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Serological Assays for SARS-CoV-2 Infectious Disease: Benefits, Limitations and Perspectives

Maria Infantino[1], Arianna Damiani[2], Francesca Li Gobbi[3], Valentina Grossi[1], Barbara Lari[1], Donatella Macchia[4], Patrizia Casprini[5], Francesca Veneziani[6], Danilo Villalta[7], Nicola Bizzaro[8], Piero Cappelletti[9], Martina Fabris[10], Luca Quartuccio[11], Maurizio Benucci[3] and Mariangela Manfredi[1]

[1]Immunology and Allergy Laboratory Unit, San Giovanni di Dio Hospital, Florence, Italy
[2]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy
[3]Rheumatology Unit, S. Giovanni di Dio Hospital, Florence, Italy
[4]Allergy and Clinical Immunology, San Giovanni di Dio Hospital, Florence, Italy
[5]Laboratory of Clinical Pathology and Immunoallergy, Florence-Prato, Italy
[6]Laboratory of Clinical Pathology, San Giovanni di Dio Hospital, Florence, Italy
[7]Immunology and Allergy Unit, S. Maria degli Angeli Hospital, Pordenone, Italy
[8]Laboratory of Clinical Pathology, San Antonio Hospital, Tolmezzo, Italy
[9]SIPMeL, Castelfranco Veneto, Veneto (TV), Italy
[10]Institute of Clinical Pathology, University of Udine, Udine, Italy
[11]Clinic of Rheumatology, ASUFC, Department of Medicine (DAME), University of Udine, Udine, Italy

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Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first appeared in Wuhan, China, in December 2019 and is now spreading worldwide.

SARS-CoV-2, formerly known by the provisional name 2019 novel Coronavirus (2019-nCoV), shares some characteristics with two other coronaviruses, which previously caused epidemic respiratory syndromes: severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome (MERS-CoV) in 2012.

Coronavirus is a positive-sense single-stranded RNA virus. It is a large pleomorphic spherical enveloped particle [Figure 1]. The viral envelope consists of a lipid bilayer where the membrane (M), envelope (E), and spike (S) structural proteins are anchored [1]. The S glycoprotein is a large type 1 transmembrane protein containing two functional subunits S1 and S2. S1, responsible for binding to the host cell receptor, comprises a receptor-binding domain (RBD). S2 contains elements needed for the fusion of the virus [2-6].

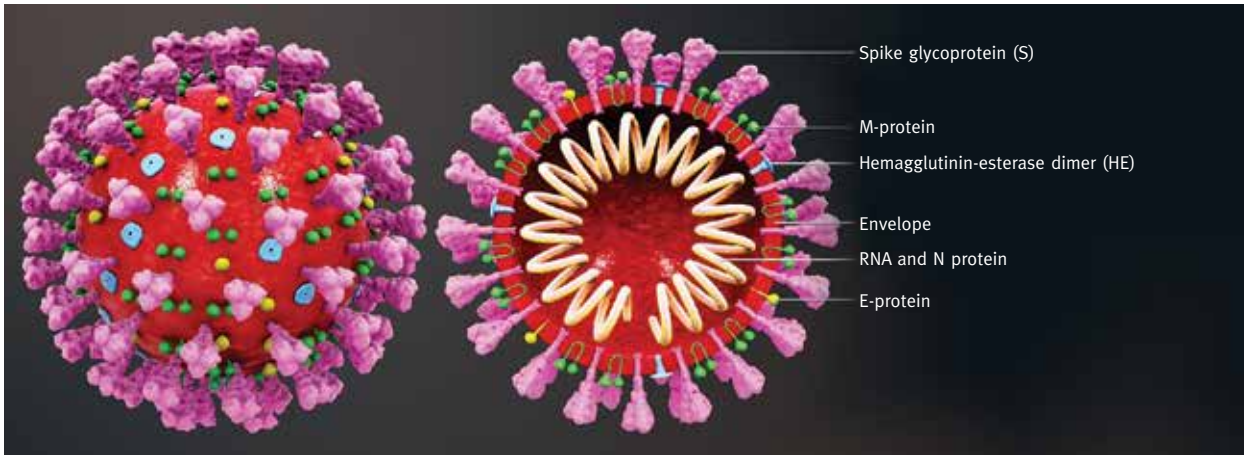
S glycoprotein forms homotrimers protruding from the viral surface [7]. A subset of coronaviruses (in particular betacoronavirus gender) also have a shorter spike-like surface protein called hemagglutinin esterase (HE) [8]. Inside the envelope there is the nucleocapsid, which is formed from multiple copies of the nucleocapsid (N) protein. This protein is bound to the single-stranded RNA genome [9]. The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell [10]. Moreover, the first two-thirds of the viral genome encode non-structural proteins (NSPs), and in particular, the RNA polymerase represents the main part of the transcription/replication machinery, well conserved among different CoV species.

