Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Internal Medicine 107 (2023) 7–16

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Review Article

Clinical usefulness of testing for severe acute respiratory syndrome coronavirus 2 antibodies

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A B S T R A C T

In the COVID-19 pandemic era, antibody testing against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has proven an invaluable tool and herein we highlight some of the most useful clinical and/or epidemiological applications of humoral immune responses recording. Anti-spike circulating IgGs and SARS-CoV-2 neutralizing antibodies can serve as predictors of disease progression or disease prevention, whereas anti-nucleocapsid antibodies can help distinguishing infection from vaccination. Also, in the era of immunotherapies we address the validity of anti-SARS-CoV-2 antibody monitoring post-infection and/or vaccination following therapies with the popular anti-CD20 monoclonals, as well as in the context of various cancers or autoimmune conditions such as rheumatoid arthritis and multiple sclerosis. Additional crucial applications include population immunosurveillance, either at the general population or at specific communities such as health workers. Finally, we discuss how testing of antibodies in cerebrospinal fluid can inform us on the neurological complications that often accompany COVID-19.

1. Introduction

The infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggers both B-cell and T-cell responses directed against all viral antigens including the Nucleocapsid (N) and Spike (S) proteins. S protein is essential for viral entry into host cells and N protein is the most abundantly expressed immunodominant protein. Following the initial contact with the virus and fueled by pro-inflammatory cytokines an antibody response is mounted. Specific antibody tests can reliably detect the presence of these antibodies in biological fluids including serum, plasma, saliva [1], urine, human milk [2] and cerebrospinal fluid (CSF) [3]. Depending on the type and timing of test as well as on the kind of fluid tested different clinical information can be procured. In this review article, we will succinctly describe some of the clinical applications of anti-SARS-CoV-2 antibody testing with a view to the future of the still evolving COVID-19 pandemic.

Several studies indicate that most immunocompetent persons develop an adaptive immune response following contact with the virus, irrespectively of disease severity. Antibodies including those of the IgM, IgA and IgG classes against N and S proteins can be detected in the serum as early as 1–3 weeks post-infection, whereas IgM decay rapidly, IgG and IgA can persist for several months (Fig. 1). Nevertheless, the titer and exact duration of the anti-SARS-CoV-2 antibodies persistence in the circulation after the clearance of the infection varies and is likely donor-specific while it also depends on disease severity [4,5]. Specifically, antibody titers in most cases correlate with disease severity as subjects with more severe COVID-19 raise higher titers (see below) and exhibit longer persistence [6].

Different assays can be used to *a.* measure the titer of antibodies and their binding to specific SARS-CoV-2 antigens (e.g., N or S proteins) and/or *b.* determine their specific neutralizing activity. Binding tests fall into two broad categories. Point-of-care tests are performed in any setting e.g., hospital ward, nursing home or workplace and they usually are lateral flow devices that detect antibodies in a blood drop. Laboratory tests require specialized personnel and include methods such as ELISA (Fig. 2) and chemiluminescence assays (CIA/CLIA) that detect the antibodies from serum, plasma, dried blood spots or CSF (Fig. 2). In total 85 tests have received EUA for serology from the FDA (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-serology-and-other-adaptive-immune-response-tests-sars-cov-2). This approval categorizes laboratories in three categories, namely H for meeting requirements to perform high complexity tests, M for meeting requirements to perform moderate complexity tests and W which are patient care settings.

Neutralizing assays are a proxy of the capacity of antibodies against S

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https://doi.org/10.1016/j.ejim.2022.11.009
Received 25 July 2022; Received in revised form 11 October 2022; Accepted 8 November 2022
Available online 10 November 2022

to block viral binding to its cognate receptor i.e., the angiotensin converting enzyme 2 (ACE2) and thus entry in human cells (Fig. 2). Types of tests in this category include virus neutralization, pseudo-virus neutralization and competitive neutralization [7]. The first two types require more time, specialized personnel and facilities for handling pathogens, while the latter (in a plate format) is commercially available and easy to set-up and perform in a standard wet lab. Currently, only two tests have received EAU from the FDA (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-serology-and-other--adaptive-immune-response-tests-sars-cov-2).

Serological assays that can reveal humoral immune responses against SARS-CoV-2 play a critical role in informing public health providers as well as in directing health care decisions and policies. Currently, antibody tests are mostly performed in central clinical laboratories with a limiting broad access to diverse populations. Moreover, it is important to provide highly sensitive assays that can discriminate between SARS-CoV-2 infection and vaccination. To this end various novel methods are still under development including a multiplexed nano plasmonic biosensor [8] or a microfluidic cartridge based devise [9]; these can be deployed as point-of-care (PoC) antibody profiling methods even in non-specialized places such as the workplace or a nursing home.

2. Clinical applications

Several clinical applications can be informed by antibody testing after careful consideration of the analyte measured (type of Ig and/or kind of antigen, see Table 1) and the biological fluid used.

2.1. Antibody testing should not be used to diagnose acute SARS-CoV-2 infection

Antibody testing against any viral antigen is not as sensitive and (in some cases) specific as molecular (i.e., PCR detection of SARS-CoV-2 genes) testing. Nevertheless, antibody testing can be for instance useful to identify infected subjects in vaccinated populations. To this end, specialized testing is being developed to simultaneously detect antibodies against S, RBD and N proteins and even neutralizing capacity of antibodies [10] in the blood or in saliva. SARS-CoV-2 antibodies in saliva serve as first line of defense against the virus. They are present in the mucosa, more precisely in saliva, after a recovered infection. Reportedly, antibody persistence in plasma and in saliva was shown in up to 15 months after mild COVID-19 [11]. Notably, salivary IgA and IgG antibodies could be detected earlier in patients with mild COVID-19 symptoms as compared to severe cases [12]. However, severe COVID-19 triggered higher salivary antibody and blood antibody titers than asymptomatic or mild infections [13]. Salivary IgA titers quickly decreased after 6 weeks in mild cases but remained detectable until at least week 10 after severe COVID-19. In conclusion, assays for both IgA and IgG have high specificity and sensitivity for the confirmation of current or recent SARS-CoV-2 infections and evaluation of the IgA and IgG immune responses [12].

