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Could SARS-CoV-2/COVID-19 simply fade away?

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Since its emergence in Wuhan, China, on November 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been progressively invading every corner of the world. As of today (April 30), it is responsible for more than 3.2 million confirmed cases and more than 220 thousand deaths in 186 countries [1]. SARS-CoV-2 belongs to the enveloped, positive-sense, single-stranded RNA (+ssRNA) *Coronaviridae* family of viruses, which includes at least 49 different species [2]. Coronaviruses are known to infect both birds and mammals, usually producing either respiratory or gastrointestinal diseases [3]. Two previous highly pathogenic outbreaks of coronavirus infections have occurred during the last decades: severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak which started in China in 2003, and Middle East Respiratory Syndrome coronavirus (MERS-CoV), first identified in Saudi Arabia in 2012 [4, 5]. Both of those had a fast expansion and a relatively high case fatality rate (CFR), but after being subject to crucial public health interventions to control their dissemination disappeared rapidly. Before a vaccine could be developed, both diseases tended to fade away.

Like other RNA viruses, coronaviruses have a high mutation rate, around two orders of magnitude higher than DNA viruses [3]. Their genomic mutation rates, estimated by the average number of mutations each offspring will have compared to the parental (or ancestral) genome, are higher. By some estimates, a typical SARS-CoV-2 strain could have around 25 mutations per year, somewhat less than seasonal flu, which has a mutation rate of almost 50 mutations per year [6]. On a per-site level, DNA viruses typically have mutation rates on the order of 10^{-8} to 10^{-6} substitutions per nucleotide site per cell infection; for RNA viruses, however, that range would be between 10^{-6} and 10^{-4} [3]. Some of these mutations would be lethal, and the virus would be unable either to replicate or to infect the host. The possibility of a mutation that would increase the already very high pathogenic capacity of the virus must have happened, perhaps only once, in the evolutionary history of SARS-CoV-2, but would currently be meagre. Many mutations would have little or no effect on the infective capacity of the virus, and would simply explain the genomic variations identified in different strains worldwide [7]. But some of this high mutation rate might be associated with what has been described as mutational degeneration in RNA viruses, which has been studied in SARS since 2002 [8, 9]. Increasing the rate of mutation accumulation ("lethal mutagenesis") could be a pharmacological mechanism to control viral epidemics by accelerating strain extinction [9, 10].

There could be, we speculate, a number of these mutations that could compromise the aggressive behaviour of the virus, leading either to a reduction in the effective reproductive rate (R_t) or in the amount of systemic injury in infected humans. That would explain the apparent progressive de-

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crease of the daily growth rate that has been described worldwide, and which has led to a relatively constant number of new confirmed cases of the Coronavirus Disease 2019 (COVID-19) during the last month (Figure 1). This theoretical speculation is also based on long-term evidence that supports the concept of viral natural and genetic attenuation through mutation of RNA viruses [8, 9]. This concept has been previously proposed as an explanation for the evolutionary behaviour of other RNA viruses, such as the H1N1 influenza A virus. H1N1, in particular, has experienced multiple extinction events during its circulation in the human population [8-11].

In Latin America, a region significantly affected by the COVID-19, a slow but progressive decline in the daily growth rate is noticeable both in countries like Colombia or Chile, which entered a strict quarantine and physical distancing policy early in the epidemic, as well as in Mexico or Brazil, which have had a much more liberal approach (Figure 2) [12-14].

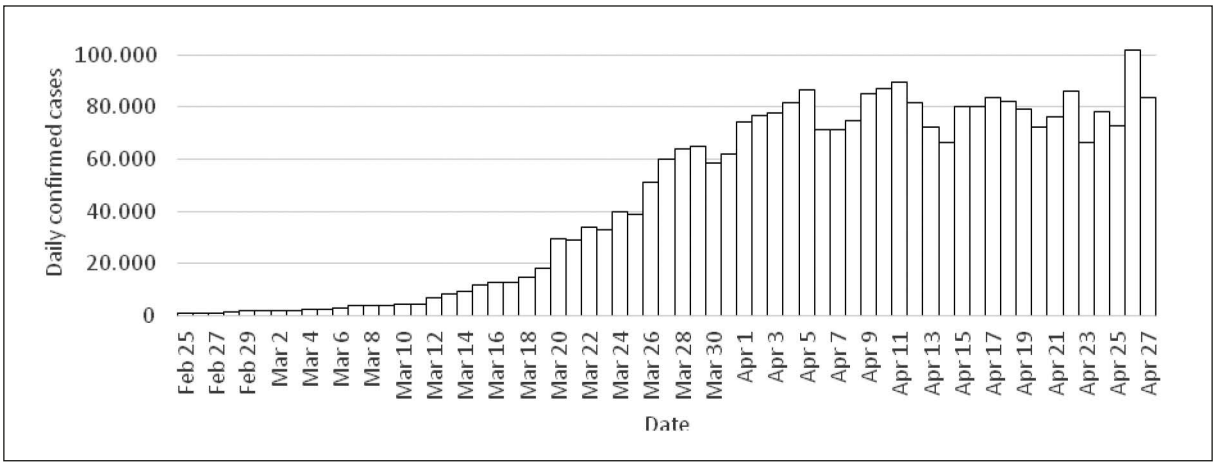

Figure 1 - Number of new daily confirmed cases of COVID-19 worldwide. *Source: Coronavirus Resource Center (1).*

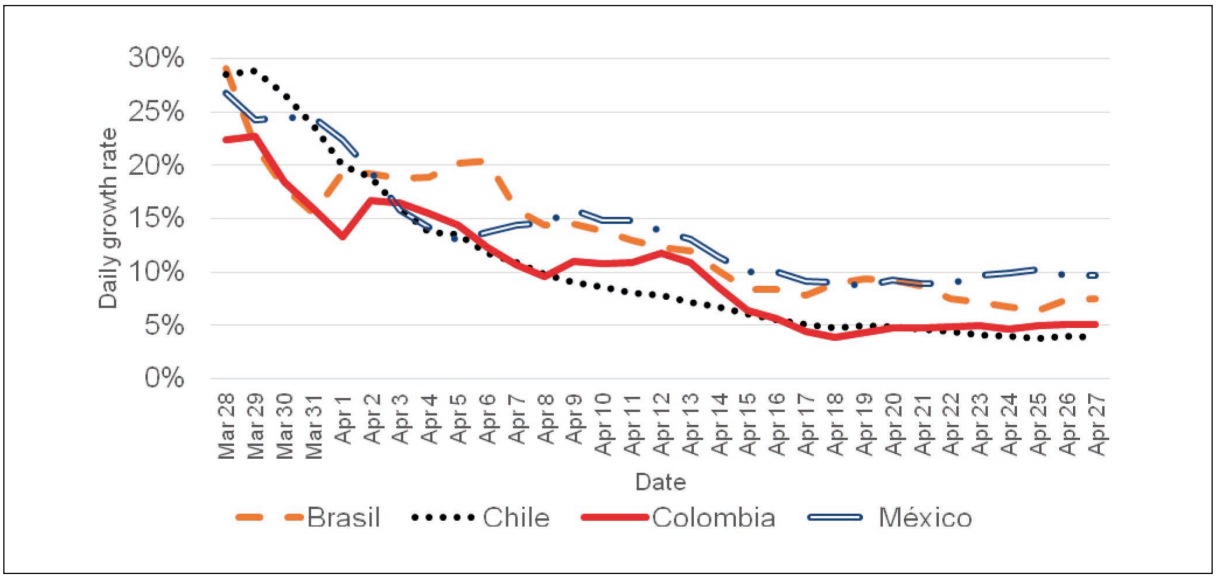

Figure 2 - Daily growth rate (%) of confirmed cases in four Latin American countries during the period March 28 to April 27, 2020.

From an evolutionary perspective, a less aggressive behaviour related mutation could be reproductively successful. The co-existence of different mutations in patients could also perhaps explain the influence of the viral load on the aggressive behaviour observed in some circumstances, as a variety of mutated viruses could include a higher mix of virulent specimens [11, 15].

In conclusion, the reduction of the growth rate of COVID-19 could be explained through deleterious (from the virus perspective) mutations. This would not imply necessarily relaxing epidemic-control strategies but would give a word of hope. While almost every country faces this first COVID-19 wave, options are that the virus drops its lethality over time, and even goes through to temporal extinction periods over the course of years ahead. If we just hold on to current social distancing measures, the problem would just perhaps, go away by itself.

Conflict of interest
None

Funding
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Should ICU COVID-19 patients empirically receive therapeutic doses of anticoagulant?

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Incidence of thrombotic complications in Covid-19 severe patients admitted to intensive care unit (ICU) is reported up to 31%, and it is associated with increased mortality [1, 2]. In patients with severe Covid-19, mild thrombocytopenia, prolongation of the prothrombin time and elevation of D-dimer levels are very common [3]. The increase of LDH and ferritin levels are relevant for the coagulation balance too, as in thrombotic microangiopathy. Helms et al. diagnosed 25 pulmonary embolisms in 150 ICU patients, while only 4 subjects (of whom 2 had a trauma) presented haemorrhagic complications [4]. We do not yet know why haemostasis is such a major issue in Covid-19. However, direct endothelial cell infection and endotheliitis may have a trigger role in Covid-19 thromboembolic complications. SARS-CoV-2 uses the pneumocytes angiotensin converting enzyme 2 (ACE2) receptor to infect the lungs, and ACE2 is also widely expressed by endothelial cells. Varga et al. diagnosed SARS-CoV-2 inclusion in the endothelial cells of kidney by electron microscopy, and diffuse endothelial inflammation of lungs, heart, intestine in 2 COVID post-mortem analysis cases [5].

Endothelial cells infection and/or inflammation might cause microvascular dysfunction with vasoconstriction and subsequent organ ischaemia, associated tissue oedema, and procoagulant state. Moreover, hypoxia itself results in vasoconstriction of pulmonary capillaries and induces activation of hypoxia-inducible factors (HIFs), that modify the expression of several genes, including tissue factor (TF) and plasminogen-activator inhibitor-1 (PAI-1). Therefore, SARS-CoV-2 infection alters the haemostatic balance among procoagulant and antifibrinolytic factors and reduce the capacity to cleave and remove fibrin deposits. This corresponds with presence of exudates, constituted of fibrin and proteinaceous material, which blocks normal gas exchange and fosters diffuse alveolar damage after infection. In a condition such as Covid-19, where excessive inflammation, hypoxia, and immobilisation predispose to both venous and arterial thromboembolic disease, planning the intensity of thromboprophylaxis is very important, especially for patients admitted to the ICU, who are at highest thrombotic risk. Prophylactic low-dose heparin should be used to reduce the risk of venous thrombosis in all the ICU Covid-19 patients, even if life-threatening pulmonary embolism has occurred despite full-dose anticoagulation with heparins [5].

We should also contemplate interactions among drugs used for COVID-19 treatment, which have impact on coagulation: *e.g.*, apixaban and rivaroxaban should not be used with tocilizumab since it increases cytochrome P450 enzyme activity (for a list of drug interactions, please check: *covid19-druginteractions.org* by the University of Liverpool).

The International Society of Thrombosis and Haemostasis (ISTH) recommends to: "measuring D-dimers, prothrombin time, and platelet count (in decreasing order of importance) in all patients who present with COVID-19 infection" [6]. Measuring fibrinogen can be useful too, even

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if this is probably more difficult in many hospitals. In 2017 Iba et al. published the sepsis-induced coagulopathy (SIC) score, specifically designed for assessing coagulation disturbances in sepsis. SIC score is based on simple parameters (platelet count, prothrombin time and Sequential Organ Failure Assessment (SOFA) score) and may be useful to stratifying COVID patients who needs heparin treatment [7]. Tang et al. reported that heparin treatment was associated with lower mortality in patients with SIC score ≥4 (40.0% vs 64.2%, p=.029), but not in those with SIC score <4 (29.0% vs 22.6%, p=.419) [2]. In this 449-case retrospective evaluation, low molecular weight (LMWH) was the most commonly used anticoagulant, and it was mostly given at the prophylactic dose (i.e. 40 mg enoxaparin/die) [2]. Since all the ICU mechanically ventilated patients are at high risk for thrombotic complications and should receive a prophylactic dose of LMWH unless of increased haemorrhagic risk, the point here is not about the prophylactic treatment but the benefits and risks of a stronger anticoagulation, which are yet undetermined. In fact, the primary function of procoagulant response is sequestering and eliminating the microbes, while, on the other hand, compromised organ circulation may benefit from anticoagulant therapy. This is why ASH (American Society of Hematology) will develop clinical practice guidelines, addressing four distinct populations of people with COVID-19: acutely ill hospitalized patients; critically ill hospitalized patients, *e.g.*, patients in the intensive care unit; patients after hospital discharge; and non-hospitalized patients (8).

In conclusion, seriously ill COVID-19 patients should not receive therapeutic doses of anticoagulant empirically (i.e. in the absence of confirmed venous thromboembolism), but yet a more aggressive strategy might be required in selected cases, under strict monitoring and surveillance.

Conflict of interest
None

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Emerging coronaviruses: first SARS, second MERS and third SARS-CoV-2. Epidemiological updates of COVID-19

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SUMMARY

Since December 2019, the emergence of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) infection has been reported unexpectedly in Wuhan, China, with staggering infection speed across China and around the world. To date, seven known strains of HCoVs belonging to four genera (i.e., α–, β–, γ, and δ-CoV) have been recognized; the latest one has been identified as the SARS-CoV-2. Although the common transmission routes of SARS-CoV-2 is the respiratory tract, it seems that other routes such as the gastrointestinal tract may be effective for the entry of the virus in the body. Although there are no biological markers to predict the susceptibility of humans to COVID-19, several risk factors have been identified to predict the susceptibility of patients to COVID-19. Initial data revealed that males, pregnant women, elderly, and underlying conditions predispose patients to higher morbidity or mortality and also might be at risk for a severe infection of COVID-19. There is a greater need to better understand the mechanisms and risk factors of transmission routes. To date, despite the whole world effort to review various aspects of SARS-CoV-2, including epidemiology, clinical manifestations, diagnosis, and treatment options, there are still gaps in the knowledge of this disease and many issues remain unclear. Therefore, there is an urgent need for update data on SARS-CoV-2. Here, this study provide the current epidemiological status (transmission routes and risk of transmission, possible origins and source, mortality and morbidity risk, and geographical distribution) of the SARS-CoV-2 in the world in 2020.

Keywords: Coronaviruses, COVID-19, SARS-CoV-2, pandemic.

INTRODUCTION

Coronaviruses (CoVs) are a large family of single-stranded RNA (+ssRNA) viruses that were first discovered in the 1960s [1]. Some CoVs co-infect both humans and vertebrate animals such as camels, cattle, cats, and bats. Human coronaviruses (HCoVs) usually are associated with the common cold and more severe diseases such as pneumonia and bronchiolitis. In immunocompromised, elderly, and child patients, HCoVs can cause life-threatening pneumonia and bronchiolitis that in turn may also cause enteric and neurological diseases [2].

To date, seven known strains of HCoVs belonging to four genera (i.e., α–, β–, γ–, and δ–CoV) have been recognized:

1) HCoV-NL63;
2) HCoV-229E (belonging to α-CoVs);

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3) HCoV-OC43;
4) HCoV-HKU1 (belonging to β-CoVs);
5) Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV);
6) Middle East Respiratory Syndrome Coronavirus (MERS-CoV);
7) the latest one, which has been identified as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [3, 4].

Among Betacoronavirus, MERS, SARS, and the new SARS-CoV-2 are considered as emerging zoonotic transmissions that have caused epidemics in humans. SARS-CoV was first reported from China in 2002-2003. MERS first emerged in September 2012 from a male Saudi Arabian patient in Saudi Arabia. In December 2019, the emergence of the SARS-CoV-2 infection was reported unexpectedly in Wuhan (China) with staggering speed across China and around the World. The World Health Organization (WHO) called the current infection outbreak caused by SARS-CoV-2: coronavirus disease 2019 (COVID-19) [5, 6].

So far, SARS-CoV-2 has affected more than 4,618,821 patients in 210 countries/area and has become a public health emergency of international concern. On March 11th, 2020, WHO declared COVID-19 as a pandemic. This is the first known pandemic caused by the emergence of a new coronavirus. Early studies indicated that most cases of infection were related to the seafood and wild animal markets and the majority of the earliest cases have been infected through zoonotic or environmental contacts and showing possible animal-to-human transmission. In recent weeks, it is now clear that human-to-human transmission of SARS-CoV-2 has been rising dramatically and has been confirmed through droplets or direct contact [7, 8].

After 5 months from the onset of the COVID-19 outbreak, this infection is still known as a public health concern with no vaccine or definite treatment and also with some unidentified epidemiological aspects related to it. Strategies to prevent COVID-19 depend on providing epidemiological information about this infection.

To date, despite the whole world's effort to review various aspects of SARS-CoV-2, including epidemiology, clinical manifestations, mortality and morbidity and diagnosis, there are still gaps in the knowledge of this disease and many issues remain unclear. Therefore, monitoring and periodical investigation of this emerging infection in an epidemiological study seems to be essential. The present study provide the current epidemiological status (i.e., possible origins and source, transmission routes and risk of transmission, mortality risk, potential risk factors, and geographical distribution) of the SARS-CoV-2 in the world in 2020.

TRANSMISSION ROUTES

The unexpected pneumonia infection caused by SARS-CoV-2 has probably evolved from Wuhan to other provinces and countries. WHO declared a global health emergency over this global pneumonia outbreak on January 30th, 2020.

The contact with animals and the consumption of wild animals were suspected as the routes of disease origin. Therefore, the possible role of animals in COVID-19 infection most not be ignored [9]. Also, since the main symptoms of COVID-19 patients are fever and respiratory disorders, the spread of SARS-CoV-2 through food is improbable [10].

According to previous reports, the predominant transmission route of SARS-CoV-2 is a human-to-human transmission that includes contact transmission through contact with oral, nasal, and eye mucous membranes and direct or indirect transmission via cough, sneeze, and respiratory droplets [11]. Although the common transmission route of SARS-CoV-2 is the respiratory tract transmission, it seems that other routes such as unprotected eyes may be the effective route for the entry of the virus in the body.

Lu et al. suggested that exposure of unprotected eyes to SARS-CoV-2 could cause acute respiratory infection [11]. In this regard, Xia et al. reported that tears and conjunctival secretions of a patient were positive for SARS-CoV-2. Also, the sputum of a sample was detected positive for SARS-CoV-2 [12].

In another study, Wang *et al.* reported that saliva contains live viruses that may allow a person-to-person transmission, as a direct or indirect route of spread [13]. Interestingly, Rothe et al. also described that even contact with asymptomatic patients may transmit COVID-19 infection [14].

According to the Public Health Agency of Canada, the airborne transmission may occur under environments related to critical care and anesthesia clinicians. In experiences associated with

SARS outbreaks, there was the possibility of airborne transmission under certain circumstances.
Wang et al. investigated the concern about the person-to-person transmission routes in dental clinics and hospitals [15]. Previous study in dental fields showed that many dental procedures produce droplets and aerosols, which are contaminated with the virus [5]. The transmission of SARS-CoV through droplet and aerosol are critical concerns in dental offices. Commonly, during dental practice, the patient's saliva, aerosol and droplet, and even blood are possible routes to virus transmission. Also, in close contact, the materials of patient, and the contaminated dental instruments or environmental surfaces may be considered as a possible route to the spread of SARS-CoV-2.
According to recent reports, SARS-CoV-2 RNA was identified in a feces specimen [7, 15]. These reports indicated that fecal-oral transmission may be considered as the route of spread [16]. One of the most important reasons for this phenomenon is that angiotensin-converting enzyme 2 (ACE2) protein, as a cell receptor for SARS-CoV-2, is highly expressed in the glandular cells of gastric, duodenal, and rectal epithelia. In this regard, Xiao et al. detected 39 (53.42%) stool samples positive for SARS-CoV-2 RNA. In another study on the SARS-CoV-2 shedding, 66.67% of patients were positive for SARS-CoV-2 RNA in stool specimens [16]. These findings suggested that fecal-oral transmission could be an additional route for transmission of SARS-CoV-2.
In a large study on 651 COVID-19 patients, 11.4% of them had gastrointestinal (GI) symptoms such as nausea, vomiting, and diarrhea. Taken together, the patients with COVID-19 without respiratory signs and fever presented a great tendency to show GI symptoms. Therefore, this point should be taken into account to control the further spread of the virus [18].
So far, SARS-CoV-2 nucleic acid has been detected in milk, blood, and urine. However, there has been no document on the transmission of the virus to humans through these routes [19, 20]. Moreover, some studies reported that none of the urine and serum samples were tested positive for SARS-CoV-2 RNA [21].
In an animal model study for SARS-CoV-2 virus transmission, SARS-CoV-2-infected animal shed virus in feces, saliva, nasal washes and urine up to 8 days post-infection [22]. Yuen et al. propounded the undeniable possibility of transmission of SARS-CoV-2 through sewage, waste, contaminated water, air condition system, and aerosols [23]. However, additional examinations are needed to investigate the role of these transmission routes in these cases [23].
To date, there has been no evidence on the occurrence of a human-to-animal transmission [24].

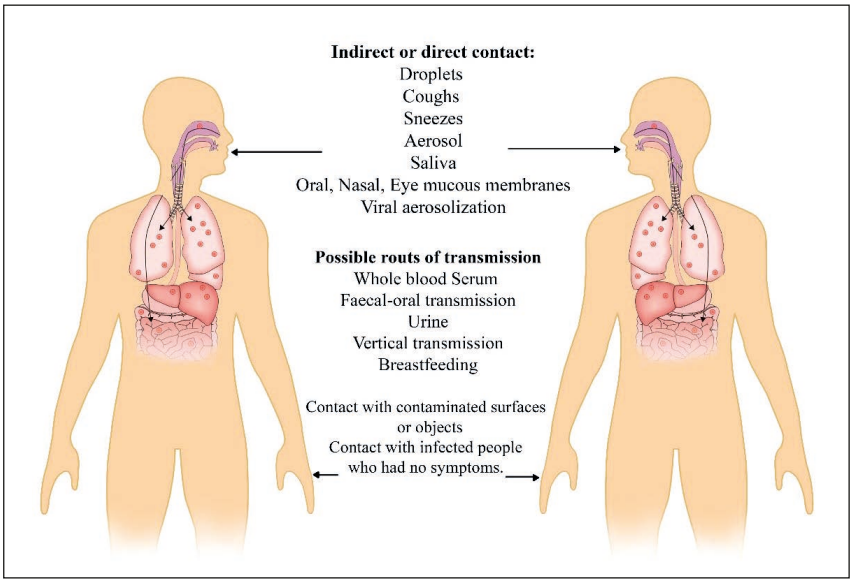

Figure 1 - Transmission rout of SARS-Cov-2.

Moreover, there are no documents that pet animals can be the origin of SARS-CoV-2 for humans or other animals [24]. Different patterns of transmission route of SARS-CoV-2 human-to-human transmission are presented in Figure 1.

RISK FACTORS FOR TRANSMISSION

Since the transmission modes of SARS-CoV-2 is not yet entirely known, we investigated several new and important findings around the world.
Commonly, several factors such as physical features, virological aspects (viral loading, location of virus receptor, etc.), environmental factors, and behavioral patterns can affect the transmission of viruses. In this regard, Cai et al. showed that asymptomatic infected people may spread SARS-CoV-2 through virus aerosolization and contact with contaminated objects and thus are considered as a carrier [25]. In a shopping mall survey in Wenzhou, COVID-19 cases probably were contaminant via asymptomatic. This significant finding is associated with the increased risk of disease transmission. In this regard, Rothe et al. showed the risk of transmission during the incubation period of asymptomatic patients [14].
Therefore, early detection and isolation of SARS-CoV-2 can be effective and even essential in reducing virus transmission. Accordingly, close and continuous monitoring in crowded places is critical, especially in asymptomatic or very mild symptomatic cases of COVID-19. Bi et al. surveyed 391 SARS-CoV-2 cases and 1286 cases with close contacts. According to their results, household members' contacts and those traveling with a coronavirus case are at higher risk of infection than other close contacts [26].
Moreover, Wang et al. indicated that saliva specimens of COVID-19 patients contained live viruses. Therefore, the transmission rate of the virus is high and may allow easy transmission via saliva. Hence, it can be suggested that the risk of transmission of SARS-CoV-2 via saliva directly or indirectly even among patients without respiratory symptoms is greater than other transmission routes.
So far, there are no strong documents on fecal-oral transmission and thus further studies are needed [12]. Recently, Xiao et al. provided evidence of gastrointestinal infection of SARS-CoV-2 and showed the risk of virus transmission via the fecal-oral route, which can be as a driver for SARS-CoV-2 transmission [18]. Consequently, concerning the approved and investigated transmission route of SARS-CoV-2, it seems that the risk of transmission of the virus is greater than what we think.
To date, no evidence of the vertical transmission of mother-to-infant has been reported. In this regard, several studies have investigated possible mother-to-infant transmission. For example, Zhu et al. analyzed the clinical characterization of 10 cases of neonates born to mothers with COVID-19 infection. According to their findings, although COVID-19 infection may have adverse effects on newborns, there is no sufficient evidence for the risk of vertical transmission of SARS-CoV-2. Moreover, Chen et al. found no evidence of vertical transmission in women with COVID-19 pneumonia in late pregnancy. These originals findings are in line with the vertical transmission of SARS-CoV and MERS-CoV, for which there was no supporting evidence. Therefore, it can be concluded that the risk of vertical transmission in pregnant women with COVID-19 is very low. However, this hypothesis needs to be further investigated.

NOSOCOMIAL TRANSMISSION

Consistent with SARS-CoV and MERS-CoV, the nosocomial transmission is a severe problem in COVID-19 and even worse. Nosocomial transmission of COVID-19 is facilitated by mobile phones of health care workers and hospital equipment [27]. According to several studies, the nosocomial transmission has been a hallmark of COVID-19 infections. Analysis of data in the Hong Kong Special Administrative Region suggested that COVID-19 is not spread by an airborne route. Moreover, the results showed that nosocomial transmissions could be prevented through vigilant basic infection control measures, including hand and environmental hygiene, and wearing of surgical masks [28]. A retrospective study showed that a total of 1716 health workers were infected by the virus, accounting for 3.84% of total cases. This finding is consistent with the person-to-person transmission of this novel coronavirus in hospital settings [29]. In another study in the Zhongnan hospital of the Wuhan University, 29.0% (n=40) of medical staff involving with COVID-19 during hospitalization was reported

[19]. Therefore, the greatest risk for COVID-19 is transmission to healthcare workers. COVID-19 has been detected in a neonate born to a pregnant woman with COVID-19 infection 36 h after birth at Wuhan Tongji Hospital. So, it is reasonable to assume that a newborn could be infected by COVID-19 and hence, newborns should be placed in separate rooms to avoid exposure to any source of infection [30]. In this regard, there is no evidence of perinatal infection of COVID-19 during pregnancy [31, 32].

MORTALITY

During the 2002-2003 SARS-CoV epidemic, more than 8,000 people were infected, of which 774 died representing a mortality rate of 10%. Later, in 2012, MERS-CoV infected more than 857 cases with 334 deaths resulting in a mortality rate of 35%. At the end of 2019, the epidemic of COVID-19 occurred. This outbreak is expanding with remarkable morbidity and mortality in the last 4 months. As recorded by the WHO, by May 18th, 2020, there had been more than 4,618,821 confirmed cases and more than 31,2000 deaths due to COVID-19, with an average mortality of about 4.08% [33]. Therefore, it seems that the mortality rate of COVID-19 is higher than influenza, especially seasonal influenza. Although regarding the rapid spread of COVID-19, it is still too early to estimate the mortality rate, there are several reports on the mortality rate in different studied populations. Su Yu et al. reported 14-15% death in hospitalized COVID-19 patients [34]. Huang et al. and Wang et al. reported mortality rates of 14.6% [35] and 4.3% [13] , respectively. Moreover, in a study conducted in China, the mortality of the 27 included patients infected by SARS-CoV-2 was 37%.

However, these mortality rates do not represent the actual death rate. The most important reason for this discrepancy is undetectable data on asymptomatic cases or patients with very mild symptoms that are not notified.

Overall, the mortality of COVID-19 is associated with underlying health conditions. Similar to an outbreak caused by SARS, several host factors may be associated with mortality in the COVID-19 outbreak including older age (>60 years), smoking history, pre-existing pneumonia, and significant comorbid illnesses (such as immunocompromised states, chronic heart, lung, and kidney disease, and diabetes mellitus) [36].

Accordingly, there is strong evidence to suggest that diabetes might be associated with mortality, while there is not sufficient evidence to display that hypertension might be associated with an increased risk of mortality [37]. Leung et al. suggested the possible role of cardiovascular, cerebrovascular, and pulmonary disease at a higher risk of mortality [37, 38]. Peng et al. concluded that fulminant inflammation, lactic acid accumulation, and thrombotic events are associated with a higher risk of mortality in COVID-19 patients [39].

According to previous studies, acute respiratory distress syndrome is the major cause of death in patients with COVID-19. This syndrome is the major indication for transferring patients to the Intensive Care Unit (ICU). Hence, the delay of hospital admission of patients with COVID-19 is significantly associated with a higher mortality rate [37, 40]. Leung et al. showed that although 67.4% of all death cases were male, gender was not associated with mortality [37].

Fever and cough are the most frequent symptoms associated with death. However, there is not sufficient documentation to show the association of this fatality with fever [37].

In a hospital-based case-cohort study, comorbidities, older age, lower oxygenation index, the serum urea nitrogen, AST/ALT ratio, TBIL, LDH, and several markers of extrapulmonary organ injuries were positively associated with death risk of COVID-19 patients [41]. In this study, among dead cases, 80% had at least one of comorbidities including hepatic disease, diabetes, cardiac disease, hypertension, and chronic pulmonary disease. There is a significant correlation between comorbidities and elevated death risk of COVID-19 patients [41].

In a prospective cohort study, four potential risk factors including age ≥65 years, preexisting concurrent cardiovascular or cerebrovascular diseases, cardiac troponin I ≥0.05 ng/mL and CD3+ CD8+ T cells ≤75 cell/μL were identified as predictors for mortality of COVID-19 patients with pneumonia [42].

MORBIDITY

Although there are no biological markers to predict the susceptibility of humans to COVID-19,

several risk factors have been identified to predict the susceptibility of patients to COVID-19.

Initial data revealed that males, pregnant women, elderly, and underlying conditions are often associated with higher morbidity and mortality and also might be at risk for a severe infection of COVID-19 [34, 35, 43]. The most predominant related comorbidities are old age, smoking, diabetes, and pulmonary disease.

Previous reports have found that the disease tends to develop quicker in elderly male people [44]. In this regard, in a retrospective cohort study, Shi et al. investigated host risk factors associated with severe COVID-19. According to their findings, among 487 studied patients, elder age, male, and presence of hypertension are independently related to severe disease at admission. In comparison, COVID-19 is much more predominant among males, with a male to female ratio of 2.7:1 [1]. Moreover, the concurrency of hypertension, diabetes, cardiovascular diseases, and malignancy was higher among severe cases at admission [45].

In a meta-regression study, it was reported that hypertension is related to ~2.5-fold-increased risk of both increased mortality and severity [46]. Moreover, hypertension should be accounted for as a clinical predictor of COVID-19 severity among older individuals [46].

