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A SYSTEMATIC ANALYSIS OF LABORATORY-GUIDED DIAGNOSIS AND MANAGEMENT OF COVID-19: CHALLENGES AND RECOMMENDATIONS

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ABSTRACT

We conducted a comprehensive analysis of patient demographics and laboratory tests encompassing Real Time PCR (RT-qPCR) and serology for SARS-CoV-2 in addition to blood components and clinical blood markers for COVID-19 disease. All relevant literature was included up to 15 July 2020 and multiple studies were analysed in tandem to correlate findings. For RT-qPCR, nasopharyngeal swabs are the most suitable samples based on detection rates by sample, but may require repeat testing. One-week post-symptom onset, serological testing is a more stable marker. Antibody titers have been linked to disease severity. Several clinical blood components and markers have been reported to be prognostically useful; however, care is needed when making interpretation owing to the association between raised levels of these being found for the co-morbidities that predispose to worse prognosis in COVID-19. Challenges in the current study when finding the information presented in this paper suggest the need for a quality assured database that outlines the complete set of results, anonymised patient data for each entry and a set of internationally-agreed guidelines for complete laboratory testing, documentation and open-access reporting. We suggest that such information would be useful to help with patient diagnosis and management, worldwide.

Contribution/Originality: This review compiles a large set of data on the efficacy of different laboratory tests in predicting severity of sequelae post COVID-19 infection and it recommends that anonymized patient results might be added to an international database from the time a pandemic is announced to help with patient management, worldwide.

1. INTRODUCTION

There have been significant pandemics already in the first two decades of the 21st century. For example, by July 2003, the severe acute respiratory syndrome (SARS) pandemic had resulted in 8,096 reported cases, including 774 deaths in 27 countries [1]. Certain severe acute respiratory syndrome coronavirus (SARS-CoV)-like viruses found in bats have recently been shown to be able to infect human cells without adaptation, suggesting the potential for SARS to re-emerge [2].

In May 2015, a single person returning from the Middle East started a nosocomial outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV) in South Korea that involved 16 hospitals and 186 patients [3]. By

July 2017, 2,040 MERS-CoV laboratory-confirmed cases, resulting in 712 deaths, were reported globally. According to Pavia, despite the progress in pandemic preparedness, gaps remain including important scientific questions, adequate resources and importantly, the ability to rapidly deliver highly effective vaccines [4]. In December 2019 a group of patients with pneumonia of unknown cause were confirmed to be infected with a novel coronavirus, known as 2019-nCoV and then COVID-19, in Wuhan, Hubei province, China, which had previously not been detected in humans or animals [5]. This current SARS pandemic had, by 12 July 2020, resulted globally in more than 12.7 million cases worldwide and in over 560,000 deaths [6].

The gold standard test used to diagnose COVID-19 cases is a Real Time Polymerase Chain Reaction (RT-qPCR) laboratory test, designed to detect at least one SARS-CoV-2 virus-specific sequence, incorporating an internal control in each test and performed on nasal, throat or nasopharyngeal swabs, sputum or bronchoalveolar lavage samples. RT-qPCR can be used to detect virus from samples from other sites such as from faeces. Notably, over 60% of errors occur in the preanalytical phase of any diagnostic process [7]. Ideally, a swab should be taken at the time of symptom onset when highest viral load occurs in COVID-19 to optimise virus detection [8]. The use of a chest computed tomography (CT) scan to diagnose COVID-19 in patients showing moderate symptoms where the RT-qPCR test result has been negative has also been recommended [9].

Cases noted as being critical, severe or non-severe have been useful for determining the notable risk factors relating to differences among a population relating to age, gender and co-morbidity. For example, Chinese cases are characterised based on the Guideline on the management of COVID-19, version 6 published by National Health Commission of the People's Republic of China. According to these guidelines, 'mild' or 'asymptomatic' cases are defined by the lack of pneumonia in imagining results. 'Moderate' cases are characterised by the presence of a fever as well as pneumonia in imaging. 'Severe' cases are classified based on respiratory distress, where a respiratory rate of $\geq$ 30 per minute, b) saturation $\leq$ 93%, and c) PaO_2 / FiO_2 $\leq$ 300mmHg; 'Critical' was classified if one of the following was present: a) respiratory failure requiring mechanical ventilation, b) shock, and c) co-existing multiple organ failure requiring close monitoring in the Intensive Care Unit (ICU). The early diagnosis of severe forms of COVID-19 is important for appropriate care of patients and for early decisions regarding assignment of intensive care unit beds.

The patient's immune response to the virus is determined through IgM (acute) and IgG (chronic) anti-SARS-CoV-2 antibody level detection. The management of patients with COVID-19 disease is supported by a range of standard-use laboratory tests including white blood cell counts, neutrophil and lymphocyte counts, platelet counts, fibrinogen levels and non-specific proinflammatory markers that include, for example, C-reactive protein (CRP), pro-calcitonin and D-dimer. Sette and Crotty have concluded from their analysis of a number of studies that SARS-CoV-2 pre-existing immune T cell mediated reactivity exists to some degree in the general, unexposed population [10]. It is hypothesised that this might be due to immunity to common cold coronaviruses. The authors note that this might have implications for COVID-19 disease severity, herd immunity and vaccine development. It is unknown whether T memory cells are involved. Specifically, in COVID-19 patients, it has been shown that circulating SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cells were identified in approximately 70 and 100% of patients, respectively [11]. $CD4^+$ T cell responses to SARS-CoV-2 spike protein, which is the main target of most current vaccines in development, correlated with the degree of the anti-SARS-CoV-2 IgG and IgA antibody titers [11]. However, further studies are required using samples from acute patients and patients with complicated disease courses in order to fully elucidate the intricacies of the T cell response for the duration of the disease. In addition, further information regarding the longevity of the SARS-CoV-2 immunological memory will need to be determined using samples from recovered COVID-19 patients.

The purpose of the current study was to review the range of laboratory tests that are useful to predict carriage, infection, prognosis and infectivity with SARS-CoV-2 and to investigate whether a policy for early publication of a

wide range of laboratory test results on a longitudinal study for a cohort of patients with a pandemic disease might be helpful in severity prediction, treatment and management of patients as the disease spreads elsewhere.