Another novel method for seroprevalence studies employs SARS-CoV-2 IgG FcγR ELISAs, methodically combining antigen-antibody binding in solution and isotype-specific detection of immune complexes, allowing for the long-term detection of anti-SARS-CoV-2 IgG antibodies in populations with a challenging immunological background and/or in populations which S-protein-based vaccine programs have been rolled out [14]. Antibody testing can also be potentially useful for long-COVID detection, a yet poorly defined clinical entity. Specifically, it has been shown that 42–53% of subjects with long COVID, but without detectable SARS-CoV-2 antibodies, nonetheless had detectable SARS-CoV-2 specific T cell responses; these findings demonstrate the diagnostic complexity of long COVID and how is compounded in many patients who were or might have been infected with SARS-CoV-2 but not tested during the acute illness and/or are SARS-CoV-2 antibody negative [15]. Additionally, machine learning approaches that input parameters such as serum pro-inflammatory, anti-inflammatory and anti-viral

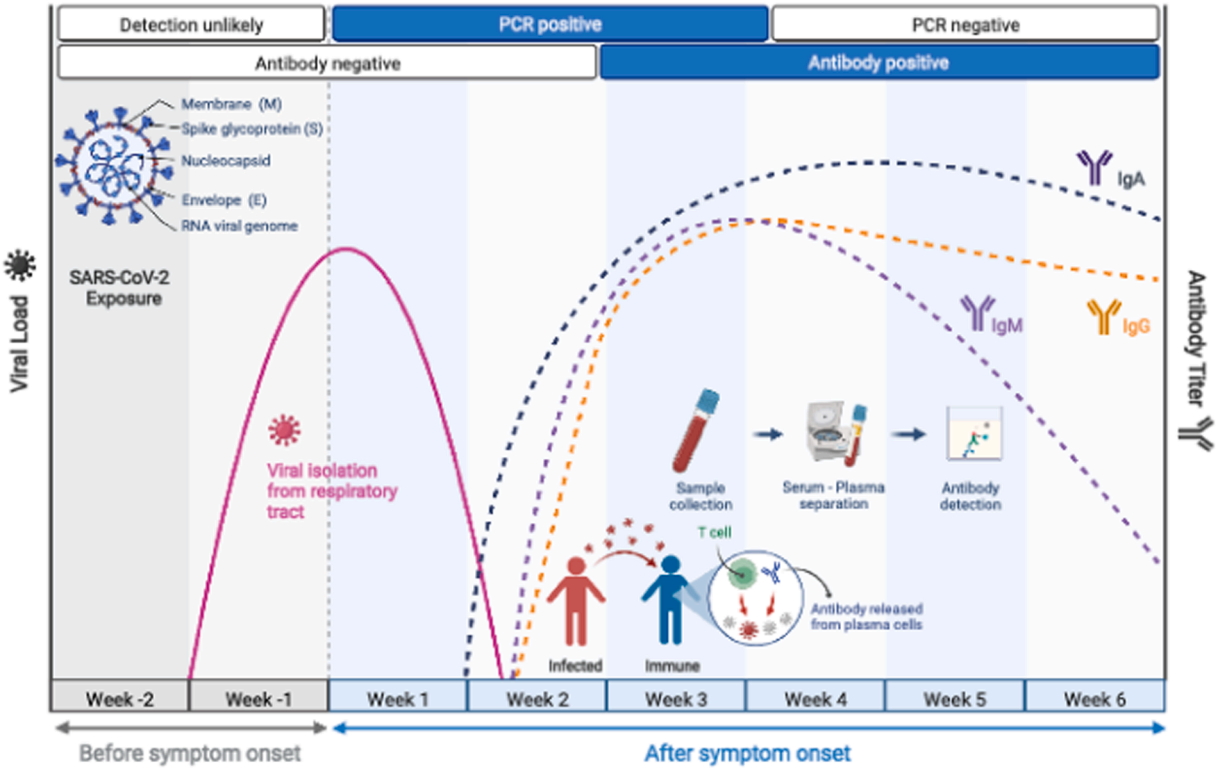

Fig. 1. Time course of SARS-CoV-2 infection, IgG, IgM and IgA antibody production and possible test positivity. IgA and IgM responses are the first to emerge, while IgG and IgA persist for longer.

cytokine and anti-SARS-CoV-2 antibody measurements can also help stratify patients at hospital admittance into high and low risk clinical categories with distinct cytokine and antibody profiles that may guide personalized therapy [16].

Given the ongoing anti-COVID-19 vaccination campaign in many countries globally, monitoring of breakthrough infections is also a matter of great importance. Since COVID-19 vaccines induce immune responses against the S protein, which is the main sero-surveillance target to date, alternative targets are being explored to distinguish infection from vaccination. The sensitivity of N seropositivity was 85% for mild COVID-19 in the first two months following symptoms onset but sensitivity was lower in asymptomatic individuals (67%). N-specific IgG concentrations were not affected by vaccination in infection-naïve participants therefore serological responses to N may prove helpful in identifying SARS-CoV-2 infections post-vaccination [17]. Similarly, in a cohort of adult paramedics in Canada, the performance of N antibodies detection was investigated to identify previous COVID-19 infections and compare differences among vaccinated and unvaccinated donors. It was found that vaccinated and unvaccinated groups require different thresholds to achieve optimal test performance, especially for detecting SARS-CoV-2 infection within the preceding 9 months [18].