Specifically, SARS-CoV and SARS-CoV-2 account for the same receptor used to penetrate into human cells and start replication: angiotensin-converting enzyme 2 (ACE2), which is mostly expressed by type II pneumocytes, as well as by endothelial, myocardial, and gut mucosa cells [11]. It has been hypothesized that the high affinity of CoV-2 Spike (S) protein Receptor Binding Domain (RBD) for ACE2 is responsible for the elevated contagious power of the new coronavirus. S protein is also among the most immunogenic structural peptides of the virus, along with nucleocapside (N) protein [12]. Thus, produced antibodies may have an important role in interrupting viral entrance into cells, as well as in its opsonization [13] and represent the target of neutralizing antibodies [14].

The immune response against the virus is determinant in the evolution of COVID-19 disease but it has yet to be fully understood. Nevertheless, reasonable hypotheses can be made based on knowledge from MERS-CoV and SARS-CoV [15].

As is true for every infectious disease, the host immunity has a role both in fighting against pathogen invasion and in determining severe organ damage and bad prognosis. COVID-19 accounts for various clinical phenotypes, from asymptomatic carriers to interstitial pneumonia, to acute distress respiratory syndrome (ARDS), multiorgan failure (MOF), and death. Both

Figure 1. Structure of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)



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innate and adaptive immunity are involved in the natural history of COVID-19.

First, monocytic-macrophagic cells are activated by the viral expression of pathogen-associated molecular patterns (PAMPs), such as its RNA. This provides interferon-1 (IFN-1) secretion and, therefore, an increase in several proinflammatory cytokines and chemokines such as interleukine (IL)-1, IL-6, IP-10, monocyte chemoattractant protein-1 (MCP-1), MIP-1A, and tumor necrosis factor alpha (TNFα), by activating the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway [16].

This initial phase is clinically characterized by the appearance of cough (due to viral invasion of respiratory tract mucosa), fever, generalized malaise, asthenia, arthralgia and myalgia. Anosmia and ageusia are also often reported. Laboratory tests at this time may show neutrophilia, with normal or reduced lymphocytes count, elevated C-reactive protein (CRP), and IL-6. Procalcitonin is classically normal in absence of over-infections [17].

