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Overview

How Useful is COVID-19 Antibody Testing — A Current Assessment for Oncologists

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic due to infection by a new human coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has seriously disrupted the provision of oncology services and their uptake. Antibody testing, both at an individual level and of populations, has been widely viewed to be a key activity for guiding the options for treatment of high-risk individuals, as well as the implementation of safe control of infection measures. Ideally, the detection of a specific antibody should signify that all individuals tested have been infected by SARS-CoV-2 and that in the case of specific IgG that they are immune to further infection. This would enable SARS-CoV-2-infected individuals to be appropriately managed and healthcare workers shown to be immune to return to work where they would no longer pose a risk to their patients or be at risk themselves. Unfortunately, this is not the case for COVID-19, where it has been shown that immunity may not be protective, and seroconversion delayed or absent. The variability in antibody test performance, particularly that of lateral flow assays, has caused confusion for the public and healthcare professions alike. Many antibody test devices have been made available without independent evaluations and these may lack both adequate sensitivity and specificity. This review seeks to educate healthcare workers, particularly those working in oncology, of the current benefits and limitations of SARS-CoV-2 antibody testing.

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Keywords: Antibody; COVID-19; lateral flow device; oncology; SARS-CoV-2

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic originated in China towards the end of 2019, when a cluster of cases of viral pneumonia were linked to a Huanan seafood market in Hubei province [1]. The causative agent was rapidly identified as a novel coronavirus and, within a few months, this virus, now designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2], had been reported from 190 countries, with 332 218 laboratory-confirmed cases [3].

Antibody detection using point of care test devices has proven to be extremely useful for diagnosing several infections [4] and they offer significant advantages compared with traditional laboratory testing. These advantages include the rapidity of test results, the capacity to self-test and to have testing undertaken in non-laboratory settings. Detection of a specific antibody as a marker of infection and subsequent immunity following SARS-CoV-2 infection is considered essential for the effective implementation of pandemic control measures [5]. An appreciation of coronavirus virology and immunology, and the accumulating data relating to SARS-CoV-2, highlights the need for extensive evaluation and caution in the application of SARS-CoV-2 immunity testing [6].

Endemic Coronaviruses and the Common Cold

The first human coronavirus strains, B814, 229E, OC38/43, were designated in 1968 [7] following reports by groups in the UK [8] and the USA [9,10]. These human coronaviruses were primarily associated with the common cold and
several studies [11–13] showed them to be responsible for periodic outbreaks/endemics of infection, with peaks of infection during autumn, winter and spring. Currently, four endemic human coronaviruses have been identified — HCoV 229E, HCoV NL63, HCoV OC43 and HCoV HKU1, and it is now recognised that they are responsible for both upper and lower respiratory tract infections [14,15] and can cause severe disease [16,17], particularly in immunocompromised individuals [18,19]. The taxonomy of coronaviruses is undergoing review at present; however, according to existing approved taxonomic classifications, strains HCoV NL63 and HCoV 229E are alphacoronaviruses and strains HCoV OC43 and HCoV HKU1 are betacoronaviruses [20].

**The First Severe Acute Respiratory Syndrome Epidemic**

Over the period 16 November 2002 to 9 February 2003, a total of 305 cases of SARS of unknown origin occurred in Guangdong province in southern China [21]. By 26 March 2003, a total of 1323 suspected and/or probable SARS cases had been reported to the World Health Organization (WHO) from 14 locations, including Hong Kong, Vietnam, Thailand, Taiwan and the USA [22]. A mortality of 4.0% was reported and a particular trait of this epidemic was the disproportionate number of healthcare workers who developed disease. The causative agent was shown to be a novel coronavirus [23], which is now classified as a betacoronavirus [20]. The SARS epidemic came under control by July 2003 and a total of 8422 cases were reported from 32 countries [24]. Later in 2003 it was shown that SARS-CoV-like viruses were present in Himalayan palm civets found in live animal markets, leading to the proposal that these venues provided an opportunity for transmission to humans [25]. Subsequently, it has been proposed [26] that the SARS-CoV-like virus isolated from Chinese horseshoe bats has common ancestry with the civet strain and that these probably constitute the principle reservoir for interspecies transmission culminating in SARS-CoV infection in humans. The SARS-CoV epidemic of 2002/2003 has been comprehensively reviewed by Cheng and colleagues [27], who reached the following conclusion: ‘The presence of a large reservoir of SARS-CoV-like viruses in horseshoe bats, together with the culture of eating exotic mammals in southern China, is a time bomb’. Another human coronavirus epidemic did occur, starting in 2012, which was unrelated to China and was due to the Middle East respiratory syndrome (MERS) coronavirus, which was derived from different interspecies transmission events [28].