2.2. Antibody testing to predict the degree of disease severity and individual protection from infection and re-infection

Several studies aim to predict COVID-19 severity in unvaccinated individuals by using anti-SARS-CoV-2 serological responses. To this end the kinetics of the serological responses along with the correlation between the antibody titers and disease outcome have been assessed. It has been found that antibody titers gradually increased for up to 3 weeks since the onset of symptoms for patients requiring oxygen supplementation with significantly higher antibody titers for patients requiring invasive ventilation [13]. Antibody titers on admission were also significantly higher in severely ill patients and serology performed well in predicting the necessity of invasive ventilation [19]. Similar results were obtained from another study that showed that high IgG levels against S positively correlated with biomarkers of immune activation and inflammation, while they were negatively correlated with pulmonary function and the extent of pulmonary CT abnormalities. It was thus proposed that S-specific IgG levels serve as a useful immunological surrogate marker for identifying at-risk individuals with persistent pulmonary injury who may require intensive follow-up care after COVID-19 [20]. Regarding protection, in the coronavirus efficacy (COVE) phase 3 clinical trial, vaccine recipients were assessed for neutralizing and binding circulating antibodies as correlates of risk for COVID-19 disease and as correlates of protection. Antibodies were measured at the time of second vaccination and 4 weeks later. It was found that vaccine recipients with post-vaccination 50% neutralization titers 10, 100, and 1000 had estimated vaccine efficacies of 78%, 91% and 96%, respectively, suggesting that measuring neutralizing activity is a strong predictor of vaccine efficacy and can be used to inform vaccination strategies [21]. To apply this concept in the community, i.e., that neutralization is a proxy for actual protection, several efforts are under way to provide lateral flow Point of Care (PoC) tests that can measure levels of RBD-ACE2 neutralizing antibody (NAb) from whole blood, with a result that can be determined by eye or quantitatively on a small instrument [22]. Also significant is the demonstration that such tests can show high correlation with conventional neutralization tests [23].

Convalescent individuals who previously recovered from COVID-19 have enhanced immune responses after vaccination (hybrid immunity) compared with their naïve-vaccinated peers; however, the effects of post-vaccination breakthrough infections on humoral immune response and predicted levels of protection remain to be determined. This was addressed in a study where neutralizing antibody responses were measured in 104 vaccinated individuals, including those with breakthrough infections, hybrid immunity, and no infection history. It was shown that immune sera after breakthrough infection and vaccination after natural infection broadly neutralize SARS-CoV-2 variants to a

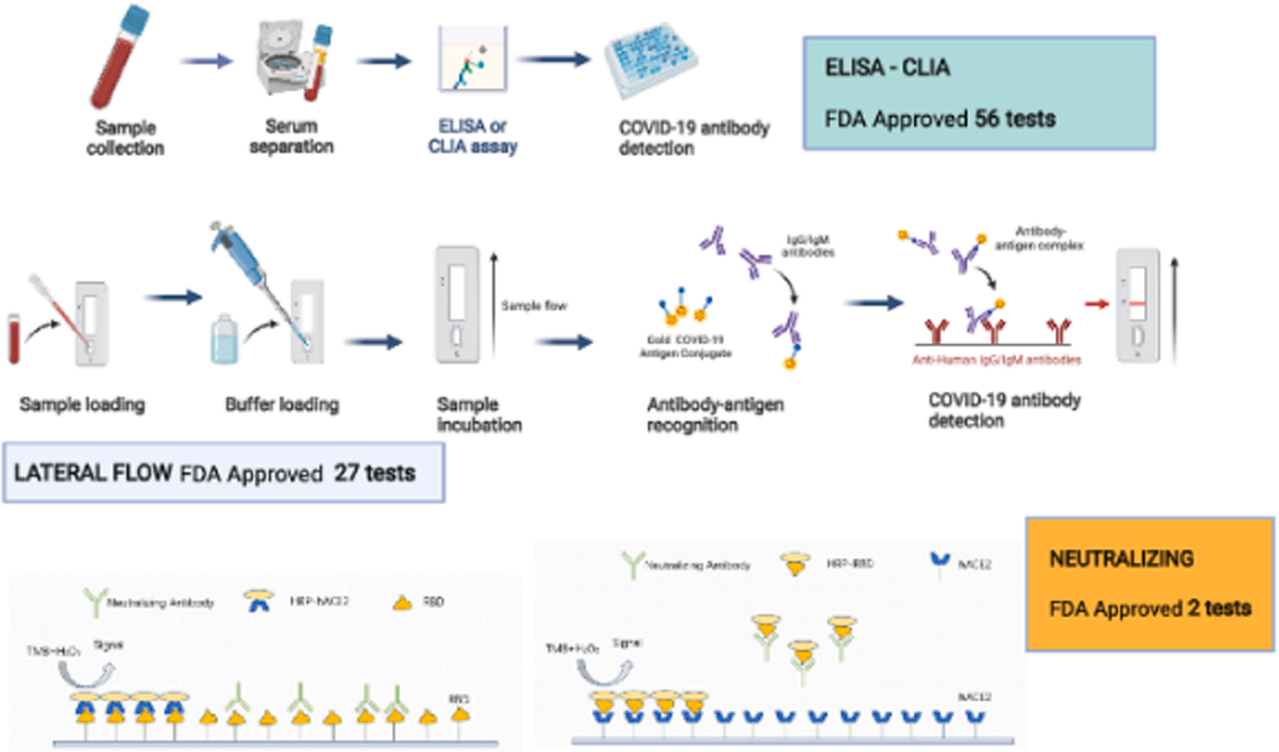

Fig. 2. FDA approved tests and methodologies. Testing with laboratory methods as represented e.g., ELISA or CLIA provides higher specificity and sensitivity, while point-of-care testing e.g., with lateral flow methods is particularly useful in specialized settings such as nursing homes or workplaces. Neutralizing antibody detection methods are based on the principle that antibodies can block the RBD-ACE2 interaction.