1.1. Methodological Explanation

Every effort was made to provide a comprehensive assessment of the peer-reviewed published evidence (PubMed, date of last search: July 15, 2020). There is international variability in testing and reporting methodologies and in particular, owing to the speed of the expansion of this pandemic, it should be noted that published studies relating to the virus detection were not accompanied by performance characteristics for the testing methods used. Multiple papers were reviewed on COVID-19 detection and the relationship between disease severity and variations in a variety of laboratory test measurements. It was deemed important to consult multiple papers, if available, on any topic to try to provide a balanced view, particularly as performance characteristics tended not to be defined for novel assays to detect the virus or immune response to the virus.

2. RESULTS

2.1. Investigation of the Symptoms of Patients with COVID-19 Disease

Table 1 shows symptoms and signs among patients diagnosed with SARS-CoV-2 infection. The table has been compiled from the findings of multiple studies [12-24]. It should be noted that the category denoted 'All' is higher than the sum of 'Non-severe', 'Severe' and 'Critical' categories owing to some of the reports that were included in the analysis not differentiating for severity.

Table-1. Symptoms by percentage occurrence of COVID-19 patients, some of which were stratified by disease severity.

Symptom	All (n=2891)	Non-severe cases (n=1043)	Severe cases (n=255)	Critical cases (n=252)
Fever	88.62%	88.11%	87.45%	93.65%
Cough	69.15%	64.24%	82.75%	74.21%
Fatigue	34.69%	35.95%	35.69%	51.19%
Sputum production	27.88%	30.11%	25.49%	21.83%
Shortness of breath	20.79%	13.42%	34.51%	53.17%
Headache	11.21%	14.09%	14.51%	6.35%
Myalgia/arthralgia	10.31%	12.85%	11.76%	14.29%
Diarrhoea	9.96%	4.03%	11.76%	17.46%
Sore Throat	9.55%	12.56%	11.37%	1.59%
Nausea or vomiting	5.43%	4.12%	4.71%	9.13%
Chills	4.77%	9.68%	10.98%	0.79%
Nasal congestion	3.36%	4.51%	2.35%	0.40%
Tonsil swelling	0.86%	1.63%	2.35%	0.79%
Throat congestion	0.80%	1.63%	0.78%	1.59%
Conjunctival congestion	0.55%	0.48%	1.57%	0.00%
Haemoptysis	0.48%	0.58%	1.96%	0.00%
Enlargement of lymph nodes	0.07%	0.10%	0.39%	0.00%
Rash	0.07%	0.00%	0.78%	0.00%

Source: Table compiled by the authors from publications numbered 12-24 in the references section.

2.2. Investigation of the Stability of the Virus in Respiratory Samples

Definitive diagnosis of COVID-19 disease has relied upon detection of the virus in patient respiratory samples. The stability of the virus in the patient sample over time, if there is to be a delay in testing, has been a consideration. Rogers, et al. [25] conducted an examination of storage parameters of liquid samples for the detection of virus by RT-qPCR by spiking high-titer SARS-CoV-2 remnant patient specimen into pooled SARS-CoV-2 RNA-negative specimen remnants for various media types: VCM, UTM®-RT, ESwabTM, M4 media and Normal Saline (0.9% NaCl). Samples stored at 18°C to 25°C, 2°C to 8°C and -10°C to -30°C were tested regularly over 14 consecutive days. Specimens consistently yielded amplifiable RNA with mean RT-qPCR crossing point

(CP) differences of <3 for all sample types and storage conditions, indicating high stability of the virus in stored samples [25].

2.3. Analysis of Published Literature for Timelines for Detection of SARS-Cov-2 RNA in Nasopharyngeal Swabs and Faeces and Antibodies to SARS-Cov-2 Virus in Patients' Blood Samples

Figure 1 displays the average values from collation of patient datasets from published reports [26-34]. It should be noted that the asymptomatic PCR % positive dashed line, showing results from testing throat swabs for the presence of virus, reflects patients who were asymptomatic following contact tracing after their being in close contact with a case (represented by day 0 on the x-axis, in this case) and who subsequently tested positive for the virus [34] with or without eventual symptoms of COVID-19 disease.

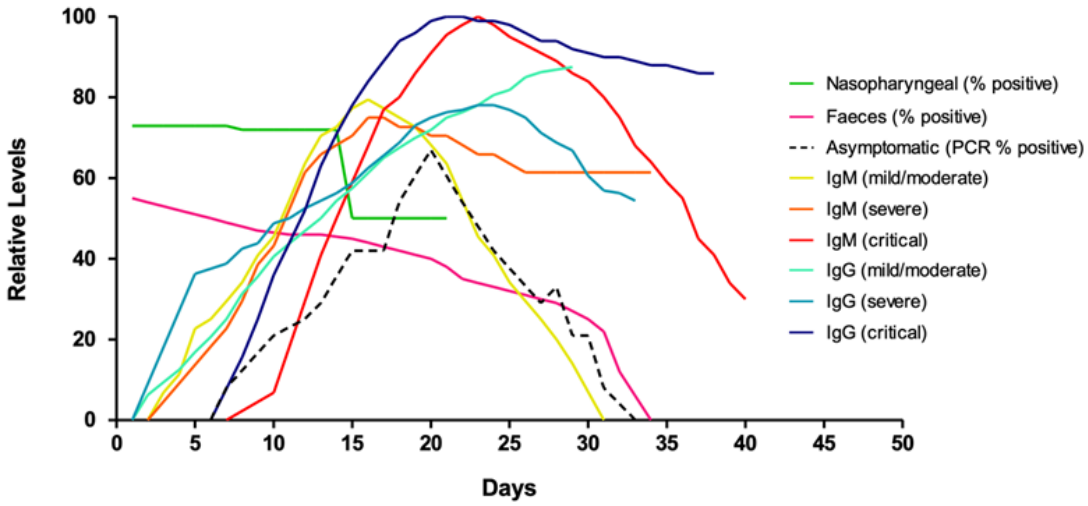


Figure-1. Timeline for detection of SARS-CoV-2 as a percentage of the infected population in different human sample types and distinguished for those mildly or severely affected by the infection where IgM and IgG are detected from blood samples.
Source: Figure compiled by the authors from publications numbered 26-34 in the references section.

The first antibodies produced during a typical immune response to infection are IgM antibodies followed by IgG. In SARS-CoV-2 infection, different types of seroconversion have been reported; these comprise IgM seroconversion before IgG, IgM seroconversion later than IgG, or synchronous seroconversion of IgM and IgG [26, 28, 32, 35-38]. It is important to consider that these observed differences could be due to variation in the assays used to detect and quantify antibody levels in these respective studies.