Within approximately one week from infection, adaptive immunity is expected to rise. That means the intervention of B lymphocytes, with the production of specific antibodies and of CD8+ T cytotoxic lymphocytes to eliminate infected cells. In addition, CD4+ T helper lymphocytes have a role in guaranteeing the modulation and coordination of all these processes to obtain the inhibition of viral replication, a decreasing of viremia and, in the best case, a restitutio ad integrum. In fact, as observed with SARS-CoV and MERS-CoV [18], it is likely that the virus has the ability to reduce IFN pathway efficiency, interfering with downstream gene expression. This, along with individual risk factors [19] such asolder age; male sex; comorbidities like diabetes, hypertension, obesity, cardiopathy, and genetic predisposition, result latency of viral control and persistence of innate cells activation, without an appropriate transit to adaptive immunity. This factor could be the main cause of COVID-19 complications, occurring at about day 12 from first symptoms presentation. At that time, circulating proinflammatory cytokines increase and inflammatory cells build up in target organs, basically the lungs, causing tissue damage without providing any control over the infection. Patients with such a course often experience dyspnea, reflecting severe interstitial pneumonia and respiratory failure, persistent fever, and progressive worsening of their general condition. Laboratory data in these cases evidence lymphopenia, elevated level of IL-6, CRP, ferritin, lactate dehydrogenase (LDH), serum creatinine, creatine kinase (CK), and D-dimer. Differences in these values between patients with good and bad prognoses are statistically significant in virtually all available reports. Viremia is constantly high. Intensive care is required at this point, supporting the patient with invasive ventilation, fluid resuscitation, and, in the worst cases, extra-corporeal membrane oxygenation, with a very elevated mortality rate.

The emerging role of drugs like tocilizumab, an inhibitor of IL-6 receptor, approved for the treatment of rheumatoid arthritis and giant cell arteritis in controlling alveolar inflammation in COVID-19, seems to corroborate the crucial pathogenetic role of the overwhelming activation of the immune system in the disease [20]. It is worth noting that most of the aforementioned risk factors may share a part in damaging immune efficiency.

Regarding the role of genetics, it is known that human leukocyte antigen (HLA) I and II haplotypes contribute in determining the ability or inability to raise an immune response against a specific pathogen. This result is because genetic sequences expressed by HLA result in a specific receptor for T and B lymphocytes (TCR and BCR) and so it is in the faculty of these cells to recognize peptides associated with the specific pathogen and to develop an adaptive reaction [21]. Preliminary

studies aimed at assessing the best SARS-CoV-2-associated peptides target for a vaccine seemed to show that genetic haplotypes, which best recognize SARS-CoV-2 sequences, may not be so widely represented in the general population [22]. From a pharmacologic point of view, this leads to the choice of other more frequent, although maybe less fitting, epitopes in vaccine preparation. Moreover, the peculiarity of the coronavirus epitopes might also explain why a small but still significant percentage of young and healthy people contract COVID-19 in a severe form.

Another source of information on COVID-19 pathogenesis can be represented by histological observations. Findings from post-mortem biopsies were described in a case report published in *Lancet Respiratory Medicine* [23]. In that study, both lungs of the patient, who died from cardiac arrest on day 14 of the disease, presented with interstitial mononuclear inflammatory infiltrates. Multinucleated syncytial cells with atypical enlarged pneumocytes were also found, with no evidence of viral inclusions in observed cells. In liver biopsies, moderate microvesicular steatosis and mild lobular and portal activity were evidenced. The peripheral blood samples showed reduced and phenotypically hyperactivated CD8+ and CD4+ cells, thus confirming the massive, although ineffective, activity of the immune system.

Accumulated proinflammatory cells and absence of wound-healing macrophages were also found in an in vivo/in vitro study, examining hematoxylin and eosin (H&E) staining of lung specimens derived from three patients who died from SARS-related acute lung injury. Interestingly, levels of anti-S neutralizing antibodies in the sera of those patients were significantly higher and had a more precocious peak in comparison to recovered ones. While aimed at facilitating viral clearance, these antibodies showed to abrogate wound-healing responses and promoted in vitro activation of monocyte/macrophage with production of MCP1 and IL-8. Thus, the authors speculated that anti-spike IgG might have a direct role in damaging pulmonary tissue and this hypothesis could also be extended in COVID-19 pathogenesis [24].

Reduced, although not hyperactivated as observed in Xu's study [24], CD8+ and CD4+ lymphocytes were associated to severe COVID-19 disease even in a retrospective study on 452 patients by Qin C et al. [25]. The cell reduction included milder affected patients, but in those cases it was significantly lower in comparison to worse clinical phenotypes. Moreover, an increased amount of neutrophils and a higher neutrophil-to-lymphocyte ratio (NLR) were noted in the same cohort [25].