Some studies have demonstrated that smoker cases are related to higher expression and the potential of upregulating the ACE2, which is known as the receptor of SARS-CoV- 2 and may be considered as a risk factor [47]. In this regard, Zhang et al. found that there was an association between smoking and the severity of COVID-19 [48]. Moreover, Liu et al. reported that in patients with the progression of COVID-19 pneumonia, a history of smoking was significantly higher in comparison with improvement patients. They suggested that smoking may be related to disease progression [49]. In a recent systematic review, it was concluded that "smoking is most likely associated factor with negative progression and adverse outcomes of COVID-19" [50].

However, according to the literature review, there are no reliable and strong data to support that smoking is a predisposing factor in men or another subgroup for infection with SARS-CoV-2 [51].

Although several studies revealed the clinical characteristics of pregnant women with COVID-19 infection are compatible with those reported for non-pregnant adults, some studies described that clinical characteristics of pregnant women are atypical [52].

Liu et al. investigated clinical and CT imaging features of the COVID-19 among pregnant women. They showed that the clinical symptoms of pregnant women were atypical and they had high complication rates compared with the non-pregnant women [52].

In a systematic review, the clinical symptoms and maternal and perinatal outcomes of COVID-19 were assessed during pregnancy. Among 108 survey pregnant cases, most mothers were discharged without any main complications; however, severe maternal morbidity as a result of COVID-19 and perinatal deaths were reported, as well [53].

Accordingly, despite the lack of any maternal deaths, one intrauterine death and one neonatal death were observed. Therefore, there is evidence on the possibility of severe maternal morbidity requiring ICU admission and perinatal death with COVID-19 infection in pregnancy [53].

Studies have shown that children are less affected than adults and clinical attack rates in the 0-19 age group are low and usually present as a mild disease [52]. Reports suggest that children are infected from the household transmission of adults. However, neonates and elderly people need more attention, due to their effete immune system and chronic underlying diseases.

Recently, it has been reported that blood group A had a significantly higher risk for COVID-19. Zhao et al. investigated the relationship between the blood group and the COVID-19 among 2.173 patients and compared them with normal patients in Wuhan and Shenzhen, China. The results showed that the proportion of blood group A in COVID-19 patients was significantly higher such that it is accounted as a risk factor [54].

Devarajan et al. studied the single-nucleotide polymorphism rs12252-C/C in the gene IFITM3 as a factor associated with severe influenza in patients with COVID-19. According to their results, this polymorphism may be a risk factor in COVID-19 patients. However, they suggested that further examination of the IFITM3-rs12252-C/C allele in a large population is needed.

Among the host factor, the platelet count can be a simple and commonly available biomarker in as-

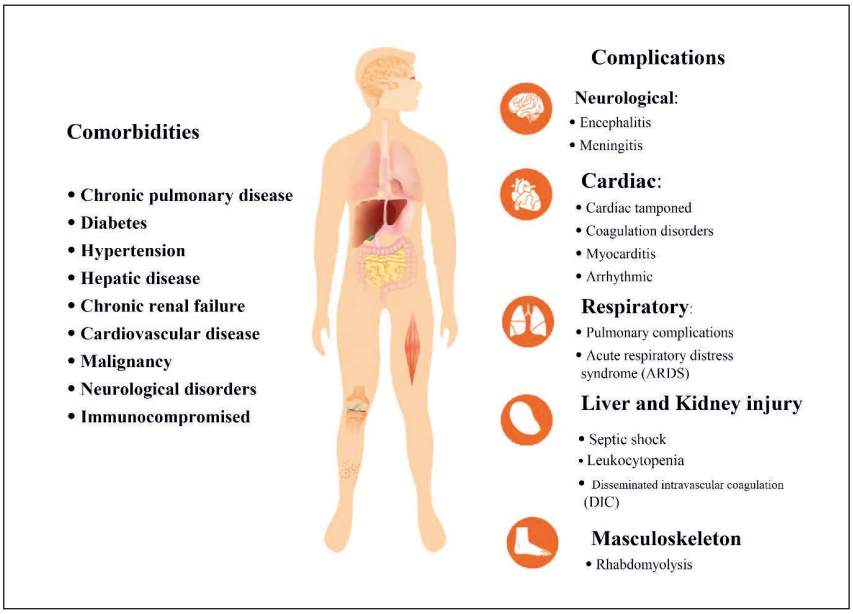

Figure 2 - Comorbidity and complication related to COVID-19.

sociation with disease severity. In the SARS outbreak, thrombocytopenia was recognized as an important risk factor for mortality.

In a meta-analysis, Lippi et al. showed that the level of platelet was remarkably lower in patients with more severe COVID-19. Therefore, thrombocytopenia could be a clinical indicator and is also considered as a risk for severe disease and mortality in patients with COVID-19 [55]. The more prevalent comorbidity and complication related to COVID-19 are presented in Figure 2.

POSSIBLE ORIGINS, SOURCES AND RESERVOIRS

The disease caused by SARS-CoV-2 in humans is a public health emergency of international concern. However, so far, the origin and the source of the causative virus and its intermediate host of the virus is yet to be fully determined [2].

CoVs of bat origin have caused tree pandemics in 21th century. SARS-CoV, MERS-CoV and SARS-CoV-2, all three originated from bats [11]. Previous studies revealed that the SARS-CoV spreading from bats to palm civets to humans and the MERS-CoV spreading from bats to camels to humans and also like many other coronaviruses, the SARS-CoV-2 may have been transmitted to humans by an intermediate animal host. To date, a large number of studies suggested, on the basis of phylogenomic analysis of the recently released genomic data of SARS-CoV-2 that the human was the most similar to Bat coronavirus isolates such as BaT-CoV RaTG13 with 96.2% identical in complete genome sequence [3, 56]. Their findings suggesting that the bats' CoV and the human SARS-CoV-2 share a recent common ancestor and SARS-CoV-2 might be transmitted from bats via unknown intermediate animal hosts (such as pangolins) to humans. According to the report, the SARS-CoV-2 virus, which is responsible for the current outbreak of COVID-19, did not come directly from pangolins. However, due to incomplete sequence of pangolin coronavirus published in GenBank, they cannot exclude that other pangolins from China may contain coronaviruses that exhibit greater similarity to the SARS-CoV-2 [57, 58]. Summary of the possible reservoir, intermediate and target hosts for SARS-CoV, MERS-CoV, and SARS-CoV-2 is presented in Figure 3.

SARS-CoV-2 binds to ACE2 with high affinity as an entry receptor to infect humans. However, some amino acid residues are different in the receptor-binding domain (RBD) of SARS-CoV compared to SARS-CoV-2. It seems that humans are infected with the virus directly from intermediate animal hosts via contact [59-61]. It is clear now that the animal may serve as a key interme-

diate host for the recombination and evolution of SARS-CoV-2. Nevertheless, further investigation and analysis may be needed to find the intermediate hosts and other sources.
Frequent host-shifting cases likely characterize coronaviruses, whether they are animal-to-animal, animal-to-human (zoonosis), or human-to-animal (reverse zoonosis). Many studies speculated that snake is a possible reservoir for SARS-CoV-2 but it was dismissed by other scholars [61-63]. In other more advanced molecular analysis and virological studies, it was shown that bats are the primary reservoir of SARS-CoV and MERS-CoV [63-65]. A similar study suggests that pangolin species are natural reservoirs of SARS-CoV-2-like CoVs, but there is no conclusive evidence that SARS-CoV-2 has a specific wildlife host as a virus reservoir [62, 66].

INCUBATION PERIOD

The incubation period of an infectious disease is the time interval between the exposures to an infectious agent until signs and symptoms of the disease appear. The incubation period of a disease can widely vary from one person to another. The incubation period data are used in estimating the size of the transmission potential and the epidemic. These data also help assess the effectiveness of entry screening and contact tracing. The reported estimate of the novel coronavirus incubation time is based on limited case data. Using data from many public reports, the incubation period for the novel coronavirus is estimated to be in the range of 2-14 days; however, two cases with an incubation period of 19 and 27 days have been reported [67, 68]. The median incubation period is 6 (interquartile range of 3 to 8) days and also the median time from the first visit to a doctor to confirm the diagnosis is about 1 (interquartile range of 1 to 2) day [69, 70]. Besides, the median time from onset of symptoms to dyspnea was 5 days, hospitalization was 7 days, and acute respiratory distress syndrome (ARDS) was 8 days [71].

GLOBAL DISTRIBUTION

WHO has described four levels of COVID-19 transmission with varying social measures and public health based on the evolution of the COVID-19 pandemic in countries or local areas with:
1) no cases reported;
2) sporadic cases;
3) clusters of cases (grouped in time and place);
4) community transmission [72].
On 29 December 2019, the first four cases of COVID-19 were reported in Wuhan City, Hubei Province, China, where the outbreak was believed to have begun at a wildlife market. Immediately after, it quickly spread to other parts of the world. Due to the lack of drugs against COVID-19, the

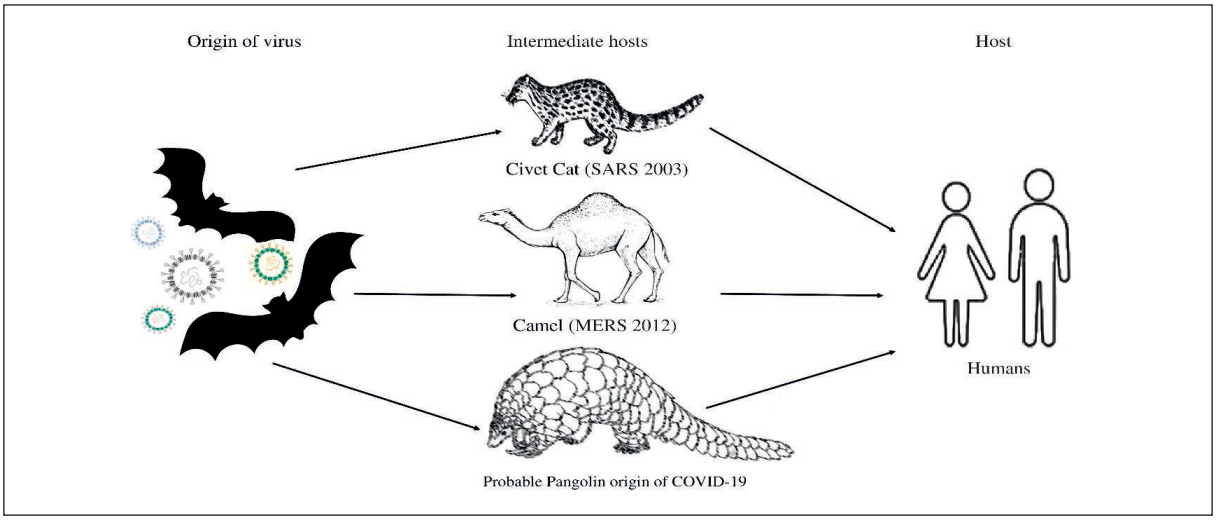

Figure 3 - Summary of potential interspecies transmission cycle of SARS-CoV, MERS-CoV, and SARS-CoV-2.

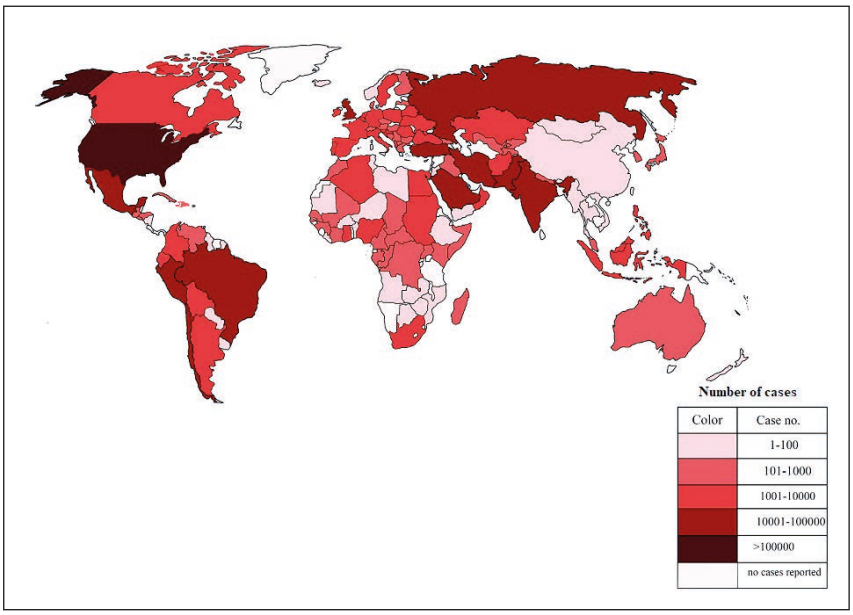

Figure 4 - The global distribution of COVID-19 patients (18 May 2020).

disease spreads rapidly and the fatality rate is relatively high [73]. In China, 82,052 were confirmed as cases and 3,339 were total deaths in 34 provinces as of 12:25, 13 April 2020. In the early days, the highest rate of spread and mortality was in mainland China where the outbreak began. But, the vast majority of cases and deaths of coronavirus are now being reported in the United States, which is currently a global hotspot, Spain, Italy, and France [74].

Studies based on modeling revealed that the transmissibility of SARS-CoV-2 was higher than the MERS in the Middle East countries, similar to SARS, but lower than MERS in the Republic of Korea [75]. The latest update in April, the global distribution of COVID-19 patients is summarized in Figure 4.

CONCLUSIONS

This study is a picture of the current research on epidemiology in response to the outbreak of COVID-19. In this review, we summarized the latest reports of transmission rout and risk of transmission, mortality and morbidity risk factor and clinical features caused by SARS-CoV-2 infection. However, further research on all aspects of the disease is needed to better understand the infection.

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Conflicts of Interest

None

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COVID 19 diagnostic multiplicity and its role in community surveillance and control

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SUMMARY

Diagnosis of persons exposed to/infected with severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) is central to controlling the global pandemic of COVID-19. Currently, several diagnostic modalities are available for COVID-19, each with its own pros and cons. Although there is a global consensus to increase the testing capacity, it is also essential to prudently utilize these tests to control the pandemic. In this paper, we have reviewed the current array of diagnostics for SARS-CoV-2, highlighted the gaps in current diagnostic modalities, and their role in community surveillance and control of the pandemic. The different modalities of COVID-19 diagnosis discussed are: clinical and radiological, molecular based (laboratory based and point-of-care), Immunoassay based (ELISA, rapid antigen and antibody detection tests) and digital diagnostics (artificial intelligence based algorithms). The role of rapid antigen/antibody detection tests in community surveillance has also been described here. These tests can be used to identify asymptomatic persons exposed to the virus and in community based seroprevalence surveys to assess the epidemiology of spread of the virus. However, there are few concerns about the accuracy of these tests which needs to evaluated beforehand.

Keywords: COVID-19, Coronavirus, Diagnosis, RT-PCR, Artificial intelligence, Surveillance.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the seventh coronavirus that has crossed the species barrier and has emerged as a global health emergency [1]. The first case of coronavirus disease (COVID-19) was reported in December 2019 at Wuhan, Hubei Province, China [2]. On 11th March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic [3]. There were only 11953 cases of COVID 19 with 259 reported deaths till 1st Feb 2020. This has exponentially increased to more than 3 million cases with 0.2 million deaths as of 30th April 2020 (Figure 1) [4].

SARS-CoV-2 is an enveloped positive single stranded RNA virus belonging to *Betacoronavirus* genus, of *Orthocoronavirinae* subfamily in the *Coronoviridae* family of order *Nidovirales* [5]. Like other betacoronaviruses, SARS-CoV-2 has Spike glycoprotein (S), Matrix proteins (M) and outer envelope (E) encapsulating the RNA and nucleoprotein (N) (Figure 2). Apart from these, the viral genome also encodes for proteins like RNA dependent RNA polymerase (RdRp) and 6 accessory ORF1ab, ORF3a, ORF6, ORF7a, ORF7b, and ORF8 proteins [6]. Genomic analysis has shown that SARS-CoV-2 has 79.6% sequence identity to SARS-CoV and 96% identity with bat coronavirus (BatCoV RaTG13)

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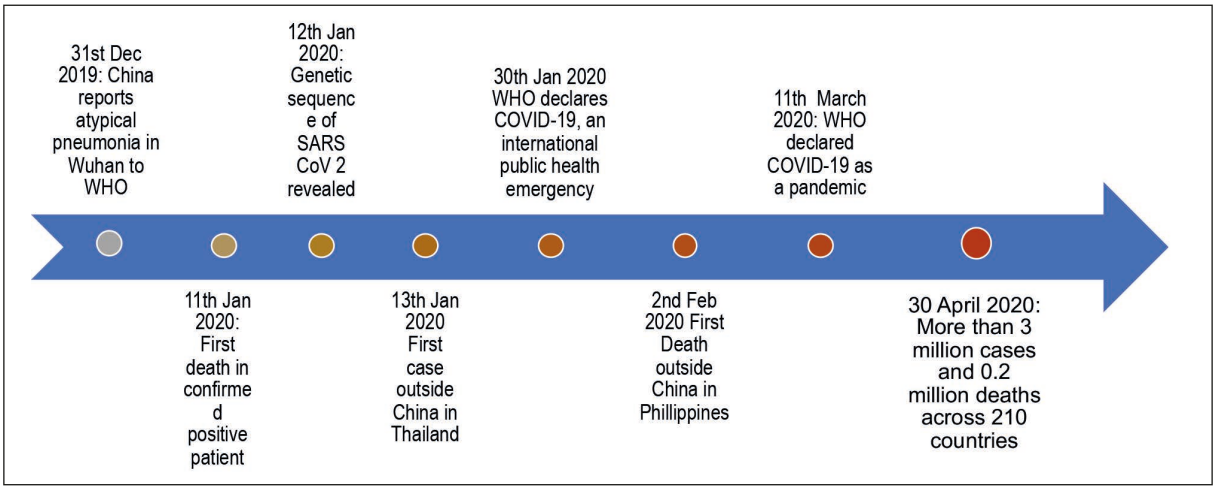

Figure 1 - Timeline of COVID-19 epidemiology (Source: World Health Organization).

[7, 8]. The virus entry is via the respiratory route where S protein mediates viral binding onto cells expressing ACE2 (angiotensin converting enzyme 2) receptor. Cellular serine protease TMPRSS2 present on the host cell is used by SARS-CoV-2 for S protein priming [8, 9]. After receptor mediated endocytosis the viral genome is released in the cytosol that translates replicase polyproteins. These polyproteins subsequently get cleaved and further assemble to form replicase transcriptase complex to help in RNA replication and sub-genomic RNA transcription SARS-CoV-2 has evolved into 2 strains designated as L and S strains [10]. L strain is more aggressive and was prevalent during early stages of the epidemic in Wuhan [11].

Screening is our window into the pandemic and its spread. Diagnosis of persons exposed to/infected with SARS–CoV-2 is central to controlling

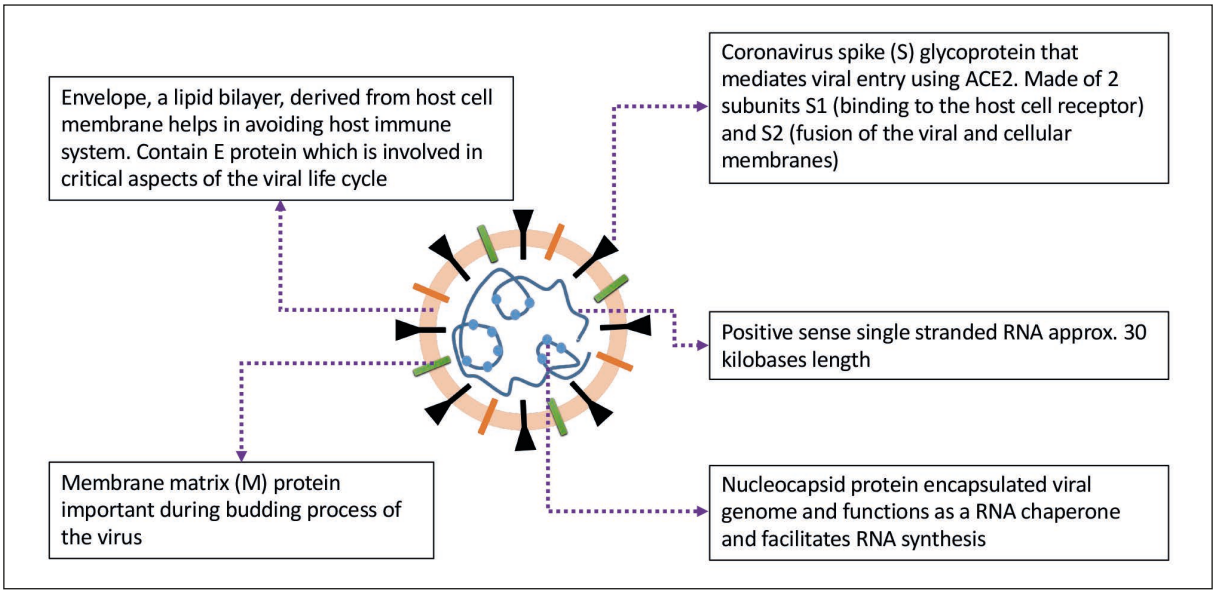

Figure 2 - Diagrammatic representation of the structure of SARS CoV 2. SARS CoV-2 has outer envelope encapsulating the RNA & nucleoprotein (N). Spike glycoprotein (S) & matrix protein (M) are transmembrane proteins embedded in the envelope [9, 50, 51].

the global pandemic of COVID-19. Few countries have upscaled diagnostic testing on a massive scale to successfully contain the spread of the pandemic. In contrast, poor resource countries like India have prioritized testing for specific groups of persons. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) based assays are considered the reference standard for COVID-19 diagnostics. But the test protocol is complex and expensive, however, and is mainly suited to large, centralized diagnostic laboratories. This has inhibited upscaling of testing capacity. To overcome this barrier, point-of-care technologies and serologic immunoassays are rapidly emerging. But the performance of these have not been evaluated adequately. These challenges are even greater in low-resource settings.

Currently, several diagnostic modalities (Clinical, molecular, immune-based and digital) are available for COVID-19, each with its own pros and cons (Figure 3). Although, there is a global consensus to increase the testing capacity, it is also essential to prudently utilize these tests to control the pandemic. In the current scenario of information overload in the field of COVID-19 diagnostics, we have reviewed the current array of diagnostics for SARS-CoV-2, highlighted gaps in current diagnostic modalities, and their role in community surveillance and control of the pandemic.

Different Modalities of COVID-19 Diagnostics

Clinical and radiological diagnosis of COVID-19

COVID 19 presents with 3 clinical stages of infection after incubation period of 2-14 days. Stage 1 - asymptomatic, Stage 2 - Upper airway and conducting airway response and Stage 3 - Hypoxia, ground glass infiltrates, and progression to Acute Respiratory Distress Syndrome (ARDS) [12]. The stages and severity varies depending on the age, immune status of the individuals and associated co-morbidities [13]. High viral load can be an important marker for severity of the disease and such patients also have long virus shredding period [14].

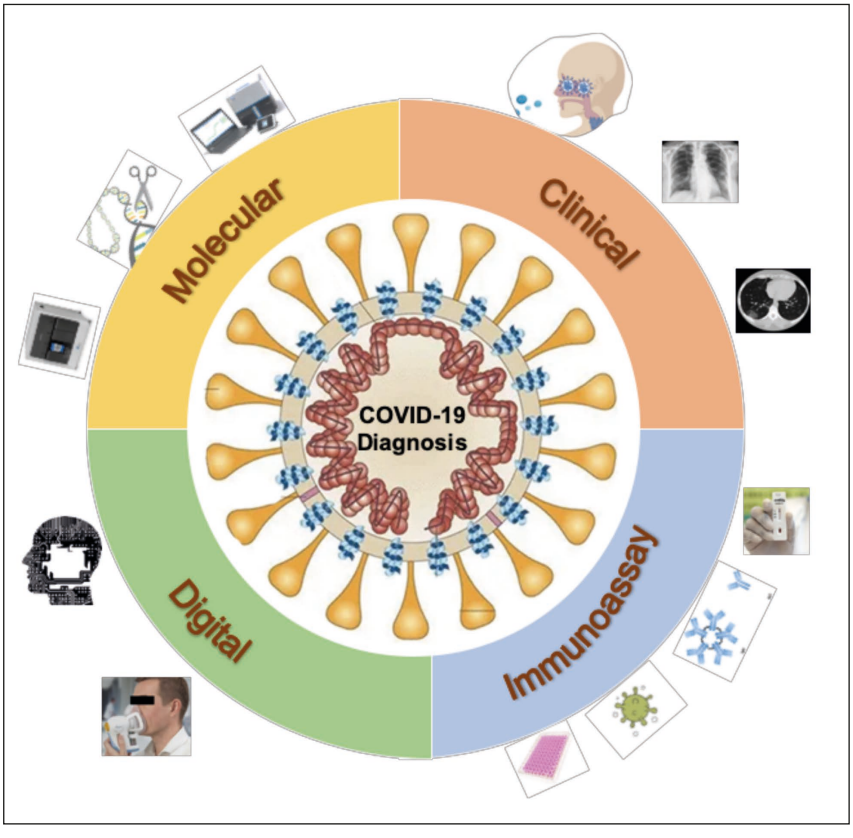

Figure 3 - Various COVID-19 diagnostic modalities.

The clinical diagnosis of SARS-CoV-2 is fever, dry cough and shortness of breath and may led to severe form such as respiratory distress and failure [15]. Respiratory failure that necessitates mechanical ventilation and support in an intensive care unit (ICU), can further cause multi-organ and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes. A case study by Li et al shows that the mean age of suffering from COVID- 19 was around 59 years ranging from 15 to 89 years [16]. Patients with comorbidities (cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancers) had higher case-fatality rates (10.5%, 7.3%, 6.5%, 6.0%, and 5.6%, respectively) than those without comorbidities (0.9%) [17]. Based on the presentation of symptoms and respiratory parameters, disease severity is divided into mild to moderate, severe and critical.

- Mild disease: non-pneumonia and mild pneumonia; this occurred in 81% of cases.
- Severe disease: dyspnea, respiratory frequency ≥30 min, blood oxygen saturation (SpO2) ≤93%, PaO2/FiO2 ratio or P/F [the ratio between the blood pressure of the oxygen (partial pressure of oxygen, PaO2) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO2)] <300, and/or lung infiltrates >50% within 24 to 48 hours; this occurred in 14% of cases.
- Critical disease: respiratory failure, septic shock, and/or multiple organ dysfunction or failure; this occurred in 5% of case [17].

CDC has added six new symptoms to its list for COVID-19: chills, muscle pain, headache, sore throat, repeated shaking with chills and a loss of taste or smell [18]. Kaye et al. reported anosmia in 73% of patients prior to COVID-19 diagnosis and was initial symptom in 26.6% of patients [19].

COVID-19 infection causes a severe lower respiratory tract infection with bilateral, basal and peripheral predominant ground-glass opacity, consolidation or both as the most common reported chest radiological findings. These findings peak around 9-13 days and slowly begin to resolve thereafter [20].

Laboratory based Molecular Diagnostics

Laboratory based molecular diagnostics are the hallmark of diagnosis of COVID-19. Currently, the diagnosis of COVID-19 is based on testing the nasopharyngeal or oropharyngeal samples collected from suspected patients. RT-PCR based tests are the standard reference for diagnosis of COVID-19. A study by Wang et al. showed higher positivity in nasopharyngeal swabs than oropharyngeal swabs, especially among hospitalized patients [21]. A nasopharyngeal swab is the preferred choice for swab-based SARS-CoV-2 testing but sometimes oropharyngeal, mid-turbinate and anterior nares samples are also tested. A study by Wu J et al. found that positivity of SARS-CoV-2 nucleic acid in the sputum of 132 patients with COVID-19 was higher than that of nasopharyngeal swabs, and viral nucleic acids were also detected in blood and digestive tract (faecal/anal swabs) [22]. Detection of SARS-CoV-2 nucleic acid in nasopharyngeal swab alone does not yield high positivity, multi-sample SARS-CoV-2 nucleic acid detection can improve the accuracy, reduce false-negative rate and better guide clinical treatment [22]. Samples should be collected using flocked swabs to increase the collection of viral load and release of cellular material. Certain specific swabs are not used for the collection of viral loaded samples such as those containing calcium alginate, wood or cotton because they contain material that inhibits PCR assays.

RT-PCR is capable of providing relatively fast results through amplification of low viral RNA with high sensitivity and specificity. The oligonucleotide primers and probes for SARS-CoV-2 detection are usually derived from RNA-dependent RNA polymerase (RdRp) gene in open reading frame (ORF), nucleocapsid (N), envelope (E) regions of the virus [23]. RT-PCR assay can be either a one-step or a two-step assay. In a one-step assay, conversion of RNA to cDNA and further PCR amplification are performed in single reaction tube. Although, this assay provides quick and reproducible results, optimizing the protocol is a challenging step. In contrast, the two-step assay is carried out sequentially in two separate tubes. In comparison to one step PCR assay, this format is more sensitive, but time-consuming [24, 25].

Limited evidence suggests that the viral load peaks during the first week of illness, then gradually declines over the second week [26]. Viral presence has also been noted in some patients 28 days after onset of symptoms. High viral load during the early phase of illness suggests that patients could be most infectious during this period,

and this might account for the high transmissibility of SARS-CoV-2.

Though, RT-PCR provides a highly sensitive and specific method for detection of infectious diseases, these methods are typically restricted in a specialized clinical laboratory and are not suitable for quick, easy, point of care diagnostic applications. Currently, reverse transcription loop-mediated isothermal amplification (RT-LAMP) is in development and testing phase for SARS-CoV-2 detection [27]. This highly specific technique uses DNA polymerase and specially designed primers that recognize distinct target sequences on the target genome. In general, there are two inner primers and two outer primers designed to synthesize new DNA strands [28]. The reaction occurs in less than an hour under isothermal conditions at 60-65°C. The approach is much more efficient while still obtaining a high level of precision, less background signal, convenient visualization for detection and does not need sophisticated equipment [28].

CRISPR-based detection can also provide a rapid, highly sensitive and specific approach for molecular based diagnostics. CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique for the detection of COVID-19 uses a variant of Cas9 called Cas13 that gets activated by binding to SARS-CoV-2-specific guide RNA [29]. Detection is through fluorescent signal produced by Cas13 mediated cleavage of fluorophore-quencher probes. Another CRISPR-based DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay uses Cas 12a to provide a faster alternative to real-time RT–PCR assay [30].

There are several other additional novel diagnostic methods in developmental phase or in evaluation. The Foundation for Innovative New Diagnostics (FIND) is conducting independent evaluations of molecular tests and immunoassays available for COVID-19 diagnostics, in collaboration with the WHO, the University Hospitals of Geneva (HUG) and others (Supplementary Table 1 and 2). Results for the first round of independent evaluation of COVID-19 PCR Based tests has been released and depicted in Table 1.

Point-of-Care Molecular Diagnostics

Rapid (results within 1 hour) point-of-care molecular assays for SARS-CoV-2 will be critical in expanding reliable point-of-care testing. These platforms are cartridge based assays, which include the Abbott ID NOW (Abbott Laboratories),

Table 1 - Evaluation of COVID-19 PCR based test.

