positive (previously admitted in Intensive Care), and 50 patients at their first access at ED with suspected COVID-19 infection (with fever and respiratory syndrome). In COVID-19 positive previously hospitalized group, on serum samples obtained at a median 7 days (range 4-11) after the first COVID-19 positive, 63.3% were positive for both IgM and IgG, 16.7% negative per both IgM-IgG, 16.7% weakly positive for both IgM-IgG, and only 1 patient was positive for IgM alone. Interestingly, on acute patients evaluated in ED, 24% were negative for COVID-19 by RT-PCR (1 was positive for COVID-19 IgM/IgG test), while 76% resulted positive for COVID-19 RT-PCR. Of these, only 18.4% showed a positive or weak positive serology for IgM and/or IgG, while majority (81.6%) was tested negative for the rapid serology assay, suggesting a sensitivity of the COVID-19 IgM/IgG rapid test of 18.4%, specificity 91.7%, negative predictive value (NPV) 26.2%, and positive predictive value (PPV) 87.5% in patients enrolled from emergency area. This study confirmed that the research for IgM and/or IgG antibodies has poor utility at the time of presentation of symptoms suggestive of acute COVID-19 infection, with useless utility to diagnosis of acute disease, while seroconversion with comparison of antibodies may be useful later, during hospitalization, or for the purposes to evaluate the previous contact with the COVID-19 virus in the population.[7-9]

In this regard, Chinese authors showed that during hospitalization of patients affected by

acute SARS-CoV-2 disease, the presence of antibodies was <40% among patients within 1 week of onset, and rapidly increased to 100.0% (Ab), 94.3% (IgM), and 79.8% (IgG) by day 15 after onset; whereas RNA detectability decreased from 66.7% in samples collected before day 7 to 45.5% during days 15-39. Furthermore, these authors found that an higher titration of Abs was independently associated with a worse clinical classification, suggesting that the antibody detection may be used as an indicator of the stage of COVID-19 progression to aid disease management.[10]

In this regard, some researchers have speculated that antibodies can be biomarkers of advanced disease with worse cytokine storm; in fact, it was observed higher antibody concentrations in patients with severe disease than in those with mild disease; in particular in one study conducted in 222 patients, a greater proportion of patients with high IgG concentrations had severe disease than did those with low IgG concentrations (52% *vs.* 32%, P=0.008).[11, 12]

After reviewing international literature, we can conclude that both molecular and serological tests are intended for patients at different stages of the disease process with RT-PCR useful for detection of current active infection, and serology tests for later stages since these assays detect the presence of SARS-CoV-2 antibodies produced by the bodies humoral immune system.[2] Serologic testing enables the understanding of how patients produce antibodies to SARS-CoV-2, and assays can detect IgA, IgM, IgG, or total antibody. Profiling of early humoral response in several studies of patients with COVID-19 determined that IgA and IgM antibodies can be detected as early as 5 days after new infection, with higher levels detected in the second and third week,[13, 14] though probably optimal detection of IgM antibodies towards the end of the first week that peaked during the third week after symptom onset and may correspond to the emergence of negative results by RT-PCR tests as the viral load is cleared;[15-17] whereas IgG antibodies become detectable later in the infection course around 7-14 days after symptom onset and levels remained relatively high until 6 weeks,[7, 8] until 100% of patients tested positive for IgG within 20 days after symptom onset.[18]

Interestingly, Long *et al.* found that asymptomatic patients have significantly longer duration of viral shedding, lower IgG and neutralizing serum antibody levels compared to the symptomatic group, suggesting that asymptomatic patients may mount a weaker antibody response and immunity may diminish within months of infection.[19]

In conclusion, to date the indications evaluating the seroconversion are: serosurveys in high-prevalence populations, understanding of the antibody responses mounted upon SARS-CoV-2 infection and after vaccination, antibody functionality (*e.g.* virus neutralization) extremely useful to evaluate immune protection from possible reinfection (individuals with antibodies to SARS-CoV-2 are probably less susceptible to reinfection, reducing the risk of severe COVID-19 and also limiting the possibility of spreading the virus), and duration of the immune responses. In the emergency area the finding of a positive antibody titer leads to the classification of the COVID-19 disease in a phase after the acute phase, and this is clinically relevant because international guidelines confirm that these patients have lower risk to develop worse forms of COVID-19 disease and reduced ability to spread the infection.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.—Luigi Petramala and Massimo Gentile have given substantial contributions to the conception or the design of the manuscript, Giuliana Guerrizio and Marianna Calabretto to literature evaluation. All authors read and approved the final version of the manuscript.

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Comment on: Cerini G, Lagi F, De Stefano G, Nencioni A, Ottaviani M, Colao MG, *et al.* Sensitivity of IgM and IgG serology for SARS-COV-2 plus physicians' gestalt or rRT-PCR for COVID-19 diagnosis. Ital J Emerg Med 2021;10:000–000. DOI: 10.23736/S2532-1285.21.00073-2.

History.—Manuscript accepted: March 29, 2021. - Manuscript received: March 23, 2021.

(*Cite this article as:* Petramala L, Guerrizio G, Calabretto M, Gentile M; Sapienza MEU Group. Clinical indications for anti-COVID-19 antibodies use. Ital J Emerg Med 2021;10:30-3. DOI: 10.23736/S2532-1285.21.00084-7)