Table 1
Antibody testing & clinical usefulness.

Analyte	Diagnosis of infection - Naive	Diagnosis of Infection - Vaccinated	Response to vaccine	Community seroprevalence	Neurological COVID (CSF testing)
IgG Spike	+++	–	+++	+++	+++
IgM Spike	+++	–	+	+	+
IgA Spike	+	+	–	+	–
IgG N proteins	+++	+++	–	+++	N/A
IgM N proteins	+++	++	–	+	N/A

(+++, ++, +) Level of clinical usefulness per analyte.
(-) Detection of analyte is not applicable.
N/A Data not available.

similar degree. These data suggest that the additional exposure to antigens derived from natural infection substantially boosts the quantity, quality, and breadth of humoral immune response regardless of whether it occurs before or after vaccination [24]. Nevertheless, the molecular details of hybrid immunity warrant additional future studies. Omicron variants share some distinct characteristics that are different from initial SARS-CoV-2 mutants and thus protection of prior infection against reinfection with omicron ranged from 18.0% for patients infected in the first wave of COVID-19 to 69.2% for those infected in the Delta wave [25].

Across the same lines of research, the extent to which Omicron infection, with or without previous vaccination, elicits protection against the previously dominant Delta variant was investigated and it was shown that vaccination combined with Omicron/BA.1 infection hybrid immunity is likely protective against Delta and other variants. In contrast, infection with Omicron/BA.1 alone offered limited cross-VoCs protection despite moderate enhancement [26]. Interestingly, long-term studies (up to 18 months) have showed persistent circulating antibodies even after mild infection, indicating that further work into the detailed immunological mechanisms that govern persistence is required [27]. Also, as mentioned above, higher titers of antibodies have been reported in patients suffering from more severe forms of the disease. These high titers were also detected in deceased patients, compared to asymptomatic patients indicating that fatal infection is not associated with defective humoral response [28].

3. Antibody testing to assess the effects of immunosuppressive drugs and modify therapeutic regimes to obtain an optimal vaccine response in sensitive populations

Healthy individuals respond adequately to full vaccination, although this response is often gender- and age-dependent and is reduced with time [29–32]. However, either binding or neutralizing antibody responses after vaccination in healthy people is reduced against the different mutants, especially against omicron; therefore the administration of a booster dose is of value [33–36]. To address the issue of vaccine response in populations receiving immunomodulatory drugs several studies have been conducted to date. The studies discussed below use binding titers against S protein as the key measure, except from a few were we specifically state that a neutralizing assay was used. Methodologically, the use of either ELISA or electrochemiluminescence is of equal value, as both type of methods are FDA-approved.

Studies following immunosuppression, include patients with rheumatic diseases, multiple sclerosis and cancer. Reportedly, COVID-19 vaccine-induced antibody responses were altered in patients with inflammatory bowel disease on commonly used immunosuppressive drugs. More specifically, patients on six different immunosuppressive treatment regimens (thiopurines, infliximab, a thiopurine plus infliximab, ustekinumab, vedolizumab, or tofacitinib) were recruited along with healthy control participants from nine centers in the UK. Eligible participants had received two doses of COVID-19 vaccines and antibodies were measured at 53–92 days post-second vaccine dose. The immunogenicity of COVID-19 vaccines varied according to drug, and was attenuated in recipients of infliximab, infliximab plus thiopurines, and tofacitinib [37].

In another prospective observational multicenter study, that included 478 patients with rheumatoid arthritis (RA), systemic lupus erythematosus (systemic sclerosis (SSc), cryoglobulinemic vasculitis and a miscellanea of 13 systemic vasculitis a significantly lower neutralizing antibody response was shown in patients *versus* controls. Increased prevalence of non-response to vaccine was attributed in those treated with glucocorticoids, mycophenolate-mofetil or rituximab [38]. Also, in patients with autoimmune rheumatic diseases under therapy with methotrexate (MTX) it was shown that MTX reduces the immunogenicity of SARS-CoV-2 vaccination in an age-dependent manner. It was suggested that holding MTX for at least 10 days after vaccination significantly improves the antibody response in patients over 60 years of age [39].

The fact that rituximab (an anti-CD 20 monoclonal antibody) interferes with vaccine efficacy is supported by another study of chronic rituximab treated patients which showed only 36% seroconversion after vaccination [40]. Notably, in ANCA-associated vasculitis patients, and despite the lack of a measurable humoral immune response, B-cell depleted patients mounted a similar vaccine induced antigen-specific T-cell response compared to B-cell recovered patients and normal controls [41]. Whether more vaccine doses can change this outcome was tested in RA patients on rituximab in a prospective, cohort study, where patients with insufficient serological responses to two doses were allotted a third vaccine dose. This third dose given 6–9 months after a rituximab infusion still did not induce a robust serological response, but was considered to boost the cellular T-cells immune response [42]. These studies in patients treated with rituximab clearly call for a re-evaluation of the 6-month interval between treatment and vaccination [43].

Similarly, the antibody levels in multiple sclerosis (MS) patients on anti-CD20 therapy (either rituximab or ocrelizumab), were assessed. It was found that 14.0%, 37.7%, and 33.3% were seropositive after the first, second and third vaccination, while no difference was found in antibody levels after the second and third dose. These findings suggest the need for clinical strategies to allow B cell reconstitution before boosting vaccination [44]. In another multicenter prospective study in MS patients, seroconversion was lowest in patients on anti-CD20 monoclonal antibodies followed by patients on sphingosine-1-phosphate-receptor-modulators [45]. This finding was independently confirmed in two other studies [46], including a large study from Israel where apart from showing that fingolimod- or ocrelizumab-treated patients had diminished humoral responses it was also found that fingolimod compromised cellular immune responses, with no improvement after the third boosting dose. Nevertheless, vaccination following >5 months since ocrelizumab infusion was associated with better seropositivity [47] while $CD4^+$ and $CD8^+$ T-cell responses were preserved [48,49].