Company	*Gene target*	*Copies / reaction*	*Avg Ct value*	*Clinical sensitivity*	*Clinical specificity*	*Supplier recommended Ct cut-off*
Altona Diagnostics	E	1–10	35.45	92%	100%	None
	S	1–10	35.99	92%	100%	
BGI Health (HK) Co. Ltd	ORF1	1–10	32.43	100%	99%	≤38
Boditech Med. Inc	E	10–50	34.9	100%	100%	≤42
	RdRP	50–100	33.46	90%	100%	
DAAN Gene Co. Ltd	ORF1	1–10	38.76	100%	96%	≤40
	N	1–10	36.97	100%	98%	
GeneFirst Limited	ORF1	1–10	35.45	100%	99%	≤37
	N	1–10	36.72	98%	100%	
KH Medical Co. Ltd	S	1–10	37.94	100%	100%	≤40
	RdRP	10–50	36.74	100%	100%	
SD Biosensor Inc.	E	1–10	37.43	100%	97%	≤41
	ORF1	1–10	36.99	100%	99%	
Tib Molbiol	E	1–10	33.34	100%	100%	>2-4 cycle higher than Ct value of 10 copies

BioFire FilmArray (bioMérieux), Cobas Liat (Roche Diagnostics), and GeneXpert (Cepheid) [31].
The Xpert Xpress SARS-CoV-2 test (Cepheid) (FDA Emergency Use Authorization) utilizes the GeneXpert platform, which is widely used for tuberculosis and HIV testing, especially in low- and middle-income countries. This capacity might be useful to scale up testing across the world, especially in resource poor settings.

Antigen detection tests
One type of RDT detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a respiratory sample. If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip and generate a visually detectable signal, typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, such tests are recommended to identify acute or early infection.
The performance of these tests depends on the time from onset of illness, the concentration of virus in the specimen, the quality of the specimen collected from a person and how it is processed. Other antigen-based RDTs for other respiratory viruses such as influenza have demonstrated the sensitivity of these tests to vary from 34% to 80% [32].
Based on this information, half or more of COVID-19 infected patients might be missed by such tests. With the limited data now available, WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for clinical decision making, although research into their performance and potential diagnostic utility is highly encouraged.
According to Seo G et al., field-effect transistor (FET)-based biosensing device for detecting SARS-CoV-2 can be used in clinical samples [33]. The sensor was fabricated by coating graphene sheets of the FET with a precise antibody against SARS-CoV-2 spike protein. The functioning of the sensor was determined using antigen protein, cultured virus, and nasopharyngeal swab specimens from COVID-19 patients. The FET device could sense the SARS-CoV-2 spike protein at concentrations of 1 fg/mL in phosphate-buffered saline and 100 fg/mL clinical transport medium [34]. Monoclonal antibodies against the nucleocapsid protein of SARS-CoV-2 have also been generated, which might form the basis of a future rapid antigen detection test [35].

Antibody detection tests
It is a known fact that identification of IgM/IgG antibodies is a much less complex process than molecular identification of virus [36]. The assays can be performed on the samples collected from blood or saliva. The "serological" tests which rely on detection of antibodies are usually against the nucleocapsid or spike proteins in the sample. A negative result in the serological assays will not assure the absence of infection. Sometimes, cross-reactivity of the non-SARS-CoV-2 coronavirus protein is also a potential problem [37]. These IgM/IgG detection assays are more reliable in conditions where patients present to the hospital in the late stage of infection, when RT-PCR may be falsely negative due to decrease in the viral shedding [38].
After SARS infection, IgM antibody could be detected in patient's sample after 3-6 days and IgG after 8 days [39]. However, the antibody response to SARS-CoV-2 has shown different profile as per limited serological studies. IgM and IgG appear 2-4 after the onset of symptoms with the median number of days for seroconversion being 10-13 days. Detection of IgM against SARS-CoV-2 tends to indicate recent exposure, whereas the detection of IgG indicates prolonged exposure to the virus. The detection of both IgM and IgG could provide useful information on the virus infection time course. These antibody kits could be IgM, IgG or combined IgM/IgG detection kits.
Apart from these rapid kits, many ELISA based antigen or antibody kits have been approved for diagnostic or research purpose, with several others in the process of development (Supplementary Table 1 and 2). Unlike rapid test kits, ELISA provide quantification of antibodies and are less vulnerable to false-positive and false-negative reactions.

Digital diagnostics
In this era of machine learning, digital diagnostics has come up as a new innovation in medical field as a complimentary tool for standard screening and diagnostic tests. Current COVID-19 outbreak provided another opportunity for Artificial Intelligence (AI) application to prove it's worth in health care settings. Two such examples are

Infervision and Intrasense Myrian, which are algorithm based AI technologies developed to read clinical images [40, 41]. These algorithms distinguish between lung lesions of COVID-19 and other respiratory infections. They basically measure volume, shape and density and compare changes of multiple lung lesions from an image to provide quantitative report in order to assist healthcare workers make quick decisions. Another, AI-based deep learning structure COVIDiagnosis-Net, showed a high accuracy of 98.3% in processing and analysing X-ray image for the early stage detections of the COVID-19 cases [42]. Another digital diagnostic tool which is in development is AiroStotleCV19, a breath test for volatile organic compounds (VOCs). Being a viral infection, COVID-10 induces oxidative stress. Developers are working on the identification of oxidative stress biomarkers during breath test for early diagnosis COVID-19.

Digital technologies are highly sensitive, specific, non-invasive and cost-effective. They can help in reducing the timeframe and workload needed in dealing with high number of cases, hence minimizing the risk of transmission to other patients and hospital staff [43].

Community surveillance and control

Being resource intensive and costly, current molecular based tests are used for confirmation of COVID-19 among possible suspects, most often the symptomatic patients. However, apart from transmission from symptomatic patients, pre-symptomatic and asymptomatic transmission plays a key role in driving disease transmission across communities, especially due to the hidden nature of the spread.

Pre-symptomatic transmission: The incubation period for COVID-19 is around 5-6 days, lasting up to 14 days. During this period, also known as the pre-symptomatic period, people can be contagious and transmission can occur. Pre-symptomatic transmission has been documented through contact tracing efforts and enhanced investigation of clusters of confirmed cases [44-46]. Data suggests that some people can test positive for COVID-19 from 1-3 days before they develop symptoms which makes it more likely that people infected with COVID-19 could transmit the virus before significant symptoms develop [45].

Asymptomatic transmission: An asymptomatic laboratory-confirmed case is a person infected with COVID-19 who does not develop symptoms. Asymptomatic transmission refers to transmission of the virus from a person, who does not develop symptoms. A recent study in *NEJM* reported that a viral load detected in an asymptomatic patient was similar to that detected in symptomatic patients, indicating the potential for transmission in asymptomatic patients [47]. On January 24, *The Lancet* reported a familial cluster of SARS-CoV-2 infection with a travel history to Wuhan, with their asymptomatic child presenting with no fever, respiratory tract symptoms or diarrhoea but had ground-glass lung opacities seen on radiography [48]. Subsequently, several asymptomatic patients were confirmed to have COVID-19 in many Chinese cities with most of them having an epidemiological history with a potential of infecting others. A study showed that during the outbreak of SARS-CoV, of all exposed health care workers, 7.5% were asymptomatic SARS-positive cases [49].

Early detection and isolation of these hidden cases is necessary to reduce the size of the outbreak of SARS-CoV-2. Current strategies have focused on identifying COVID-19 suspect/symptomatic, testing and isolating them. However, we are missing out on asymptomatic transmission that is a major driver of community transmission of the corona virus accounting as high as 80% of transmission. Widespread testing of populations can play a key role in identifying asymptomatic people and isolating them, thus, curbing further transmission. Countries such as South Korea have successfully controlled the pandemic by testing aggressively to identify possible carriers of infection and isolating them effectively (Figure 4). However, in resource-poor settings, where up-scaling of conventional RT-PCR is cumbersome, use of rapid test kits can be a feasible option for population-wide testing.

Rapid diagnostic tests (RDTs) are simple standalone antigen/antibody detection tests that can be used at the point of care outside the laboratory/hospital by minimally trained staff and can provide test results within 15 minutes. They are attractive for decentralized testing particularly in low resource settings. These rapid tests can be used to broaden the criteria for testing and include asymptomatics with probable exposure to the virus. In India, RDTs have been approved

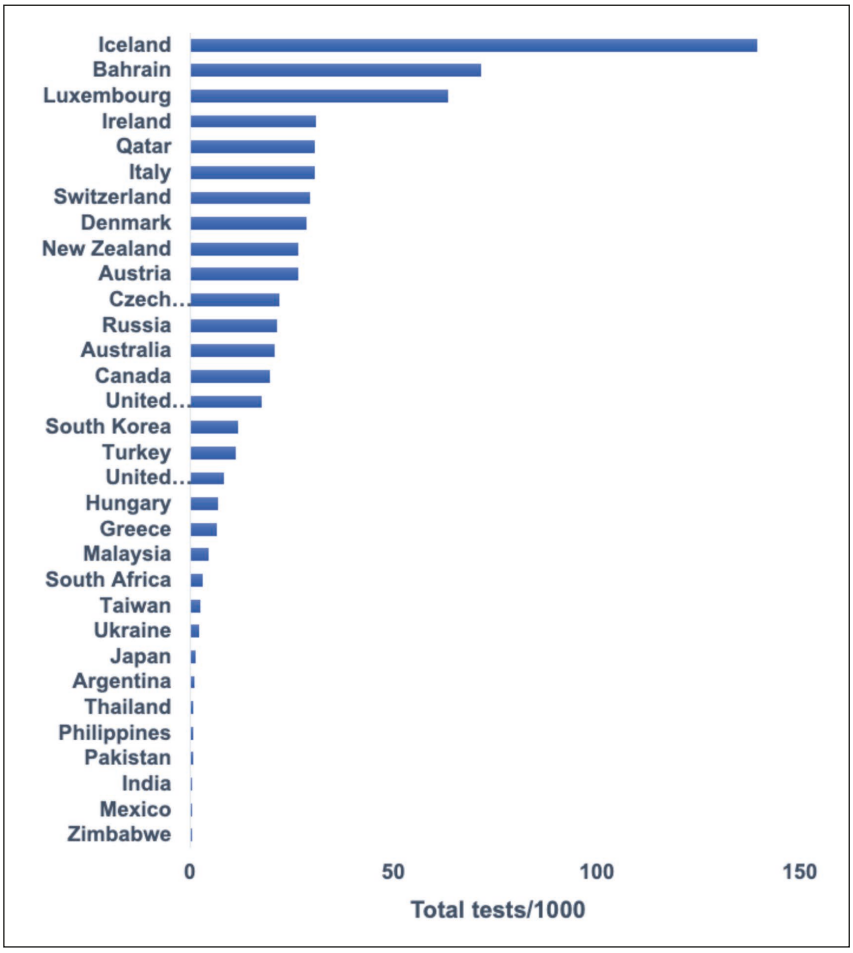

Figure 4 - Comparison of number of tests done for COVID-19 per 1000 population across several countries.

for use in hotspots/cluster containment zones to identify asymptomatic persons exposed to the virus and isolating them to prevent further community transmission. However, because of its low specificity, RDT negatives are further confirmed by RT-PCR.

The use of rapid antibody tests is manifold. RDTs could be used in seroprevalence surveys to understand the dynamics of spread of the virus in the community, assess attack rates and extent of an outbreak. It can verify the immune response to vaccines during clinical trials, or be used in contact tracing weeks or longer after a suspected infection, help inform public policy makers about the burden of asymptomatic cases in a population. This is useful for the purpose of community surveillance and understanding the epidemiology of COVID-19 in the country.

A positive test result in the convalescent phase indicate that they will be safe from another infection for at least some time which mean they could return to work or work as a shield for the vulnerable population till we achieve herd immunity. However, there is no evidence that people who have recovered from COVID-19 and have antibodies are protected from a second infection. There have already been some reported cases of re-infection with corona virus.

CONCLUSIONS

Rapid and early detection of the SARS-CoV-2 virus is key to prevent the spread of the virus and control the pandemic. The first line of defence against any outbreak is always developing the diagnostic assays for identification of confirmed

cases and isolating them. Immunoassays against the antigen or antibodies provide the second line of diagnostics and complement nucleic acid tests.

Worldwide lockdown with strict social distancing and use of masks was adopted by most countries to curtail the spread of COVID-19. However, not doubting the efficiency of lockdown, there are high chances of secondary waves of epidemic following the end of this lockdown. Thus, prompt and reliable diagnostic facilities along with appropriate non-pharmacological interventions and vaccines is the need of the hour. The future development of portable assays such as isothermal amplification, barcoding and microfluidic technologies and application of artificial intelligence algorithms could enable point-of-care testing and multiplex assays to be rapidly implemented in an outbreak situation. This approach can reduce mortality and help in curtailing the spread of zoonotic pathogens.

Authors contributions

SCT and VD were involved in conception of the idea, literature review, drafting the manuscript, editing and finalization of the manuscript. JPT was involved in conception of the idea, literature review, critical review, editing and finalization of the manuscript. AP was involved in literature review, critical review of the manuscript and finalization of the manuscript. All the authors gave their approval to the final submitted version of the manuscript.

Conflict of interest

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Bacterial and fungal infections among patients with SARS-CoV-2 pneumonia

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SUMMARY

We reviewed studies reporting bacterial and fungal co-infections in patients with COVID-19. The majority were retrospective studies with poor quality data biased with short follow-up and selection of patients. Septic shock was reported in 4% to 33.1% of patients. Seventy-one to 100% of patients received antibacterial treatments. Invasive pulmonary aspergillosis seems to be an increasingly observed complication in critically ill patients with SARS-CoV-2 infection as previously reported in patients hospitalized in ICU with severe influenza. High quality prospective studies are urgently needed to verify the incidence of bacterial and fungal infections and their role on the outcome of COVID-19.

Keywords: COVID-19, SARS-CoV-2, bacteremia, fungal infections, aspergillosis, candidemia.

SARS-CoV-2 pandemic which started in Wuhan, China at the end of December, 2019 has been responsible for over 5 million cases and 341,722 deaths worldwide as of May 24, 2020 with USA and western Europe recording the majority of cases. In this short period of time a huge amount of information encompassing the characteristics of this new virus, the mechanism of infection, its pathophysiology, clinical manifestations, diagnosis, possible therapeutic approach and autopsy findings have been generated by the medical and biological community [1-11]. However, despite the fact that a significant number of hospitalised patients with SARS-CoV-2 pneumonia require Intensive Care Unit (ICU) admission, data regarding bacterial and fungal infections, especially in critically ill patients are very limited and generally overlooked even in large case series [2-5]. From this point of view it seems unexpected that the role, if any, of superimposed infections, on hospital stay, clinical outcome and deaths can be considered marginal with respect to other well described risk factors such as hypertension, obesity, diabetes and the development of ARDS, myocardial damage and thromboembolic events [12]. It is well known that viral respiratory infection such as influenza can be complicated by bacterial and fungal co-infections and the SARS outbreak was characterised by an high rate of nosocomial transmission of drug-resistant microorganisms [13]. However, reported antibiotic use among patients hospitalised with COVID-19 infection is high ranging from 71% to 100% (Table 1) [14-26]. Although the principles of antimicrobial stewardship have been highlighted it should be pointed out that in the emergency situation caused by the COVID-19 pandemic with overwhelmed wards and ICUs as well as the therapeutic uncertainty it became hardly difficult to apply all the interventions routinely done in the pre-pandemic time [27, 28].

Secondary or hospital-acquired infections has been recorded in 5.1% to 38.9% among Chinese patients and in 4.8% to 27.4% of patients in Western countries but all the data are biased by the limited follow-up, especially for those patients hospitalised in ICU [3-5, 14, 17, 19, 23-25]. Only three Chinese studies reported bacteremia with a prevalence

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Table 1 - Study regarding SARS-CoV-2 with diagnosis of bacterial and fungal infections.

Author/Country/ Reference	*Typology of study*	*N° patients*	*Antibacterial treatment*	*Sepsis/Septic shock*	*Bacteremia (BSI)/ Microorganisms*	*Bacterial or fungal Pneumonia/VAP*	*Hospital acquired infection*
Zhou/China/4	Retrospective multicentre cohort	191	185 (95%)	112 (59%)/38 (20%)	NR/NR	NR/10/32 (31%)	28 (14.6%)
Yang/China/14	Retrospective single centre	52 (critically ill)	49 (94%)	NR/NR	1 (2%)/KPC	7 (13%)2 KPC; 1 *Aspergillus flavus*; 1 *A. fumigatus*; 1 *Pseudomonas aeruginosa*; 1 *Serratia marcescens*	9 (17.3%)
Chen/China/3	Retrospective single centre	99	70 (71%)	NR/4(4%)	NR/NR	NR/1 (1%) *Acinetobacter baumannii* MDR	5 (5.1%)
Huang/China/5	Prospective single center	41 (13 ICU)	41 (100%)	NR/NR	NR/NR	NR/NR	4 (10%)/ ICU 4 (31%)
Wu/China/15	Retrospective cohort study single center	201	196 (97.5%)	NR/NR	NR/NR	0/148 (0%)	NR
Guan/China/2	Extracted data from 552 hospitals	173 (severe); 33 ICU	139 (80.3%)	NR/11 (6.4%)	NR/NR	NR/NR	NR
Goyal/USA (New York)/16	Retrospective case series	393	NR	NR/NR	19 (5.6%); 15 (11.9%) ICU/NR	NR/NR	NR
Arentz/USA (Washington)/17	Case series	21 (ICU)	NR	NR/NR	1 (4.8%)/ *P. aeruginosa*	NR/NR	1 (4.8%)
Li/China/18	Cohort study	548	NR	NR/NR	42 (7.7%)/NR	NR/NR	NR
Barrasa/ Spain/19	Consecutive case series	48 (ICU)	42 (88%)	NR/NR	-	NR/NR	6 (12.5%)
Wang/China/20	Consecutive case series	344 (ICU)	266 (77.3%)	NR/114 (33.1%)	NR/NR	NR/NR	NR
Bhatraju/ USA/21	Consecutive case series	24 (ICU)		NR/NR	0	0	0
Alattar/Qatar/22	Consecutive case series*	25 (ICU)	NR	NR/NR	0	4 (16%): 2 *Klebsiella pneumoniae*, 1 *P. aeruginosa*, 1 *Staphylococcus aureus*	4 (16%)
Morena/Italy/23	Consecutive case series*	51 (9 ICU)	39 (76%)	NR/NR	14 (27.4%)/8 *Enterococcus* spp.; 5 KPC; 5 ESBL+KP; 3 *Candida* spp.; 4 ConS;3MRSA; 1 *P. aeruginosa*, *Escherichia coli*; *Enterobacter aerogenes*§	NR/NR	14 (27.4%)
Yu/China/24	Multicenter prospective observational	226 ICU	168 (74.3%)	NR/33 (14.6%)	2 (0.9%)/NR	NR/48 (21.2%)	49 (21.7%)
Cao/China/25	Retrospective case series	18	18 (100%)	NR/NR	NR/NR	NR/NR	7 (38.9%)
Du/China/26	Retrospective observational study (two hospitals)	85	77 (90.6%)	28 (32.9%)/16 (19.7%)	NR/NR	3 positive fungal cultures	NR

ICU, intensive care unit; NR, not reported; KPC, *Klebsiella pneumoniae* carbapenemase producing; KP ESBL+, Extended spectrum beta-lactamase (ESBL) *Klebsiella pneumoniae*; ConS, coagulase-negative staphiloccoci; MRSA, methicillin-resistant *Staphylococcus aureus*; *Treated with Tocilizumab; §31 episodes in 14 patients.

ranging from 0.9% to 7.7% but the pathogens responsible was described only in one study [14, 18, 24]. Outside China, bacteremia was registered in 4.8% to 27.4% with *Enterococcus* spp. responsible of more than half of the cases [16, 17, 23].
A picture of sepsis has been reported in 59% of patients in the study by Zhou et al. and they hypothesised that such clinical condition could be attributed to SARS-CoV-2 [4, 29]. Septic shock ranged from 4% to 33.1% in China but all the studies lacked of any information regarding the responsible microorganisms [2-4, 20, 24, 26].
As far as invasive fungal diseases among hospitalised patients with COVID-19, Gangneux and coworkers raised the concern that several risk factors (i.e., ICU admission, corticosteroid therapy, intubation/mechanical ventilation, underlying respiratory disease, cytokine storm) associated with the aggressive features of SARS-CoV-2 to the lung tissue can be responsible of an increase of invasive fungal infections (IFIs) and mortality in this setting [30]. Chen et al. reported positive fungal culture from respiratory samples in five out of 99 patients (5%): *Aspergillus flavus* in one patient, *Candida glabrata* in one patient and *C. albicans* in other three patients [3]; however, the role of *Candida* as a respiratory pathogen is doubtful even among critically ill patients and should be regarded as a colonizer. Yang et al. found *A. flavus* and *A. fumigatus* among two out of seven patients with hospital acquired pneumonia (13.5%) identified among 52 critically ill patients admitted to ICU in Wuhan [14]. In another retrospective study conducted in two hospitals of Wuhan regarding 85 fatal cases of COVID-19, fungal culture from sputum obtained from 9 patients were reported positive in 33.3% of cases with eight (9.4%), three (3.5%) and 2 (2.4%) patients receiving voriconazole, fluconazole and caspofungin [26]. However, in all the studies from China fungal infections were poorly defined and for such reason it appears difficult to make any inference. Interestingly, Antinori and coworkers reported an high rate of candidemia (6.9%) among 43 patients treated with tocilizumab, a recombinant humanized anti-human IL-6 receptor monoclonal antibody that has been suggested to be active against the cytokine storm described in patients with severe COVID-19 [31]. The authors speculated on the possible role of the suppression of IL-6 response on the high incidence of candidemia since previous studies conducted in interleukin-6 deficient mice showed that they were more susceptible to systemic *Candida albicans* infection, had a decreased survival and an increased fungal load in their organs when compared with IL-6 positive controls [31-33].
Invasive pulmonary aspergillosis (IPA) has been considered an illness of severely immunocompromised patients, especially those with severe neutropenia, hematologic malignancies and those undergoing solid organ transplants [34, 35]. Among critically ill patients other risk factors such as diabetes, chronic obstructive pulmonary disease, burn injury and influenza infection have been also described [36]. In the last years the observation that severe influenza pneumonia resulting in acute respiratory distress syndrome (ARDS) can be complicated by *Aspergillus* co-infection has been increasingly reported [37, 38]. Influenza virus causes alveolar epithelial and endothelial damage together with impaired mucociliary activity. SARS-CoV-2 is responsible of a severe pneumonia (COVID-19) complicated by ARDS in 14.8% of hospitalised patients [2]. Pathologic findings of COVID-19 pneumonia include pulmonary oedema, hyaline membrane formation, multinucleated syncytial cells with atypical enlarged type II pneumocytes [39,40].The diagnosis of Invasive Pulmonary Aspergillosis (IPA) in ICU patients is considered difficult for several reasons and even if available algorithms are applied they show variable and generally low performance with sensitivities ranging from 23 to 85% and specificities from 70 to 80% [41-45]. Moreover, CT-scan demonstrating findings suggestive for invasive fungal disease are rarely observed in mechanically ventilated patients [44, 45]. Up to now, thirty-three cases of probable (or putative)/possible SARS-CoV-2 associated IPA have been published (Table 2) [46-54]; all the patients had been hospitalised in ICU and the diagnosis of IPA was made a median of 5 days post-ICU admission with an overall mortality of 67%. The median age was 70 years with a predominance of male gender (81.8%) and 21.2% of patients were affected by chronic obstructive pulmonary disease and 27.7% by diabetes mellitus. *Aspergillus fumigatus* was cultured in the majority of cases either from tracheal aspirate of bronchoalveolar lavage; galactomannan antigen performed on serum was positive only in 23.1% of cases whereas it performed better on respiratory samples (71.4% positive).

Table 2 - Case reports and case series of invasive pulmonary aspergillosis in patients with COVID-19.

Author/Country/ Reference	*Age/Sex*	*Underlying disease*	*Chest X-Ray*	*Days post-ICU admission to diagnosis for IPA*	*Microbiology/ GMAg/β-D-glucan*	*Antifungal treatment*	*Classification**	*Outcome*
Prattes/ Austria/46	70/M	COPD Gold 2; diabetes type 2; hypertension; obesity	Bilateral ground-glass opacities with a crazy paving appearance; reversal halo sign	2	*Aspergillus fumigatus* (endotracheal aspirate culture); PCR positive for *A. fumigatus*; microscopy; *Aspergillus* LFD positive/Negative (serum)/Negative (serum)	Voriconazole	Putative	Death (illness day 19)
Blaize/France/47	74/M	Myelodisplastic syndrome; hypertension; Hashimoto's thyroiditis	NR	4	*A. fumigatus* (tracheal aspirate); PCR positive for *A. fumigatus* (430 cp/ mL); microscopy positive/Negative (tracheal aspirate); Negative (serum)	No	Putative	Death (illness day 9)
Lescure/ France/48	80/M	Previous thyroid cancer	Bilateral ground-glass opacities, pleural effusion, alveolar condensations	NR	*A. flavus* (tracheal aspirate culture)/NR	Voriconazole switched to isavuconazole	Putative	Death (illness day 24)
Van Arkel/The Netherlands/49	83/M	Cardiomiopathy	NR	3	*A. fumigatus* (tracheal aspirate)/ Negative (serum)	§	Possible	Death (illness day 12)
	67/M	COPD Gold 3	NR	3	*A. fumigatus* (tracheal aspirate)/ ND/ND	§	Possible	Death (illness day 11)
	75/M	COPD Gold 2a	NR	5	*A. fumigatus* (bronchoalveolar lavage)/Positive (BAL) index 4.0/ND	§	Putative/ Probable	Death (illness day 12)
	43/M	None	NR	14	NR/Positive (BAL) index 3.8; negative (serum)/ND	§	Putative/ Probable	Alive
	57/M	Asthma	NR	5	*A. fumigatus* (BAL)/ Positive (BAL) index 1.6; negative (serum)/ND	§	Putative/ Probable	Death (illness day 20)
	58/M	None	NR	28	*A. fumigatus* (multiple sputum cultures)/ND/ND	§	Possible	Alive
Koehler/ Germany/50	62/F	COPD Gold 2; hypertension; obesity	Bilateral ground-glass opacities with crazy paving; peripheral nodular consolidation	NR	*A. fumigatus* (BAL); PCR positive for *A. fumigatus*/Negative (serum); Positive (BAL) (index >2.5)/ ND	Voriconazole	Putative/ Probable	Death

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Author/Country/ Reference	*Age/Sex*	*Underlying disease*	*Chest X-Ray*	*Days post-ICU admission to diagnosis for IPA*	*Microbiology/ GMAg/β-D-glucan*	*Antifungal treatment*	*Classification**	*Outcome*
Koehler/ Germany/50	70/M	None	Ground-glass opacities with small nodular infiltrate	NR	*A. fumigatus* PCR positive (BAL)/ Positive (serum) (index 0.7) positive (BAL) (index >2.5)/ND	Isavuconazole	Putative	Death
	54/M	Diabetes mellitus; hypertension	Bilateral ground-glass opacities, diffuse nodular opacities, cystic cavities (partly air crescent sign)	NR	*A. fumigatus* (tracheal aspirate) PCR positive (BAL)/ Negative (serum); positive (BAL) (index >2.5)/ND	Caspofungin followed by voriconazole	Putative	Death
	73/M	COPD Gold 3; hypertension	Ground-glass opacities and consolidation with nodular infiltrates	NR	*A. fumigatus* (tracheal aspirate)/Negative (serum)/ND	Voriconazole	Putative	Death
	54/F	None	Bilateral ground-glass opacities, crazy paving, small nodular infiltrates	NR	Negative (tracheal aspirate)/Positive (serum) (index 2.7 and 1.3)/ND	Caspofungin followed by voriconazole	Putative	Alive
Antinori/Italy/51	73/M	Diabetes mellitus; hypertension; obesity; atrial fibrillation	Interstitial pneumonia	4	*A. fumigatus* (BAS)/ Positive (serum) (index 8.6)/ND	Liposomal amphotericin B	Putative (autopsy confirmed)	Death (illness day 13)
Alanio/France/52	53/M	Hypertension; obesity; ischaemic heart disease	Typical COVID-19	NR	BAL: negative/ Positive (BAL) index 0.89; negative serum/ Positive (523 pg/mL)	None	Putative	Alive
	59/F	Hypertension; obesity; diabetes	Typical COVID-19	NR	*A. fumigatus* (BAL)/ Negative (BAL & serum)	None	Putative	Alive
	69/F	Hypertension; obesity	Typical COVID-19	NR	*A. fumigatus* (BAL)/ BAL: ND; negative (serum)/Negative (7.8 pg/mL)	None	Putative	Alive
	63/F	Hypertension; diabetes mellitus; ischaemic heart disease	Typical COVID-19	NR	BAL: negative/ Negative (BAL); Positive (serum)/ Positive (105 pg/mL)	None	Putative	Death (illness day 1)
	43/M	Asthma	Typical COVID-19	NR	*A. fumigatus* (BAL)/ Negative (BAL & serum)/Negative (7 pg/mL)	None	Putative	Alive
	79/M	Hypertension	Typical COVID-19	NR	*A. fumigatus* (BAL)/ Negative (BAL & serum)/Negative (23 pg/mL)	None	Putative	Alive
	77/M	Hypertension; asthma	Typical COVID-19 emphysema	NR	*A. fumigatus* (BAL)/ Positive (BAL) index 3.9; negative (serum)/ Positive (135 pg/mL)	Voriconazole	Putative	Death (illness day 18)

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Author/Country/ Reference	*Age/Sex*	*Underlying disease*	*Chest X-Ray*	*Days post-ICU admission to diagnosis for IPA*	*Microbiology/ GMAg/β-D-glucan*	*Antifungal treatment*	*Classification**	*Outcome*
	75/F	Hypertension; diabetes mellitus	Typical COVID-19	NR	*A. fumigatus* (BAL)/ Negative (BAL & serum) Positive (450 pg/mL)	Caspofungin	Putative	Death (illness day 11)
	47/M	None	Typical COVID-19 + one peripheral nodule	NR	*A. fumigatus* (BAL)/ ND(BAL) negative (serum)/Negative (14 pg/mL)	None	Probable	Death (illness day 3)
Rutsaert/ Belgium/53	86/M	None	NA	9	*A. flavus* (tracheal aspirate)/ND (BAL); negative (serum)	None	Probable	Death (illness day 17)
	38/M	Obesity	NA	6	*A. fumigatus* (BAL); histology positive/ Positive (BAL) index 2.4; negative (serum)/ND	Voriconazole, isavuconazole	Proven	Alive (ICU day 28)
	62/M	Diabetes mellitus	NA	16	*A. fumigatus* (BAL); histology positive/ Positive (BAL) index 2; negative (serum)/ ND	Voriconazole	Proven	Death (illness day 27)
	73/M	Hypertension; obesity; diabetes mellitus	NA	5	*A. fumigatus* (BAL); histology positive/ Positive (BAL) index 2.65; negative (serum)/ND	Voriconazole	Proven	Alive (ICU day 24)
	77/M	Hypertension; diabetes mellitus; CKD	NA NA	2	*A. fumigatus* (BAL); histology positive/ Positive (BAL) index 2.79; negative (serum)/ND	Voriconazole	Proven	Alive (ICU day 21)
	55/M	Hypertension; HIV	NA	13	Negative/histology negative; Negative (BAL); positive (serum) index 0.8/ ND	Voriconazole, isavuconazole	Possible	Death (illness day 27)
	75/M	Acute myeloid leukaemia; IPA (2012)	NA	8	*A. fumigatus* (BAL)/ Positive index 2.63; ND (serum)/ND	Voriconazole	Possible	Death (illness day 11)
Lahmer/ Germany/54	80/M	Pulmonary fibrosis	Typical COVID-19	5	*A. fumigatus* (BAL)/ Positive (BAL) index 6.3; positive (serum) index 1,5/ND	Liposomal amphotericin B	Putative	Death
	70/M	None	Typical COVID-19	6	*A. fumigatus* (BAL)/ Positive (BAL) index 6.1; negative (serum)/ND	Liposomal amphotericin B	Putative	Death

ICU, intensive care unit; NR, not reported; ND, not done; NA, not available; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; Gold, global initiative for chronic obstructive lung disease; BAS, bronchoalveolar aspirate; CKD, chronic kidney disease; IPA, invasive pulmonary aspergillosis; *According to Blot et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med 2012;186:56-64. GMAg, galactomannan antigen; LFD, lateral flow device; §Five patients of this case series received voriconazole plus anidulafungin and one patient was treated with liposomal amphotericin B.