Some data are available confirming an increased incidence of liver abnormalities in COVID-19 affected patients, which may indicate the effect of SARS-CoV-2 on the liver or be a drug-induced damage [26]. Acute renal injury has also been reported and explained as a possible consequence of the virus itself, or of the infection-induced inflammation, or both in a synergistic way [27]. Myocardiocytes expression of ACE2 receptors as well as the cardiovascular system's sensitivity toward circulating proinflammatory cytokines, may be responsible for the COVID-19-associated myocarditis, which has been described in some reports, along with heart attack and fatal arrhythmias precipitating clinical condition of subjects with previous ischemic heart disease [28].

The impressive burden of the disease, in term of needing dedicated wards, medical personnel, and above all, beds in the intensive care units, makes it extremely important for accurate diagnostic tools to be available to rapidly identify affected patients and appropriately treat them.

It has been shown that the viral RNA can be detected from nasal and pharyngeal swabs, bronchoalveolar lavage, and blood plasma using real-time reverse-transcription polymerase chain reaction (RT-PCR) [29-31].

The identification of the SARS-CoV-2 viral sequences led to the rapid availability of RNA tests RT-PCR diagnostic assays as a fundamental tool in the SARS-CoV-2 diagnosis [32,33]. Routine identification of cases of SARS-CoV-2 virus is based on the detection of unique sequences of virus RNA by nucleic acid amplification tests (NAAT) such as RT-PCR, with confirmation by nucleic acid sequencing, when necessary.

Recently, the World Health Organisation (WHO) provided interim guidance to laboratories showing the strategic use of diagnostic testing in different transmission scenarios of the COVID-19 outbreak, including how to justify testing when prioritizing patients due to lack of proper facilities. The WHO document specifies the conditions necessary to consider a case laboratory-confirmed by NAAT for areas with no known or established SARS-CoV-2 circulation [34].

In the first case, for areas with no SARS-CoV-2 virus circulation, one of the following conditions needs to be met: a positive NAAT result for at least two different targets on the SARS-CoV-2 genome, of which at least one target is preferably specific for SARS-CoV-2 using a validated assay. One positive NAAT result for the presence of betacorona virus and SARS-CoV-2 further identified by sequencing partial or whole genome of the virus, as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used, is needed.

In the second case, for areas where SARS-CoV-2 virus is widely spread, a simpler algorithm might be adopted where, for example, screening by RT-PCR of a single discriminatory target is considered sufficient; however, one or more negative results do not rule out the possibility of SARS-CoV-2 infection.

A number of factors could lead to a negative result in an infected individual, including poor quality of the specimen containing little patient material, the specimen was collected late or very early in the infection, the specimen was not handled and shipped appropriately, or technical reasons inherent in the test (e.g., virus mutation or RT-PCR inhibition).

In fact, RT-PCR tests are plagued by many limitations. They have a long turnaround time (on average over 2 to 3 hours to generate results, even if methods with a lower time than 90 minutes are now available) and are labor-intensive, complex, costly, technically intricate, easily contaminated. They require first-rate certified laboratory facilities with restrictive biosafety levels and ad hoc trained personnel. In addition to these intrinsic methodological limits, RT-PCR tests may also produce negative results early on as infection originates in the alveolar cells, but swabs are administered to the upper respiratory tract.

The positive rate in swabs samples varies by sample site [35-40] but since data are today controversial it is not possible to accurately assess sensitivity and the diagnostic impact of combining oropharyngeal and nasopharyngeal tests. However, one of the largest studies [37] reported that oropharyngeal swabs detected the SARS-CoV-2 less frequently than nasopharyngeal swabs and should not be used in place of nasopharyngeal swabs, particularly from day 8 of symptom onset. During this current public health emergency of international concern, screening and diagnosing patients quickly to aid containment is a priority and these limits make RT-PCR unsuitable for use in the field. Consequently, new tools, such as serological tests capable of tracking the virus through each phase of the disease, are in great demand Mapping the serology on a large-scale population may help epidemiologists to suppress human-to-human transmission of the 2019 novel coronavirus and provide prompt therapy for patients.