2.4. The Management of Patients Diagnosed with COVID-19

Blood parameter findings, presented in Figure 2, shows the collation of the results from 10 studies of the most-commonly used blood tests used to monitor the progression of COVID-19 disease in patients: Zhou, et al. [24] (n=21); Guan, et al. [13] (n=1099); Wang, et al. [20] (n=125); Kim, et al. [15] (n=28); Chen, et al. [39] (n=99); Wu, et al. [40] (n=80); Zhang, et al. [41] (n=138); Lo, et al. [16] (n=10); Wan, et al. [17] (n=135); and Chen, et al. [12] (n=274). It should be noted that for fibrinogen levels the study population is low, due to a limited availability of patient data where fibrinogen levels were reported.

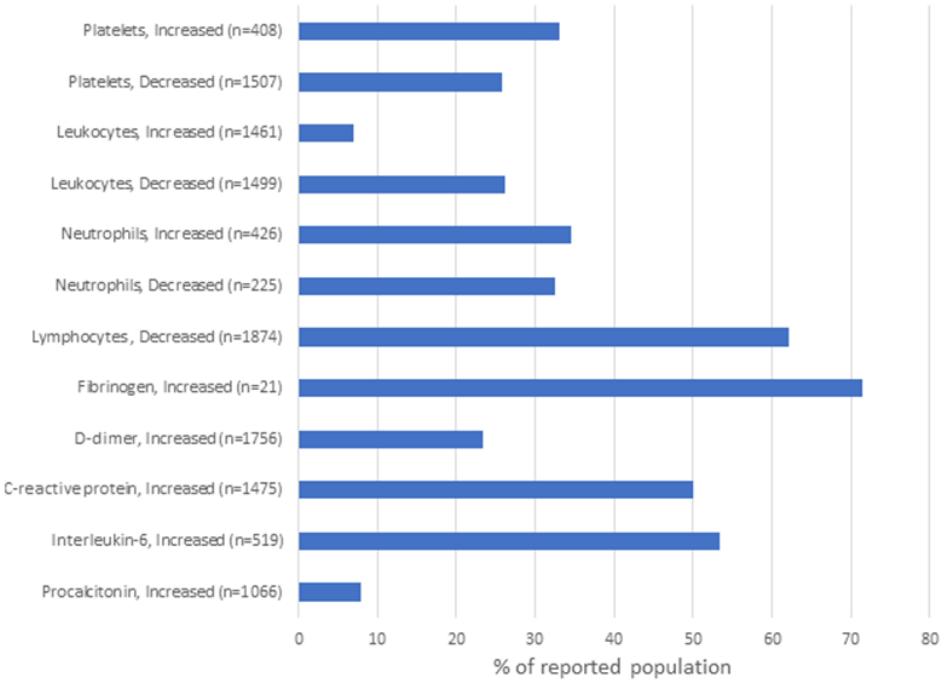
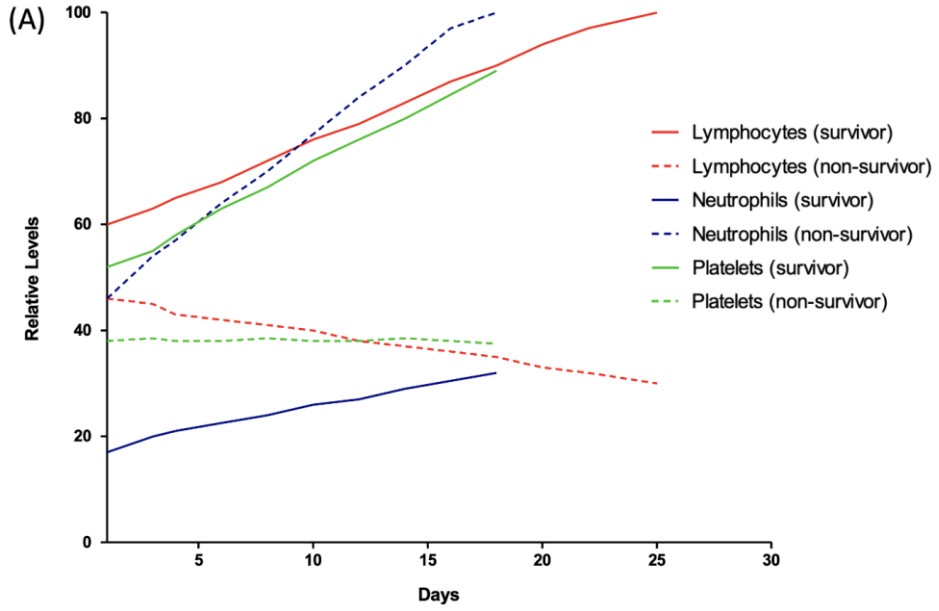


Figure-2. Prevalence of blood markers above or below the reference range from a study of reports on a total of 2,108 patients diagnosed with COVID-19 disease.
Source: Figure compiled by the authors from publications numbered 12, 13, 15, 16, 17, 20, 24, 39, 40 and 41 in the references section.

Figure 3 shows relative levels of blood cells, components and serum markers over time according to disease severity of COVID-19 disease as a further expansion of the total percentages shown in Figure 2. In Figure 3a distinct differences have been shown in the lymphocyte, neutrophil and platelet counts for survivors and non-survivors of COVID-19 disease for the cohort of patients who showed these signs. Figure 3B shows distinct differences between CRP, D-dimer, IL-6, LDH and ferritin levels for mild and severe cases in the first three weeks post-symptom onset.