In conclusion, data regarding bloodstream and respiratory bacterial and fungal infections among patients with COVID-19 are generally of poor quality with missed information about involved microorganisms and their profile of sensitivity to antimicrobial agents. Prospective high quality studies evaluating the role as well as the incidence of co-infections among patients with COVID-19 are urgently required.

Conflict of Interests
None

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COVID-19 and gastrointestinal injury: a brief systematic review and data from Bulgaria

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SUMMARY

In December 2019, a new Coronavirus (SARS-CoV-2) emerged in China, causing the pandemic disease COVID-19. The clinical presentation is variable, but the predominant symptoms are those of the upper respiratory tract.
Aim: The aim of the current study is to describe the incidence and type of the gastrointestinal injury (GI) in COVID-19, as well as their prognostic value.
Materials and Methods: We conducted a coincidental search on this topic in PubMed, Web of Science and EMBASE. We also followed a group of 31 Bulgarian COVID-19 patients throughout the course of their disease and analyzed their symptoms (catarrhal and other) and outcome.
Results: The publications concerning our survey followed a total of 1509 COVID-19 patients. In the Bulgarian cohort, only 14 from the 31 patients were laboratory-confirmed COVID-19 cases. Approximately 1/3 of the infected individuals presented with GI. In some patients this was the first, or only, symptom of the disease. It was also indicative of a more severe disease course. Conclusion: GI may be an important symptom and prognostic factor in COVID-19. Therefore, patients with acute gastrointestinal symptoms must be actively tested for SARS-CoV-2.

Keywords: COVID-19, gastrointestinal injury, diarrhea, vomiting, Bulgaria.

INTRODUCTION

At the moment, the world is struggling with a pandemic caused by a novel Coronavirus, called Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). The disease caused by this virus - COVID-19, first appeared in December 2019 in Wuhan, Hubei, China [1]. Since then, the infection has spread rapidly to over 200 countries around the globe. So far there are approximately 4.5 million infected and over 300. 000 deceased worldwide. Bulgaria is one of the mildly affected European countries, with nearly 2.500 confirmed cases and 116 deaths. However, the number of new cases grows steadily by 3% every day [2, 3]. The International Health organizations and the Governments of all affected countries took urgent quarantine and hygiene measures [4, 5]. Nevertheless, the diverse, nonspecific clinical presentation and the high number of non-symptomatic virus-carriers lead to controversial results. [6-8]. Similar to the other known Corona Viruses, the respiratory manifestations of SARSCoV-2 are most common: fever, cough, rhinitis, myalgia and dyspnea [6, 9]. For this reason, physicians and epidemiologists aim to diagnose and isolate patients with these typical symptoms. With the growing number of cases worldwide, more data regarding gastrointestinal involvement is being col-

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lected, with more frequent reports of symptoms like nausea, vomiting and diarrhea. Most authors explain this phenomenon with the fact that both the angiotensin converting enzyme 2 (ACE-2) and transmembrane serine protease 2 (TMPRSS2) are found both on the alveolocyte and the enterocyte cell membrane. Their co-expression on the cell membrane surface is crucial for the virus invasion [10, 11].

MATERIALS AND METHODS

We conducted a coincidental search on the topic in PubMed, Web of Science and EMBASE (search date: April 15th, 2020). We used the following keywords: coronavirus, COVID-19 and SARS-CoV-2 in combination with gastrointestinal symptoms, diarrhea, nausea and vomiting.

We also analyzed prospectively 31 Bulgarian patients (average age =28.2 years; age interval =0-72). All of them have been evaluated with at least one COVID-19 diagnostic test – either a RT-PCR of a nasopharyngeal swab (NPS), or a commercial rapid test. The rapid tests that we used were: Biopanda COVID-19 Rapid Test IgM/IgG, UK (sensitivity 88%, specificity 98.3%) and BioMedomics COVID-19 IgM/IgG Rapid test, UK (sensitivity 89%, specificity 91%). The patients' clinical and laboratory data was documented and analyzed.

The research with the participating Bulgarian patients was conducted in accordance with the Declaration of Helsinki, 2000 and was approved by the hospital Ethical Committee.

RESULTS

Our research project was targeted and concise, due to the pandemic state of emergency and the need of definite and prompt solutions to be presented to the scientific community.

The literature search with the corresponding key words gave a total of 87 original publications with free access, 5 of which we evaluated as appropriate and in accordance with our topic of interest. A total of 1509 patients with provided clinical and laboratory data were described in these papers.

In the study of Jin et al. in February, 2020, 74 (11.4%) out of 651 patients with confirmed COVID-19 had at least one gastrointestinal symptom. 53 of them had diarrhea, and 22 presented with nausea and vomiting [12]. Pan *et al.* analyzed 204 patients with COVID-19 in January and February 2020. Although the majority had fever or respiratory symptoms, the authors describe 103 (50.5%) patients with superimposed GI. Interestingly, 6 (2.9%) patients presented with gastrointestinal symptoms only [13].

Wan et al. retrospectively analyzed data from 232 COVID-19 patients, admitted between February and March 2020 in several Chinese provinces, including Hubei. Most of them were hospitalized with the classic catarrhal symptomatology and radiographic evidence of interstitial pneumonia. 49 (21%) out of all patients, included in this study, had diarrhea of varying intensity. This symptom was more frequent in elderly patients and in those with a more severe disease course [14]. A similar tendency could be seen during the SARS epidemic in 2003. Patients with diarrheal syndrome were put more oftenly on mechanical ventilation in contrast to these that did not have any GI symptoms [15].

Research from the USA also shows a relatively high frequency of GI in patients with COVID-19. However, they also conclude that patients with prevailing gastrointestinal symptoms are being tested late in their disease course. Individuals with catarrhal presentation are usually being tested for SARSCoV-2 between 3-5 days of symptom onset, while those with GI are tested between day 7 and day 9.

Another intriguing conclusion, made by Nobel et al., is that patients with gastrointestinal symptoms have a lower risk of severe disease course (0.0% severe cases with gastrointestinal symptoms vs 5.0% sever cases amongst patients without such, p=0,03) [16]. This data contradicts most of the results from China [12-14].

Booth *et al.* described 144 COVID-19 patients, admitted to 10 hospitals in Toronto. Like most other authors, they described fever (99.3%) and cough (69.4%) as the most frequent symptoms. Regarding the dyspeptic symptoms, the authors concluded that they are more common among the elderly and occur in combination with a catarrhal syndrome [17] (Table 1).

Among the investigated by us 31 Bulgarian patients, between 21 March and 09 April 2020, 14 (45.16%) had a laboratory confirmed COVID-19 infection. All of them were diagnosed with RT-PCR of NPS, while 3 patients also had a positive rapid IgM test. Most patients had mild or moderately severe infection. Only one individual was criti-

Table 1 - Incidence of the gastrointestinal symptoms in patients with COVID-19.

	Patients with SARS-CoV-2 infection	*Diarrhea*	*Nausea / vomiting*	*Notes*
Jin, et al. [12]	651	53 (8.14%)	22 (3.37%)	Average duration diarrhea is 5 days and is self-limiting.
Pan, et al. [13]	204	35 (17.2%)	8 (3.9%)	Patients with GI were admitted significantly later than those with catarrhal symptoms.
Wan, et al. [14]	232	49 (21.12%)	10 (4.31%)	Bloody faeces were observed in 4% of patients.
Nobel et al. [16]	278	56 (20.14%)	63 (22.67%)	Probably GI are associated with a more indolent form of COVID-19.
Booth et al. [17]	144	34 (23.6%)	28 (19.4%)	Some patients (4.2%) are hospitalized with fever and diarrhea only. No cough.

cally ill and necessitated mechanical ventilation. He was 38 years old, a chronic alcoholic abuser with untreated severe arterial hypertension. Out of the 14 evaluated patients, 9 were admitted to the Infectious Disease Units and 5 were treated on an outpatient basis by their general practitioner. In the other 17 patients with suspected COVID-19 the diagnosis was excluded, following two consecutive RT-PCR tests. Table 2 presents the clinical and laboratory characteristics of the patients.

Table 2 - Clinical and laboratory data of patients from Bulgaria.

	Patients with SARS-CoV-2 infection	*Patients without SARS-CoV-2 infection*
	N = 14	*N = 17*
Age	45.7	38.2
Hospitalization	9	13
Fever	14	17
Cough	14	16
Shortness of breath	7	4
Diarrhea	4	1
Nausea / vomiting	10	15
WBC $\times 10^9$ cells/L	3.47	4.22
Lymph. $\times 10^9$ cells/L	1.09	1.81
Platelets $\times 10^9$ cells/L	195.4	233.8
Alanine aminotransferase, U/L	71.4	26.1
C-reactive protein, mg/L	85.1	62.4

DISCUSSION

Our research is aimed at not only defining the incidence of gastrointestinal symptoms in COVID-19 patients, but also determining their type and eventual prognostic value. This problem gained popularity with the description of the virus tropism towards enterocytes, the presence of SARSCoV-2 in feces and the possibility of fecal-oral transmission [18-20]. Our research of the accessible literature showed that nearly 1/3 of the patients in all age groups presented with some GI. The classic symptoms -diarrhea, nausea and vomiting-, can occur independently or in combination and can present at any time during the disease course.

Cases of COVID-19 patients, presenting initially with GI, are particularly important. Research by Chen et al. described 9 patients in whom the infection began with diarrhea and fever, whereas the catarrhal symptoms appeared approximately 2 -5 days later [21].

Another key feature is the likelihood of some patients with mild forms of infection to remain afebrile during the whole course of the disease while having only prevailing GI. There is a small number of reported cases of patients with only diarrhea and nausea/vomiting without any respiratory symptoms [22].

Most authors do not take into account the age of the patients when describing the frequency of GI [14,16]. According to one international team, the incidence of diarrhea among elderly patients is statistically more significant [23]. The majority of research papers demonstrate an association between

GI and a more severe disease course, especially the need for mechanical ventilation. In critically ill patients, elevated levels of the hepatic enzymes are being detected, as well as a lower monocyte count and hemostatic disorders [14]. Nobel et al. is one of the few research teams that state the opposite [16]. Based on the observations of our patients with confirmed COVID-19, it can be said that GI is relatively common. Nausea and vomiting, in particular, are especially common, although they may also result from the general intoxication of the organism, caused by the virus, as they can be observed with the same frequency in patients with a negative RT-PCR test. Rather higher average values of Alanine aminotransferase can be noticed, however, in RT-PCR confirmed COVID-19 cases, which corresponds to some level of hepatic injury. Some authors also find evidence of direct hepatic injury by SARSCoV-2 [16, 23]. According to others, this is not caused by the virus itself, but is rather a result of a host autoimmune reaction against the liver cells [10, 24].

CONCLUSION

Secondary to the catarrhal symptoms and fever, GI is one of the most common symptoms in patients with COVID-19. There is controversial data regarding its prognostic value and association with the severity of the disease. However, its importance regarding the epidemiology of the infection is indisputable. Patients with GI should be actively tested for SARSCoV-2. Clinicians should be prepared for the plausibility that a SARSCoV-2 infected individual might present with diarrhea or nausea/vomiting only. It is of great importance to take into consideration the fact that the virus is detectable in feces longer than in respiratory secretions when discharging patients.

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Conflict of interest
The corresponding author states that there is no conflict of interest.

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Neurologic aspects of COVID-19: a concise review

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SUMMARY

In addition to the conventional respiratory symptoms, patients with COVID-19 can exhibit neurological complications. In this concise review, we aim to report the most frequent neurologic manifestations related to Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV2) infection.

SARS-CoV2 can reach the central nervous system from the bloodstream or olfactory pathway by binding ACE-2 receptor and the spike protein protease TMPRSS2. Headache is reported in more than 10%; of affected patients and loss of smell and taste disturbance are reported in a slightly smaller percentage of cases.

Acute cerebrovascular events are diagnosed in 3%; of COVID-19 patients, but those with more severe manifestations have cerebrovascular events in more than 6%; of the cases, as reported by two retrospective studies from Italy and China. Moreover, five cases of large-vessel stroke have been described in low-symptomatic COVID-19 patients aging less than 50 years suggesting that SARS-CoV2 can be associated with an increase of the risk of stroke in relatively young people.

Peripheral nerve diseases can be observed after an apparently uneventful SARS-CoV2. Based on a literature review, nine patients experienced Guillain-Barré; syndrome (GBS) and 6 of these needed mechanical ventilation. Two more cases have been described with Miller-Fisher syndrome or polyneuritis cranialis, both had rapidly resolving symptoms.

In conclusion, nervous system symptoms can be observed during SARS-CoV2 infection of which headache and smell and taste disturbance are the main symptoms reported. Cerebrovascular complications can complicate the course of COVID-19 in apparently low-risk patients. GBS is a life-threatening manifestation of COVID-19.

Keywords: Covid-19, neurologic aspects.

Since April 2020 every two weeks 1 million new COVID-19 patients have been reported worldwide totalling 5 million by the end of May with a reported number of deaths of over 300.000 by mid-May. During this period physicians and scientist all over the world have started to explore the disease and report on the clinical characteristics, pathophysiology and outcome of the disease [1, 2]. Although initially the reports on clinical characteristics focused on the severe pneumonia and need for mechanical ventilation that is the hallmark of COVID-19 infection, it quickly became clear the disease has multiple non-pulmonary features. A study investigating SARS-CoV-2 viral load in autopsy tissues demonstrated that significant viral amounts can be detected in kidneys, liver, heart and brain confirming preliminary investigations [3]. It has now become clear that there is a massive activation of the coagulation system through the severe inflammatory response [4]. This does not only cause deep venous thrombosis, pulmonary embolisms and renal failure, but also results in cerebral infarctions [5]. Besides strokes, every week new reports and reviews on neurological complications in COVID-19 are being published. Here we provide a

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summary of these findings now the first wave in Europe appears to have waned.

In the clinical presentation of patients infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) headache was reported in 11-34%, with the largest series reporting 14% [1, 6]. So far it is unclear what causes the headache, but hypoxia, metabolic disturbances or systemic inflammation may all be considered to contribute. Two studies available through the bioRxiv preprint platform suggest that SARS-CoV-2 can reach the Central Nervous System from the bloodstream or olfactory pathway by binding ACE-2 receptor and the spike protein protease TMPRSS2, but the clinical relevance of such brain invasion is unclear. An experimental model of SARS-CoV-1 infection did not report brain inflammation [7-9]. Direct infection of the central nervous system by SARS-CoV2 is considered unlikely, since cerebrospinal fluid (CSF) analysis is often normal. In the experience from our institutions at least eight patients with headache and PCR proven COVID-19 infection showed normal CSF composition (leukocyte count, total protein and glucose concentration) with negative COVID-19 PCRs in CSF. This complies with other case series in the literature [10]. So far, one case of acute necrotizing encephalitis (ANE) has been published, which is considered a post-infectious inflammatory syndrome rather than a direct infection of the brain by the virus [11]. Pending further publications on ANE following COVID19 a causal relationship is unsure.

A typical finding of COVID-19 is the loss of smell (or anosmia), which has been described in 40% of cases in a Spanish case control study and 34% in a Italian study [12, 13]. Although smell and taste disorders have been reported before in studies on other viral infections, the rate in COVID-19 patients is quite substantial. Although it has been theorized to be due to direct invasion of the olfactory nerve by the virus, further studies are needed to support this.

Infection is considered an important risk factor for stroke, based on large population-based studies and it is to be expected that a higher incidence of stroke during the weeks following a COVID-19 could be reported in otherwise low risk patients [14]. A large retrospective study from Wuhan investigating the neurologic aspects of COVID-19 found a total 2.8% incidence of acute cerebrovascular events, with higher rate (6%) in patients with severe COVID-19 [15]. A similar incidence was reported in an Italian study where cerebral infarctions were diagnosed in 9 (2.5%) of 362 patients [5]. In only 2 of these 9 cases a definite risk of stroke (atrial fibrillation) was present. Two patients received systemic thrombolysis and one patient underwent a successful mechanical thrombectomy, with no relevant side-effects. These findings suggest that stroke has a significant incidence in COVID-19 and stroke treatment can be similar as in COVID-19 unrelated cases [5].

A recent study from New York, described 5 cases of stroke in COVID-19 patients aging less than 50 years during a 2-week period. Clot retrieval was performed in 4 cases and no procedure-related side-effect was reported. Comparing the incidence with the previous 12 months the authors found that the stroke incidence was about 6 times higher in the under 50 years of age group (0.73 cases every 2 weeks), suggesting an association between COVID-19 outbreak and stroke incidence in apparently low-risk cases [16]. The suggested explanation for the increased risk of stroke includes direct damage of the virus on the vascular endothelium and activation of coagulation through the systemic inflammatory response.

In addition to direct effects of the viral infection associated with stroke, post-infectious inflammatory neurological syndromes have also been identified after COVID-19. In Guillain-Barré Syndrome (GBS) an aberrant immune response triggered by a recent infection results in peripheral nerve injury. An association between GBS and COVID-19 has been suggested in a recent publication of 9 GBS cases in whom 8 patients developed GBS 5-10 days after fever and respiratory symptoms due to COVID-19 infection, while one case presented with ongoing fever and GBS [17]. Severe symptoms with respiratory failure needing mechanical ventilation were reported in 6 cases. No case tested positive for SARS-CoV-2 by PCR on cerebrospinal fluid and all patients had a positive nasopharyngeal PCR test and chest imaging characteristic of COVID-19. All cases received intravenous immunoglobulins. Similar findings were previously reported in 4 patients affected by SARS that developed symptoms more than 20 days after primary infection [17, 18].

Miller Fisher Syndrome (MFS) and polyneuritis cranialis, which are variants of GBS causing cra-

nial nerve and pharyngeal and facial weakness, have also been described in COVID-19. A Spanish study described two patients developing MFS and polyneuritis cranialis, respectively, 5 and 3 days after the symptoms of mild COVID-19 developed. PCR was positive in both cases by nasal swabs but was negative by CSF examination. Neurological features resolved in both cases within 2 weeks without relevant sequelae [19].

In conclusion, neurologic symptoms are frequently reported in COVID-19 patients, but no impact of SARS-CoV-2 as direct causative agent of an inflammatory disease of the brain has been currently demonstrated. Headache and anosmia are reported with the highest frequency and appear to improve with COVID-19 symptoms disappearance. The direct effect of the virus on the endothelium and the inflammatory cascade activation after COVID-19 increase the risk of stroke, which is also relevant in young patients and in those without any risk for cerebrovascular infection. In these cases, endovascular and systemic treatments have so far not been associated with an increased risk of bleeding. Also, peripheral nerve disease can be triggered by SARS-CoV-2 infection, but the low amount of data does not permit to draw specific conclusion in term of its prognosis and treatment.

Conflict of interest
None to declare

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COVID-19 and pregnancy: a review of current knowledge

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SUMMARY

Background: Since December 2019, coronavirus disease 2019 (COVID-19) has become a major health problem that is spreading all over the world. Several viral infections such as SARS, MERS, and influenza have been associated with adverse pregnancy outcomes. The question arises whether pregnant women are at greater risk of complications related to COVID-19 compared to other people What complications should we expect in the fetuses whose mothers were infected?
Aims: This review aims to provide a summary of studies on symptoms of COVID-19 and the possible risks of COVID-19 among pregnant women, as well as complications in fetuses and neonates whose mothers were infected with COVID-19.
Methods: The included data were provided from Web of Science, Cochrane, PubMed, and Scopus which are extracted from the published studies in English until April 2nd, 2020 that contained data on the risk of COVID-19 in pregnancy.
Results: The early symptoms of patients with COVID-19 were fever, cough, dyspnea, myalgia, and fatigue; while production of sputum, headache, hemoptysis, and diarrhea were other symptoms which were less common. There is no evidence of vertical maternal-fetal transmission in pregnant women with COVID-19.
Conclusions: The clinical findings in pregnant women with COVID-19 are not significantly different compared to other patients, and pregnant women with COVID-19 are not at a higher risk of developing critical pneumonia compared to non-pregnant women. Although, there has been no sign of vertical infection in infants, but maternal infection can cause serious problems such as preterm labour and fetal distress.

Keywords: COVID-19, pregnancy, SARS, neonates, coronavirus.

INTRODUCTION

Coronaviridae, Arteriviridae, and Roniviridae are the three families of the *Nidovirales* order [1]. The *Coronavirinae* family is divided into two subfamilies which include the *Coronaviridae* and the *Torovirinae*. The *Coronavirinae* is also divided into four categories by phylogenetic clustering which are comprising the alpha, beta, gamma and delta coronaviruses [1]. The envelope and a non-segmented positive-sense RNA are the characteristics of viruses in *Nidovirales* order. While all of these viruses possess large genomes, *Coronavirinae* is considered to contain the largest RNA genome that is identified [1]. Family of coronaviruses consists of viruses which can lead to several symptoms including fever, dyspnea, and pneumonia [2]. Coronavirus is responsible for important public health problems that led to the global epidemics including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19) [3]. Rather than SARS-CoV-1 and MERS-

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CoV, genetic features of SARS-CoV-2 is found to be more similar to bat-SL-CoVZC45 and bat-SL-CoVZXC21 which are SARS-like coronaviruses derived from the bat [4].

First identified in December 2019, COVID-19 became an epidemic in Wuhan, Hubei Province, China [5]. Based on the World Health Organization, 159 countries and regions have been affected between 31 December 2019 and 17 March 2020 [6]. Moreover, 184,976 cases were confirmed within this period with 7,529 deaths [6]. Since there are suggestions regarding similarity about the pathogenesis of COVID-19 and SARS-CoV-1, the potential risk of vertical maternal-fetal transmission of COVID-19 may be as low as SARS-CoV-1 [7]. Viral infections such as SARS, MERS, and influenza have been related to the adverse pregnancy outcomes [8-10]. To reduce fetal rejection, some physiological changes happen in pregnant women for reducing immune responses [11]. Therefore, pregnant women may be at a higher risk of complications after COVID-19 infection.

Multiple studies concerned about the effects of COVID-19 on pregnancy. Thus, this review aims to summarize the symptoms and possible risks of COVID-19 infection in pregnant women. We also provide a summary of studies as of April-2nd, 2020on complications in fetuses or neonates whose mothers were infected with COVID-19.

Data sources

The included data were provided from Web of Science, Cochrane, PubMed, and Scopus which were extracted from the published studies in English until 2 April 2020 that contained data on the risk of COVID-19 in pregnancy with the following medical cases heading terms and/or text words: pregnancy, pregnancy outcomes, pregnant women, COVID-19, vertical transmission, and 2019-nCoV.

SARS-CoV-1 and pregnancy

Wong *et al.* [10] reported that the SARS tests on neonates who were born to mothers with SARS were negative; however, out of seven pregnant women with SARS who were in the first trimester, four patients had a spontaneous miscarriage. In addition, out of five pregnant women with SARS who were after 24 weeks, four patients underwent preterm delivery, and intrauterine growth restriction was observed despite women's recovery before delivery [10]. Another investigation on the effect of SARS-associated coronavirus among five neonates who were born to pregnant women with SARS during the outbreak in Hong Kong showed that all performed tests, including viral cultures, reverse transcriptase-polymerase chain reaction, and paired serologic titers were negative in those infants [12]. However, one preterm neonates developed jejunal perforation shortly after birth, and another one suffered from ileal perforation and necrotizing enterocolitis [12]. Five pregnant women who were infected with SARS in their second or third trimester delivered their neonates with no evidence of SARS infection [13]. Stockman *et al.* also reported that samples of cord blood and placenta of one patient were negative for antibodies to SARS-CoV while her serum samples were positive at the time of delivery [14]. Furthermore, breast milk samples, that were tested on days 12 and 30 of postpartum, were negative for the antibodies. The reverse transcription-polymerase chain reaction was also negative for viral RNA in stool samples of the neonates [14].

MERS-CoV and pregnancy

MERS-CoV infection clinical presentations vary from asymptomatic infection to more serious forms such as, acute respiratory distress syndrome, multi-organ failure, septic shock, and even death [15-18]. The disease shows nonspecific symptoms at the early stages, including headaches, malaise, low-grade fever, chills, myalgia, nonproductive cough, and dyspnea [19, 20]. Similar to SARS-CoV, patients infected by MERS-CoV may also suffer from some gastrointestinal symptoms which include abdominal pain, anorexia, nausea, vomiting, and diarrhea [18]. Since acute respiratory distress syndrome occurs more frequently in MERS patients rather than patients with SARS, the mortality rate of MERS-CoV patients was higher (~36%) than SARS patients (~10%) [21].

A study indicated that one pregnant woman infected by MERS-CoV presented with respiratory failure and admitted to ICU,delivered a healthy infant [22]. Another investigation demonstrated that all five pregnant women with MERS-CoV required ICU care; one women delivered a stillborn infant at 34 weeks and another infant died 4 hours after delivery [8]. One pregnant woman whose polymerase chain reaction test was positive for MERS-CoV presented an abrupt vaginal

bleeding; results of several tests were all negative for MERS-CoV, indicating that this woman fully recovered from MERS without transmitting the infection to her baby [23].

Clinical and laboratory characteristics of pregnant women with COVID-19 infection

Huang *et al.* reported that the early symptoms of patients with COVID-19 were fever, cough, dyspnea, myalgia, and fatigue [24]. Production of sputum, headache, hemoptysis, and diarrhea were other less common symptoms [24]. However, some patients did not show at first any signs of fever [24, 25]. Another study also reported that fever and cough were the most common symptoms of pneumonia onset in patients with COVID-19 [26]. Wan *et al.* study on 135 patients with COVID-19 have indicated that fever, cough, and fatigue were the most common symptoms and chest CT images of all patients showed bilateral ground-glass opacity or patchy shadows in the lungs [25]. Ground-glass opacity has been reported to be the most frequent early finding in chest CT images of 15 pregnant women with COVID-19 [26].

In a study on three pregnant patients with confirmed COVID-19 infection, fever has been observed as a symptom; while, there were no signs of significant lymphocytopenia or leukopenia [27]. Chen et al. observed that patients had decreased lymphocyte count and increased hypersensitive C reactive protein [28]. Consolidations and crazy paving pattern are two other findings which were observed with the progression of COVID-19 infection [26]. Zhu et al. observed that, in addition to cough and fever, diarrhea occurred in one patient out of nine patients [29]. Notable, in Liu et al. study, lymphocytopenia has been considered as the most frequent abnormal findings in laboratory tests [26]. Some other clinical characteristics have been observed in pregnant women with COVID-19 infection, including myalgia, sore throat, malaise, lymphocytopenia, and increased concentrations of aminotransferase [30]. Liu et al. found that leukocytosis, lymphopenia, increased neutrophil ratio, and initial normal body temperature were more common in 41 pregnant women with COVID-19 who were clinically-diagnosed or laboratory-confirmed compared to non-pregnant patients [31]. They indicated that it is more common for some pregnant women to have mixed or complete consolidations compared to non-pregnant patients. Whereas, ground-glass opacity occurred less frequently in the pregnant group compared to non-pregnant group [31]. Interestingly, Liu *et al.* noted that the initial identification of pregnant women with COVID-19 infection may be more challenging due to their atypical clinical findings [31]. Collectively, these studies have suggested that clinical characteristics of pregnant women with COVID-19 infection are similar to non-pregnant patients [27, 30, 32].

Table 1 - Clinical and laboratory characteristics of pregnant women with COVID-19.

Cases	*Age (range/ average)*	*Gestational week (range + days)*	*Cough*	*Fever (before delivery/ postpartum)*	*Fatigue/ malaise*	*Myalgia*	*Diarrhea*	*Dyspnea*	*Sore throat*	*Lymphopenia*	*Elevated C-reactive protein*	*Reference*
15	23–40	12–38	9	13 / 1	4	3	1	1	1	12	10	[26]
17	29.5/ 28.7 *	≥ 37 **	4	4	1	-	1	1	-	5	7	[32]
9	26-40	36 - 39+4	4	7 / 6	2	3	1	1	2	5	6	[30]
9 ***	30	31-39	5	9	-	-	1	-	1	-	-	[29]
3	-	-	-	1 / 2	-	-	-	-	-	0	-	[27]
16	-	37.9 ± 1.6	-	-	-	-	-	-	-	-	-	[33]
41 ****	22-42	-	15	16 / 14	5	-	0	-	-	25	27	[31]
7	29-34	37- 41+2	1	6	-	-	1	-	-	-	7	[38]

* Age ranges belong to pregnant women who received epidural anesthesia and general anesthesia, respectively.
** Gestational ages belong to 14 patients out of 17 pregnant women. The three other women gestational age was less than 37 weeks.
*** The authors reported the first symptoms in pregnant women and some symptoms probably haven't been reported.
**** The number of cases include both laboratory-confirmed and clinically-diagnosed patients.

Table 2 - Neonatal outcomes in infants who were born to women with COVID-19.

Cases	*Severe asphyxia*	*Death*	*1-min/5-min Apgar scores*	*Low birth weight*	*Premature delivery*	*Reference*
11	0	0	8 / 9	-	-	[26]
17	0	0	9 / 10	0	3	[32]
9	0	0	8-9 / 9-10	2	4	[30]
7	0	0	8-9 / 9-10	0	-	[38]
3	-	0	-	1 *	1	[27]
10	-	1	7-10 / 8-10	2**	6	[29]

*Authors reported that one premature infant was transferred to the neonatology department due to the low birth weight but we are not aware whether the rest of the neonates had a low birth weight or not.
**Two infants were small-for-gestational-age and 1 was a large-for-gestational-age.

What problems do the COVID-19 cause for pregnant women and their delivery?

As a retrospective study reported, there have been no significant differences in blood loss during the delivery (cesarean section) of 16 pregnant women with COVID-19 infection and 45 pregnant women without COVID-19 infection [33]. CT images that were taken before and after delivery of 11 pregnant women with COVID-19 infection demonstrated that delivery did not lead to the pneumonia aggravation [26]. Moreover, other symptoms of the patients did not aggravate because of pregnancy or childbirth [26]. Liu et al. study indicated that all of 15 pregnant patients recovered from COVID-19 pneumonia; although, some of them did not receive antiviral agents [26]. Chen et al. demonstrated that 17 pregnant women with COVID-19 delivered their babies safely through cesarean section with epidural or general anesthesia [32]. They also reported that intraoperative hypotension has occurred in 12 patients out of 14 patients who received epidural anesthesia [32].

How COVID-19 affects the neonates who were born to women with COVID-19 infection?