Another important aspect to consider is its potential use in the asymptomatic population or those presenting with mild symptoms (including healthcare workers). A substantial group of people who are not tested for viral RNA (for practical reasons) thereby masking a population's true rate of infection. Ultimately serology offers the greatest potential to understand the true scale of human-to-human transmission of the 2019 novel coronavirus and, for this reason, serologic tests have been broadly implemented in clinical laboratories to provide a much wider application than molecular tests.

Nevertheless, diagnostic performance and predictive value of SARS-CoV-2 serological testing have not been systematically evaluated and large studies are lacking. Currently there is an increasing number of in vitro diagnostic companies (mainly Chinese) that are developing or have developed tests for antibodies (see https://www.finddx.org/covid-19/pipeline/). Five of the 17 antigen-detection rapid diagnostic tests and 26 of the 53 antibody detection tests reported on the website have been selected for the first round of evaluation. Additional tests will continue to be reviewed on a rolling basis. However, there is little data published about these tests. They are often controversial and mainly involve small Chinese cohorts. It is also worth noting that given the urgency to share scientific knowledge very rapidly some of the published studies should be viewed with caution and not be used to guide clinical practice since they have not been peer-reviewed (untraceable on PubMed Central).

As it is for pathogenetic information, most of our knowledge on diagnostics comes from previous studies on SARS CoV. Since SARS-CoV-2 belongs to the same large family of viruses as those that caused the MERS and SARS outbreak, we could assume that its antibody generation process is similar.

During the outbreak of SARS-CoV, different serological assays, including immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), and Western blot (WB) analysis, were developed. The first assays used in serological diagnosis of SARS-CoV [41-45) were virus-based; in particular IFA was based on virus-infected African green monkey kidney cells spotted on glass slides and ELISA on extracts or supernatant of infected cells. Some studies suggested that virus-based IFA and ELISA were highly sensitive (85–100%) but lacked specificity. False positive results were due to antigens well-conserved among different CoV species [46,47] and to cross-reaction with autoantibodies in autoimmune diseases [48]. Later, serological assays based on recombinant antigens derived from both S and N proteins were widely used in laboratory diagnostics. The use of recombinant antigens offers the advantage of working without the need for biosafety level 3 containments and are also more appropriate for assay standardization. In particular, the N protein is easy to clone into prokaryotic or eukaryotic expression plasmids due to its small size and the absence of glycosylation sites. Researchers [49-54) suggested that recombinant protein based WB and ELISA were highly sensitive (sera from 73–100% of previously diagnosed SARS cases reacted with the complete or partial N protein) and with a low to moderate specificity as well as previous studies performed with viral lysates. Since S protein is difficult to express into prokaryotic in its full length protein, only fragments can be used in immunoassays. Several studies showed different reactivity of SARS patient sera with S protein, ranging from very low (13%) to 100%, depending on the method used. In fact, patients who develop antibodies that recognize conformational epitopes or glycosylation-dependent epitopes of the S protein could present as false negative where denatured non-glycosilated linear forms are used. Moreover, a rate of false positive results have been shown (0–30%) using ELISA and WB assays [49,54,55]. The structural similarity and the sequence conservation of the immunogenic proteins of related CoV species that share common structural epitopes, can elicit cross-reactive antibodies in the host. Since cross-reactivity is more likely to occur with a high level of conservation of the proteins, this might explain why N protein-based serological assays were more often associated with cross-reactivity than S protein-based assays. Analysis of complete N sequences revealed that SARS-CoV N protein shared 25–29% identity with α-CoV and 33–47% with related β-CoV. The complete S protein sequence showed a lower degree of conservation,

sharing 23–25% an identity with α-CoV and 29% with related β-CoV. Walls et al. [56] reported that polyclonal antibodies derived by SARS-CoV were able to neutralize SARS-CoV-2, preventing the entry into cells. Most SARS-CoV neutralizing antibodies target the S domain and in particular the RBD domain. Since S domains of SARS-CoV and SARS-CoV-2 share 75% amino acid sequence similarity, further studies will be necessary and useful to evaluate whether SARS-CoV antibodies could neutralize the newly emerged SARS CoV-2.