**SARS-CoV-2: the Agent of the Current Severe Acute Respiratory Syndrome Epidemic — COVID-19**

Structurally, coronaviruses are spherical, enveloped particles, 120–160 nm in diameter, with a characteristic fringe of petal-shaped surface projections (peplomers/spikes). They are positive-sense, single-stranded RNA, enveloped viruses belonging to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae. Four genera have been described, alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus. The seven human coronaviruses belong to the alphacoronavirus (HCoV-229E and HCoV-NL63) and betacoronavirus genera, which also include many bat species. Within the betacoronavirus genus there are several subgenera [29], including Embecovirus (includes HCoV-OC43 and HCoV-HKU1), Sarbecovirus (includes SARS-CoV and SARS-CoV-2) and Merbecovirus (MERS-CoV). It has been reported [30] that SARS-CoV-2 shows very high genetic relatedness compared with coronavirus isolated from Malayan pangolins, which are trafficked in wet food markets. Similar to the previous epidemic of 2002/2003, in which civets were the intermediate host, it is most likely that recombination with bat coronaviruses has taken place [31].

Clinically, SARS-CoV-2 infection can range between asymptomatic/subclinical disease [32] and acute respiratory failure with ensuing death [33]. Older males appear to be more likely to develop severe disease requiring intensive support [34], as do those with comorbidities including hypertension and diabetes [35]. Children appear to be less likely to be infected than adults and the disease appears to be milder [36]. Overall mortality from COVID-19 has been reported to be 2.3% [37]; however, this varies with population demographics and other factors, such as the availability of adequate supportive treatment and the implementation of infection control measures [38]. The incubation period is typically 5 days and 90% of individuals are expected to have developed symptoms within 2 weeks of active monitoring or quarantine [39]. In most cases [40], the clinical presentation includes fever (83–98%), dry cough (76–82%) and fatigue or myalgias (11–44%).

**Coronavirus Antibody Testing**

Detection of a specific antibody can be useful to evidence infection, through showing either the presence of IgM or rising titres of IgG. Furthermore, the detection of a specific IgG can infer immunity and potential resistance to future infection. Finally, the detection of a specific IgG can facilitate the epidemiological monitoring of the spread of infection. In the case of coronaviruses, all this was carried out during the first SARS epidemic; so what has been learned? A key requirement for the development of antibody assays is the need to be able to detect sufficient true positives who have had the disease (sensitivity) and true negatives who have not had the disease (specificity). Sensitivity will be influenced by the time taken to seroconvert and the longevity of the antibody response, so there will be a window when individuals with the disease appear negative (false negatives), particularly because the test used fails to detect low levels of specific antibody. Specificity will be influenced by cross-reactions (false-positives), particularly with, but not limited to, other human coronaviruses.
The detection of seroconversion is an accepted diagnostic method for virus infections, and it is usually recommended that acute and convalescent sera are taken 2 weeks apart. In a prospective study of SARS, Peiris and colleagues [41] reported a mean time to seroconversion of 20 days following the onset of symptoms, with 93% sensitivity at 30 days. In a separate study, Hsueh and colleagues [42] reported a mean time to seroconversion of 12.3 days, and it was also noted that SARS-CoV RNA could persist for some time in patients who had seroconverted. Subsequently, it was shown [43] that the antibodies produced following SARS-CoV infection were neutralising, remained at high levels for at least 5 months, but did NOT confer protection against the development of symptoms. In a separate study, Hsueh and colleagues [44] reported seroconversion to commence as early as 4 days following the onset of illness and that worsening of disease occurred despite high antibody levels, which they attributed to ‘an over-exuberant immune response’. The innate and adaptive immune responses to SARS-CoV infection have been shown to be atypical [45] and poor clinical outcome is associated with hypercytokinaemia [46].