There were no differences between birth weight, fetal distress, neonatal asphyxia, and preterm birth of the neonates who were born to women with or without COVID-19 [33]. Assessments of the placentas, that were delivered from pregnant women with confirmed COVID-19 infection, demonstrated different degrees of fibrin depositions both around and inside the villi, as well as increased local syncytial nodules. Data showed that one of the placentas had severe infarction and another one presented aconcurrent chorionic hemangioma morphology. Meanwhile, none of the three placentas showed pathological changes in chorioamnionitis and villitis [27]. Records of 15 pregnant women with COVID-19 indicated that there were no neonatal death, neonatal asphyxia, and stillbirth [26]. Another study showed that three out of 17 neonates who were born to women with COVID-19 were premature; however, no death or neonatal asphyxia were reported [32]. Zhu et al. found that six out of ten neonates who were born to women with COVID-19 were premature and Pediatric Critical Illness Score (PCIS) of six neonates were less than 90 [29]. They reported that perinatal infection with COVID-19 may lead to some problems, including premature labour, thrombocytopenia, which is accompanied by abnormal liver function, fetal distress, respiratory distress, and death [29]. Chen et al. concluded that 1-min and 5-min Apgar scores of neonates born to women with COVID-19 were 8-9 and 9-10, respectively [30]. Wang et al. also reported that a pregnant woman with COVID-19 delivered an infant with an uneventful postpartum and neonatal course [34]. As studies investigated, all of the samples collected from neonates who were delivered by women infected with COVID-19 were negative for COVID-19 nucleic acid [34]. Altogether, these findings suggest that there is no evidence of vertical transmission in pregnant women with COVID-19.

What should be considered when administering medicines for pregnant women?

Based on interim guidance provided by the World Health Organization on 13 March 2020, no specific anti-COVID-19 treatments are recommended. However, several clinical trials are investigating potential antivirals medications to treat COV-

ID-19 [35]. Chloroquine and remdesivir (GS-5734) are two antiviral drugs that have shown promising inhibitory effects on SARS-CoV-2 replication in cell culture [36]. Chloroquine has shown adverse effects on the fetal development [37]. Meanwhile, Zhou *et al.* suggested that hydroxychloroquine is a better potential therapeutic agent compared to chloroquine because of its safety profile in pregnant women [37]. It is observed that a higher dose of carbetocin or carboprost tromethamine has been used during cesarean section of pregnant women with COVID-19 compared to women without COVID-19 for treating uterine contraction fatigue. Thus, it is suggested that prophylactic administration of uterotonic drugs may lead to the less postpartum hemorrhage in women [33].

CONCLUSIONS

There are a few studies concerning with the effects of COVID-19 on pregnant women and their neonates. However, there is not enough evidence to draw a definitive conclusion. As the COVID-19 is spreading further all over the world, more studies are needed to be performed on the pregnant patients and their neonates. We discussed the clinical, laboratory, and radiological characteristics of pregnant women with COVID-19 infection and concluded: (1) clinical findings in pregnant women with COVID-19 are not significantly different compared to other patients; (2) pregnant women with COVID-19 are not at a significantly higher risk of developing critical pneumonia compared to non-pregnant women; (3) and there has been no sign of vertical infection in infants, but maternal infection can cause serious problems such as preterm labour and fetal distress.

Competing interests
The authors declare that they have no competing interests.

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The rationale for Low-Molecular Weight Heparin (LMWH) use in SARS-CoV-2 infection

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SUMMARY

In spite of many ongoing attempts to repurpose existing antivirals, no drugs have emerged yet with the desirable activity against SARS-CoV-2. Hydroxychloroquine, lopinavir/ritonavir, remdesivir, umifenovir, favipiravir, ribavirin and β-interferon-1 gave rise to variable but still inconsistent proof of clinical efficacy in the treatment of COVID-19. Pathogenetic studies have shown significant differences between commonly defined viral pneumonia and COVID-19 pulmonary disease. In severe forms, immune/inflammatory alterations reminiscent of disease forms like Macrophage Activation Syndrome (MAS) have been described, and therapeutic options other than anti-infective have been proposed and implemented, such as anti-inflammatory and anticoagulative agents. The thrombotic phenomena described in the pulmonary vascular bed of patients with severe COVID-19 suggest the administration of low-molecular weight heparin (LMWH) as standard measure in hospitalized patients with COVID-19.

Keywords: SARS-CoV-2 infection

Following the appearance and worldwide circulation of SARS-CoV-2, the etiologic agent of COVID-19, a number of existing drugs with some putative antiviral effects were administered to patients in spite of the lack of any significant evidence of a possible therapeutic effect [1]. With no existing drugs of proven efficacy, in a sort of emergency experimental scenario, a series of drugs like hydroxychloroquine, lopinavir/ritonavir, azithromycin, umifenovir, faviparivir and remdesivir have been used both in a compassionate manner and in comparative clinical trials [2]. The intention to repurpose existing drugs is not new in viral diseases, as testified by the successful use of lamivudine and tenofovir (both TDF and TAF) in both HIV and HBV infection [3, 4]. However, unlike the case of bacteria, the target specificity of antiviral drugs is mostly species-specific and the results gathered in these months are so far rather disappointing.

Hydroxychloroquine use in COVID-19 patients was described in observational studies including thousands of patients, with hard endpoints like intubation and death [5]. No benefit was associated to hydroxychloroquine use but instead a higher risk of death was found to be associated to the intake of both hydroxychloroquine alone and in combination with a macrolide. While much criticism was expressed around these observational studies [6], especially concerning some apparent inconsistency of data analyzed, no data from randomized controlled trials on hydroxychloroquine are yet available.

Lopinavir/ritonavir, still a second line antiretroviral drug, was tested in a small-sized open randomized trial in COVID-19 patients with minor degrees of respiratory failure, but the non-significant limited benefit recorded in lopinavir/ritonavir recipients has discouraged its further use in COVID-19 patients [7].

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Umifenovir and favipiravir were compared in a randomized study with no control arm. Although an overall better outcome was recorded in favipiravir recipients (7 day recovery rate), the lack of a control arm made it impossible to draw any meaningful conclusion about the possible role of these anti-influenza drugs in COVID-19 patients [8].

In a randomized double-blind comparative trial the use of remdesivir was not found to be associated to a significant improvement when compared to placebo [9]. In the same study, the use of remdesivir did not even provide a faster viral clearance from upper airways, thus casting doubts about its real antiviral effect against SARS-CoV-2. In a further numerically larger (538 *vs* 521 patients) double-blind comparative trial vs placebo remdesivir was instead found to be significantly associated to a shorter time to recovery and with a reduction in mortality, although not statistically significant (7.1% with remdesivir and 11.9% with placebo) [10]. These findings suggest that a very early administration of remdesivir might (mildly) impact on the clinical course of SARS-CoV-2 infection, although more insights into its real antiviral action are required.

More recently, the findings of a small-sized open randomized trial comparing β-interferon 1b in association with ribavirin and lopinavir/ritonavir vs lopinavir/ritonavir alone disclosed an advantage for the β-interferon 1b group in terms of a shorter time to viral clearance as established by nasopharyngeal swab [11].

Albeit some recognizable effects did actually emerge from few of such studies, this multifaceted drug-repurposing initiative is far from providing the desired results. This challenge is made methodologically more difficult by the relatively low mortality rate attributable to COVID-19, which makes mandatory the implementation of very large clinical trials with careful patients recruitment and stratification.

Newer findings concerning the pathogenesis of COVID-19 do suggest that pneumonitis developing in SARS-CoV-2-infected patients behaves differently as compared to viral pneumonia due to other respiratory pathogens [12]. A number of findings look atypical when compared to what is commonly known about conventional viral pneumonia, and two are particularly striking. The first concerns the rather short duration of fever in spite of developing pneumonitis. Patients requiring hospitalization are often admitted with fever, which often spontaneously subsides in spite of multiple still expanding infiltrations in the lungs. A second surprising point is the fast-developing pulmonary fibrosis, which is the pathologic landmark associated to respiratory failure and need for assisted ventilation [13]. A reappraisal of the pulmonary pathogenesis of COVID-19 has shed some light on a possible multistep mechanism taking place in the wide anatomic interface involving type II pneumocytes, interstitial space, microcirculation and macrophages [14]. SARS-CoV-2 was found to be able to infect type II pneumocytes through binding to ACE2 receptors, which are abundantly expressed in these resident pulmonary cells [15]. Infection of type II pneumocytes occurs in close anatomical connection with both the pulmonary microvascular network and lung stromal cells, including lymphocytes undergoing activation. This leads to macrophage recruitment and activation with associated release of proinflammatory and procoagulant molecules. In such a low blood pressure setting with thin vessel walls, immunothrombosis follows due to high local cytokines levels, tissue factor synthesis and eventual vessel injury [16]. Despite intensive fibrinolytic reaction microthrombi formation takes place, with ensuing pulmonary infarction, hemorrhages and pulmonary hypertension. The widespread hemorrhagic phenomena taking place in the lungs are then followed by extensive fibrotic reaction, which challenges to various extent the full recovery of respiratory function. Such a pathogenetic hypothesis well matches with the higher risk of severe disease forms in patients with pre-existing risk factors for cardiovascular diseases. This severe inflammatory response is reminiscent of the cytokine storm associated to the Macrophage Activation Syndrome (MAS), also termed secondary haemophagocytic lymphoblastocytosis (sHLH) [17], and these similarities prompted the promising experimental clinical use of anti-cytokine therapy in the treatment of severe forms of COVID-19 [18]. It is thus apparent, according to this pathogenetic hypothesis, that COVID-19 actually begins as a viral respiratory disease, but its major pathologic findings are the result of a so far incompletely disclosed delayed inflammatory/immune reaction [12]. As a consequence, while an early antiviral therapy (once available) might actually reduce the chance of a

subsequent severe progression (by possibly reducing viral replication in the airways), the treatment approach to COVID-19 should also be oriented to drugs avoiding thrombotic phenomena and mitigating the phlogistic processes.

Following both clinical experience and according to autopsy studies, coagulopathy is being perceived as increasingly important in the pathogenesis of severe COVID-19 disease [19]. In a Chinese case series in-hospital mortality was associated to D-dimer blood levels >1 μg/mL and coagulopathy was much commoner in patients who died (27/54, 50%) than in survivors (10/137, 7%, p<0.0001) [20]. However, the local (pulmonary) rather than systemic nature of coagulation abnormalities was apparent in these patients as, unlike disseminated intravascular coagulation (DIC), both platelets level and prothrombin time were found to be both in the normal range [14, 20]. D-dimer seems to actually play the role of a key parameter in estimating the severity of COVID-19 associated pulmonary disease. Although still debated, the administration of prophylactic heparin has gradually gained consent as standard measure to be applied to hospitalized COVID-19 patients, unless contraindicated [21]. In a retrospective investigation of 449 patients the mortality rate at 28 days was lower in heparin intakers when D-dimer levels were six times upper the normal limit of normality, and the same applied to those with sepsis-induced coagulopathy scores > 4 [22]. It must also be noted that heparin action in case of COVID-19 patients might not be limited to its anticoagulative effects, as interference with viral spike protein (binding to ACE2 receptors) and down-regulation of >IL-6, which

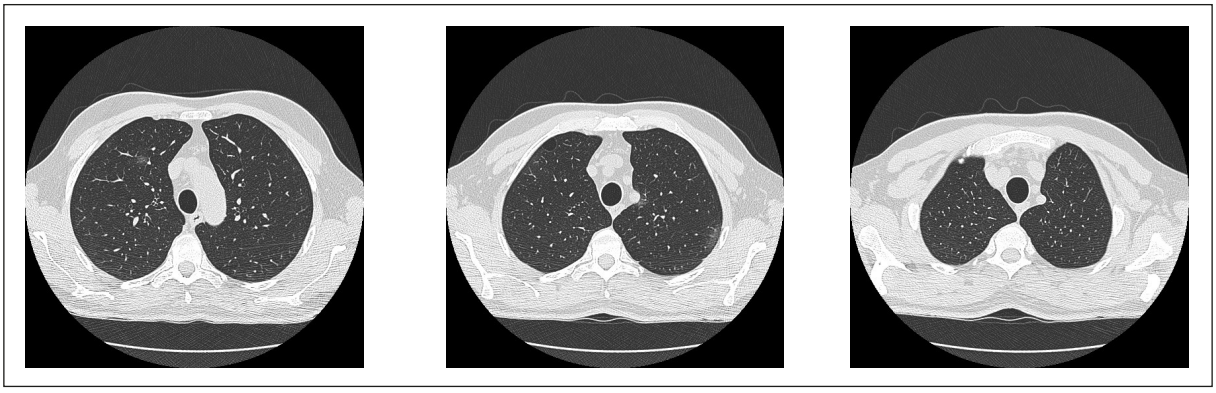

Figure 1a - Ct-Scan picture of a 52-male admitted for mild COVID-19 made on admission.

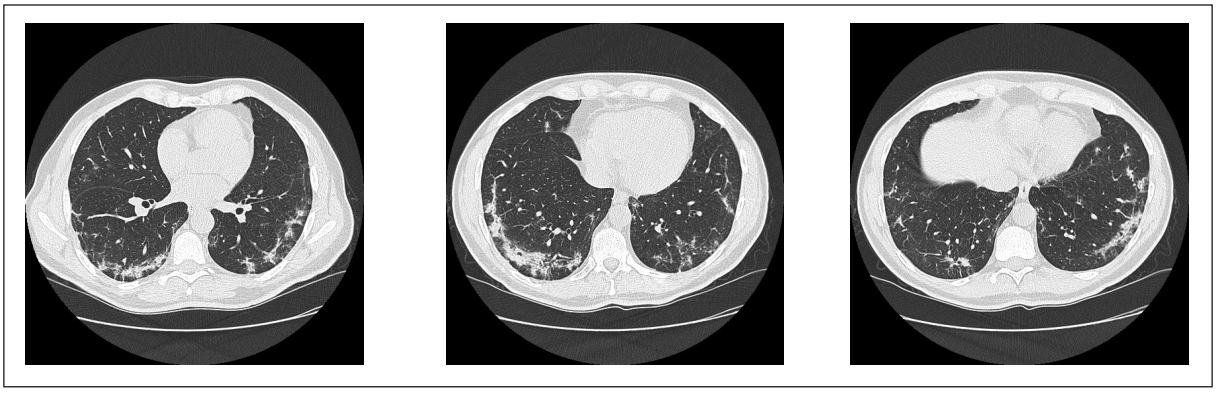

Figure 1b - Control CT-Scan picture made 15 days after hospital discharge in the same patient (who cleared his PCR signal for SARS-CoV-2 infection) showing nely appeared fibrotic lesions in the posterobasla lateral portions of the lungs. A further CT-Scan made a month later showed unaltered findings.

is part of the cytokine storm, have been both described [21, 23]. Timing and doses of heparin are still being discussed, and a randomized clinical trial with high-dose of the low-molecular weight heparin (LMWH) enoxaparin is ongoing in order to verify whether early LMWH treatment might impact on COVID-19 outcome [24]. The role of LMWH prophylaxis deserves attention also in clinical cases with limited evolution, as persistent pulmonary lesions with possible long-term impact on respiratory function have been described in patients who eventually recovered from COVID-19 [25].

The example here shown (see Figure 1) concerns a 52-year old male subjects who experienced mild respiratory failure during a 12-day hospitalization for COVID-19 in Italy. The patient was one of the first COVID-19 cases hospitalized in Torino, Italy, and no prophylactic LMWH was administered. He was admitted to the hospital following three days of cough, high fever, diffuse muscular aches and general malaise. Fever subsided after two days and an uneventful recovery took place, with first negative PCT test for SARS-CoV-2 infection at hospital discharge. Saturation was 91% on admission but rose to 97% four days afterwards. The patient had a mild disease, as also testified by his first CT-scan picture taken on admission. Once discharged he underwent a control visit after 15 days to confirm negativity of PCR testing for SARS-CoV-2 infection and for CT-Scan control. Testing for SARS-CoV-2 infection was confirmed as negative, but surprisingly, the CT-Scan disclosed new fibrotic pulmonary lesions in posterolateral-basal portions of the lung. These lesions remained unaltered at a further control made 20 days later, with saturation persistently above 97% and no additional signs or symptoms. LMWH prophylaxis was not given as such practice was standardized later in the course of the Italian COVID-19 epidemic, and the question here is whether its administration would have reduced the development or the size of these lately appearing pulmonary fibrotic lesions [26]. Whatever the answer, since residual fibrotic pulmonary lesion might impact on pulmonary function in recovered patients, these findings actually deserve attention, as such kind of post-recovery fibrotic morbidity might be less rare than otherwise expected. The analysis of a 70-patient series in China revealed that as much as 94% of patients (66/70) had residual disease on their final CT-Scans, with ground-glass opacities as the prevalent pattern [25].

While the final position of LMWH in the management of COVID-19 has still to be defined, the prophylactic use of LMWH, also considering its favorable risk/benefit ratio, seems warranted in patients requiring hospitalization.

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None

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Comprehensive review of mask utility and challenges during the COVID-19 pandemic

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SUMMARY

Masks are widely discussed during the course of the ongoing COVID-19 pandemic. Most hospitals have implemented universal masking for their healthcare workers, and the Center for Disease Control currently advises even the general public to wear cloth masks when outdoors. The pertinent need for masks arises from plausible dissemination of the SARS-CoV-2 through close contacts, as well as the possibility of virus transmission from asymptomatic, pre-symptomatic, and mildly symptomatic individuals. Given current global shortages in personal protective equipment, the efficacy of various types of masks: N95 respirators, surgical masks, and cloth masks are researched. To accommodate limited supplies, techniques for extended use, reuse, and sterilization of masks are strategized. However, masks alone may not greatly slow down the COVID-19 pandemic unless they are coupled with adequate social distancing, diligent hand hygiene, and other proven preventive measures.

Keywords: mask efficacy, universal masking, coronavirus, COVID-19, N95 respirators.

INTRODUCTION

On April 3, 2020, the Center for Disease Control (CDC) issued an advisory that the general public have to wear cloth face-masks when outside, particularly those residing in areas with significant Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) community transmission [1]. Recent research reveals several factors related to the nature of the virus as well as the epidemiological spread of the illness that may have led to this decision. However, controversy prevails whether this recommendation will alleviate or aggravate disease progression. Since hospitals across America lacking sufficient Personal Protective Equipment (PPE) and scrambling for supplies, universal masking may create more chaos- especially with certain states imposing monetary fines on individuals spotted outdoors without a mask. As new information being discovered each day about the Coronavirus Disease 2019 (COVID-19), it is more imperative than ever to update existing strategies and formulate more effective methods to flatten the contagion curve.

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AIRBORNE VS. DROPLET TRANSMISSION OF THE DISEASE

In a scientific brief released by the World Health Organization (WHO), there have been studies with mixed evidence and opinions regarding the presence of SARS-CoV-2 ribonucleic acid (RNA) in air samples. Santarpia *et al.* from the University of Nebraska Medical Center detected viral RNA in samples taken from beneath the patient's bed and from the window ledge, both areas where neither the patient nor health care personnel had any direct contact. They also found that 66.7% of air samples taken from the hospital hallway carried virus-containing particles [2, 3]. It is worth noting that certain Aerosol-Generating Procedures (AGP) may increase the likelihood of airborne dissemination. Whether airborne transmission is a major mode of SARS-CoV-2 spread in the community and in routine clinical settings (with no aerosol-generating procedures) is still a debatable question with no definitive answer.

We should consider the epidemiology of COVID-19 thus far in the pandemic, to determine if transmission patterns are more consistent with that of other common respiratory viral pathogens, or more consistent with that of the agents we classically consider to be transmitted by the airborne route (measles, varicella zoster virus, and *Mycobacterium tuberculosis*). The attack rates in various settings (household, healthcare, and the public) as well as the expected number of secondary cases from a single infected individual in a susceptible population (basic reproduction number or R_0) are more consistent with those of a droplet spread pathogen. For measles, the R_0 is 12-18, and the secondary household attack rates are ≥90%. In the case of the varicella zoster virus, the R_0 is ~10, and the secondary household attack rate is 85% [4, 5]. The R_0 for pulmonary tuberculosis is up to 10 (per year) and the secondary household attack rate has been reported to be >50%. With SARS-CoV-2, the R_0 is around 2.5 -3 and secondary household attack rates are 10-30% from the data available so far (Figure 1) [6, 7]. A systematic review of reported reproductive numbers from previous seasonal influenza outbreaks and pandemics by Biggerstaff et al. shows a median R_0 of 1.28 [8]. This data suggests that droplet transmission may be more likely. The dichotomy of airborne versus droplet mode of spread may be better described as a continuum rather, as pointed out in a recent article in the Journal of the American Medical Association (*JAMA*). Infectious droplets form turbulent gas clouds allowing the virus particles to travel further and remain in the air longer [9]. The neces-

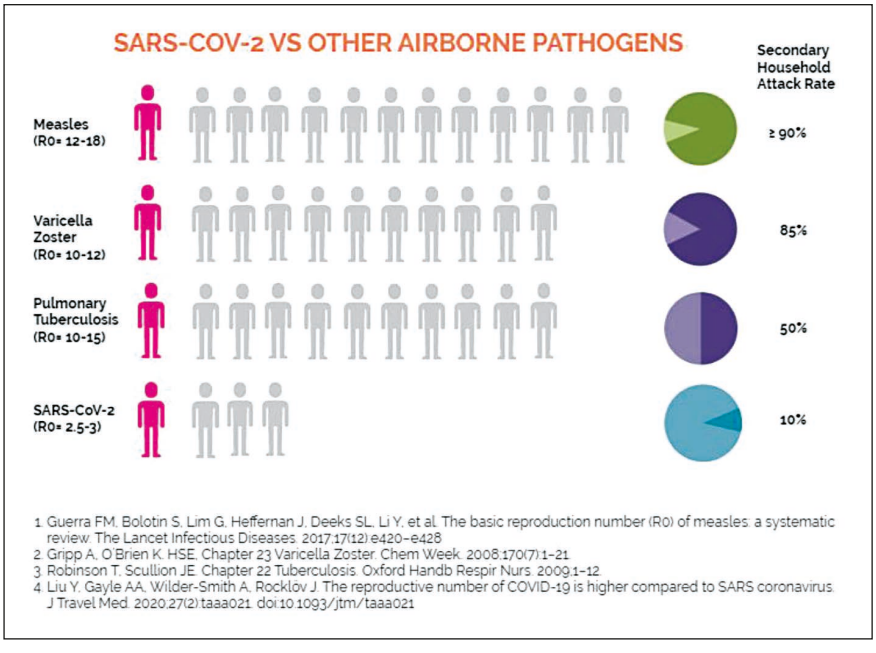

Figure 1 - Infographic comparing basic reproduction number (R_0) and secondary household attack rate of SARS-CoV-2 vs that of known airborne pathogens (measles, varicella zoster, pulmonary tuberculosis) based on historical data.

sary precautions for an airborne illness should be chosen over droplet precautions, especially when there is concern for an AGP.

UNIVERSAL MASKING: RISKS AND BENEFITS

The idea of universal masking has been debated extensively since the initial stages of the COVID-19 pandemic. According to public health authorities, significant exposure is defined as "face-to-face contact within 6 feet with a patient with symptomatic COVID-19" in the range of a few minutes up to 30 minutes [10]. The chance of catching COVID-19 from a passing interaction in a public space is therefore minimal, and it may seem unnecessary to wear a mask at all times in public. Randomized clinical studies performed on other viruses in the past have shown no added protection conferred by wearing a mask, though small sample sizes and noncompliance are limiting factors to their validity [11]. On the contrary, it has been enforced in many parts of Asia including Hong Kong and Singapore with promising results [10]. Leung et al. state that the lack of proof that masks are effective should not rule them as ineffective. Also, universal masking would reduce the stigma around symptomatic individuals covering their faces. It has become a cultural phenomenon in many southeast Asian countries and has been cited as one of the reasons for successful containment in Singapore, South Korea, and Taiwan. The most important benefit of universal masking is protection attained by preventing spread from asymptomatic, mildly symptomatic and pre-symptomatic carriers [12].

In a study carried out by Park *et al.* to estimate viral loads during various stages of the disease, it was found that asymptomatic patients had similar viral loads to symptomatic patients, thereby suggesting high potential for transmission [13]. Furthermore, numerous cases are being reported concerning the spread of illness from asymptomatic carriers [14-17]. In an outbreak at a skilled nursing facility in Washington described by Kimball et al., 13 of 23 residents with positive test results were asymptomatic at the time of testing out of whom 3 never developed any symptoms [17]. Many hospitals are now embracing the policy of universal masking. A mask is a critical component of the Personal Protective Equipment (PPE) clinicians need when caring for symptomatic patients with respiratory viral infections, in conjunction with a gown, gloves, and eye protection. Masking in this context is already part of routine operations in most hospitals. There are two scenarios in which there may be possible benefits. One scenario is the lower likelihood of transmission from asymptomatic and minimally symptomatic healthcare workers with COVID-19 to other providers and patients. The other less plausible benefit of universal masking among healthcare workers is that it may provide some protection in the possibility of caring for an unrecognized COVID-19 patient. Rhee et al. mention that the prevalence of asymptomatic infection in the general population is only 1-2% in most areas but among confirmed cases, is around 20-50%. Given the 70% sensitivity rate for nasopharyngeal swab polymerase chain reaction testing and high number of affected individuals who test negative initially, undue caution is undeniably warranted [18].

Universal masking should be coupled with other favorable practices like temperature checks and symptom screening on a daily basis to avail the maximal benefit from masking. Despite varied opinions on the outcomes of universal masking, this measure helps improve health care workers' safety, psychological well-being, trust in their hospital, and decreases anxiety of acquiring the illness. On the other hand, universal masking may give a false impression of protection and may result in increased face touching.

EFFICACY OF VARIOUS TYPES OF MASKS

The World Health Organization (WHO) recommended in February that surgical masks should suffice when treating COVID-19 patients, and N95 respirators or PAPRs should be used only in case of aerosol generating procedures. The CDC, however, insisted that N95 respirators be used by all medical professionals coming in contact with COVID-19 patients. Once hospitals suffered shortages, surgical masks were also permitted. Rhee et al. pose the question: are the CDC's recommendations "driven by supply shortages rather than science" [18]? How different are the levels of protection conferred by N95 respirators as compared to surgical masks? With the possibility of airborne transmission of the virus, are cloth masks truly helpful in preventing infection

in the public? A study by Ma et al. demonstrates 99.98%, 97.14%, and 95.15% efficacy for N95, surgical, and homemade masks respectively in blocking the avian influenza virus (comparable to coronavirus in size and physical characteristics). The homemade mask was created using 1 layer of polyester cloth and a 4-layered kitchen filter paper [19]. N95 masks (equivalent to FFP/P2 in European countries) are made of electrostatically charged polypropylene microfibers designed to filter particles measuring 100-300nm in diameter with 95% efficacy. A single COVID measures 125 nm approximately. N99 (FFP3) and N100 (P3) masks are also available, though not as widely used, with 99% and 99.7% efficacy respectively for the same size range. Though cloth masks are the clear-cut last resort for medical professionals, a few studies state no clinically proven difference in protection between surgical masks and N95 respirators [20. 21]. Even aerosolized droplets (<5 μm) were found to be blocked by surgical masks in a study by Leung et al. in which 4/10 subjects tested positive for coronavirus in exhaled breath samples without masks and 0/10 subjects with masks [22]. On the contrary, Bae et al. found in their study of four COVID-19 positive subjects that "neither surgical masks nor cloth masks effectively filtered SARS-CoV-2 during coughs of infected patients." In fact, more contamination was found on the outer surface of the masks when compared to the inner surface, probably owing to the masks' aerodynamic properties [23]. Due to limitations present in the above-mentioned studies, further research is necessary to conclusively determine which types of masks are efficacious in preventing infection by the virus. In a scarcity of surgical masks and respirators for healthcare personnel, sub-optimal masks can be of some use provided there is adherent use, minimal donning and doffing, and it is to be accompanied by adequate hand washing practices [21]. Furthermore, even the most effective mask is useless if not worn correctly or fitted properly. Though healthcare workers may feel falsely safe or protected while wearing a mask (particularly loose fitting industrial masks), minimal air leakage, regular fit-testing and seal checks with N95 respirators are of paramount importance.

In case of severe infections with high viral loads or patients undergoing aerosol-generating procedures, Powered Air-Purifying Respirators (PAPRs) are also advisable as they confer greater

Table 1 - Summary table comparing features, benefits, and drawbacks of various types of masks currently being use.

	Features	*Benefits*	*Drawbacks*
N95 Respirators	- Tight fitting (filtration rate >95%) - To be used by healthcare workers	- Greater protection against aerosols and droplets	- Requires regular fit-testing and seal check - Diminishing supplies - Higher cost than surgical masks
Surgical Masks	- Loose fitting, provides physical barrier - To be used by healthcare workers	- Cheaper, more easily available - Can be layered over N95 masks	- Air leakage (cannot be used during aerosol-generating procedures) - Disposable, meant for one-time use
Cloth Masks	- Loose fitting, usually made of polyester or cotton - Can be layered with filter paper - For use by general public	- Can be homemade, washed and reused - Use can prevent hoarding of medical masks	- Insufficient protection from aerosols
Powered Air-Purifying Respirators (PAPRs)	- Loose head-top with battery powered blower to filter air - For use during aerosol-generating procedures	- Greater protection compared to N95 - Does not require fit-testing, can be worn with facial hair - More comfortable	- Expensive, limited availability - High cost and difficulty of maintenance

Source: Respiratory Protection During Outbreaks: Respirators versus Surgical Masks
https://blogs.cdc.gov/niosh-science-blog/2020/04/09/masks-v-respirators/

protection than N95 respirators. Despite being more comfortable for long-term use and accommodative of facial hair, their use is limited due to high cost and difficult maintenance [24] (Table 1). 3-D printing is also being utilized to combat the current shortage of masks worldwide. However, virological testing for leakage between the two reusable components and contamination of the components themselves after one or multiple disinfection cycles is essential before application in real-life situations [25].

ONGOING ISSUES

WHO estimates a monthly requirement of nearly 90 million masks exclusively for healthcare workers to protect themselves against COVID-19 [26]. In spite of increasing the production rate by 40%, if the general public hoards masks and respirators, the results could be disastrous. Personal protective equipment is currently at 100 times the usual demand and 20 times the usual cost, with stocks backlogged by 4-6 months. The appropriate order of priority in distribution to healthcare professionals first, followed by those caring for infected patients is critical. In the US alone, a survey conducted by the Association for Professionals in Infection Control and Epidemiology revealed that 48% of the healthcare facilities that responded were either out or nearly out of respirators as of March 25, 2020. The gravest risk behind the universal masking policy is the likely depletion of medical resources [27, 28]. A possible solution to this issue could be to modify the policy to stagger the requirement based on the severity of community transmission in that area of residence. In the article appropriately titled "Rational use of face masks in the COVID-19 pandemic" published in the Lancet, Feng et al. describe how the Chinese population was classified into moderate, low, and very low risk of infection categories and advised to wear a surgical or disposable mask, disposable mask, and no mask respectively [29]. This curbs widespread panic and eagerness by the general public to stock up on essential medical equipment when it may not even be necessary.

In the hospital setting, there is need for a clear consensus on when N95 respirators are indicated versus surgical masks. Amidst CDC's shift in recommendations to battle diminishing supplies, certain hospitals and professional societies have accelerated their infection control protocols to be extra cautious. This includes expanding the definition of AGPs "based on theoretical concerns rather than documented transmissions" [18].

REUSE, EXTENDED USE, AND DECONTAMINATION

Several studies have been conducted to identify the viability of the COVID-19 on various surfaces [30, 31]. CDC and National Institute for Occupational Safety and Health (NIOSH) guidelines state that an N95 respirator can be used up to 8 hours with intermittent or continuous use- though this number is not fixed and heavily depends upon the extent of exposure, risk of contamination, and frequency of donning and doffing. Though traditionally meant for single-time usage, after 8 hours, the mask can be decontaminated and reused. CDC defines extended use as the "practice of wearing the same N95 respirator for repeated close contact encounters with several patients, without removing the respirator between patient encounters." Reuse is defined as "using the same N95 respirator for multiple encounters with patients but removing it ('doffing') after each encounter. The respirator is stored in between encounters to be put on again ('donned') prior to the next encounter with a patient." It has been established that extended use is more advisable than reuse given the lower risk of self inoculation. Furthermore, health care professionals are urged to wear a cleanable face shield or disposable mask over the respirator to minimize contamination and practice diligent hand hygiene before and after handling the respirator. N95 respirators are to be discarded following aerosol-generating procedures or if they come in contact with blood, respiratory secretions, or bodily fluids. They should also be discarded in case of close contact with an infected patient or if they cause breathing difficulties to the wearer [32]. This may not always be possible given the unprecedented shortage of PPE, hence decontamination techniques and repurposing are the need of the hour.