Nisreen et al. [57] validated and tested various antigens (RBD, N, S1) in different in-house and commercial ELISA methods, showing that among the spike protein antigens tested, the S1 was more specific than S in detecting SARS-CoV-2 antibodies, while N protein was more sensitive than S1. Amanat et al. [58] described an ELISA method based on reactivity to the immunogenic S protein of the virus, generating two different versions of the S protein, one expressing a full-length version of the S protein, the other only the smaller fragment RBD. They suggested that antibodies mounted after infection target both the full-length S protein as well as the RBD. Compared to full viral antigens, assays using recombinant proteins could be more easily standardized. In fact, in a serological assay it would be preferable to use a high immunogenic recombinant antigen with specific epitopes and with a low grade of cross-reactivity. In addition, kinetic and qualitative/quantitative aspects of seroconversion are still to be accurately defined.

Zhao et al. [59] enrolled 173 patients with confirmed SARS-CoV-2 infection using ELISA kits showing a seroconversion rate for total (IgA/IgG/IgM), IgM, and IgG antibodies of 93.1% (161/173), 82.7% (143/173), and 64.7% (112/173), and a median time of 11, 12, and 14 days, respectively. Not taking into account the quicker seroconversion of total antibodies possibly attributed to the double-antigen sandwich, it is interesting to note how the median times of IgG and IgM seroconversion are quite similar (at day 12 or 14) to data reported in the literature on the previous SARS-CoV, in which IgG and IgM antibody levels increased to detectable levels at the second week of illness [60]. Another important finding was the suggestion of combined use of the nucleic acid tests and serological tests to markedly improve diagnostic sensitivity for COVID-19 patients during different phases of the disease, ranging from 78.7% to 100%.

Guo et al. [61] analyzed 82 confirmed and 58 probable COVID-19 cases (RT-PCR negative but with typical clinical manifestation). They focused on the early humoral response of IgM and IgA antibodies (median duration = 5 days, IQR 3–6). Compared to the ELISA titers at days 0–7, the IgM and IgA antibodies levels increased both between days 8–14 but did not increase further between days 15–21 or after day 21, whereas the IgG antibodies increased on days 8–14 but continued to rise until days 15–21 and plateaued by day 21.

Similar to Zhao's study, Guo's group also showed a positive detection rate significantly increased (98.6%) when combined IgM assay with nucleic acid tests compared with the single RT-PCR test (51.9%). Okba et al. [62] also underlined the high sensitivity of the IgA compared to the IgG antibodies but few researchers have currently studied all the three antibodies (IgG, IgM, and IgA), even though IgA importance was already well known in the profiles of patients with pneumonia SARS-CoV [60].

Interestingly, Luo et al. [63] investigated the seroconversion both post-exposure and post-symptoms onset using three different immunoassays: ELISA, colloidal-gold lateral-flow immunoassays (LFIA), and chemiluminescence immunoassay (CLIA). Even though it was not the aim of the study, the authors showed comparable diagnostic performances among the three methods. The median seroconversion time for IgM and IgG antibodies was 18 and 20 days post-exposure and 10 and 12 days post-symptom onset, respectively. The antibody levels increased rapidly after 6 days post-onset but together with a decrease of the viral load pointing out their complementarity.