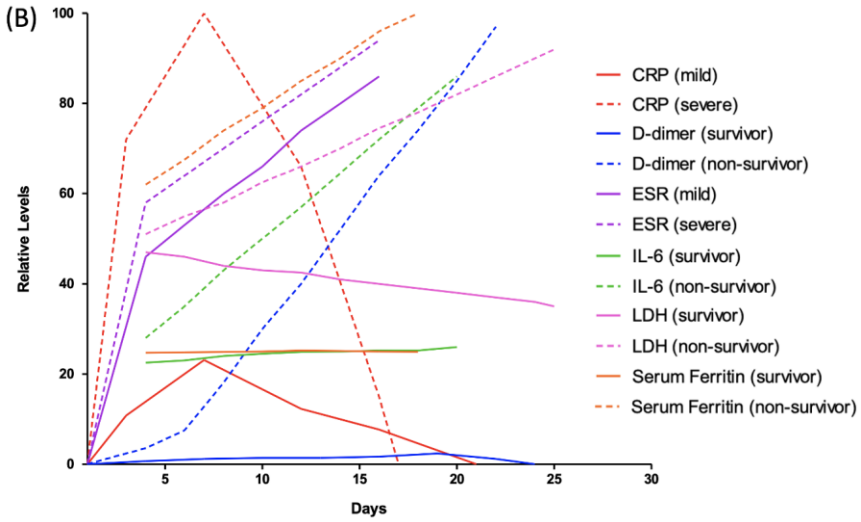


Figure-3. Timeline of increased and decreased blood cells (A) and serum markers (B) among patients who have been classified variously as being either mildly or severely affected by COVID-19 or as survivors or non-survivors of COVID-19.
Source: Figure compiled by the authors from publications numbered 15, 18 and 23 in the references section.

Figure 4 differentiates the percentage of each variable by severity using data collated from several studies [12, 13, 16, 17, 24, 41-45]. It should be noted, however, that decreased neutrophils where the severity of the patients was noted was not found in any publication and that fibrinogen measurement specifically for non-severely affected patients was not reported in a way that could be extrapolated accurately for this paper. This figure represents average percentages derived from tests taken at different stages of illness. It should be noted that there were differences in the stratification of patients between Figure 3 and 4 where the patient populations were divided into survivor and non-survivor versus non-severe and severe/critical, respectively, for the purpose of analysis.

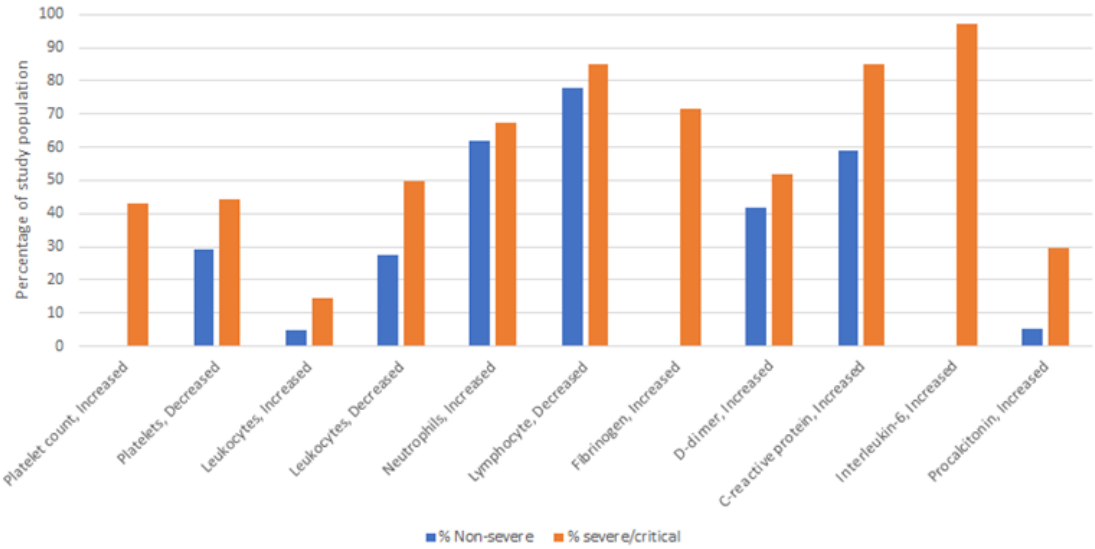
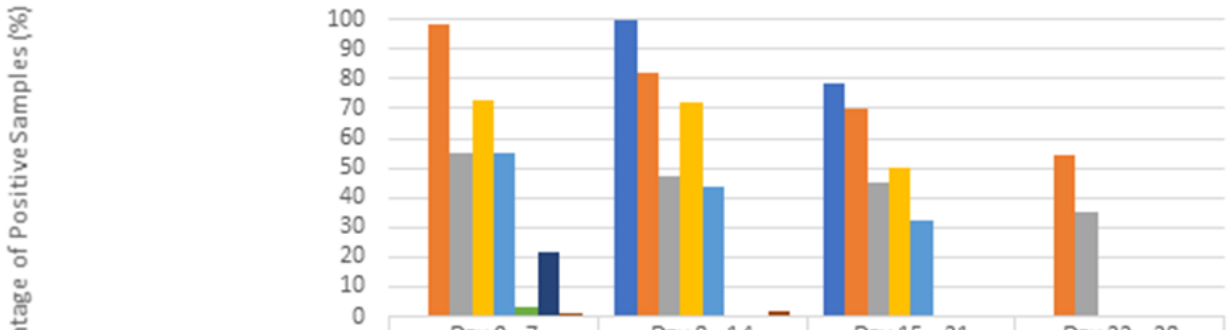


Figure-4. The prevalence of blood markers above or below the reference range for non-severe patients (n=1164) and severe/critical patients (n=453) following diagnosis with COVID-19 disease.
Source: Figure compiled by the authors from publications numbered 12, 13, 16, 17, 24, 41, 42, 43 and 44 in the references section.

Figure 5 denotes percentage of total positive test results recorded for different sample types within published literature based on an amalgamation of data across different studies. The table also represents the percentage positivity of recorded samples arranged in weekly intervals for each sample type where these were available. Figure 5 shows the relative detection levels of COVID-19 by RT-qPCR. Bronchoalveolar lavage data consist of an amalgamation from three studies [27, 29, 31]. Using this method, 89% of these COVID-19-containing samples

were detectable upon analysis, while weeks 2 and 3 showed both 100% detectability and 78.6% detectability, respectively. Note that blank boxes within the figure denotes data that is not available.



	Day 0 - 7	Day 8 - 14	Day 15 - 21	Day 22 - 28
Bronchoalveolar Lavage		100	78.6	
Sputum	98	82	70	54
Faeces	55	47	45	35
Nasopharyngeal Swabs	73	72	50	
Oropharyngeal (Throat) Swabs	55	44	32	
Tears/Conjunctivital Secretions	3	0	0	
Blood	22	0	0	
Urine	1	2	0	

Figure-5. Hierarchy of usefulness of different sample types and the associated percentages of positivity over time for the diagnosis of COVID-19 post-symptom onset using RT-qPCR.
Source: Figure compiled by the authors from publications numbered 27, 29 and 31 in the references section.