In dialysis patients, plasma samples were analyzed from 130 hemodialysis and 13 peritoneal dialysis patients after two doses of BNT162b2 or mRNA-1273 vaccines. It was found that 35% of the patients had low-level or undetectable IgG antibodies to S and that neutralizing

antibodies against the vaccine-matched SARS-CoV-2 and Delta were low or undetectable in 49% and 77% of patients, respectively. In these cases, antibody responses must be continuously monitored to adopt the best prophylactic and/or therapeutic strategy [50].

In patients after allogeneic stem cell transplantation vaccination efficacy might be impaired depending on the immune reconstitution. It has been shown that most patients did develop a high antibody titer (138 out 182 patients, 75.8%); while patients undergoing allogeneic stem cell transplantation have been excluded from the initial registration trials, this real-world study showed that most patients do have an adequate response to mRNA vaccines [51]. On the contrary, seroconverted kidney transplant recipients showed impaired neutralization against emerging variants of concern after standard two-dose vaccination [52]. Comparing kidney to liver transplant patients after vaccination showed that in liver transplant recipients, IgG levels against every S epitope tested increased significantly compared to the kidney transplant recipients. It seems that vaccination elicits a stronger antibody response in liver than in kidney transplant recipients, a phenomenon that cannot entirely explained by the different immunosuppression employed [53].

In people infected with HIV (PLWH) and receiving suppressive antiretroviral therapy binding circulating antibodies against RBD were measured one month following the first and second COVID-19 vaccine doses, and again 3 months following the second dose. It was shown that PLWH with well-controlled viral loads and $CD4^+$ T-cell counts in a healthy range generally mounted strong initial humoral responses to dual COVID-19 vaccination [54].

Overall, cancer patients with COVID-19 have reduced survival. While most cancer patients, have an almost 100% rate of seroconversion after SARS-CoV-2 infection or vaccination, patients with hematological malignancies have lower or even minimal seroconversion rates. A study from Florida [55] revealed that in 515 cancer patients seropositivity after two vaccination doses was 90.3% but was significantly lower among patients with hematologic cancer (84.7%) *vs.* solid tumors (98.1%) and was lowest among patients with lymphoid cancer (70.0%). Importantly, patients receiving vaccination within 6 months after anti-CD20 monoclonal antibody treatment had a significantly lower seroconversion (6.3%) compared with those treated 6 to 24 months earlier (53.3%) or those who never received anti-CD20 treatment (94.2%). In another prospective observational study immunogenicity was assessed in 85 patients treated with immune checkpoint inhibitors (ICIs) for a broad range of solid tumors. Despite the relatively poor responses following the priming dose, the seroconversion rates significantly increased after the second dose; the administration of a third booster dose remarkably optimized antibody responses [56]. Similarly, in a cohort of patients with hematologic malignancies, 76.3% of patients developed humoral immunity, and the cellular response rate was 79%. Hypogammaglobulinemia, lymphopenia, active hematologic treatment, and anti-CD20 therapy during the previous 6 months were associated with an inferior humoral response. A significant dissociation between the humoral and cellular responses was observed in patients treated with anti-CD20 therapy; in these cases, the humoral response was 17.5%, whereas the cellular response was 71.1%. In these patients, B-cell aplasia was confirmed while T-cell counts were preserved [57]. Finally, in a cohort where patients had received bone marrow transplantation or CAR-T cells, significantly lower anti-S antibodies were noted to the Wuhan strain following 2doses of the BNT162b2 mRNA vaccine, with proportional lower cross-recognition against Beta, Delta, and Omicron S-RBD proteins. Both cohorts neutralized the wildtype WA1 and Delta variants but not the Omicron variant [58].

The titers of neutralizing antibodies were also determined in patients with Multiple Myeloma (MM) or Waldenström macroglobulinemia (WM) after vaccination. Patients with MM produce lower amounts of neutralizing antibodies against SARS-CoV-2 after full vaccination, even after two booster doses, especially those under treatment with anti-CD38 or anti-BCMA therapies [59–62]. In MM, vaccine-mediated antibody production is affected by race, disease, vaccine, and treatment characteristics [63]. In WM, the data suggest that vaccination with either 2 doses of the BNT162b2 or 1 dose of the AZD1222 vaccine led to lower production of neutralizing antibodies in patients compared to controls. Moreover, active treatment with either rituximab or Bruton's tyrosine kinase inhibitors was proven to be an independent prognostic factor for suboptimal antibody response after vaccination, even after a booster vaccine dose [64,65]. In patients with myeloid malignancy, including 46 patients with acute myeloid leukemia (AML) and 23 patients with myelodysplastic syndrome (MDS), seroconversion rates were 94.7% and 100% respectively, with no significant difference from healthy controls. Nevertheless, patients with MDS showed a significantly lower antibody titer than that found in healthy controls or AML patients. This study demonstrates that patients with myeloid malignancies may be more responsive to vaccines than patients with lymphoid malignancies [66]. In patients with chronic lymphocytic leukemia or B cell non-Hodgkin lymphoma, and multiple myeloma the use of a third vaccine dose is supported by evidence, even though some of these patients will still demonstrate vaccine failure [67]. Finally, in another cohort, and despite the absence of humoral immune responses in fully vaccinated anti-CD20-treated patients with lymphoma, their $CD8^+$ T-cell responses reach similar frequencies and magnitudes as controls [68].