Li et al. [64] studied samples collected from 397 RT-PCR confirmed COVID-19 patients and 128 negative patients from eight different clinical centers using a rapid lateral flow immunoassay searching for IgM and IgG antibodies. The IgM-IgG combined assay showed better sensitivity compared to the single IgM or IgG test. In fact, among the COVID-19 patients it was found that 64.48% (256 of 397) had both IgM and IgG positive test results, while 18.13% (72 of 397) and 6.04% (24 of 397) had only IgM, and only IgG positive results, respectively.

Jia et al. [65], evaluated 57 patients suspected of SARS-CoV-2 infection, 24 of whom had positive nucleic acid tests and 33 had negative. The positive detection rate with combined IgM and IgG for patients with SARS-CoV-2 negative and positive nucleic acid test was 72.73% and 87.50%, respectively, using the fluorescence immunochromatographic assay method. The results were significantly higher than the nucleic acid or IgM, IgG singly, confirming the study's conclusion that the highest overall sensitivity comes from an IgM-IgG combined assay.

As with most existing studies on the diagnostic performance of the SARS-CoV-2 antibodies, the great limitation of these two latter studies is that they contain few medical and clinical details about the specific sample collection of the patients studied, such as when the patient was infected or how long symptoms had been present before the blood sample was collected. Moreover, they have a great bias when considering the various time lapses between the patient's initial exposure to the virus and the blood exam confirming detection. However, it is important to be aware of how difficult it is to identify the exact time of contagion during a pandemic infection because there are so many possible sources of contagion (consider

healthcare workers, for example). As a consequence, data are still of little use today, both when considering the different isotypes and comparing single or double positivity of different isotype antibodies.

It is worth noting the reported low rate of isolated IgM antibody positivity in the majority of the studies. We can speculate that this could be a false negative due to low antibody concentrations or to their short duration. Certainly, the post-pandemic retrospective studies, especially those conducted on asymptomatic carriers, will provide us with more precise information on the detection of single antibodies and on the virus infection time.

As these tests become more and more available, it is increasingly difficult to distinguish quality and clinical efficacy. In the absence of specific references, there is consequently an ongoing scientific and political debate on the most proper use of serological diagnostics, and on the best time to use them. We actually do not know if they perform better as a screening test (to be confirmed through NAAT testing), as a diagnostic tool alongside molecular diagnostics to achieve the greatest accuracy, or with the epidemiologic aim of getting a real picture of the pandemic at its end, thereby evaluating the immunization state of the population. In any case, it will be necessary to individuate people who have had contact with the virus (often completely asymptomatic) and have gained immunity, those who are still infectious although paucisymptomatic, and those who have no antibodies and are therefore potentially susceptible to contagion. This status is particularly important with the prospect of returning to work, especially for healthcare practitioners. Moreover, it could be helpful in isolating new outbreaks and for epidemiological purposes. Despite the urgency to get these tests in the field, laboratories should always be reminded that the kits used require formal, essential recognition, such as the CE mark, and that there must be strong evidence of the kits' reliability (accuracy, precision, specificity, and sensitivity).

In Italy, as well as in Spain, we have been seeing a different approach by individual regions and private/public bodies (e.g., companies, pharmacies, family doctors) in procuring and executing rapid tests that might be full of risks that could cause erratic results, bad health decisions, and risky plans for individuals and the community as a whole.