Several laboratory assays have been used to detect antibodies to coronaviruses [47]. They can be divided into live/inactivated virus (e.g. neutralisation assay/slide immunofluorescence) or virus protein/peptide [e.g. recombinant enzyme-linked immunosorbent assay (ELISA)/peptide array] assays. Neutralisation assays have the advantage of presenting virus antigens in their native state; however, they are very technically demanding, difficult to quality control and subject to large margins of error. Recombinant ELISA assays have proven popular for SARS immunity [48] and seroprevalence studies [49] and have used as the antigen either the nucleocapsid or the spike proteins of SARS-CoV. The nucleocapsid protein is an immunodominant antigen [50] and although genomic studies [51] have shown low (<33%) amino acid sequence homology with other coronavirus nucleocapsid proteins (including human coronaviruses), there are regions of high conservation. False-positive results due to antibodies from other human coronaviruses have been reported with recombinant nucleocapsid ELISA [52]. The SARS-CoV surface spike protein plays a key role in virus infection of the host by binding to the host cell receptor, angiotensin-converting enzyme 2, thereby mediating virus entry [53]. The spike protein is immunogenic, producing neutralising and protective antibodies [54]. It appears to be an ideal candidate for the antigen for ELISA-based assays; however, in longitudinal studies using a spike protein recombinant ELISA, Zhao and colleagues [55] observed a loss of sensitivity (82%) compared with an nucleocapsid protein recombinant ELISA (100%) in convalescent sera taken 2–3 months post-recovery. A limitation of spike protein recombinant ELISAs is that only fragments of spike protein are used as the antigen and native structure is not retained, which may account for assays using complete spike protein (e.g. immunofluorescent antibody assays) having greater specificity than those using linearised/non-conformational spike protein (e.g. recombinant ELISA). Conversely, recombinant ELISA assays are easier to standardise and are not subjective compared with immunofluorescent antibody assays.

**SARS-CoV-2 Antibody Testing**

The primary diagnostic test for COVID-19 is SARS-CoV-2 nucleic acid detection by reverse transcriptase polymerase chain reaction (RT-PCR) or alternative nucleic acid detection technologies [56]. What role has antibody testing in the diagnosis, treatment and control of the COVID-19 pandemic? From the SARS pandemic (2002–2004), it has been shown that the immune response to the closely related SARS coronavirus is complex, generates neutralising antibodies, but may not be fully protective and there is a risk of severe damaging immune pathologies in some individuals. Furthermore, for SARS-CoV-2 there are very few data to date evaluating the performance of different antibody assays and establishing the time-course of antibody responses, and determining the extent and consequences of reinfection. The mortality of the SARS pandemic was less than 10 000, whereas it is reasonable to assume that the mortality of the COVID-19 pandemic will be at least 10 times higher. Estimating population immunity is an imperative for understanding disease transmission and monitoring the effectiveness of intervention measures targeted at controlling the spread of infection [57]. Additionally, determining individual COVID-19 immunity has been proposed [58] as a means of re-establishing a workforce that can undertake activities with reduced risks from SARS-CoV-2 infection to themselves or to others.