In a consensus generated by members of the European Multiple Myeloma Network it was confirmed that a suboptimal anti-SARS-CoV-2 humoral immune response, means that a proportion of patients are likely unprotected. Factors associated with poor response are uncontrolled disease, immunosuppression, concomitant therapy, more lines of therapy, and CD38 antibody-directed and B-cell maturation antigen-directed therapy. These facts suggest that monitoring the immune response to vaccination in patients with multiple myeloma might provide guidance for the administration of additional doses of the same or another vaccine, or even treatment discontinuation [69].

Overall, clearly, the subtype of hematologic malignancy and B-cell depleting treatment may predict a poor immune response to vaccination. Recently, antiviral drugs and monoclonal antibodies for pre-exposure or post-exposure prophylaxis and for early treatment of COVID-19 have become available. These therapies should be offered to patients at high risk for severe COVID-19 and vaccine non-responders, including patients with hematologic malignancy [70]. Evidence suggests that patients with hematologic cancer and those who are receiving immunosuppressive treatments may need additional vaccination doses [55]. There is clearly a need to develop guidelines to direct vaccination schedules and protective measures in oncology patients, differentiating those with hematological malignancies and those in an immunocompromised state [71].

4. Antibody testing to assess seroprevalence evaluation in the community post-infection and/or vaccination, can inform public health policies in a population level or in specialized settings

Public health decisions require surveillance testing to obtain accurate epidemiological data for COVID-19 pandemic. Surveillance testing may be random sampling of a population to determine incidence and prevalence. To this end, testing need to be able to discriminate immunity from active infection *versus* from vaccination. In a population level, determining true rates of infection can inform us on the effectiveness of measures used for the restrain of the pandemic i.e., vaccination and social distancing. Similarly, such serological surveys can be performed in places of importance such as hospitals, nursing homes, critical workplaces and universities [72]. Regarding methods, these studies (unless otherwise stated) employ anti-S or anti-RBD determination with FDA-approved tests.

4.1. Population studies

In a population level, interesting data emerged from Australia. As of

mid-2021, Australia's only nationwide COVID-19 epidemic occurred in the first 6 months of the pandemic. In Australia's largest national SARS-CoV-2 serosurvey from 11,317 specimens only 71 were positive for SARS-CoV-2-specific antibodies while no seropositive specimens had neutralizing antibodies, thus the study concluded that Australia's seroprevalence was extremely low (<0.5%) and highlighted the population's naivety to the virus and the urgency for vaccine protection [73]. In another national-wide study from Mexico, and from 9640 blood samples, seroprevalence was estimated by socioeconomic and demographic characteristics. The national seroprevalence was 24.9% being lower for adults 60 years and older. Higher seroprevalence was found among urban and metropolitan areas, low socioeconomic status, low education and workers. Among seropositive people, 67.3% were asymptomatic. These data suggested that social distancing, lockdown measures and vaccination programs need to consider that vulnerable groups are more exposed to the virus [74].

In order to estimate the prevalence of unidentified SARS-CoV-2 infection in the general population of Hong Kong, a prospective cross-sectional study was conducted after each major wave of the COVID-19 pandemic. The study enrolled 4198 participants. Only six participants were confirmed to be positive for anti-SARS-CoV-2 IgG; the adjusted prevalence of unidentified infection was 0.15%. Extrapolating these findings to the whole population, indicated that there were fewer than 1.9 unidentified infections for every recorded confirmed case and it was estimated that the overall prevalence of SARS-CoV-2 infection in Hong Kong before the roll out of vaccination was less than 0.45% [75]. In a Norwegian population-based cross-sectional study, a total of 110,000 people aged 16 years or older were randomly selected during November-December 2020 (before vaccine introduction) and were invited to complete a questionnaire and provide a dried blood spot sample. National weighted and adjusted seroprevalence was 0.9%. In this paradigm, seroprevalence was comparable to virologically detected cases [76]. In Greece, a serosurvey was conducted between March and December 2020. It was designed as a cross-sectional survey repeated at monthly intervals. Of 55,947 serum samples collected, 705 (1.26%) were found positive for antibodies, with higher seroprevalence (9.09%) observed in December 2020. Highly populated metropolitan areas were characterized with elevated seroprevalence levels as compared to the rest of the country [77].

Interestingly, surveillance data in high-income countries have reported more frequent SARS-CoV-2 diagnoses in ethnic minority groups. To further test this, the cumulative incidence of SARS-CoV-2 was estimated in six ethnic groups in Amsterdam, the Netherlands. Compared to Dutch-origin participants (15·9%), cumulative SARS-CoV-2 incidence was higher in participants of South-Asian Surinamese, African Surinamese, Turkish, Moroccan and Ghanaian background. Also, SARS-CoV-2 incidence was higher in the largest ethnic minority groups of Amsterdam, particularly during the second wave.

SARS-CoV-2 antibody seroprevalence can also add crucial epidemiological information about population infection dynamics [78]. To assess the evolving SARS-CoV-2 seroprevalence related to the first national lockdown in Belgium, a nationwide seroprevalence study was performed, using 3000–4000 residual samples during seven periods. Seroprevalence increased from 1.8% to 5.3% over a period of 3 weeks during lockdown (start lockdown mid-March 2020). Thereafter, seroprevalence stabilized. This showed that during lockdown, an initially small but increasing fraction of the Belgian population showed serologically detectable signs of exposure to SARS-CoV-2, which did not further increase when confinement measures eased and full lockdown was lifted [79]. In Germany, a federal state-wide cross-sectional seroprevalence study named SaarCoPS, representative for the adult population was performed. Serum was collected from 2940 adults via stationary or mobile teams during the 1st pandemic wave steady state period. They estimated an adult infection rate of 1.02%, an under-reporting rate of 2.68-fold and infection fatality rates of 2.09% in all adults including elderly individuals. These type of studies are important because they can provide a valuable baseline for evaluation of future pandemic dynamics and impact of public health measures on virus spread and human health [80]. Such studies have also been published among others from Cyprus [81] and Malawi [82]. These kind of studies can help determine how previous SARS-CoV-2 infection and the time since vaccination should be considered when planning booster doses and the design of COVID-19 vaccine strategies [83].