According to the WHO-China joint report on COVID-19, sputum is produced in 33.4% of all patients with COVID-19. Of 690 patients who produced sputum, these samples were analysed across 5 different studies [27, 29, 31, 33, 43] and overall, 77% were detectable for the virus. When examined longitudinally, 98% detectability was observed in week 1 (days 0-7), 82% detectability was observed in week 2 (days 8-14), 70% detectability was observed in week 3 (days 15-21) and 54% detectability was observed in week 4 (days 22-28).

Faecal sample positivity data collected from three studies [29, 30, 33] showed an average detection rate of 41% for samples tested. Three separate studies that investigated longitudinal examination, however, saw 55%, 47%, 45% and 35% detectability, respectively, during analysis in weeks 1, 2, 3 and 4. While detectable virus was found from days 29-33, a prevalence value was not reported in the literature for the study population. A collection of three population studies [27, 29, 31] comprising 4,918 COVID-19 patients resulted in detection of the virus in 40% of nasopharyngeal swabs. However, for four studies that conducted longitudinal testing, 73%, 72% and 50% of samples were positive for the virus across weeks 1, 2 and 3, respectively. Oropharyngeal (throat) swab sample data were gathered from four studies [27, 29, 31, 46] a total percentage detectability of 39% was observed, however, during a longitudinal study a 55%, 44% and 32% detectability was recorded across weeks 1, 2 and 3, respectively. Tears/conjunctivital secretions accounted for a total detectability of 3%, and when examined longitudinally, a 3% detectability was observed in week 1 [43]. Two case studies were examined when analysing blood samples and a total of 5/316 samples (2%) were detectable for the virus [29, 46]. A 22% detectability in blood samples was observed in week 1. Urine samples were the least useful method in detecting COVID-19, as a total percentage detectability of 1% was observed across 2 studies [29, 33]. During longitudinal analysis, a 1% and 2% percentage detectability was observed in weeks 1 and 2, respectively.

3. DISCUSSION

COVID-19 presents common dominant symptoms in the literature as seen in Table 1. The most common symptom is fever, noted in 88.62% of cases. Cough is also reported commonly at 69.15% of cases. Shortness of breath shows a higher frequency of reporting as case severity increases, with 13.42% in non-severe cases, 34.51% in severe cases and 53.17% in critical cases [12-24].

Other symptoms commonly reported included headache and fatigue as shown in an American study [47] and sore throat [15]. Most patients also experience more than one symptom [20]. Less dominant symptoms include nasal congestion, myalgia, arthralgia, chills, throat congestion and tonsil swelling [24]. Additionally, diarrhoea has been reported to be an uncommon finding in patients with COVID-19, for example appearing in 3 of 125 patients in one study [20] and in 3 of 28 patients in another [15]. When different studies were combined the overall prevalence of diarrhoea as a symptom in patients diagnosed with COVID-19 was 10% Table 1. A more focused study on GI symptoms, that found it to be more common, determined that 24.2% of 58 patients studied reported diarrhoea [48] with 61.1% of patients in this study showing some form of GI symptoms, including anorexia (17.9%) and nausea (17.9%). Interestingly, 11 (11.6% of the study population) of the COVID-19 positive patients showed GI symptoms exclusively and had no CT imaging features of COVID-19 pneumonia. The presence of GI symptoms in this study were not linked to outcome, however. This study by Lin, et al. [48] does suggest that multiple types of testing may be needed for diagnosis of COVID-19 disease in any population, considering transmissibility concerns. Bronchoalveolar lavage shows the highest level of virus detectivity among the sample types used for RT-qPCR Figure 5, although its necessitating a more invasive procedure for sample collection than the other sample types used means that it is used on a minority of patients.

The molecular detection of SARS-CoV-2 virus in patient samples depended on the development of new assays (of which many were developed commercially in the first weeks and months of the pandemic), in contrast to the use of routinely-used tests for assaying blood components or markers. The sensitivity, specificity, positive predictive and negative predictive values were not quoted for the assays that were analysed in the current study for the detection of SARS-CoV-2 virus, so the sensitivities or accuracies of any of the assays used to generate these data are unknown. Encouragingly however, one recent study by Van Kasteren, et al. [49] that compared seven manufacturers' RT-qPCR assays for the detection of SARS-CoV-2 RNA found that while sensitivity levels for the assays varied six-fold, there was no-cross-reactivity noted and 96.4% of symptomatic individuals had viral loads above the lowest limit of detection found. Figure 1 shows reported levels of detection of virus in throat swabs post symptom onset.

From a detection perspective salivary viral load was highest during the first week post-symptom onset [37]. Slow viral RNA clearance (≥15 days after illness onset), is associated with male gender, advanced age, hypertension, severe illness at admission and corticosteroid treatment [50]. In one longitudinal study of 23 patients. Liu, et al. [51] have found that patients with severe COVID-19 tend to have a high viral load and a long virus-shedding period, a finding mirrored by that of Zheng, et al. [33] but not by Lavezzo, et al. [52] when the latter were investigating viral loads in symptomatic and asymptomatic cases. Liu, et al. [51] found that the mean viral load of severe cases was around 60 times higher than that of mild cases. The assay Cycle Threshold (Ct) values of severe cases remained significantly lower for the first 12 days after onset than those of corresponding mild cases [51]. The findings of Chen, et al. [53] has shown that those in a high-transmission setting had a higher viral load, higher temperature and lower lymphocyte count compared with those from a low-transmission area, indicating enhanced immune responses and exhaustion of immune cells due to persistent and large-scale viral infection [53]. Overall, these findings suggest both that the Ct figure should be reported by the laboratory rather than the standard reporting as virus detected or virus not detected as this may help prognostically and that repeated RT-qPCR testing may be helpful for guiding patient management. It has been shown by Rogers, et al. [25] that viral RNA is stable over time should there be a delay between collection of any respiratory sample and testing. Furthermore,

Amanat, et al. [54] compared reactivity of heat-treated and non-heat-treated serum samples and found that heat inactivation (56°C for 1 hour) did not negatively impact assay performance. This is an important observation as one hazard associated with serological testing of COVID-19 patients is that the SARS-CoV-2 virus could still be present in the sample, particularly if the sample is taken during the acute phase of infection. They also found no significant difference between serum and plasma samples, suggesting both specimen types are suitable for serological testing [54].