**Lateral Flow/Rapid Test Devices for the Detection of SARS-CoV-2 Antibodies**

Lateral flow devices (LFDs) have been available for about 50 years and for antibody detection, sandwich and competitive assay formats have been used [59]. In the field of infectious diseases, LFDs can perform with high sensitivity and specificity [60] and they have contributed to the effective control of disease outbreaks [61,62]. LFDs offer the capacity to test finger-prick blood or oral fluid samples at the point of care, dispensing with the need for laboratory facilities and they can provide results within a matter of minutes. LFDs can be mass-produced, and at a relatively low cost, for example, in the context of the COVID-19 pandemic certain manufacturers have advertised the capacity to produce 50 000 tests per week at a cost as low as $3.60 per device. Potential limitations of LFDs include batch to batch variation, subjectivity of reading, limited quantitative capacity and when carried out outside the laboratory there is little quality assurance, no access to pathologist review and no original sample left for confirmatory or additional testing.

Since the WHO declaration of the COVID-19 pandemic on 11 March 2020, several manufacturers have rapidly made available LFDs (Figure 1) for antibody testing in an attempt
to address the need for additional diagnostic capacity, provide back to work immunity screening and deliver sero-epidemiological surveillance [63]. Because of the need for SARS-CoV-2 immunity testing and the current short timeframe of the pandemic, few validations have been published and users have needed to rely on manufacturers’ claims of test performance.

Frequently, sensitivities and specificities in excess of 90% have been reported by manufacturers in test-associated literature; however, such claims require further investigation [64] for reasons including the following. In many instances, small numbers of samples have been tested, and rarely have samples documented positive for other human coronaviruses been included in evaluation panels. Furthermore, in these evaluations, the inclusion of samples from cases of COVID-19 confirmed by SARS-CoV-2 RT-PCR has been adopted as a convenient gold standard in preference to a comparison with a reference assay (e.g. plaque reduction neutralisation). In the few published reports of the performance of LFDs [65–68], the reported test sensitivities compared with commercial evaluations for specific IgG have generally been lower (Table 1) and specific IgM test results have lacked sensitivity and are subject to potential false positivity [69].

**Current Benefits and Limitations of SARS-CoV-2 Antibody Testing**

Unlike the previous SARS epidemic, in which data were available from multiple assay formats, enzyme immunoassays (ELISA) or chemiluminescence immunoassays have been the mainstay of serological data pertaining to the current COVID-19 pandemic. Several studies [70–73] have shown that the rate of SARS-CoV-2 IgG detection by immunoassays increases with time from 1 week after the onset of symptoms in COVID-19 patients, whereas the sensitivity of SARS-CoV-2 nucleic acid detection peaks and then declines [73,74]. Similar to SARS, two types of immunoassay have been developed, those using nucleocapsid or...
nucleoprotein as the antigen [71,72,75], which is highly immunogenic, and those using spike protein [70,75], which has neutralising activity. In practice, there is little to choose between the performance of these assays, which can be misleading. Currently, there is a paucity of published evaluations of SARS-CoV-2 antibody assays using appropriate reference assays for comparison. Organisations such as Public Health England have published evaluations [96]; however, to our knowledge the data presented have not been subject to peer review and reference assays have not been used similar to those published during the previous SARS epidemic [97]. Understandably, there is a reluctance to use neutralisation assays in which SARS-CoV-2 needs to spread from cell to cell through a population and possibly provide a route to specialist service provision?

**COVID-19 Antibody Testing for Oncologists**

The COVID-19 pandemic has had a devastating impact on both the provision of cancer services and their uptake [93,94]. Serological tests appear to hold the key for widespread testing of populations but also for cancer patients and those working in treatment centres. It has been recommended that all cancer patients undergo serological testing for COVID-19 infection [95]. It was hoped that serology could determine the extent of the disease transmission through a population and possibly provide a route out of social distancing measures. Detecting the presence of antibodies to SARS-CoV-2 does not necessarily mean an individual is immune from future infection. The market has been flooded by COVID-19 antibody detection devices, many of them without the required specificity and sensitivity. These have been used in a haphazard manner and often by self-administration. The lack of authoritative, independent evaluations by national public health authorities has left users of such devices reliant on manufacturers’ claims of performance, which to non-specialists can be misleading. Currently, there is a paucity of published evaluations of SARS-CoV-2 antibody assays using appropriate reference assays for comparison. Organisations such as Public Health England have published evaluations [96]; however, to our knowledge the data presented have not been subject to peer review and reference assays have not been used similar to those published during the previous SARS epidemic [97]. Understandably, there is a reluctance to use neutralisation assays in which SARS-CoV-2 needs to spread from cell to cell through a population and possibly provide a route to specialist service provision?