However, the current utilization of serological tests could provide more information about the kinetics of different immunoglobulin isotypes during the disease. We could then explore the existence of any correlation between the antibodies time of appearance and titers, and clinical features such as disease severity. Hence, it would be better to use quantitative antibodies detection (e.g., ELISA, CLIA) rather than qualitative (chromatographic immunoassay) methods. Although there is little and contrasting data available on this topic, antibodies may provide a mirror of how immunity is acting, giving clinicians a valid prognostic as well as diagnostic tool. If it is true that improper immune activation and a failure to develop virus-targeted adaptive immunity seem to be determinant in clinical precipitation, an observed delay in antibodies formation may elicit an alert on those patients and induce stricter monitoring. Nevertheless, knowledge from other infectious, as well as from autoimmune diseases, suggests that specific antibodies may contribute to determine organ damage. For example, immunoglobulins can activate immune cells as macrophages both in a pro-inflammatory and anti-inflammatory way through their interaction with different subtypes of cellular FcγRs. This topic is particularly interesting when considering the development processes of vaccines. In fact, it is a common observation that some attempts at active immunization fail because of their paradoxical effect of increasing infection-mediated injury [66]. A hypothesis is that diverse viral epitopes could generate antibodies with different behavior toward monocytic-macrophagic cells, also showing differences in response kinetics. This is consistent with the findings of Zhang et al. [67], where sera from patients who had recovered from SARS-CoV infection revealed a slower but significantly more persistent seroconversion after the first phase of viral-mediated lung injury, while deceased patients developed very high antibodies titers just in the early stage of infection but with an equally rapid decremental slope. So, when facing the same pathogen, the immune system of recovering patients might recognize those viral peptides leading to the production of more efficient antibodies in terms of timing, activity on immune cells as well as neutralizing power, whereas patients with the poorest prognosis could react against other epitopes, leading to a praecox and dysfunctional response.

In this complex scenario, once experience and comprehension have been gained in using SARS-CoV-2 antibodies, the tests could be added on to other known biomarkers such as D-dimer, LDH, and CK to more accurately predict the cytokinic storm, thereby allowing timely intervention with a pharmacological blockade. In this regard, there is a medical debate on when it is best to administer drugs such as tocilizumab so as not to depress the immune response too early while preventing its non-returning degeneration.

Nevertheless, what is fundamental to keep in mind, for clinicians as well as for laboratory doctors and public health administrators, is that no laboratory test, neither RT-PCR nor serology, could ever substitute clinical observation and practical experience. If there is clinical suspicion for COVID-19, a negative response from tests cannot exclude the presence of the disease. This finding is true for every medical condition, and even more so for such a critical situation, when a missed positive patient means a missed opportunity to adequately treat him/her and to accurately contain the devastating cascade of contagion.

COVID-19 diagnostics is now a critical public health tool and must be performed by authorized laboratories with highly

specific capabilities, also in terms of evaluating the kits to be used in the epidemic control chain.

For these reasons, we have tried to overview the little data currently available in the literature, to emphasize the need for a broader validation scale in the various subpopulations, especially in the asymptomatic carriers and convalescent patients.

Something that would seem to represent an opportunity might then become a dangerous weapon at a time when screening is actually the strongest tool we have to fight COVID-19 infection.

Correspondence
Dr. M. Infantino
Immunology and Allergy Laboratory Unit, San Giovanni di Dio Hospital, Florence 50143, Italy
Phone: (39-055) 6932502, **Fax:** (39-055) 6932289
email: maria2.infantino@uslcentro.toscana.it

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Capsule

New ideas about neoantigens

Tumors with a low mutational burden are thought to have fewer neoantigens available for T cells to respond to and thus are not necessarily considered for checkpoint blockade therapy. **Subudhi** and colleagues treated patients with metastatic, castration-resistant prostate cancer, which has a relatively low mutation burden, with the cancer drug ipilimumab. Patients that responded to the treatment had a T cell response signature and detectable neoantigen immunity. Checkpoint blockade therapy with ipilimumab can thus instigate T cell responses to tumor neoantigens, despite the low tumor mutational burden status.

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Eitan Israeli

Capsule

Airway-hugging macrophages

Effective immune defense in the lungs relies on myeloid cells that phagocytose, process, and present foreign substances that enter the airways, including pathogens. **Ural** and co-authors studied a subset of pulmonary interstitial macrophages predominantly found within the lungs that serve an immunoregulatory role during responses to lung inflammation. These findings provide a deeper insight into the specialized myeloid subsets that contribute to maintaining pulmonary homeostasis.

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Eitan Israeli