RT-qPCR of faeces samples has been shown to be useful in some cases, albeit being diagnostic of fewer COVID-19 patients than respiratory sampling allows (Figure 5) although diarrhoea was noted as a symptom in only 10% of patients, overall (Table 1). This suggests that diarrhoea should not be the deciding factor for testing faeces for SARS-CoV-2 carriage. Disease classification and age of patients appears to be highly correlated. Severe cases are more likely to be older than non-severe cases, by a medium of 7 years in one study [13]. A further difference in age was noted in a study which compared severe and mild cases, where an average age of 37 years was noted in mild cases while the average age of severe cases was 61 years [16].

More males than females are noted to be affected by COVID-19 and the outcomes for men appear to be worse than for women [13, 20, 24]. The proportion of females diagnosed with COVID-19 disease in a study population has been shown to range from 25.8% [33] to 41.9% [13]. A meta-analysis which focused on the outcomes of patients by gender in China which included data from the 2003 SARS outbreak found that men were 2.3 times as likely as women to die from SARS-CoV-2 infection [14].

Co-morbidities are also very common among the critically ill, with 76% having at least one in one study [24]. The most common co-morbidity in all studies is hypertension, the prevalence of which increases between severity class [13, 21]. Diabetes has been shown to be a co-morbidity that includes a higher likelihood of gastrointestinal symptoms and a higher mortality rate [19]. It has also been noted that patients with severe COVID-19 and diabetes have increased leukocytes, neutrophils and decreased lymphocytes when compared to patients that are severe but not diabetic [22]. It has been shown that asymptomatic cases are on average younger and less likely to present with clinical anomalies such as lymphopenia and leukopenia [55] although significantly higher levels of creatine kinase-MB have been found in asymptomatic groups [56]. Evidence of asymptomatic transmission to co inhabitants has also been reported [55].

Diagnosis using serological tests is most useful for patients presenting after disease onset, in particular those with mild illness, or those presenting late as these patient populations will have a low viral load which may be below the limit of detection for RT-qPCR. These tests are also important diagnostic tools for identifying individuals who have seroconverted and also those that could donate blood for convalescent plasma treatment [57]. In order to be diagnostically useful, it is important to chart the dynamics of the host antibody response that occurs following SARS-CoV-2 infection in order to ensure samples for serological testing are taken at the correct time to avoid false negatives. As this is a new virus that has not been encountered in the clinic before, the antibody response is still being characterised. Indeed, the time course for SARS-CoV-2 antibody development varies between published studies and the method used for antibody detection and quantification. However, several studies have permitted a general timeline to be extrapolated and this is shown in Figure 1 and differentiated on the basis of clinical severity of disease. It should be noted that the majority of studies focus on IgM and IgG antibody levels, with limited information regarding the role or kinetics of secretory IgA antibody levels in response to SARS-CoV-2 infection.

Guo, et al. [26] investigated the kinetics of the antibody response to SARS-CoV-2 in 140 infected patients. An ELISA-based assay (using purified recombinant nucleocapsid proteins as coating antigens) was used to measure IgA, IgM and IgG antibody levels in 208 plasma samples; 82 confirmed cases and 58 probable (patients tested negative by RT-qPCR but exhibited other suggestive manifestations) cases. Of the 208 plasma samples tested, 90.4% (188/208) and 93.3% (194/208) were positive for IgM and IgA, respectively. Furthermore, analysis of the 41

acute phase plasma samples (collected 1-7 days PSO) showed that IgM and IgA antibodies were detected in 85.4% (35/41) and 92.7% (38/41) of samples, respectively. IgM and IgA antibody levels increased from days 0-7 to days 8-14 with no further increase between days 15-21 or day 21 onwards. IgG antibodies were detected on days 0-7, increased on days 8-14, continued to increase on days 15-21, and plateaued by day 21. In a study conducted by Zhao and colleagues [32] the seroconversion rate of 173 patients was determined. This patient population consisted of 51.4% females (89/173) and 48.6% males (84/173), with a median age of 48 years. All patients enrolled in the study presented with acute respiratory infection syndrome and/or CT abnormalities, in addition to a positive RT-qPCR test for SARS-CoV-2 from an upper respiratory tract sample. Antibody levels were measured using ELISA; the specificity of the assays was 99.1% (total antibodies), 98.6% (IgM) and 99.0% (IgG). The seroconversion rate for total antibody was 93.1% (161/173), 82.7% (143/173) for IgM, and 64.7% (112/173) for IgG. Twelve patients (6.9%) remained seronegative for total antibody, possibly due to the unavailability of serum samples for testing at the later stage (<13 days PSO) of their illness. The median time for seroconversion was day 11 (total antibodies), day 12 (IgM) and day 14 (IgG) PSO. Antibodies were detected in <40% of patients 1 week following onset, however this increased to 100% (total antibodies), 94.3% (IgM) and 79.8% (IgG) 15 days after onset. Interestingly, in this study there was no significant difference in the rate of seroconversion between severe and mild patients [32].

Stratification of COVID-19 patients into mild/moderate, severe and critical groups showed a difference in antibody kinetics [28]. IgM levels of the non-critical group rose steadily from day 5, before peaking at day 16 and then gradually declining. A different pattern is observed for the critical group, where IgM levels begin to rise steadily from day 10 and peak on day 23 before steadily decreasing. A similar observation was made for IgG levels, where the non-critical groups exhibited a gradual increase from day 5. Indeed, IgG levels of patients in the mild/moderate group were still increasing on day 28. For the critical group, IgG levels rose steadily from day 7 and levels peaked on day 20. Overall, patients in the critical group exhibited a stronger antibody response compared to non-critical patients over the time period examined [28]. Several studies have shown that a higher antibody titer is associated with a more severe clinical classification, suggesting that a high antibody titer could be considered a risk factor for severe COVID-19, independent of age, gender and comorbidities [28, 32, 36]. In contrast, To, et al. [37] found no correlation between serum antibody levels and clinical severity. Furthermore, a study by Chen, et al. [58] found no significant difference in antibody levels between adults and paediatrics (median age 14.5 years). Levels of IgM were 1.22$\pm$0.39 g/L (paediatrics; n=12) versus 1.03$\pm$0.41 g/L (adults; n=20) [p=0.2515] and levels of IgG were 10.86$\pm$1.44 g/L (paediatrics; n=12) versus 11.85$\pm$4.51 g/L (adults; n=20) [p=0.9805].