Dr. Nathan of Northeastern University Feinberg School of Medicine recommends recycling four masks in series using one per day, keeping the mask in a dry clean environment and then repeating the first mask on the 5th day, second on the 6th day, and so forth. This ensures clearance of the

virus particles by the next use. Alternatively, respirators can be sterilized between uses by heating to 70ºC (158ºF) for 30 minutes. Liquid disinfectants such as alcohol and bleach as well as ultraviolet rays in sunlight tend to damage the mask [33]. Steam sterilization is the most commonly utilized technique used in hospitals. Other methods include gamma irradiation at 20kGy (2MRad) for large-scale sterilization (though the facilities may not be widely available), vaporized hydrogen peroxide, ozone decontamination, ultraviolet germicidal irradiation, and ethylene oxide [34]. Though a discussion on various considerations of decontamination techniques is out of the scope of this paper, detailed guidelines have been published by the CDC and the COVID-19 Healthcare Coalition [35, 36].

CONCLUSIONS

A recent startling discovery by Sanche *et al*. shows that the basic reproductive number (R_0) is actually much higher than previously thought. Using expanded data, updated epidemiological parameters, and the current outbreak dynamics in Wuhan, the team came to the conclusion that the R_0 for the novel coronavirus is actually 5.7 (95% CI 3.8-8.9) compared to initial estimate of 2.2-2.7 [37]. Concern for transmissibility demands heightened prevention strategies until more data evolves. The latest recommendation by the CDC regarding cloth masking in the public may help slow the progression of the pandemic. However, it is of paramount importance to keep in mind that masks alone are not enough to control the disease and must be coupled with other non-pharmacological interventions such as social distancing, quarantining/isolation, and diligent hand hygiene.

Conflict of interest
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Preparing for emerging respiratory pathogens such as SARS-CoV, MERS-CoV, and SARS-CoV-2

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SUMMARY

Preparing for emerging respiratory pathogens is a fundamental requirement for enhancements of the safeguard in healthcare settings. We are facing an increasing pressure to be prepared more than before. Healthcare organizations should be ready to deal with such emerging infectious disease. Here, we share some points that are essential to be considered while we prepare our institutions to prevent the transmission of emerging respiratory pathogens such as MERS-CoV and the recently emerging pandemic of SARS-CoV-2, the causative agent of COVID-19.

Keywords: Preparedness; emerging respiratory infections; MERS-CoV; COVID-19; SARS-CoV-2.

INTRODUCTION

Preparing for emerging respiratory pathogens is a fundamental requirement for enhancements of the safeguard in healthcare settings. For a long-time, we had feared the emergence of a novel pathogen that would result in a pandemic. The question is not if it will happen or not, but when it is going to happen. In 2002, we had witnessed the emergence of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in Guangdong Province, China [1,2]. Then cases were described in multiple countries including Vietnam, Hong Kong, Canada, United Sates, Ireland, Vietnam, and Singapore [1, 3-10]. All the mentioned cases were linked to a patient who stayed in hotel M in Hong Kong [11]. In 2012, we had seen the emergence of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [12-14]. MERS-CoV was associated with multiple healthcare associated outbreaks and this became a hallmark of MERS-CoV [15-17]. In December 2019, the 2019-novel coronavirus (nCoV), later termed COVID-19, emerged in Wuhan city, China, and this virus is called SARS-CoV-2. There are multiple factors for the emergence and amplification of infectious diseases as outlined by the World Health Organization [18, 19]. Thus, we are facing an increasing pressure to be prepared more than before. Here, we share some points that are essential to be considered while we prepare our institutions to prevent the transmission of emerging respiratory pathogens such as the 2019 nCoV (SARS-CoV-2), the etiologic agent of COVID-19.

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Administrative support

Infection Prevention and Control (IPC) encompasses the administrative level as well as the healthcare workers. Involving the top hospitals'

management staff such as the chief executive officer, the chief medical, nursing and operating officers to ensure appropriate and timely support for all IPC preventive measures and plans. These administrators are expected to allocate adequate resources and personnel to the infection control department.

Communication plans

It is imperative to have an excellent communication plan that covers multiple aspects of the preparedness. In a study of pandemic influenza preparedness, the preparation and risk communication scored 48% [20]. Health risk communication in the event of emerging infectious diseases is important to mitigate negative consequences and requires a coordinated efforts [21]. Effective communication should ensure active internal reporting system between the hospital departments and infection prevention and control staff. This communication should also include an active reporting system within the overall health system in a country in relation to suspected or confirmed cases. Emerging infectious disease epidemics and pandemics similar to other crises necessitate unique forms of communication [22]. In addition, it is important to have consultation with the public and key stakeholder in the development of planning strategies for communication [23]. This activity is further enhanced by the rapidly growing social media and these media could be used to disseminate information quickly and widely [24].

Personal Protective Equipment (PPE)

Personal protective equipment is a part of standard precautions and includes the use of gowns, gloves, and protective mask or goggles. The availability of all required IPC supplies with easy access to all staff should be ensured at all the times. Healthcare organizations may need to develop a stringent plan of communications to ensure achieving of personal protection.

"Zero tolerance" policy regarding IPC measures violation

It is important to implement and strictly observe a "zero tolerance" policy regarding the non-adherence to IPC measures and that they should not tolerate violation among staff. Additionally, adopting a zero-tolerance approach to IPC measures violation is an achievable and an imperative goal in the setting of increasing health-care associated transmissions of multiple organisms and the occurrence of outbreaks [25]. This approach had recently received criticism as the occurrence of one infection after a period of zero infection had resulted in blaming the infection control program for such occurrence. Thus, the initial concept was associated with positive outcomes, it later got more negative connection necessitating the need to express the message in a more positive way [26].

The emergence of MERS-CoV was linked to the multiple healthcare associated outbreaks and this is a hallmark of MERS [15]. Many of these outbreaks were brought under control with the basics of infection control measures. So, it is very important to deliver a positive message for adherence to infection control standards at all time and avoid the blame game and such program had been implemented to target zero tolerance to hand hygiene non-compliance [27].

Visual or Numerical Triage Scoring System

For effective and immediate isolation of patients suspected to have infectious diseases, healthcare workers (HCWs) should have a high index of suspicion. Such suspicion is improved by specific triage policies and procedures such as the utilization of visual alertness to prompt HCWs to further screen suspected patients for fever, respiratory symptoms, and epidemiologic links for early detection and isolation. Visual or numerical triage scoring system utilizes a scoring system based on the case definition and assign a relative score for each epidemiologic link, clinical link and signs or symptoms. Such a triage system was used by the Saudi Ministry of Health MERS-CoV as shown in table 1 and 2 [28]. Visual triage or otherwise known as respiratory triage should be efficient and utilized as visual clues to alert HCWs on the case definitions and can be used in emergency room (ER), hemodialysis unit, and urgent care units. Such visual triage was used in the case of MERS-CoV in Saudi Arabia [29, 30]. The main purpose of such visual triage is the identification of possible cases meeting the case definition through the application of evidence from objective observation of the patient's characteristics to prioritize emergency treatment. One study found that visual triage scoring system to have sensitivity and specificity of this cutoff score

Table 1 - Visual triage showing clinical symptoms and signs in one section and the second section showing epidemiologic link to MERS-CoV.

	A. Clinica/ symptomlsign	*Points*	*Score*
1	Fever (≥38°C)	2	
2	Cough (New or worsening)	2	
3	Shortness of breath (New or worsening)	2	
4	Nausea, vomiting, diarrea	1	
5	Sore throat and/or runny nose	1	
6	DM. Chronic renai failure. CAD/heart failure	1	
	B. Risk of exposure to MERS		
7	Exposure to a confinned MERS case in last two weeks	3	
8	Exposure to carnei or products (Direct or indirect*) in the last two weeks	2	
9	Visit to health care facility thai has MERS case in last two weeks	1	
	Total Score		

*Patient or household.
DM = Diabetes Mellitus.
CAD = Coronary Artery Disease.
MERS = Middle East Respiratory Syndrome Coronavirus infection.

of 74.1% and 18.6%, respectively for MERS-CoV infection [30].

Emerging Infectious Disease Drill

In a study evaluating infectious disease drills for MERS, measles and Ebola cases, 40% of hospitals failed at least one drill [31]. In addition, the drill identified lapses in infection control such as: hand hygiene (36%), PPE use (74%), and posting of isolation signage (70%) [31]. These drills utilized unannounced mystery patient drills to test preparedness for MERS and measles and other drills utilized patients imitating smallpox infection or anthrax exposure [31-33]. Recently, the Central Board of Accreditation of Healthcare Institutions (CBAHI) in Saudi Arabia had launched such a program to test the preparedness of hospitals to recognized and manage patients suspected to have COVID-19. Such unannounced inspections are thought to strengthen hospitals' infection control measures and reduce risk of infectious disease transmission [34]. In a previous study of unannounced mystery patient simulating avian influenza attending emergency departments and public health centers showed that 89% did not respond correctly [34]. It is important to realize

Table 2 - Respiratory Triage Checklist for MERS-CoV and COVID-19 from the Saudi Ministry. of Health.

Risks for Acute Respiratory lllnesses	*Score*	
A. Exposure Risks	*Any Patient (Adult or Pediatric)*	
A history of travel abroad during the 14 days prior to symptom onset. OR Visiting or being a resident of a high-risk area for COVID-19 in the kingdom during the 14 days prior to symptom onset*. OR A close physical contact with a confirmed case of COVID-19 or MERS-CoV in the past 14 days. OR An exposure to camel or camel's products (direct or indirect**) in the past 14 days.	3	
B. Clinical Signs and Symptoms and Medical History	*Pediatric*	*Adult*
1. Fever or recent history of fever.	1	2
2. Cough (new or worsening).	1	2
3. Shortness of breath (new or worsening).	1	2
4. Nausea, vomiting, and/or diarrhea.	–	1
5. Chronic renai failure, CAD/heart failure, Immunocompromised patient.	–	1
Total Score		

*As determined and announced by the Ministry of Interior or Ministry of Health.
**Patient or household.
A score ≥4, ask the patient to perform hand hygiene, wear a surgical mask, direct the patient through the respiratory pathway and inform MD for assessment. MRSE-CoV OR COVID-19 testing should be only done according to case definitions.

geographic/cultural variations in holding such drills. In addition, drills are valuable to identify those failures so that specific corrective action can be taken and thus doing drills actually leads to improved outcomes.

Staff accommodation for isolation or quarantine

Quarantine is the separation or restriction of movement of exposed well persons for the duration of the incubation period. The origin of the word quarantine comes from the Latin *quadragina* or the Italian *quaranta*, meaning 40, as sailors were observed 40 days before disembarkation of ship during the bubonic and pneumonic plague [35]. This procedure could be accomplished at home or in a designated quarantine location. These spacesshould be available and ready to be used at any time with availability of all required supply**.** During emerging respiratory illnesses, there will be a need to isolate and quarantine staff. During SARS, there were government quarantine facilities, and those needing quarantine were positioned in individual rooms and meals were delivered [35]. For healthcare workers who had unprotected exposure to MERS-CoV, for example, it is required to quarantine themselves and SARS exposed staff were quarantined after exposure [36, 37]. In Hong Kong, 131,132 persons (50,319 close contacts and 80,813 travelers) were placed in quarantine [35].

Routine Audits and Rounds by the Infection Prevention and Control Staff

Infection control risk assessment through routine audits and rounds is essential to monitor and protect healthcare facilities. These activities are very important to monitor compliance with infection control practices but also important as IPC staff would utilize these rounds for education and training on the case definitions. Thus, more routine and more frequent rounds/visits by IPC staff to all hospitals areas/departments especially for high risk areas such as critical care, emergency rooms, hemodialysis, and burn units are recommended. It is important to ensure the best utilization of the IPC link nurses/staff to support the staff with this regards mainly after working hours. Hospitals had long been doing audit and feedback on fundamental concepts in infection control such as hand hygiene with the development of an improvement plan to increase compliance [38-40]. Thus, it is also very important to maximize these audits to include case definitions and understanding of emerging infectious diseases. Audit is based on five steps: choosing a topic, stipulating suitable practice standards, testing actual practice by collecting data, correcting practice, and then to show improvement in practice (closing the loop) [41, 42].

Immediate Recognitions and Isolation of Suspected Patients

It is essential that HCWs are well trained on the case definitions for any emerging infectious disease to allow prompt identification and isolation of such patients. Case definitions usually rely on the presence of symptoms and epidemiologic link. It is important to ensure proper implementation of isolation with minimum exposure to the patients. One strategy could use the Identify-Isolate-Inform tool. This tool was developed for Ebola virus disease containment and was adopted for other communicable diseases such as measles [43, 44].

Airborne Infection Isolation (AII) Rooms

Airborne Infection Isolation (AII) rooms, otherwise known as negative pressure isolation rooms, are structurally engineered spaces that contain airborne particles within it. Ensuring appropriate functioning of negative pressure isolation rooms is important in airborne infections. In a study in the USA, negative-pressure isolation rooms of surveyed hospitals with airborne precautions were available in 77% [45]. In another study in 2009 in USA, it is reported that 15% of hospitals does not have sufficient numbers of negative-pressure rooms to accommodate current isolation needs [46]. Although, hospitals might not have sufficient Airborne Infection Isolation rooms, healthcare organizations should have plans for interim AII rooms surge capacity allowing to convert rooms or areas to safely accommodate patients requiring AII on an emergent base pending the availability of a longer term AII rooms. Such surge capacity was indicated by 71% of organization in one survey [46]. SARS-CoV-2 is considered to be transmitted through contact and droplet as the mode of transmission, however, it is still strongly recommended to perform any aerosol generating procedures under negative pressure environment [47].

N-95 and Respirator Training and Availability
N-95 respirators are important parts of the personal protective equipment during the care of patients requiring airborne infection isolation (AII) precautions. The 'N' class indicates protection against non-oil-based aerosols and '95'represents that the respirator is at least 95% efficient at filtering particles with a median diameter >0.3 um [48]. Thee use of powered air-purifying respirator (PAPR) is needed for those who could not be fit tested. PAPR draws air through a filter and delivers a filtered air under positive pressure to a hood that is worn by the healthcare worker [49]. PAPR is much more expensive than N-95 respirators [50]. The use of PAPR was common during the SARS outbreak and in one study 84% preferred PAPR over N-95 respirator [50]. It is imperative to make sure that healthcare workers receive N-95 respirator fit testing or PAPR training. The purpose of fit-testing is to make sure that the healthcare worker has an N-95 respirator with the correct brand, model, and size designed that appropriately seals the face [51]. In addition, the training will focus on adequate training of donning and doffing of personal protective equipment and the practice of seal-check with each use of N-95 respirator [52]. It was found that N-95 fit testing reduced geometric mean exposures to airborne particles from 25% to 4% of ambient levels before and after quantitative fit-testing, respectively [53]. On the other hand, PAPR have high-efficiency particulate air (HEPA) filters which filter >99.97% of oil proof particles 0.3 µm in diameter [54]. Thus, it is important to have adequate supply and training on N-95. One study showed the availability of N-95 mask in 95% of Emergency departments [45]. Another study showed that there was no difference in video presentation, small group demonstration, and self-directed slide show just-in-time training modalities for N-95 fit testing [55].

CONCLUSION

The emergence of the COVID-19 pandemic had illustrated to all healthcare organizations, the need to be prepared for such occurrence. It might had been a theoretical risk but the COVI-19 had showed the reality. This review had shed some light on few areas of concern for healthcare organizations and further studies are needed to optimize preparedness.

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MERS-CoV and SARS-CoV Infections in Animals: A systematic review and meta-analysis of prevalence studies

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SUMMARY

Introduction: Coronaviruses are zoonotic viruses that include human epidemic pathogens such as the Middle East Respiratory Syndrome virus (MERS-CoV), and the Severe Acute Respiratory Syndrome virus (SARS-CoV), among others (e.g., COVID-19, the recently emerging coronavirus disease). The role of animals as potential reservoirs for such pathogens remains an unanswered question. No systematic reviews have been published on this topic to date.

Methods: We performed a systematic literature review with meta-analysis, using three databases to assess MERS-CoV and SARS-CoV infection in animals and its diagnosis by serological and molecular tests. We performed a random-effects model meta-analysis to calculate the pooled prevalence and 95% confidence interval (95%CI).

Results: 6,493articles were retrieved (1960-2019). After screening by abstract/title, 50 articles were selected for

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full-text assessment. Of them, 42 were finally included for qualitative and quantitative analyses. From a total of 34 studies (n=20,896 animals), the pool prevalence by RT-PCR for MERS-CoV was 7.2% (95%CI 5.6-8.7%), with 97.3% occurring in camels, in which pool prevalence was 10.3% (95%CI 8.3-12.3). Qatar was the country with the highest MERS-CoV RT-PCR pool prevalence: 32.6% (95%CI 4.8-60.4%). From 5 studies and 2,618 animals, for SARS-CoV, the RT-PCR pool prevalence was 2.3% (95%CI 1.3-3.3). Of those, 38.35% were reported on bats, in which the pool prevalence was 14.1% (95%CI0.0-44.6%).

Discussion: A considerable proportion of infected animals tested positive, particularly by nucleic acid amplification tests (NAAT). This essential condition highlights the relevance of individual animals as reservoirs of MERS-CoV and SARS-CoV. In this meta-analysis, camels and bats were found to be positive by RT-PCR in over 10% of the cases for both; thus, suggesting their relevance in the maintenance of wild zoonotic transmission.

Keywords: Coronavirus, SARS-CoV, MERS-CoV, serology.

INTRODUCTION

Rationale

Since 2002, the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), became an essential zoonotic pathogen, after the recorded epidemics of SARS taking place in China and other countries across East Asia. A decade later, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), originating in Saudi Arabia, emerged as the second most relevant zoonotic coronavirus [1, 2]. Currently, SARS-CoV, taxonomically, shares species level with other SARS-related coronaviruses within the subgenus *Sarbecovirus*. The subgenuses *Embecovirus*, *Hibecovirus*, *Merbecovirus*, and *Nobecovirus*, are all included within the genus *Betacoronavirus* (order *Nidovirales*; suborder *Cornidovirineae*; family *Coronaviridae*; subfamily *Coronavirinae*); while the MERS-CoV is part of the subgenus *Merbecovirus* [3-7].

As expected with other coronaviruses, SARS and MERS CoVs share many ecological and zoonotic aspects, as well as several clinical, epidemiological, and management features of the disease [8-11]. Structurally, these viruses are positive-strand RNA enveloped isolated from bats that share a high degree of sequence homology with human isolates, suggesting their role as likely natural hosts and reservoirs [4, 12-15]. The aforementioned raises the issue of the role and implications of animals as natural hosts and reservoirs for these viruses [10, 16, 17]. Thus, a better understanding of the frequency and transmission dynamics across the wild, suburban, and urban settings, from animals to humans (spillover), is of utmost importance [18-21]. Despite multiple studies, conducted mainly in humans, animal studies are still scarce, particularly addressing all available evidence on the prevalence of SARS-CoV and MERS-CoV in different animal hosts [22, 23] concisely.

Such findings would be of extreme importance to extrapolate in light of the ongoing expanding epidemics of the third highly relevant zoonotic coronavirus (SARS-CoV-2), currently causing the Coronavirus Disease 2019 (COVID-19), which is also believed to have originated from animals [22, 23], mostly bats in China [24-27]. For these reasons, we carried out a systematic review and meta-analysis to consolidate what has been found from each study assessing infection in animals with MERS-CoV and SARS-CoV by serological and molecular techniques.

It is essential to mention that still, to May 15, 2020, there is a lack of data for SARS-CoV-2 prevalence in animals. Some studies have focused on the phylogenetic analyses of SARS-CoV-2 regarding animals [28, 29], such as bats and pangolins [30]. Even more, some case reports and small series of natural infection of SARS-CoV-2, especially in cats, have also reported, and shortly a systematic review of SARS-CoV-2 disease in animals is highly expected.

Objectives

- To summarize the frequency of infection of animals reported on currently available observational studies for MERS-CoV and SARS-CoV.
- To examine the differences between the pool prevalence by technique, animals, and countries.
- To compare the significant differences in the

frequency of infection between SARS-CoV and MERS-CoV in animals by main serological and molecular techniques.

METHODS

Protocol
This protocol follows the recommendations established by the PRISMA statement [31].

Eligibility criteria
We included published peer-reviewed articles that reported infection in animals with serological or molecular confirmation of SARS-CoV or MERS-CoV. For serological tests, we considered Enzyme-linked Immunosorbent assay (ELISA), Indirect Immunofluorescence test (IFI), Immunofluorescence Antibody test (IFAT), pseudo-particle Neutralization test (ppNT), micro-neutralization test (mNT), and the MERS-COV antigen assay (MERS-CoV Ag assay). For molecular-based testing, Reverse Transcription Polymerase Chain Reaction (RT-PCR), and the Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) were included. Article language limit was not set, and we included publications from January 1, 2002, until the date the search was finalized and completed (February 1, 2020). Review articles, opinion articles, and letters not presenting original data were excluded from the study, as well as studies reporting on cases with incomplete information.

Information sources and Search Strategy
We conducted a systematic review using Medline/PubMed, Scopus, and Web of Sciences. The search terms used were as follows: "coronavirus", "SARS coronavirus 2019", "SARS-CoV", "MERS coronavirus 2019", "MERS-CoV". The searches were concluded by February 1, 2020, and four different researchers independently evaluated search results.

Study Selection
The results of the initial search strategy were first screened by title and abstract. The full texts of relevant articles were examined for inclusion and exclusion criteria (Figure 1). When an article reported duplicate information from the same patient, the data of both reports were combined to obtain complementary data, counting only as a single case. Observational studies that reported the frequency of animals infected due to SARS-CoV or MERS-CoV were included for quantitative synthesis (metanalysis).

Data collection process and data items
Data extraction forms, including information on the type of publication, the publishing institution, country, year, and date of publication, as well as the number of infected animals assessed by serological or molecular tests, were filled independently by four investigators. A fifth researcher checked the article list and data extractions to ensure there were no duplicate articles or duplicate information of the same study and also resolved discrepancies about study inclusion.

Assessment of methodological quality and risk of bias
For quality assessment, we used the Quality Appraisal of Case Series Studies Checklist of the IHE and specifically the critical appraisal tool to assess the quality of cross-sectional studies (AXIS) [32, 33]. Publication bias was evaluated using a funnel-plot. A random-effects model was used to calculate the pooled prevalence and 95% CI, given variable degrees of data heterogeneity, and given the inherent heterogeneity in any systematic review of studies from the published literature. Besides, Egger's test was performed.

Statistical approach
Unit discordance for variables was resolved by converting all units to a standard measurement for that variable. Percentages and means ± standard deviation (SDs) were calculated to describe the distributions of categorical and continuous variables, respectively. Since individual patient information was not available for all patients, we report weighted means and SDs. The baseline data were analyzed using the Stata version 14.0, licensed for Universidad Tecnológica de Pereira.
The meta-analyses were performed using Stata, and the software Open Meta[Analyst] [34] and Comprehensive Meta-Analysis ve.3.3® licensed for Universidad Tecnológica de Pereira. Pooled prevalences and their 95% confidence intervals (95% CIs) were used to summarize the weighted effect size for each study grouping variable using the binary random-effects model (the weighting took into consideration the sample sizes of the individual studies), except for median age, where a continuous random-effect model was applied

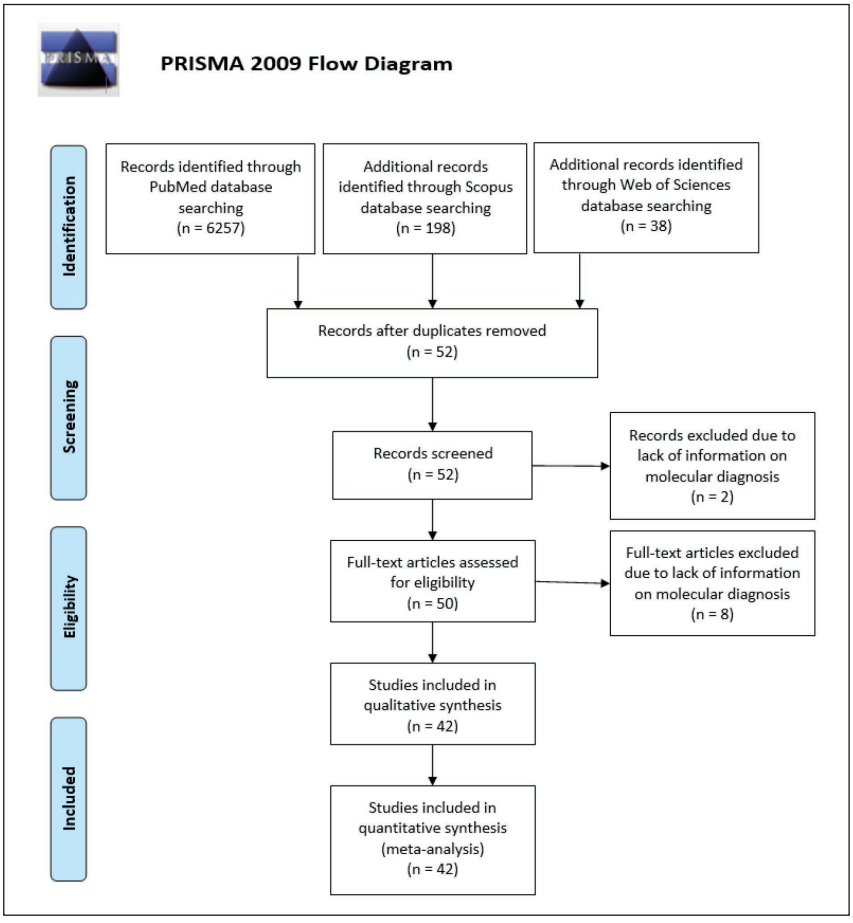

Figure 1 - Study selection and characteristics.

(DerSimonian-Laird procedure) [35, 36]. A random-effects meta-analysis model involves an assumption that the effects being estimated in the different studies are not identical, but follow some distribution. For random-effects analyses, the pooled estimate and 95%CIs refer to the center of the distribution of pooled prevalence but do not describe the width of the distribution. Often the pooled estimate and its 95%CI are quoted in isolation as an alternative estimate of the quantity evaluated in a fixed-effect meta-analysis, which is inappropriate. The 95%CI from a random-effects meta-analysis describes uncertainty in the location of the mean of systematically different prevalence in the various studies.

Measures of heterogeneity, including Cochran's Q statistic, the I^2 index, and the tau-squared test, were estimated and reported. We performed subgroup analyses by techniques, animals, and countries. And meta-analyses for each of the variables of interest. Publication bias was assessed using a funnel-plot. A random-effects model was used to calculate the pooled prevalence and 95% CI, given variable degrees of data heterogeneity, and given the inherent heterogeneity in any systematic review of studies from the published literature.

RESULTS

Study Selection and Characteristics

A total of 6,493 articles were retrieved using the search strategy. After screening by abstract and title, 50 articles were finally selected for full-text assessment. Of these, eight were excluded due to lack of information on molecular diagnosis, and 42 were finally included for final qualitative and quantitative meta-analysis (Figure 1). Our review included

42 studies that were published between January 1, 2002, and December 31, 2019, most of them from Kenya (18%), Saudi Arabia (16%), Egypt (10%), and Qatar (10%), including a total of 23,807 animals assessed by RT-PCR, and 8,604 by ELISA, 37 studies for MERS-CoV and 5 for SARS-CoV [37-77]. All the studies were cross-sectional. We analyzed 16 variables for the meta-analyses (Table 1). Publication bias was assessed with a funnel plot for the standard error by logit event, with no evidence of bias for MERS but with evidence for SARS. Additionally, the Egger test suggested that there was no notable evidence of publication bias on MERS (P=0.6708), but significant for SARS (P=0.0103).

Individual study characteristics

The mean of the number of included animals for RT-PCR per study was 384 and 374 for ELISA, with positive rates ranging from 0 to 100% in both coronaviruses.

Serological findings

Regarding the ELISA, the pool prevalence for MERS-CoV derived from 15 studies, including 7,648 animals, was 73.0% (95%CI 63.8-82.2%) (Table 1). In the case of SARS-CoV, with seven studies, with 947 animals, it was 3.0% (95%CI 0.4-5.5%) (Table 1).

The results for MERS-CoV with the IFI/IFAT techniques were similar, 83.9% (95%CI 66.0-100.0%) (no significant difference with the ELISA) (Table 1). Not enough studies with these techniques were available for meta-analyses of SARS-CoV. However, for SARS-CoV, the pool prevalence with the Western Blot, from 2 studies, with 44 animals, was 65.0% (95%CI 0.0-100.0%). Similarly, for MERS-CoV, there were not enough studies with Western Blot available for meta-analyses (Table 1).

Molecular findings

Regarding the RT-PCR, the pool prevalence for MERS-CoV derived from 34 studies, including

Table 1 - Meta-analysis outcomes (random-effects model)*.

Coronavirus, technique, animals, countries	*Number of Studies*	*Pool Prevalence (%)*	*95%CI*	*n*	*Q†*	*I²‡*	*t²§*	*p*
MERS Studies								
ELISA	15	73.0	63.8-82.2	7,648	1271.924	98.899	0.032	*<0.001*
IFI/IFAT	4	83.9	66.0-100.0	322	53.402	94.382	0.031	*<0.001*
RT-PCR	34	7.2	5.6-8.7	20,896	1719.949	98.081	0.001	*<0.001*
Camels	20	10.3	8.3-12.3	20,330	1705.777	98.89	0.011	*<0.001*
Qatar	5	32.6	4.8-60.4	177	110.178	96.37	0.01	*<0.001*
United Arab Emirates	4	16.0	5.8-26.2	8,166	100.376	97.01	0.01	*<0.001*
Saudi Arabia	5	15.4	0.0-37.2	2,509	799.239	99.5	0.01	*<0.001*
Egypt	3	7.7	0.0-16.5	4,013	175.581	98.86	0.01	*<0.001*
Kenya	13	0.4	0.2-0.6	3,830	7.724	0.0	0.01	*<0.001*
ppNT	9	26.8	6.2-47.4	1,066	6788.447	99.882	0.099	*<0.001*
Protein MicroArray	8	73.1	56.1-90.2	1,265	957.284	99.269	0.059	*<0.001*
mNT	15	41.8	21.0-62.6	4,837	9678.135	99.855	0.167	*<0.001*
SARS Studies								
ELISA	5	3.0	0.4-5.5	947	19.327	68.955	0.001	*<0.001*
RT-PCR	5	2.3	1.3-3.3	2,618	78.037	66.682	0.001	*<0.001*
Bats	2	14.1	0.0-44.6	1,004	77.578	88.37	0.005	*0.003*
Western-Blot	2	65.0	0.0-100.0	44	15.815	93.677	0.221	*<0.001*

*95% CI = 95% confidence interval. †Cochran's Q statistic for heterogeneity. ‡I2 index for the degree of heterogeneity. §Tau-squared measure of heterogeneity.
ELISA, enzyme-linked immunosorbent assay; IFI, Indirect Immunofluorescence; IFAT, immunofluorescence antibody test; RT-PCR, reverse transcription-polymerase chain reaction; ppNT, pseudoparticle neutralization; mNT, microneutralization test.