| Lateral flow device evaluation | COVID-19 positive/negative samples | SARS-CoV-2 IgG sensitivity/specificity | SARS-CoV-2 IgM sensitivity/specificity |
|--------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| Li et al. [65]                 | 397/128 venous blood              | Sensitivity = 70.5%                  | Sensitivity = 82.6%                  |
|                                |                                   | Specificity = 98.4%                  | Specificity = 91.4%                  |
| Pan et al. [66]                | 86/22 venous blood                | Sensitivity = 55.8%                  | Specificity = 54.7%                  |
|                                |                                   | Specificity = ND                     | Specificity = ND                     |
| Döhla et al. [67]              | 22/27 fingertip blood             | Sensitivity = 36.4%                  | Specificity = 36.4%                  |
|                                |                                   | Specificity = 88.9%                  | Specificity = 88.9%                  |
| Hoffman et al. [68]            | 29/124 serum                      | Sensitivity = 93.1%                  | Specificity = 69.0%                  |
|                                |                                   | Specificity = 99.2%                  | Specificity = 100%                   |

ND, not determined.

Should effective SARS-CoV-2 vaccines be developed, antibody testing will be required for immunogenicity profiling and population seroprevalence studies, preferably with the capacity to differentiate wild-type immunity from vaccine-induced immunity. Finally, in high-risk patients, such as those requiring treatment for cancer, the COVID-19 pandemic has significantly impacted upon the provision of diagnosis and treatment and a key prerequisite for the future uptake of services will be adopting measures to reassure both patients and staff that they are safe [92]. Can SARS-CoV-2 antibody testing facilitate the return of patients to specialist service provision?

Table 1

Reported sensitivities and specificities of lateral flow devices for SARS-CoV-2 IgG and IgM

- Sensitivity = proportion of true positives correctly identified
- Specificity = proportion of true negatives correctly identified

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to be cultured, and immunofluorescence and pseudo-
negative assays have been used as alternatives [98].

Several studies of COVID-19 population immunity have shown differing antibody detection rates of antibody, probably reflecting differing epidemiological circum-
stances. For example, in a study undertaken in Santa Clara County, California, USA, where 865 people were tested, a 4.65% COVID-19 antibody-positive test rate was reported [99] and in a second study from Gangelt, Germany, where 1000 people were tested, a preliminary 14% antibody-
positive rate was reported [100]. In the latter study, the town had a very high prevalence of SARS-CoV-2 infection. A large SARS-CoV-2 seroprevalence study of 578 Spanish healthcare workers [101], has documented a seroprevalence of 9.3%. Another factor impacting upon the reliability of SARS-CoV-2 seroprevalence studies is the decline of anti-
body levels following infection; for example, Patel and colleagues [102] have documented 58% of seropositive indi-
viduals reverting to seronegativity after 60 days.

Other immune mechanisms are at work to confer immu-
nity that current COVID-19 LFDs fail to detect. A recent study [103] showed that a series of 23 SARS-recovered pa-
tients in Singapore, 17 years after the outbreak, still possessed long-lasting T cell immunity, which displayed robust cross-reactivity to SARS-CoV-2. The paper concluded that understanding T cell immunity to the virus in the community and its impact on susceptibility and pathogen-
esis was the key to the management of the current pandemic. There are clearly three groups of people with immunity in any population. There are those who have recovered and recorded antibodies; another who have fought it off with T cell responses alone and finally a large group of uninfected people with residual cross-immunity triggered by previous related coronavirus infections. This is clearly more complex that the political desire to segregate society into the susceptible, the infected and the recovered.

The hope was that these tests could be used by oncology service providers to allow antibody-positive workers to return to work and potentially for cancer patients to be triaged. However, the extremely low seroconversion rate almost certainly precludes this.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clon.2020.10.008.

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