In the early phase (within 7 days PSO) RT-qPCR has the highest sensitivity compared to antibody testing. However, past this time, antibody testing proves to be more sensitive. In a study conducted by Zhao, et al. [32] samples from hospitalised patients taken 8-14 days PSO exhibited sensitivities of 89.6% (total Ab), 73.3% (IgM) and 54.1% (IgG) compared to 54% for RT-qPCR. This increased to 100% (total Ab), 94.3% (IgM) and 79.8% (IgG) compared to RT-qPCR (45.5%) 15-39 days PSO. Furthermore, patients with undetectable RNA in respiratory tract samples collected during day 1-3, day 4-7, day 8-14 and day 15-39 had total antibody detection rate of 28.6% (2/7), 53.6% (15/28), 98.2% (56/57) and 100% (30/30), respectively. Furthermore, a study by Guo, et al. [26] showed the detection efficiency by IgM ELISA was greater than RT-qPCR 5.5 days PSO and that the positive detection rate increases significantly (98.6%) when RT-qPCR and ELISA tests are combined compared to RT-qPCR alone (51.9%). However, it is important to note in this study that 18 of the 82 confirmed cases (22%) tested negative for IgM. This could be due to timing as 13 of these patients were enrolled less than 7 days after symptom onset [26]. Furthermore, although this assay exhibited no cross-reactivity against NL63, 229E, OC43 and HKU1, strong cross-reactivity was observed with SARS-CoV by Western blotting and ELISA.

Secretory IgA plays a crucial role in mucosal immunity, and mucosal surfaces are the first point of entry for several pathogens including SARS-CoV-2. A study examining the kinetics of the IgA antibody response in patients with confirmed SARS-CoV-2 infection by RT-qPCR showed that IgA levels increased from onset of symptoms and

peaked at 20-22 days PSO [59]. The IgA antibody response was stronger and more persistent than that observed for IgM, where IgM antibody levels peaked at 10-12 days and declined from day 18 PSO [59]. A study by Fourati, et al. [60] observed significantly higher levels of IgA and IgG antibody titers in ICU patients that survived compared to non-survivors. However, further longitudinal studies are needed.

The humoral immune response, in particular the generation of neutralising antibodies, plays an important role in disease surveillance and protecting individuals from future reinfection. The persistence of protective antibodies in patients that have recovered from COVID-19, and whether such patients can become re-infected, remains an important question in the field. Information can be gleaned from past studies on SARS-CoV, which showed that 11.8% of patients were positive for IgG antibodies 7 days PSO [61]. It was shown that IgG and neutralising antibody (NAbs) levels peaked at 4 months PSO and gradually decreased from 74.2% (IgG) and 83.9% (NAbs) 3 years PSO to 8.7% (IgG) 6 years PSO [62-64]. Most SARS-CoV-2 studies to date have analysed serum samples collected from patients during the acute phase of illness. However, a study by Du, et al. [65] conducted serological tests on 60 convalescent patients, where serum samples were obtained between 32 and 61 days PSO of COVID-19. Of these, 78.3% (47/60) tested positive for IgM and 100% (60/60) tested positive for IgG. The average antibody titer was 74.1$\pm$79.2AU/ml and 244.2$\pm$209.4 AU/ml for IgM and IgG, respectively. A follow up test (48-58 days PSO) on ten of these patients approximately 7 days following the first test (41-51 days PSO) showed an average reduction of 28.3% in IgM levels (126.9 AU/ml to 36.0 AU/ml) and an average reduction of 33.6% in IgG levels (393.0 AU/ml to 132.2 AU/ml), suggesting that antibody levels are not always maintained at a high level in convalescent patients [65].

Serological studies are also important in order to predict population immunity and determine the percentage of the community/population that have been infected and for how long they carry the virus (*i.e.* asymptomatic virus carriers). A recent study examining a population of Vo', a small town in Italy, found that 42.5% of patients with confirmed SARS-CoV-2 infection (RT-qPCR on samples obtained by nasopharyngeal swab) were asymptomatic [52]. Additionally, a seroepidemiological study of SARS-CoV-2 infection in Spain (ENE-COVID; 61,075 participants) by Pollán, et al. [66] showed seroprevalence was approximately 5% and 4.6% in the non-institutionalised population (between April 17 2020 and May 11 2020) using a point-of-care test or chemiluminescent immunoassay for IgG antibodies, respectively. Based on the point-of-care test, seroprevalence gradually increased with age from 1.1% in infants younger than 1 year before plateauing at approximately 6% in adults aged 45 years or above. However, seroprevalence was lowest in adults aged 85 years or more when serum samples were analysed by immunoassay. No differences between males and females was reported. Interestingly, asymptomatic patients comprised 32.7% and 28.5% of the population based on the point-of-care test and the immunoassay, respectively [66].

Several blood and inflammation markers were identified in asymptomatic cases which have previously been reported in symptomatic cases, including lower white blood cell and lymphocyte count and elevated or decreased platelet counts. Figure 3 shows that there are several blood analytes which may be predictors of more severe disease progression. Decreased leukocytes and platelets and increased D-dimer, C-reactive protein and procalcitonin were all more common among severe/critical patients than non-severe patients. Increased fibrinogen and increased IL-6 were also very common among the severely ill although a lack of data for non-severe patients prevents a comparison as does a direct comparison with levels of these markers in patients with co-morbidities associated with higher than normal levels in their own right prior to contracting SARS-CoV-2. Reports have highlighted additional blood analytes suggested to be useful in determining severity in certain patients with COVID-19 disease, including elevated levels of lactate dehydrogenase as shown in Figure 3B and creatine kinase-MB [56]. Elevated levels of D-dimer are commonly reported alongside elevated levels of C-reactive protein in severe patients [13, 24, 67]. In non-severe patients, elevated levels of C-reactive protein remain common, but D-dimer is less likely to be elevated [13, 67].

Laboratory finding of patients with different severity classifications may also be useful in determining at risk groups and evaluating prognosis. Elevated inflammatory blood protein levels are persistent in patients infected with COVID-19. D-dimer and IL-6 have been identified as possible markers for a worse prognosis due to commonality of levels above the reference range for more critically ill patients [12, 23]. A Study by Zhang, et al. [67] who examined the D-dimer levels of 343 cases found that D-dimer levels on admission greater than 2.0μg/mL were an effective predictor of mortality in patients with COVID-19, with a sensitivity of 92.3% and a specificity of 83.3%. This included 12 non-survivors with D-dimer levels above this cut off, who on admission had no severe symptoms [67].