20,896 animals, was 7.2% (95%CI 5.6-8.7%) (Table 1). From the total number of animals, 97.3% corresponded to camels, in which pool prevalence was 10.3% (95%CI 8.3-12.3). In the case of SARS-CoV, with 2,618 animals in from 5 studies, the RT-PCR pool prevalence was 2.3% (95%CI 1.3-3.3). Of them, 38.35% were bats, in which the pool prevalence was 14.1% (95%CI0.0-44.6%) (Table 1).
Comparing the findings by countries, Kenya, Qatar, Saudi Arabia, United Arab Emirates, and Egypt, reported three or more studies for MERS-CoV in animals using RT-PCR (Table 1). The highest prevalence was found in Qatar (Figure 2), with five studies, including 177 animals, with 32.6% (95%CI 4.8-60.4%), followed by United Arab Emirates (UAE) (Figure 2), with four studies, including the highest number of animals, 8,166, for a pool prevalence of 16.0% (95%CI 5.8-26.2%) (no significant differences between both countries). Saudi Arabia yielded 15.4% and Egypt 7.7% (Figure 2). The lowest pool prevalence derived from Kenya (Figure 2), with 13 studies and 3,830 animals, with 0.4% (95%CI 0.2-0.6%), significantly lower than Qatar and UAE (Table 1).

DISCUSSION

A considerable number of studies have shown that the proportion of infected animals testing positive by molecular techniques, is an essential condition to consider the relevance of individual animals as reservoirs of MERS-CoV and SARS-CoV [8, 17, 78-80]. In this meta-analysis, positivity amongst camels and bats by RT-PCR was found in more than 10% of the evaluated animals, suggesting their possible role and importance in the maintenance of wild zoonotic transmission [40].
In 2012, the MERS-CoV was first detected in humans, and it wasn't until mid-2016 that 1,733 laboratory-confirmed human cases and 628 deaths were reported to the World Health Organization (WHO) from 27 countries [61]. The majority of these cases were reported from the Arabian Peninsula, but imported cases to other countries have also caused significant hospital-linked outbreaks, such as in South Korea, in 2015. Severe respiratory disease and death rate was higher in infections among older patients and those with preexisting conditions. Dromedary camels have been identified as a potential reservoir for the virus following the detection of the virus in camels in Saudi Arabia, Oman, and Qatar, and the detection of high seroprevalence levels of MERS-CoV antibodies in camel populations from a broader range of countries including countries in the Middle East and Africa. Most MERS-CoV infections in humans are not linked to camel exposure and are thought to be due to human-to-human transmission, particularly in health-care settings. The low frequency of camel-to-human infections is supported by the fact that MERS-CoV seroprevalence among the general human population in Saudi Arabia is less than 0.5%, with significantly higher seropositivity amongst camel shepherds (2.3%) and slaughter-

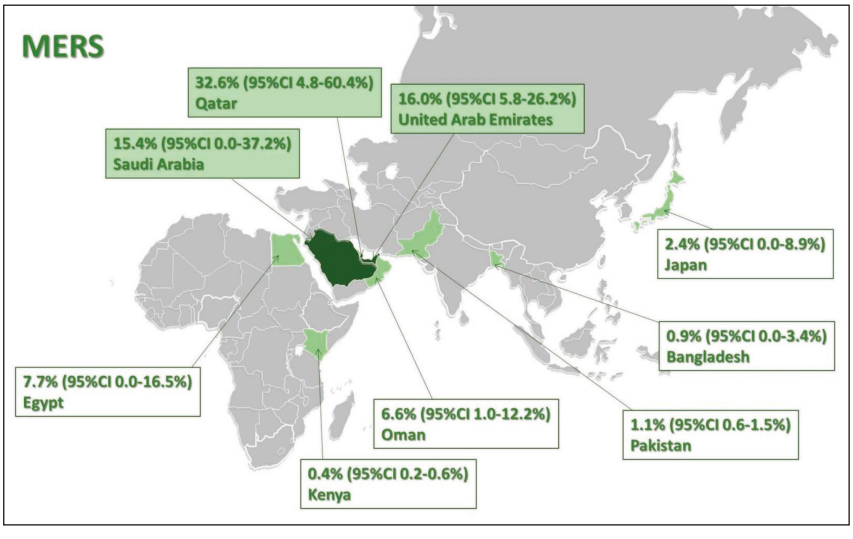

Figure 2 - Pooled prevalences of MERS-CoV in animals, by countries, obtained from the meta-analysis by the random-effects model.

house workers (3.6%) [61]. Nevertheless, results from 20 studies have shown that prevalence in camels is approximately 10.3%, ranging from 8.3 to 12.3 (95%CI); thus, incriminating camels instead as potential animal reservoirs.

According to the Food and Agriculture Organization (FAO), the world population of camels in 2001 was 19 million, of which, 17 million were dromedary camels, and approximately 65% of these were found in the eastern African countries of Sudan, Somalia, Ethiopia, and Kenya [61]. Kenya was found in this systematic review to have a pool prevalence of 0.4% for MERS-CoV, considering more than 3,800 animals. Even though the majority of dromedary camels are in Africa, no cases of MERS-CoV in humans have been reported in Africa, except for a cluster of three family members in Tunisia, back in 2013, which was linked to an imported index case with no history of exposure to camels [81]. Such findings may be related to the low frequency of infection among camels, as observed revealed in this systematic review. In such contexts, comparative genomics and phylogenetic studies focusing on viral sequences derived both from human hosts and dromedaries are essential to trace and link possible zoonotic transmission of MERS-CoV from dromedaries to humans [37].

A retrospective study carried out in Kenya detected MERS-CoV antibodies in more than 90% of camels from different regions of the country[43, 61]. A more recent study that analyzed > 1,000 human sera among pastoralists who did not keep camels reported two likely asymptomatic (< 0.2%) positive human cases for MERS-CoV by neutralizing antibodies detection [61, 82]. To better understand the risk of transmission between camels and humans living in close contact, more studies are needed, including more serosurvey or seroprevalence investigations amongst camels and humans within the same households to determine the prevalence of MERS-CoV antibodies as well as to determine the frequency of infection by molecular techniques and also establish which are the possible risk factors associated with seropositivity in camels and humans. Studies involving follow-up of herds of camels from time of calving through the first year of life with serial blood samples together with oral and rectal or fresh fecal swabs would better help define the ecology of the MERS-CoV-like virus infecting these animals and provide virus isolates for genetic characterization [53]. Another concerning issue is that the MERS-CoV is not only shed by nasal secretions and feces but also from milk (viral RNA), raising the possibility of food-borne transmission of MERS-CoV [65]. Also, a high proportion of camels presenting for slaughter in some studies show evidence for nasal MERS-CoV shedding [46], thus increasing the likelihood of potential airborne transmission. A recent systematic review, including studies published before December 31, 2018, reporting measures of seroprevalence or prevalence of hCoV-EMC or MERS-CoV RNA in dromedary populations was published [83]. Nevertheless, this systematic review did not proceed with the corresponding meta-analysis. In Saudi Arabia, they included studies ranging from 0.12-56%, which overlaps with our findings (95%CI 0-37.2%). For UAE, similarly, they reported 0-29%, also overlapping with the current report (95%CI 5.8-26.2%). And for Qatar, they reported 22-79%, while now we estimated the 95CI% in 4.8-60.4% [83]. Although consistent, our findings are more robust, pooled, and accurate.

Evidence suggests that MERS-CoV was present and circulating in camels some decades ago before MERS emerged, causing epidemics in the Middle East, as found in a study assessing blood samples from 1992, finding low-frequency antibodies (4.5%) in the Rift Valley of Kenya [43]. In another retrospective study surveying countries in Africa, (Somalia, Sudan, and Egypt) it was found that 189 archived serum samples from camels tested positive for MERS-CoV antibodies, as far as 1983, with 80% in Somalia and 86.7% in Sudan in 1984 and 85.2% in Somalia and 81.4% in 1997 in Egypt [59]. Also, camels have tested positive to MERS-CoV by serological and molecular-based methods (including genome sequencing) in different studies outside the Arabian Peninsula, and across Africa [42, 67]. In those countries, imported infected camels have also been a matter of concern, even in recent years [37]. For that reason, studies have also been carried in Australia and Japan [52]. However, preliminary data suggest that nor Australian or Japanese dromedaries are exempt from MERS-CoV infection, demanding further confirmatory studies [52, 70].

In addition to camels, the role of other animals in MERS transmission remains largely unknown. Molecular investigations have suggested that

bats in Saudi Arabia are infected with several alphacoronaviruses and betacoronaviruses. A virus isolated from 1 bat showed 100% shared nucleotide identity to a human virus from an index case-patient. An increasing body of research suggests that bats may play a role in human infection [57]. A wide range of CoV species is known to circulate among bats in Saudi Arabia [57]. Although the prevalence of CoVs was high (≈28% of fecal samples), MERS CoV was found in only one bat [57]. A 3.5% MERS CoV infection rate (n = 29; 95% CI 0–20%) in *Taphozous perforatus* bats is low compared with that for severe acute respiratory syndrome-like CoV in rhinolophid bats in China (10%-12.5%) but consistent with CoV prevalence among bats in Mexico [57, 84]. Bats are reservoirs of several viruses that can cause human disease, including rabies, Hendra, Nipah, Marburg, severe acute respiratory syndrome CoV, Ebola, rabies, and even some arboviral diseases, such as dengue and Venezuelan Equine Encephalitis viruses [57, 85-90]. Although in the current systematic review, we were not able to find enough prevalence studies of MERS-CoV in bats for a meta-analysis, we did find more studies relating bats to SARS-CoV, in which 14% a pool prevalence was found after analyzing more than 1000 specimens. Cross-species transmission from bats to humans can be direct, through contact with infected bats or their excreta, or facilitated by intermediate hosts, probably also in MERS-CoV, but especially for SARS-CoV [91].

Bat CoVs are typically host specific; however, MERS-related CoVs have reportedly been found in many bat families, including *Vespertillionidae, Molosidae, Nyteridae*, and *Emballonuridae* (sheath-tailed bats) in Africa, the Americas, Asia, and Europe [57]. In addition to bats and camels, the presence of MERS-CoV in other animals has been investigated, including the alpaca (*Vicugnapacos*), a native *Camelidae* species from South American and a close descendent of the vicuña, which has proved naturally susceptible to MERS-CoV infection. Such findings prompt future studies to determine the role of alpacas as an additional livestock reservoir for MERS-CoV in other areas of the Middle East [68] . In a survey carried out in Qatar, an endemic area for MERS, alpacas were found positive to MERS-CoV-specific antibodies with reciprocal titers ranging from 49 to 773 [68]. These findings raise essential questions regarding the possibility that some regions of South America would be suitable for MERS-CoV transmission and established endemicity, as well as for other zoonotic coronaviruses. In these same lines, the genus *Vicugna,* which includes the *V. vicugna* (vicuña), another South American camelid, also deserves further investigation regarding its possible susceptibility to infection by MERS-CoV and other coronaviruses. In some countries of South America, the llama, another camelid, widely used as a meat and pack animal by Andean cultures since the Pre-Columbian era, could also prove susceptible to MERS-CoV infection demanding a careful investigation.

In Saudi Arabia, other animals have also been scrutinized, resulting in negative to MERS-CoV, as is the case for sheep, goats, cattle, and chicken [53]. Similar results have been found in Egypt, assessing not only sheep, goats but also water buffalos and cows, testing even negatively [64]. In that same study, more than 93% of camels tested positive for antibodies by ppNT and mNT, exhibiting a high prevalence [64].

In contrast, a study in Egypt following on serum microneutralization assay (mnT) found that one serum sample from a sheep (1/51, 2%) revealed 1:640 neutralizing titer [38]. This same study found negative results from other domestic animals such as cattle, goats, donkeys, buffalo, and horses, but also bats. Using the mnT, they found 84% positivity in camels, with RT-PCR positive confirmation of around 4% [38]. Sheep were also found to test negative for MERS-CoV in a study from the United Arab Emirates [58].

Interestingly also primates, such as the *Papioanubis*, rodents such as *Acomyskempis, Acomyspercivalli, Elephantulusrufescens, Gerbilliscus robustus, Aethomyshindei, Myomyscusbrodernani, Grammonysdolichorus, and Saccostomusmeamsi*, were screened for MERS-CoV in a study from Kenya, testing all negative by RT-PCR [47]. Unfortunately, the authors of this study did not assess blood samples of those animals by serological tests. In a similar study from Kenya, using IFI/IFAT, authors found seropositivity rates as high as 94% in camels [43].

Data derived from a longitudinal study in camels performed in Saudi Arabia, provided evidence for reinfection of previously seropositive camels, suggesting that prior infection does not provide complete immunity from reinfection. This finding is relevant to camel vaccination strategies as

a means to prevent zoonotic transmission [51]. These results may be of interest for MERS-CoV and other coronaviruses in humans, as is the case of Coronavirus Disease 2019 (COVID-19), in which there is also a concern for possible reinfections in humans throughout the ongoing 2020 outbreak in China. In the specific case of MERS-CoV in camels, it appears that infections do not elicit long-lasting (mucosal) immunity [46, 56].

Besides reinfection, coinfection with other coronaviruses is also a matter of pressing concern. In a 2019 study, results revealed the occurrence of MERS-CoV and HKU8r-CoV co-circulation in camels. The study also suggested the possibility of circulation of a recombinant coronavirus virus with the spike of MERS-CoV and the nucleocapsid of an HKU8r-CoV in Kenya. However, the authors failed to provide molecular evidence of an HKU8r-CoV or a putative recombinant virus [76].

In contrast to MERS-CoV, SARS-CoV has also been detected in studies from different animals, besides bats, in China, and other countries such as Kenya, but also from pigs, implicating such species in possible zoonotic transmission [41, 60, 71, 73]. A study in China reported on the isolation of SARS-CoV from a pig during a survey aimed to determine potential routes of viral transmission short after the SARS epidemic, finding that the animal was in close contact with humans in a suburban area and its extended farming villages, Xiqing County of Tianjin, where a SARS outbreak occurred in late spring of 2003 [41].

The results of this systematic review highlight the importance of animals as reservoirs for coronavirus and their close link as zoonotic diseases, as for the case of MERS and SARS. Also, the increasing need for more field studies aimed to understand the main epidemiological features, ecological/environmental aspects, and the role of wild and domestic animals as drivers of these emerging viral infections [25, 92, 93]. Despite a growing volume of literature, further studies on many aspects of related to MERS-CoV and SARS-CoV are needed. Moreover, with the recent emergence of SARS-CoV-2 causing the COVID-19 epidemics, studies aimed at evaluating the role of animals reservoirs such as bats, camels, and other domestic animals as well as wild game, including pangolins, birds, snakes, and other reptiles and mammals, would be highly relevant, as to drawing the landscape on the origin of these coronaviruses as zoonotic pathogens, and their potential for global expansion [12, 24, 92, 94, 95].

As previously mentioned, soon will be essential to develop systematic reviews about the SARS-CoV-2 prevalence in different animals, as felines, dogs and other vertebrate seems to be susceptible according to various analyses, with still pending interpretations of the preliminary findings reported in the literature for transmission and endemicity mediated by animals of SARS-CoV-2/COVID-19 [28, 28, 96, 97].

Limitations

This review has several limitations. First, still few studies are available for inclusion, especially for SARS-CoV [98, 99]. It would be better to include as many more studies not only from the Middle East but especially from East Asia. Second, more detailed information on the collected and sample animals, particularly regarding their clinical findings and conditions during collection, was unavailable in most studies at the time of analyses; however, the data in this review permit a first synthesis of the frequency of infection due to MERS-CoV and SARS-CoV in animals, although the need to be more detailed for the last one.

CONCLUSIONS

Infection with MERS-CoV and SARS-CoV is considered crucial in animals given their reported frequency [99, 100]. These results, as mentioned, have not only implications for MERS-CoV and SARS-CoV but also the novel SARS-CoV-2, causing the COVID-19. Additional research is needed to elucidate multiple aspects of transmission, reinfection, coinfection, and many other ecological aspects of the disease, including the role of environmental issues related to their natural cycles. Future research should focus on developing studies that contribute to fully characterizing and defining the determinants of coronavirus zoonotic spillover and their linkages to make operational contributions for risk assessment [15]. The phenomenon of cross-species spillover is the defining characteristic of pathogens that transmit from vertebrate animals to humans, zoonoses, as is the case of MERS-CoV, SARS-CoV, and SARS-CoV-2. The public health burden imposed by zoonoses includes outbreaks of those pathogens that can lead to even more significant outbreaks,

as currently with the ongoing COVID-19 [29, 94]. Camels and bats are essential confirmed hosts of MERS-CoV and SARS-CoV, respectively. The role of other animals remains an entirely unanswered question, but a link between these viruses and other mammals remains a latent possibility.

Author contributions

DKBA and AJRM formulated the research questions, designed the study, developed the preliminary search strategy, and drafted the manuscript. MCCT, AGB, YHR, ICB, HABA, and LJPC refined the search strategy by conducting iterative database queries and incorporating new search terms. MCCT, AGB, YHR, ICB, HABA, LJPC, DKBA, and AJRM searched and collected the articles. AJRM and DKBA conducted the quality assessment. All authors critically reviewed the manuscript for relevant intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of interest

All authors report no potential conflicts, except AJRM. AJRM is a COVID-19 consultant for Abbott Laboratories de Colombia S.A.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Differences among confirmed and not-confirmed COVID-19 patients at "D.Cotugno" hospital, Naples (Italy): what we learned from first suspected cases?

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SUMMARY

Clinical presentation of COVID-19 is common to other respiratory infections. We compared the characteristics at hospital admission of confirmed and not-confirmed COVID-19 patients, in the early phase of the epidemic. Thirty-seven suspected patients were enrolled, and COVID-19 was confirmed in 17. Confirmed patients are older, have more frequently contact with confirmed cases. Distinctive clinical characteristics among COVID-19 were the ground-glass opacities at CT scan, and a pO2/FiO2 ratio less than 250. In not-confirmed group, Influenza represented the most frequent alternative diagnosis. This study contributes to highlight the characteristics to consider at hospital admission in order to promptly suspect COVID-19.

Keywords: COVID-19, SARS CoV-2, Hospital admission, Diagnosis.

INTRODUCTION

In Italy, an autochthonous transmission of COVID-19 was identified in late February, causing a cluster of thousands of cases mainly in Northern Italy [1, 2]. In Campania Region, Southern Italy, the first case was detected on February 26, 2020.
The spectrum of this illness is wide, ranging from mild disease to rapidly evolving severe pneumonia. The complete understanding of the clinical picture is still evolving, and new disease manifestations are continuously reported [3, 4]. At presentation, most common symptoms include fever, cough, shortness of breath and, less commonly, asthenia, arthro-myalgias, conjunctivitis, diarrhoea [3, 5, 6]. The most frequently reported symptoms are common to other infections affecting the respiratory tract, most of which are self-limiting, but some of them, especially Influenza, may also cause severe conditions [7]. In the very early phase of the COVID-19 epidemic, the differential diagnosis was challenging for clinicians, and epidemiological criteria mostly driv-

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en the clinical pathway. This situation may occur again in the future, in case of a second wave or in case of resurgence of COVID-19 in upcoming months or years.
In order to support clinicians in this diagnostic challenge, we report epidemiological, clinical, radiological and laboratory findings among suspected COVID-19 patients in the very early phase of the epidemic at "D. Cotugno" hospital in Naples, Italy, comparing confirmed and not-confirmed cases, and describe alternative diagnoses among not-confirmed cases.

PATIENTS AND METHODS

We conducted a retrospective observational study. The setting of the study is represented by two medical divisions of "D. Cotugno" hospital, a mono-specialistic infectious diseases referral centre sited in Naples, Campania, Southern Italy. The hospital has a 24h-open Emergency Department dedicated to Infectious Diseases urgencies, and a virology laboratory representing the Regional referring site for SARS-CoV-2. These divisions were selected because were the first identified and equipped, within the hospital, for the management of COVID-19.
We included in the study all patients consecutively admitted in the selected medical wards because of clinical suspicion of COVID-19 since the beginning of the epidemic to March 10, 2020. No exclusion criteria were applied. Demographical, epidemiological, clinical, radiological and laboratory data were collected consulting the electronic clinical database.
Diagnosis of COVID was performed through different RT-PCR methods targeted to different genomic region (regions RdRp, N and E) on nasopharyngeal swab (the commercial kits used changed during the study period). According to National protocols, all positive samples were confirmed by another RT-PCR, targeted to another genomic region, at Italian National Institute of Health (Istituto Superiore di Sanità, ISS) in Rome. Otherwise, among patients with negative samples, the repetition of test, at least 24 hours apart, is required to exclude the COVID-19 diagnosis. Moreover, an extensive multiplex PCR panel for other respiratory pathogens (FilmArray Pneumonia Panel Plus, Biomerieux) was performed in most patients.
All patients have been managed according to clinical standard of care, and in accordance to ethical standards. All data included in the study have been presented anonymously and collectively, in order to avoid any recognizability of single patients included.

RESULTS

All the 37 patients observed at "D. Cotugno" Hospital in Naples for clinical suspicious of COVID-19, from February 10, when the first suspected case had been admitted, to March 10, 2020, were consecutively enrolled. The demographic, clinical, radiological and biochemical data are summarized in Table 1: 24 patients (65%) were males, median age was 37 years (IQR 28). Most patients (65%) reported as risk factor travels in affected area, 22% reported contact with a confirmed case, 25% reported contact with persons from affected area, while for the remaining 2 patients the epidemiological risk factor was unclear. Out of 37 admitted patients, 17 (46%) were confirmed for COVID-19. Table 1 described the demographic, clinical, radiological and biochemical characteristics of the confirmed patients versus the not confirmed ones. There was no different gender distribution, while COVID-19 patients were older than not confirmed (p=0.001). COVID-19 patients more frequently presented as a risk factor a contact with confirmed case (47% vs 0, p<0.001) and less frequently reported a travel in affected area (35% vs 95%, p<0.001). No differences were found in the clinical presentation at the time of admission between the two groups, and no differences was evidenced in the mean time from symptoms' onset to hospitalization. Similarly, there were no differences for initial laboratory findings except for procalcitonin which was higher in not confirmed patients (0.003 vs 0.1; p=0.004, data available for 27 out of 37 patients). All patients underwent to a radiological examination: chest computerized axial tomography was performed for 18 (49%) patients, 12 (67%) in the confirmed COVID-19 patients and for 8 (43%) not confirmed patients. In the group of COVID-19 confirmed more frequently patients showed imaging related to ground-glass opacities and consolidation (GGO) respect to not confirmed patients (83% vs 37%, p=0.03). Diagnostic tests for alternative diagnosis have been performed in 27 out of 37 patients: among

11 patients in the confirmed group and among 15 patients among not confirmed. An alternative diagnosis has been established in 10 not confirmed patients, while 2 co-infections were detected among confirmed patients: in 2 confirmed COVID-19 patients there was *H. influentiae* superinfection and the 60% of not confirmed patients presented Influenza virus B infection. Other alternative diagnosis are detailed in Table 1. Confirmed COVID-19 patients most frequently had severe respiratory symptoms considering that 41% of these had PaO2/FiO2 ≤ 250 vs 0 patients in the not confirmed group, while the value of MEWS (Modified Early Warning Score), used at admission to stratify the patients' severity, showed no significant difference.

Table 1 - Demographical, epidemiological, clinical, biochemical and radiological characteristics among COVID-19 confirmed and not-confirmed patients.

Characteristics	*Total*	*A. Confirmed patients*	*B. Not-confirmed patients*	*P A vs B*
Number	37	17	20	
Demographic				
Gender (Males n, %)	24 (65)	11 (65)	13 (65)	0.980
Age (median, IQR)	37 (28)	49 (58)	29 (11)	*0.001*
Epidemiologic characteristics				
Risk factor for COVID-19:				
Contact with confirmed case (n, %)	8 (22)	8 (47)	0	*<0.001*
Travel in affected area (n, %)	25 (68)	6 (35)	19 (95)	*<0.001*
Contact with persons form affected area (n, %)	2 (5)	2 (12)	0	0.115
Other (n, %)	2 (5)	1 (6)	1 (5)	0.960
Comorbidities (n, %):	14 (38)	9 (53)	5 (25)	0.081
Time from onset of symptoms to hospitalization (mean, SD)	3.8 (+2.9)	4.7 (+2.9)	3.0 (+3)	0.089
Clinical characteristics at hospital admission				
Fever (n, %)	36 (97)	16 (94)	20 (100)	0.272
Cough (n, %)	28 (76)	12 (70)	16 (80)	0.560
Dyspnea (n, %)	11 (30)	7 (41)	4 (20)	0.160
Arthralgies (n, %)	6 (16)	3 (18)	3(15)	0.828
Conjunctivitis (n, %)	3 (8)	1 (6)	2(10)	0.647
Other (n, %)	4 (11)	0	4 (20)	0.058
Chest computerized axial tomography (n, %)	18 (49)	12 (67)	8 (43)	
Ground-glass opacities (GGO) (n, %)	13(72)	10(83)	3 (37)	*0.03*
Laboratory findings				
WBC count (mean, SD)	7891+4048	7037 (4908)	8616 (4100)	0.290
Neutrophil total count (mean, SD)	5594+3880	4745 (4716)	6317 (3957)	0.277
Lymphocyte count (mean, SD)	1480+607	1567 (625)	1406 (617)	0.437
C Reactive Protein (mean, SD)	3.1+4	3.4 (4.8)	2.8 (4.2)	0.687
Procalcitonin* (mean, SD)	0.06+0.11	0.03 (0.1)	0.1 (0.1)	*0.041*
Alternative diagnosis (n, %): **	12	2(17)	10(83)	*0.001*
Influenza A virus	1(8)	0	1(10)	
Influenza B virus	6 (50)	0	6(40)	
Haemophilus influentiae	3 (26)	2(100)	1(10)	
Parainfluentiae virus	1(8)	0	1(10)	
Streptococcus pneumonie	1(8)	0	1(10)	
Severity of clinical presentation:				
MEWS ≥3 (n, %)	7	2 (12)	5 (25)	0.306
Clinical indication to hemogasanalysis (n, %)	17	15 (88)	2 (10)	*<0.001*
PaO2/FiO2 ratio (mean, SD)	343+154	341 (140)	359 (66)	0.611
PaO2/FiO2 ≤250 (n, %)	7	7 (41)	0	*0.001*

*: performed in 27 out of 37 patients; **extensively performed in 27 out 37 patients.

DISCUSSION

In this study, epidemiological, clinical, radiological and laboratory differences among confirmed and not-confirmed COVID-19 patients were explored. No clear clinical clues peculiar of COVID-19 were not identified: the clinical picture is common to other seasonal respiratory infections. On the contrary, the reduction of PaO2/FiO2, and radiological pattern showing GGO may represent useful indications for suspecting COVID-19. The findings of this study may support clinical decisions during the initial evaluation of a suspected patients, when an etiological diagnosis is still pending, and support the decision to start promptly a specific treatment even before the diagnosis.

This study represents a window on the early phase of the epidemic in Southern Italy. We believe that similar epidemiological situations may occur again, for example in other parts of the world currently initially affected, or in the case of a COVID-19 "second wave" or resurgence after initial control.

The main limit of this study is represented by the limited number of patients. We decided to stop the enrolment on March 10, 2020 in order to give a picture of initial outbreak phase, during which some decisions were driven mostly by epidemiological factors more than criteria of clinical severity. Indeed, in the initial phase, the need for an early diagnosis and prompt isolation of suspected cases is higher, so as to limit as more as possible the community spreading of the disease: for this reason, patients with mild disease were admitted to hospital, also.

Despite these limits, some interesting remarks emerge. During this early phase COVID-19 patients are older than not confirmed patients: this finding is in line with several reports that identify older patients as those at higher risk for symptomatic and severe presentation of COVID-19 [5, 8]. Moreover, many not-confirmed patients were admitted, even with mild diseases, for the presence of an epidemiological risk factor, in particular travels to affected areas of Northern Italy: this population, mainly travelling for study and work reasons, is younger. Similarly, the differences emerged among risk factors were probably driven by the relevance attributed to epidemiological criteria at the beginning of the outbreak.

The main remark is represented by the absence of any key clinical and laboratory signs that is distinctive for COVID-19 confirmed patients at hospital admission. Procalcitonin only is higher among not-confirmed patients, as also reported in another study [9], but its level, in any case, is not clinically important. Similarly, the use of MEWS at admission is not useful, in our study, to identify COVID-19 patients. According to our observation, the radiological picture showing a GGO and the presence of a PaO2/FiO2 ratio lower than 250 are significantly predictive of COVID-19, only. Of interest is the spectrum of alternative diagnosis among not confirmed patients: 60% was infected with the influenza virus. This finding is in line with a recent similar report form Italy [10], and with the period of the study, when Influenza seasonal outbreak was still ongoing. Of note, two COVID-19 patients have a superinfection with *H. influentiae*.

In conclusion, even if the number of patients is small, we believe that this study can support clinicians to select clinical, biochemical and radiological characteristics to consider at hospital admission, and to promptly suspect COVID-19, even before the laboratory confirmation of the diagnosis. The findings of these study may be even more useful in epidemiological situation like that present in the early phase of the epidemic. Moreover, the results of this study also support the performance of an extensive diagnostic panel for respiratory pathogens, in order to exclude bacterial and viral superinfections in COVID-19 patients and to obtain etiological diagnosis in not confirmed patients.

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Conflicts of interest

All authors declare to have no conflict of interest.

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Prognosis of COVID-19: Changes in laboratory parameters

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SUMMARY

Introduction: Since December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome - Coronavirus 2 (SARS-CoV-2) emerged in China and has become a global threat. Comparison of hematological parameters between mild and severe cases of SARS-CoV 2 is so far limited, but significant differences in parameters such as interleukin-6, d-dimers, glucose, fibrinogen and C-reactive protein have been already reported.
Purpose: In this study we analyzed the changes observed in easily measured blood biomarkers in the patients and provided evidence of how these markers can be used as prognostic factors of the disease.
Methods: Demographic characteristics, detailed medical history, and laboratory findings of all enrolled SARS-CoV 2 infection positive patients who were referred to Patras University Hospital from the period of March 4th 2020 (when first confirmed case in Greece appeared in our hospital) until April 4th 2020 were extracted from electronic medical records and analyzed.
Results: We provided evidence that some very common laboratory values can be used as independent predictive factors in SARS-CoV 2 infection. Despite the retrospective nature of this study and the small number of subjects analyzed, we showed that NLR, LDH, d-dimers, CRP, fibrinogen and ferritin can be used early at the patient's first visit for SARS-CoV 2 infection symptoms and can predict the severity of infection.
Conclusion: More studies are warranted to further objectively confirm the clinical value of prognostic factors related to SARS-CoV 2 and establish an easy-to-get panel of laboratory findings for evaluating the disease severity.

Keywords: SARS CoV 2, risk factors, disease severity, laboratory tests.