4.2. Specialized settings studies, hospitals and health care workers

In October 2020 SARS-CoV-2 seroprevalence among hospital healthcare workers (HCW) of two Irish hospitals was 15 and 4. 1%, respectively. In a comparative study in the same HCW population 6 months later, measuring anti-nucleocapsid and anti-spike antibodies, seroprevalence increased to 21 and 13%, respectively; 26% of infections were previously undiagnosed. Breakthrough infection occurred in 23/4111(0.6%) of fully vaccinated participants; all had anti-S antibodies [84]. HCWs in COVID-19 patient care in Sweden have been infected with SARS-CoV-2 at a higher rate compared to blood donors. This Swedish study detected substantial variation between different IgG-assays and proposed that multiple serological targets should be used to verify past infection. Their data suggested that $CD4^+$ T-cell reactivity was not a suitable measure of past infection and does not reliably indicate protection from infection in naive individuals [85]. HCW in Switzerland, with exposure to COVID-19 patients had only a slightly higher absolute risk of seropositivity compared to those without, suggesting that the use of PPE and other measures aiming at reducing nosocomial viral transmission were effective. This study demonstrated that household contact with known COVID-19 cases represented the highest risk of seropositivity [86].

Studies of similar design have been reported from multiple places around the globe. A study in Colombian hospital workers [87] can be cited where seroprevalence was higher than measurable acute infection prevalence thus providing a way of determining true infection rates. Also such studies have been used to determine which group of healthcare providers are more prone to infection showing that those in acute medical units and those working closely with COVID-19 patients were at highest risk of infection [88]. This was also true for a Belgian hospital where seroprevalence was higher among participants in contact with patients or with COVID-19 confirmed subjects or, to a lesser extent, among those handling respiratory specimens, as well as among participants reporting an immunodeficiency or a previous or active hematological malignancy [89]. Similar findings have been reported from a Greek tertiary hospital where clinicians in contact with patients, as expected were more exposed-infected [90]. In a Japanese hospital, aiming to understand the mode of nosocomial infection, 685 HCW were recruited prior to the vaccination with anti-COVID-19 vaccine. Positive rates of HCW's working in COVID-19 wards were significantly higher than those of HCW's working in non-COVID-19 wards. By subtracting the positive rates of PCR from that of IgG (RBD), the rate of overall silent infection were estimated to be 6.0% [91]. In a cross-sectional study from hospital staff in a University Hospital in Munich, Germany, overall seroprevalence of SARS-CoV-2-IgG in 4554 participants was 2.4%. Staff engaged in direct patient care, including those working in COVID-19 units, had a similar probability of being seropositive as non-patient-facing staff. Increased probability of infection was observed in staff reporting interactions with SARS-CoV-2–infected coworkers or private contacts or exposure to COVID-19 patients without appropriate personal protective equipment [92].

SARS-CoV-2 anti-S-IgG level were also measured in 535 vaccinated healthcare workers from Israel with known previous infection status 6–8 months after the second dose and it was shown that when interpreted alongside vaccination timing, anti-S serological assays could confirm or exclude previous infections within the previous 3 months [93]. In another approach, infection rates were calculated in fixed cohorts by PCR and antibody testing of 1% of the local population and >90,000

app-based dataset. The study surveilled a catchment area of 300,000 inhabitants. Increased risk for seropositivity was detected in several high-exposure groups, especially nurses. As probably expected, contact to a COVID-19-affected person was the strongest risk factor, whereas public transportation, having children in school, or tourism did not affect infection rates [94].

Seroprevalence was assessed among health workers in five public hospitals located in different geographic regions of Ethiopia. A total of 1997 sera were collected. The overall seroprevalence was 39.6%. Of the 821 seropositive HCWs, 224 had a history of symptoms consistent with COVID-19 while 436 had no contact with COVID-19 cases as well as no history of COVID-19 like symptoms. These findings highlight the significant burden of asymptomatic infection in Ethiopian hospitals and may reflect the scale of transmission in the general population [95]. In another sensitive population, seroprevalence in children less than 6 years of age was tested in the canton of Fribourg. A total of 871 children, with a median age of 33 months were included; 412 (47%) were female. Overall, 180 (21%) of children were seropositive. The number of household members tested positive for SARS-CoV-2 (PCR test) was the main exposure risk, but the family size was not associated with an increased risk of infection. In young children, extra-familial care does not increase the risk of becoming SARS-CoV-2 seropositive, neither does the number of contacts present in extra-familial care [96].

This approach can be useful for sensitive populations such as the people experiencing homelessness (PEH). In a Danish study it was shown that the prevalence of SARS-CoV-2 antibodies was more than twice as high among PEH and associated shelter workers, compared to the background population. These results could be taken into consideration when deciding in which phase PEH should become eligible for a vaccine [97]. Another sensitive population is nursing homes. In a Belgian study, seroprevalence was determined among residents and staff randomly selected from 20 nursing homes geographically distributed in Flanders, Belgium. The seroprevalence in the 20 nursing homes varied between 0.0% and 45.0%. This study showed that nursing homes are more affected by SARS-CoV-2 than the general population. The noted large variation suggests that some risk factors for the spread among residents and staff may be related to the nursing home itself and is a sign that epidemiological data in specialized places must be interpreted with caution [98]. In another Danish study, citizens living in social housing areas of low socioeconomic status had a three times higher SARS-CoV-2 seroprevalence compared to the general Danish population. The seroprevalence was significantly higher in males and increased slightly with age. Living in multiple generations households or in households of more than four persons was a strong risk factor for being seropositive [99].