However, regarding IL-6, there are other conditions that are associated with an increased IL-6, such as obesity [68] so the usefulness of IL-6 as a predictor for severity of outcome in COVID-19 disease in patients with conditions that have associated inflammation detectable *via* IL-6 requires further study. In a detailed study, for any link between obesity and severity of COVID-19 disease, Zheng, et al. [33] found that when compared to those with non-severe COVID-19, patients with severe disease were more obese (89.5% vs. 59.6%, p = 0.021). Similar to the study by Kern, et al. [68] the study also notes that it has previously been established that increased inflammatory activity in the liver and visceral fat is correlated with increased levels of IL-6 [69] which may lead to greater severity of COVID-19. The obese severe cases in this study were also more likely to smoke. This trend is mirrored in other comparison studies [20]. The number of smokers in one study increased from 13.1% to 22.1% when comparing non-severe and severely affected populations [13].

Elevated levels of CRP and serum amyloid (data not shown) have likewise been observed in the more critically ill [20]. Elevated CRP has been shown to be much more prominent in critical cases than severe when two groups are compared as suggested in Figure 3B. For example, one such study found that 81% of critically ill patients had elevated CRP while 56% of non-severe patients had elevated CRP Guan, et al. [13]. Gonçalves and Sesterheim [70] in a study of diabetic and obese patients with COVID-19, have suggested that serum amyloid may play a key role in the pathogenesis of COVID-19, in addition to potentially having a prognostic role.

COVID-19 includes an evolving set of clinical manifestations, including stroke and cardiomyopathies associated with coagulopathy and vasculopathy, which can cause sudden death and other serious morbidities [71]. A study by Fogarty, et al. [72] has shown that race and ethnicity have major effects upon thrombotic risk, with significantly lower risk in Chinese compared to Caucasian individuals. Fogarty, et al. [72] also note that severe COVID-19 infection is associated with a significant coagulopathy in Caucasian patients that correlates with disease severity and that probably contributes to the underlying pulmonary pathogenesis [72]. This study showed similar findings to those of Zhou, et al. [24] and Guan, et al. [13] in that significantly raised fibrinogen, D-dimer and CRP were found in the poor prognosis group. Artifoni, et al. [73] go further in suggesting that D-dimer level-guided aggressive thromboprophylaxis regimens using higher doses of heparin should be evaluated in prospective studies. These authors thereby place a value on repeated testing of D-dimer for patient management during treatment.

Ferritin (Figure 3B) can be used as both a biomarker of disease progress and prognosis. However, the precise mechanism by which ferritin contributes to disease in sepsis, rheumatologic, immunologic and malignant disorders remains incompletely understood [74]. A Spanish study of cerebrovascular disease in COVID-19 has noted this complication to occur in 1.4% of cases studied (1,683 admissions of patients with COVID-19 disease of which 23 showed cerebrovascular symptoms), 17 suffering from stroke at which time ferritin levels were raised [75]. Most recently, in a paper by Ellul, et al. [76] where 96 patients with stroke have been described, patients frequently had vascular events in the context of a pro-inflammatory hypercoagulable state with elevated CRP, D-dimer, as well as ferritin. It has previously been reported that hyperferritinaemia, regardless of the underlying pathology, is associated with multiple organ dysfunction and high mortality [74]. It should also be noted however, that hyperferritinaemia has been already associated with obesity [77] and diabetes mellitus [78].

Lymphopenia is common in cases where it is reported, with 38.4% - 82.1% of patients exhibiting low white blood cells counts [13, 20, 67]. Levels have been as low as 61 cells/μL in severe-type patients [43] where the normal range in adults is 1,000-4,000/μL, and this finding has been reported to be more common in severe cases [15]. Levels of lymphopenia appears lower again in critically ill populations, where 92% of critical cases showed lymphopenia, compared to 75% of severe cases in comparison reports [24].

From this analysis it was evident that future studies would benefit from incorporating follow-up or longitudinal sampling of their patient cohorts. Predictive algorithms might be expected to be useful; Banerjee, et al. [79] have already demonstrated that machine learning and artificial intelligence can be used to help to predict SARS-CoV-2 infection from full blood counts, for example.

4. CONCLUSION

Based on analysis of the literature there is no specific symptom that can be used to predict COVID-19 disease. Analysis of respiratory sample stability over time indicates that delays in testing for up to 14 consecutive days does not impact on viral RNA quality. It was notable that performance characteristics were not reported for COVID-19 specific tests necessitating an investigation for consensus using multiple papers. There are differences both in detection rates among respiratory sample types (highest in sputum for patients producing sputum and otherwise highest using nasopharyngeal swabs) and over the course of COVID-19 disease post symptom onset. An initial negative RT-qPCR results warrants multiple samplings in suspected cases. Temporal overlap with the patient's immune response shows that in the early days post symptom onset, antibody detection (IgM and IgG; there is limited information currently for secretory IgA) testing could be informative, however. Antibody titers are higher in critical cases. Most investigators of the effect of viral load on severity of disease suggest that the higher the viral load in respiratory secretions the more severe the infection, which suggests that reporting strategies for RT-qPCR should include the Ct for the test result rather than stating solely that SARS-CoV-2 was detected. A number of standard blood marker laboratory tests have been found to be useful prognostically for patients diagnosed with COVID-19 disease. However, the co-morbidities that predispose patients to severe viral disease also cause abnormally high levels of at least some of these markers in their own right prior to contracting COVID-19.

The challenges in the current study in finding the information presented in this paper suggest the need for a quality assured database that outlines the complete set of results, anonymised patient data for each entry and a set of internationally-agreed guidelines for complete laboratory testing, documentation and open-access reporting. We suggest that such information would be useful to help with patient diagnosis and management, worldwide.

Abbreviations

COVID-19:	Coronavirus disease-2019
CP:	Crossing Point
CRP:	C-reactive protein
CT:	Computed tomography
Ct:	Cycle threshold
ELISA:	Enzyme-linked immunosorbent assay
GI:	Gastrointestinal
ICU:	Intensive care unit
Ig:	Immunoglobulin
IL-6:	Interleukin 6
LDH:	Lactate dehydrogenase
MERS:	Middle East respiratory syndrome
PSO:	Post-symptom onset

RNA:	Ribonucleic acid
RT-qPCR:	Real time polymerase chain reaction
SARS:	Severe acute respiratory syndrome
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2

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