5. Anti-SARS-CoV-2 antibodies and inflammatory markers in CSF as a proxy for neurological COVID and long-COVID

Another insightful clinical application of anti-SARS-CoV-2 antibody testing is in assessing neurological disease associated with COVID-19. To investigate the pathophysiological mechanism of encephalopathy and prolonged comatose or stuporous state in severally ill COVID-19 patients, antibodies were measured in the CSF. All eight patients assayed had anti-SARS-CoV-2 antibodies in their CSF, while 4/8 patients had high titers which were comparable to high serum values. This was suggestive of blood-brain barrier (BBB) disruption; which likely eased the entry of cytokines and inflammatory mediators into the CNS enhancing neuroinflammation and neurodegeneration [3]. In a similar study, COVID-19 antibody responses were measured in serum and CSF samples from 16 patients with neurological symptoms. IgG specific for S was found in 81% of patients in serum and in 56% of patients in CSF. Interestingly, levels of IgGs in both serum and CSF were associated with disease severity and all patients with elevated markers of CNS damage in CSF also had anti-SARS-CoV-2 antibodies in the CSF; further anti-SARS-CoV-2 CSF antibodies had the highest predictive value for neuronal damage *versus* all tested clinical variables and biomarkers [100]. In a cross-sectional study of CSF neuroinflammatory profiles from 18 COVID-19 subjects with neurological complications (stroke, encephalopathy, headache), pro-inflammatory cytokines (IL-6, TNFα, IL-12p70) and IL-10 were increased only in the CSF of stroke COVID-19 subjects; a similar increase was also observed in non-COVID-19 stroke subjects. Anti-SARS-CoV-2 antibodies were observed in the CSF of 77% of COVID-19 patients with severe disease despite no evidence of SARS-CoV-2 viral RNA and CSF-CRP was present in all subjects with critical stages of COVID-19 (7/18) but only in 1/82 controls [101].

In another study, blood and CSF samples from 11 critically ill COVID-19 patients presented with unexplained neurological symptoms including myoclonus, oculomotor disturbance, delirium, dystonia and epileptic seizures, were analyzed for anti-neuronal and anti-glial autoantibodies. All patients showed anti-neuronal autoantibodies in either serum or CSF and antigens included known intracellular and neuronal surface antigens, but also various specific undetermined epitopes. These antigens were found to localize in vessel endothelium, astrocytes and neuropil of basal ganglia, hippocampus or olfactory bulb. The notion that several COVID-19 triggered autoantibodies may lurk in the shadows due to potential molecular mimicry of SARS-CoV-2 proteins with human polypeptides is pending confirmation. Any type of autoantibody may explain certain aspects of multi-organ disease in COVID-19 [102]. When CSF-derived monoclonal antibodies were isolated from an individual with severe COVID-19 it was found that these monoclonal antibodies targeted both antiviral and anti-neural antigens, including one clone that reacted to both spike protein and neural tissue [103]. Notably, in a distinct cohort of 60 prospective patients with encephalopathy and severe COVID-19 no autoantibodies were detected. These neuro-COVID-19 patients presented elevated levels of the cytokines IL-18, IL-6, and IL-8 in both serum and CSF, while MCP1 was elevated only in CSF and IL-10, IL-1RA, IP-10, MIG and NfL were increased only in serum. The levels of 14-3-3 and NfL in CSF significantly correlated with the degree of neurologic disability in the daily activities at the following 18 months [104].

These data combined do not support direct infection of the CNS by SARS-CoV-2 or specific neuroinflammation in the pathogenesis of neurological complications in COVID-19 [105]. Thus, the role and possible neural cross-reactivity of CSF anti-SARS-CoV2 IgG antibodies remains ambiguous. Evidence from CSF profiling in COVID-19 with neurological symptoms mainly suggests BBB disruption in the absence of intrathecal inflammation, compatible with cerebrospinal endotheliopathy. In that context, persistent BBB dysfunction and elevated cytokine levels may contribute to both acute symptoms and long-COVID [106]; therefore, measurement of anti-SARS-CoV-2 circulating IgGs in the CSF likely provides a reliable biomarker for the appearance of long-COVID symptoms.

6. Conclusions

As the pandemic continues, with new strains emerging, anti- SARS-CoV-2 antibody measuring will remain an indispensable tool. As discussed, through careful and on-point testing valuable information can be extracted on disease prognosis, prevention, epidemiology and care for sensitive individuals (immunocompromised) or sensitive populations (the elderly or hospitalized). In addition, as variants of concern will most likely keep emerging, determining different serotypes, as defined by the humoral immune response adds tools in the continuing global effort. The paradigm (and representing a significant evolutionary leap) of Omicron strain and its subvariants [107] teaches that it has antigenic features that clearly distinguish it from previous SARS-CoV-2 variants; therefore, some antibody tests are less sensitive against Omicron [108]. Updating tests as necessary, will ensure that we will maintain the ability to monitor SARS-CoV-2 seroprevalence in the community post-infection and/or vaccination [109], while in individual patients monitoring humoral immune responses aids disease prognosis. Worth mentioning is however, that given the acquisition of memory (B- and T-) immune cells

post-infection and/or vaccination [110], the titers of circulating antibodies cannot entirely predict the protection levels of an individual from reinfection with an existing or a new strain. Moreover, given that tissues (including the mucosa) and not blood are the main sites of mounted immune responses upon microbial/viral infection (4) the validity of circulating antibodies in predicting overall immunity and protection against future infections should not be overestimated.

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