INTRODUCTION

In December 2019, the city of Wuhan in China became the center of an outbreak of pneumonia of unknown reason. The outbreak of Severe Acute Respiratory Syndrome - CoronaVirus 2 (SARS CoV 2) has rapidly spread throughout the world [1]. Although the outbreak is likely to have started from a zoonotic transmission, recent reports have provided evidence for person to person transmission in family and hospital settings via direct contact or through droplets spread by coughing from an infected person [2-4].
The clinical spectrum of the disease varies from asymptomatic infection, mild upper respiratory symptoms to severe pneumonia with respiratory failure and even death. The most common symptoms at onset of SARS-CoV 2 illness are fever, cough, ache, dyspnoea, haemoptysis and diarrhoea. The severe symptoms of SARS-CoV 2 are associated with an increase rate of fatalities [2]. Both clinical and epidemiological features of patients with COVID-19 demonstrate that this kind of infection can cause clusters of severe respiratory illness leading to intensive care unit admissions and high mortality rates [3].
The outbreak of SARS CoV 2-induced coronavirus disease 2019 (COVID-19) has put health authori-

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ties on high alert in China and across the globe. As a new type of highly contagious viral infection in human, the pathophysiology of unusually high pathogenicity for SARS-CoV 2 has not yet been completely understood [1]. Epidemiological and clinical characteristics of patients with SARS-CoV 2 have been reported but risk factors for mortality and clinical course of illness, including viral shedding, have not been well described.

The aim of this retrospective study was to assess the epidemiology of the disease for the first month period since the first case was identified at the largest reference center in western Greece and evaluate the related to disease blood biomarkers, as information on specific laboratory data between severe and moderate COVID-19 is limited.

MATERIAL AND METHODS

Data collection

Demographic characteristics, detailed medical history, and laboratory findings of all enrolled SARS-CoV 2 infection positive patients who were referred to Patras University Hospital from the period of March 4th 2020 (when first confirmed case in Greece appeared in our hospital) until April 4th 2020 were extracted from electronic medical records and analyzed. Written informed consent was obtained from all patients enrolled in the study.

Demographic characteristics from all study subjects were analyzed. Parameters from peripheral blood counts included white blood cells (WBC), lymphocytes absolute count (ALC) (<1100K/μL or >1100 K/μL), absolute monocyte count (K/μL), coagulation parameters (PLTs, fibrinogen, D-dimers), platelets markers (PDW and MPV), C-reactive protein, lactate dehydrogonase (LDH), creatinine kinase (CPK), and neutrophil to lymphocyte ratio (NLR). The presence of lung infiltrations are also included in the analysis from chest x-rays or CT scan where available. Patients were categorized based on sequential organ failure assessment (SOFA) score. Hospitalization was also included in the analysis and outcome.

Statistical analysis

Statistical analysis of data was performed using SPSS-25 statistical software. Statistical values were expressed as mean ± SD. The minimum value of the level of statistical significance, p-value, in all statistical tests was set at 0.05. The Pearson correlation was used for studying relationship between variables and the Mann-Whitney test was used to investigate the differences of a continuous variable to two different and independent population groups.

RESULTS

In this retrospective, one-center study, a total number of 64 adult patients (≥18 years old) with laboratory-confirmed SARS-CoV 2 using PCR were enrolled. All patients were evaluated at the Emergency Department of Patras University Hospital during the period from 4th March 2020 until 4th April 2020. SOFA score was used to categorize the patients in two groups: those with SOFA score >2 (group 1) were classified as having severe COVID-19 infection and were admitted to the hospital (67.2%) and those who had a SOFA score <2 (group 2), were considered moderate and were treated in an outpatient setting (32.8%).

The median age of all patients was 57.11±16.3 years old. 47.7% were men and 52.3% were women. 58.14% of the severe cases were male. The median age of the severe cases was significantly higher than moderate ones (62.2±13.4 vs 47.1±16.2 years old, p= 0.001). Co-morbidities were present in twenty patients (30.8%). Hematological or other malignancies were observed as the most common comorbidity (30%), followed by hypertension (25%), atrial fibrillation (15%), hyperlipidemia (10%), diabetes (10%) and multiple sclerosis (10%). The most common symptoms on admission were fever and cough. X-ray and/or CT scan abnormalities (lung infiltrations) were found in 29 patients

Table 1 - Patients' demographic characteristics.

Parameters	*Admitted patients*	*Outpatients*
Males(n)/Females(n)	25/23	6/10
Age (yrs)	62±13.4	47±16.2
Comorbidities		
Malignancies	5	-
Hypertension	3	1
Arrhythmia	3	-
Diabetes	1	1
Multiple sclerosis	1	1
Other	3	1
Heterozygous thalassaemia	6	2

(45.3%). All patients' demographic characteristics are shown in table 1.

We then examined the plasma levels of acute reactant proteins; C-reactive protein, ferritin, and fibrinogen. In the majority of the cases these factors were increased. Analysis of all patients revealed that CRP was above 0.5 mg/dL in 64.6% of patients, ferritin was above 300mg/dl in 63.9% patients, and fibrinogen levels were over 400 mg/dl in 81.1% of the subjects examined. D-dimers were above the threshold of 500μg/dl in 69.2% of the cases. Similar results were observed for LDH, which was above the normal threshold of 230 U/l in 73.6% of cases (normal limits 120-220 U/l). Then we analyzed these factors for the two patient groups separately (SOFA score>2; group 1, SOFA score <2; group 2). All factors analyzed were statistically significantly increased in group 1 compared to group 2; ferritin (1572±3512 vs 266±426, p=0.03), LDH (323±134 vs 211±55, p=0.019), d-dimers (1498±1613 vs 481±338, p:0.024), CRP (4.78±4.5 vs 2.64±4.8, p=0.01) and fibrinogen (556.5±151 vs 402.5±83, p=0.004) were increased among patients who were admitted to hospital compared to those treated at home. (Figure 1)

Further analysis was performed on complete blood counts in all patients. Lymphocyte and monocyte count, and neutrophil to lymphocyte ratio (NLR) was examined. Moderate monocytosis (500-1000 absolute monocyte count) was observed in 43.75% of the cases, and severe monocytosis (>1000 absolute monocyte count) was found in 3.1 % cases. Analysis of the absolute monocyte count between the two groups did not reveal statistically significant differences between them (group 1 vs group 2: 580±185 vs 540±231, p=0.54). It is notable that examination of peripheral blood smears of patients revealed activated monocytes in few cases, mainly in patients from group 1. NLR >3 was observed in 43.1% of patients. The NLR in group 1 was statistically significant increased compared to group 2 (4.09±2.9 vs 2.9±0.99, p:0.001). Lymphopenia (<1100 K/μL) occurred in 46.3% of patients. Absolute lymphocyte count (ALC) in both groups were as follows: group 1 vs group 2: 1792±3404 vs 1662±395 (p:0.809). Platelet count was also examined. Patients with SOFA score above 2, had slightly lower platelet count compared to group 2 (242.3±105 vs 249.3±84.2, p:0.619). All measurements are presented in Figure 2. Mean platelet volume (MPV) and platelet distribution width (PDW) were examined in all patients. There were no statistical significant differences in both MPV and PDW between the two groups. Absolute lymphocyte count <1100/μL was observed as a negative predictive factor in SARS-CoV 2 infection.

Eight patients (12.5%) had beta-thalasaemia trait,

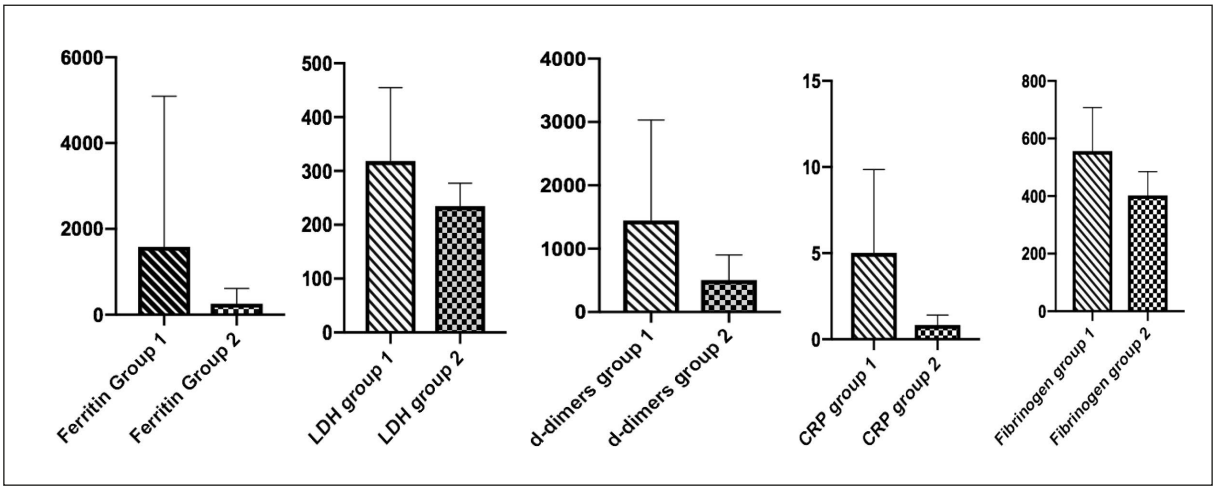

Figure 1 - Measurements of ferritin, LDH, d-dimers, CRP and fibrinogen represented per group. The box plots show the data from all analyzed patients. In every box plot, we show the mean values and the standard error of the means. In every box plot, the upper line is the highest measurement detected. All measurements are grouped as 1, 2: group 1: patients with SOFA score >2 and were admitted to hospital, group 2: patients with SOFA score < 2, and were treated at home).

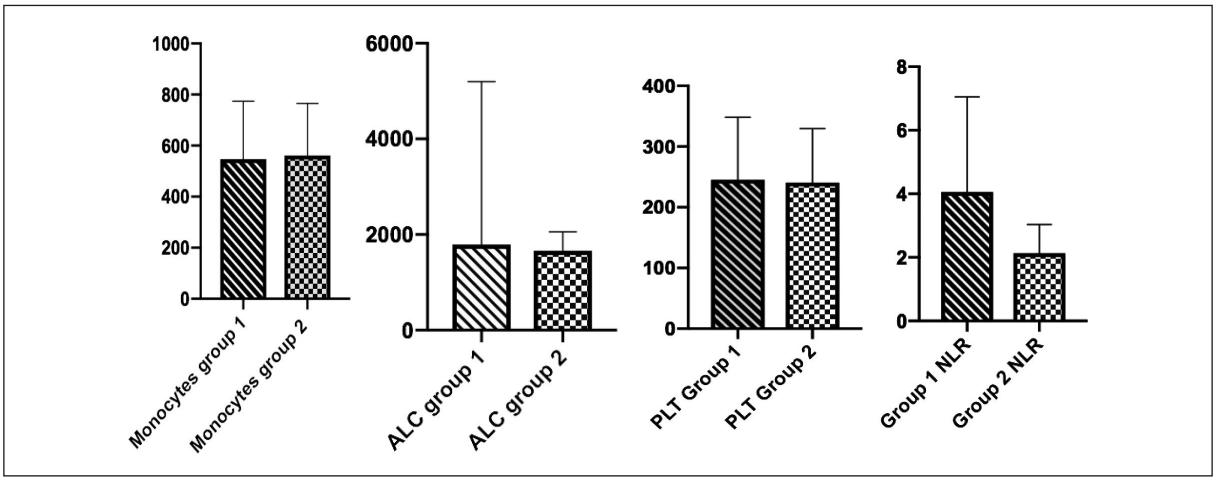

Figure 2 - Measurements of monocytes, lymphocytes absolute count, platelets and NLR represented per group. The box plots show the data from all the patients analyzed. In every box plot, we show the mean values and the standard error of the means. In every box plot, the upper line is the highest measurement detected. All measurements are grouped as 1, 2: group 1: patients with SOFA score >2 and were admitted to hospital, group 2: patients with SOFA score <2, and were treated at home).

and 6 of them (75%) were included in the admitted to Hospital group. Five of 43 severe cases (11.63%), died during the study period. The median age of deceased cases was 61.4 years old. Four were male and one female. Remarkably, in all patients who have died, absolute lymphocyte count upon admission was lower than the cut off of 1100K/μL and NLR ratio as well as LDH were very high.

We further compared NLR, CRP, and LDH with radiological findings (lung infiltrations) at first visit of all patients included in the study. These three factors were statistically significant higher in all patients with radiological findings during their first visit at the hospital compared to those with no findings (p=0.002, p=0.048 and p=0.002 respectively).

DISCUSSION

The pandemic of SARS-CoV 2 caused by the Severe Acute Respiratory Syndrome -Corona Virus 2, has led to numerous cases and deaths around the world, as the new virus is spreading far more quickly and is very contagious [5]. Although a good knowledge has been gained on clinical

Table 2 - Laboratory blood tests of all examined patients. The minimum value of the level of statistical significance, p-value, in all statistical tests was set at 0.05.

Parameters	*All patients*	*Admitted (Group 1)*	*Outpatients (Group 2)*	*P value*
CRP (mg/dL)	4.38±4	4.78±4.5	2.64±4.8	*0.01*
Ferritin (mg/dl)	1457±3327	1572±3512	266±426	*0.03*
LDH (mg/dl)	302±130	323±134	211±55	*0.019*
NLR	1.5±0.5	3.98±2.9	3.09±2.38	*0.001*
Monocytes (K/μL)	550±220	580±185	540±231	0.54
Lymphocytes	2457±8496	2677±9438	1511±938	0.809
Platelets (K/μL)	244±99	242.3±105	249.3±84.2	0.619
d-dimers (μg/dl)	1227±1464	1498±1613	481±338	*0.024*
Fibrinogen(mg/dl)	532±153	556±151	402±83	*0.004*

features of SARS-CoV 2 infection, less clear information has been provided on laboratory abnormalities [6]. In the present study we aimed to identify any possible relation between laboratory tests and the disease severity, additionally any tests that could work as potential risk factors for prediction of disease progression and severity [5].

In consistence to previous reports, the present study showed that male are more susceptible in developing severe disease [7]. It is already known that older males (>50 years), particularly those with underlying co-morbidities, may be more likely to develop severe SARS-CoV 2. Moreover, although SARS-CoV 2 infection has a relatively low mortality rate, it can be highly deadly and lethal, especially in high risk patients with co-morbidities. The reported incidence of SARS-CoV 2 infection accompanied to underlying co-morbidities in the literature were up to 26%. We reported a similar incidence rate of 30.8%, with malignancy being the most common co-morbidity (30%).

In terms of laboratory results, there were obvious differences between severe and non-severe cases in LDH, d-dimers and inflammatory markers including CRP, ferritin, fibrinogen and NLR. Our analysis revealed statistically significant elevated CRP, ferritin and NLR in the group of patients who were admitted to hospital, suggesting the close relation of SARS-CoV 2 infection and inflammation [8]. Although lymphocytopenia has been well described in a retrospective analysis of patients in Hong Kong and Singapore afflicted with SARS in 2003 and was associated with adverse outcomes and ICU admission, we did not find statistically significant differences between the two groups of patients we studied [9]. It is thought that SARS-CoV 2 infection is associated with coagulation as well. Regarding d-dimers and fibrinogen, the statistically significant elevation that was found in more severe cases, may be related to the activated and accelerated response to infection. Coagulation has also an immune function which can be hence considered another line of defense against severe infections [6]. All these findings would imply that routine blood tests may be additional useful tools for improving early prognosis and provide more intensive treatment.

In addition, platelets are important immune cells in hemostasis, coagulation, vascular integrity maintenance, angiogenesis, anti-inflammatory response Changes in their number and activity are closely related to a variety of diseases [10]. Previous studies have shown that severe infections can cause thrombocytopenia, which is characterized by a rapid platelet decline. It has also been suggested that a consistently present low grade disseminated intravascular coagulation (DIC) may cause a low platelet count in SARS. Furthermore, low platelet count is associated with increased risk of severe disease and mortality in patients with SARS-CoV 2 infection and has been suggested to serve as clinical indicator of worsening illness during hospitalization [11]. In our study, we found that platelets were slightly lower in severe ill patients compared to the moderate ones, but there were not statistically significant differences, and this can be attributed to small sample. Nevertheless, six patients had thrombocytopenia (PLT<150.000K/μL). All of them developed severe respiratory failure during hospitalization needing mechanical ventilation support. A possible explanation could be that the lung may be one of the organs in which mature megakaryocytes release platelets and that thrombocytopenia in patients with SARS-CoV 2 infection may be associated with lung damage observed in that type of infection [10]. Moreover, injury of lung tissue and pulmonary endothelial cells can lead to activation, aggregation and retention of platelets in the lung and the formation of thrombus at the injured site, which may lead to the depletion of platelets and megakaryocytes, resulting in decreased platelet production and increased consumption [10].

Due to alterations in coagulation, not only the platelet count but also the platelet function should be carefully assessed. In some studies it has been suggested that platelets distribution width (PDW) and mean platelet volume (MPV) could be useful tools to evaluate the activation of coagulation or thrombocytosis-related disease [12]. In our cases, no statistically significant differences in MPV and the PDW between severe and no-severe ill patients was found.

It is remarkable that we found higher NLR in severe ill patients and that was statistically significant related to the disease severity [8]. NLR, a well-known marker of systemic inflammation and infection, has been studied as a predictor fac-

tor of infections. The increase of NLR in our study, is consistent with the findings from another study [13] where several patients with SARS-CoV 2 infection had a rising neutrophil count and a falling lymphocyte count during the severe phase [1, 13]. In regards to hemoglobulin disorders, so far very little clinical experience of infected patients with such disorders, especially beta-thalassaemia trait, has been recorded. We reported that 8 patients with beta-thalassaemia trait were included in our study subjects, and six of them (75%) were severely ill and required hospitalization. Haemoglobin disorders are generally not associated with respiratory conditions. However, complications involving the heart, lungs and the immune system, can be present in these patients and in a SARS-CoV-2 positive patient may trigger very serious complications [14].

Our study provides a list of potential predictor markers for SARS-CoV 2 severity. We found a statistically significant correlation between SOFA score and LDH, NLR and ferritin. Especially for LDH, Tsui et al reported that elevated LDH level on admission of SARS-CoV 2 patients, was independent predictor factor of an adverse clinical outcome [15, 16]. Moreover, NLR, LDH, ferritin, d-dimers, fibrinogen and CRP were statistically significant higher in the group of patients who were hospitalized. Therefore, the combination of the above easily measured markers, in SARS-CoV 2 infected patients, even in the Emergency Department may predict more serious disease progression.

In an earlier published study, of 41 patients with laboratory confirmed SARS-CoV 2 infection, Huang et al reported a mortality rate of 15% (6 deaths among 41 patients) [7]. We reported comparable results with a mortality rate up to 11.63% (5 deaths among 43 severe ill patients).

In this study, we provide evidence for the first time to our knowledge that some very common laboratory values can be used as independent predictive factors in SARS-CoV 2 infection. Despite the retrospective nature of this study and the small number of subjects analyzed, we showed that NLR, LDH, d-dimers, CRP, fibrinogen and ferritin can be used early at the patient's first visit for SARS-CoV 2 infection symptoms and can predict the severity of infection. These markers can be used in every hospital setting while expecting confirmation of SARS-CoV 2 PCR results and guide clinicians to provide the best treatment options to these patients. More studies are warranted to further objectively confirm the clinical value of prognostic factors related to SARS-CoV 2 and establish an easy-to-get panel of laboratory findings for evaluating the disease severity.

Conflicts of interest

The authors declare no conflicts of interest.

Funding sources

None

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Risk of hepatic failure in COVID-19 patients: A systematic review and meta-analysis

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SUMMARY

Liver injury has been reported to occur during the disease in severe cases. Therefore, this meta-analysis study aims to investigate the incidence of liver injury among published literature from 2019-Jan-01 to 2020-April-03 to provide an outline for further studies on the liver injury of COVID-19.

Four databases including Pubmed, Embase, Web of Science, and the Scopus were searched for studies published from 2019-Jan-01 to 2020-April-03. Data analysis and drawing of charts were performed using the Comprehensive Meta-Analysis Software Version 2.2 (Biostat, USA).

The search yielded 450 publications, of which 64 potentially eligible studies were identified for full-text review and 21 studies fulfilling the inclusion criteria remained. A total of 4191 COVID-19 patients were included in our meta-analysis. The pooled prevalence of liver injury was 19.5% (95% CI: 14.3-26.1). According to our results, there was significant heterogeneity among the 19 studies (X^2=738.5; p<0.001; I_2 =94.34%). Among 288 death cases, the pooled prevalence of liver injury was 22.8% (95% CI: 11.7-39.8).

In summary, the COVID-19 disease itself can result in severe and even fatal respiratory diseases and even may lead to ARDS and multiple organ failure. The results of this systematic review highlight the importance of liver injury that may assist clinicians anywhere in the globe in controlling COVID-19-related infection and complications. Moreover, the prevalence of liver injury can be higher in severe cases than in mild cases.

Keywords: SARS-CoV-2, COVID-19, Liver injury, Meta-analysis.

INTRODUCTION

Since December 2019, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) emerged in Wuhan city of central China [1]. These viruses can cause respiratory, intestinal, hepatic, and neuronal diseases and may lead to acute respiratory distress syndrome (ARDS), multiple organ failure (MOF), and even death in severe cases [2, 3]. Mild cases of COVID-19 presented symptoms like fever dry cough, fatigue, vomit, and diarrhea [4]. In severe cases, respiratory distress and/or hypoxemia occurred one week after the onset of the disease and then deteriorated into ARDS, septic shock, metabolic acidosis, and even death [5]. Recent studies have reported the presence and expression of angiotensin-converting enzyme 2 (ACE2) as a functional receptor for SARS-CoV-2 in pulmonary epithelial cells. Also, it has been observed probably in cardiomyocytes and renal tubular epithelial cells [6-8]. To date, a comprehensive analysis of clinical manifestations of COVID-19 revealed that SARS-CoV-2 infection not only caused severe acute respiratory syndrome but also multiple organ injuries, including lymphocyte reduction, myocardial dysfunction, and even acute renal failure. In many clinical sur-

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veys, liver dysfunction has also been observed, indicating a possibility that in patients with COVID-19 may cause hepatic injury [9].
Recent investigations on complications of COVID-19 have revealed that the occurrence of liver injury ranged from 14.8% to 53%. Also, it is accompanied mainly by abnormal ALT/AST levels followed by slightly elevated bilirubin levels [10]. The proportion of liver injury in death cases and severe COVID-19 patients was significantly higher than that in mild patients [11, 12]. Currently, studies on the proportion of liver injury caused by SARS-CoV-2 are limited.
Thus far, several studies have investigated the characteristics of liver injury caused by SARS-CoV-2 infection; however, a larger number of systematic reviews are needed to understand the proportion of liver injury in COVID-19 patients. Therefore, this meta-analysis study aims to investigate the incidence of liver injury among published literature from 2019-Jan-01 to 2020-April-03 to provide an outline for further studies on the liver injury of COVID-19.

METHODS

Search strategies
A systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines by searching databases including Pubmed, Embase, Web of Science, and the Scopus from 2019-Jan-01 to 2020-April-03 to find relevant studies.
The search strategy was based on the following keywords: COVID-19, severe acute respiratory syndrome coronavirus 2, novel coronavirus, SARS-CoV-2, nCoV disease, SARS2, COVID19, Wuhan coronavirus, Wuhan seafood market pneumonia virus, 2019-nCoV, coronavirus disease-19, coronavirus disease 2019, 2019 novel coronavirus, Wuhan pneumonia, "Liver injury" OR "Liver abnormality" OR "Liver damage" OR "hepatic damage" OR "liver function abnormality" OR "hepatocellular injury". These keywords were used in the titles, abstracts, and keywords fields. The reference list for each selected paper and relevant review articles were checked to identify missing studies.

Selection criteria and quality assessment
Two reviewers checked the search results in the databases with relevant keywords independently and analyzed the titles, abstracts, and full texts to apply eligibility for inclusion according to inclusion criteria, and discrepancies were resolved through discussion. No restriction based on the publication language was set, but at least the abstract must be available in English.
Included studies met the following inclusion criteria: patients were confirmed and diagnosed according to the criteria recommended by WHO (i.e., epidemiological history, clinical symptoms, and laboratory or radiological findings), and the data for complication findings and liver abnormalities were included. All of the studies that reported any kind of hepatic failure were included. Duplicate publications, unpublished papers, case reports, reviews, animal studies, and letters were excluded. Studies with lacking information about patients' characteristics and complications were also excluded.
Only available data from published articles were collected. If all laboratory findings related to liver function were reported but the rate of Liver injury of any population studies was not reported, it was regarded as "not available" and excluded from the meta-analysis. The procedure of the literature search is shown in Fig. 1.

Quality assessment and data extraction
Reporting of Observational Studies in Epidemiology (STROBE) statement was used for assessing the quality of studies independently by two researchers, and any disagreements were resolved by consensus [13]. Criteria related to title and abstract, introduction, methods, results, discussion, and other information were assessed and a score was assigned to each item. Then, the following data were extracted from eligible studies by two researchers including first authors, location of study, year of publication, detection methods of SARS-CoV-2, age of patients, the sample size of confirmed COVID-19, and the incidence of liver injury. Inconsistencies between the researchers were discussed to reach consensus.

Statistical analysis
The meta-analysis was performed using the random-effects model to estimate the pooled prevalence and corresponding 95% confidence interval (CI). Heterogeneity between studies was assessed using the Cochran's Q statistic and I-square (I2) test. Publication bias was assessed graphically

using a funnel plot and mathematically using the Begg's rank correlation and Egger's weighted regression test. Through these analyses, P <0.05 was considered indicative of statistically significant publication bias. Analysis of data and construction of graphs was performed by Comprehensive Meta-Analysis Software Version 2.2 (Biostat, USA).

RESULTS

The search yielded 450 publications, of which 64 were identified as potentially eligible for full-text review. Finally, 21 studies fulfilled the inclusion criteria (Figure 1, Table 1) [3, 5, 9, 12, 14-29]. Also, except for one study performed in the USA, all studies were conducted in China. The sample size of the studies ranged from 21 to 788 patients. The real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was applied to detect SARS-CoV-2 infection. A total of 4191 COVID-19 patients were included in our meta-analysis. Moreover, 288 death cases were included.

Nineteen studies have considered liver injury among patients suffering from COVID-19. The pooled prevalence of liver injury was 19.5% (95% CI: 14.3-26.1) (Figure 2). According to our results, there was significant heterogeneity among these 19 studies (X^2 = 738.5; p< 0.001; I= 94.34%).

Also, Begg's and Egger's tests were performed to evaluate the publication bias. Based on the results of Begg's (z = 0.31, p = 0.75) and Egger's tests (=0.24, p=0.4), there was no significant publication bias (Figure 3).

Among the 288 death cases, the pooled prevalence of liver injury was 22.8% (95% CI: 11.7-39.8) (Figure 4). Based on our results, there was significant heterogeneity among the four studies (X^2=296.2; p<0.01; I^2 = 85.93%). Moreover, Begg's and Egger's tests were performed to evaluate the publication bias. Based on the results of Begg's (z=0.31, p=0.75) and Egger's tests (t=1.05, p=0.4), there was no significant publication bias. The funnel plot for publication bias did not show any evidence of asymmetry (Figures 3 and 5).

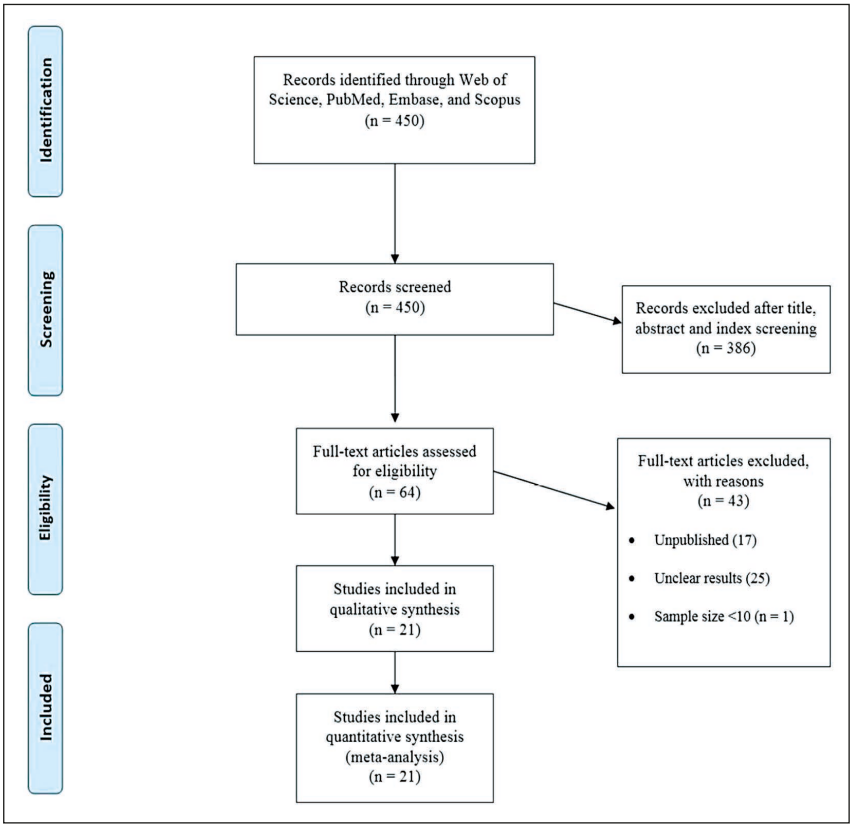

Figure 1 - Flow chart of the study selection for inclusion in the systematic review.

Table 1 - The characteristics of studies included in the meta-analysis a) active patients, b) dead patients.

a)

Number	*Author*	*Location*	*Year*	*Age (Median)*	*SARS-CoV-2 Detection method*	*Case of COVID 19 No.*	*Case with liver damage or injury after admission No.*	*Ref*
1.	Fu et al.	China	2020	-	RT-PCR	355	101	[20]
2.	Zhenyu et al.	China	2020	50.5	RT-PCR	148	41	[9]
3.	Ya et al.	China	2020	44 in older group 1.3 in younger group	RT-PCR	32	10	[21]
4.	Na et al.	China	2020	53.8	RT-PCR	40	22	[27]
5.	Chen et al. (A)	China	2020	56.3	RT-PCR	21	1	[16]
6.	Qingxian et al.	China	2020	47	RT-PCR	298	44	[15]
7.	Wu et al.	China	2020	46.1	RT-PCR	80	3	[25]
8.	Chen et al. (B)	China	2020	55.5	RT-PCR	99	43	[17]
9.	Jin et al.	China	2020	45.1	RT-PCR	651	64	[22]
10.	Chen et al. (C)	China	2020	62	RT-PCR	387	23	[18]
11.	Zhang et al. (A)	China	2020	46.6	RT-PCR	645	81	[29]
12.	Arentz et al.	USA	2020	70	RT-PCR	21	3	[14]
13.	Tang et al.	China	2020	67	RT-PCR	73	33	[3]
14.	Wang et al.	China	2020	69	RT-PCR	404	118	[5]
15.	Yang et al.	China	2020	59.7	RT-PCR	52	15	[12]
16.	Xieet al.	China	2020	60	RT-PCR	79	29	[26]
17.	Lian et al.	China	2020	68.2 in older group 41.1 in younger group	RT-PCR	788	82	[23]
18.	Liu et al.	China	2020	68 in older group 47 in younger group	RT-PCR	56	10	[24]
19.	Zhang et al. (B)	China	2020	57	RT-PCR	140	8	[28]

b)

Number	*Author*	*Location*	*Year*	*Age (Median)*	*COVID-19a detection*	*Death case of COVID 19 No*	*Case with liver damage or injury after admission No*	*Ref*
1.	Li et al.	China	2020	73	RT-PCR	25	5	[35]
2.	Chen et al.	China	2020	68	RT-PCR	113	10	[18]
3.	Wang et al.	China	2020	76	RT-PCR	65	22	[5]
4.	Du et al.	China	2020	65.8	RT-PCR	85	30	[19]

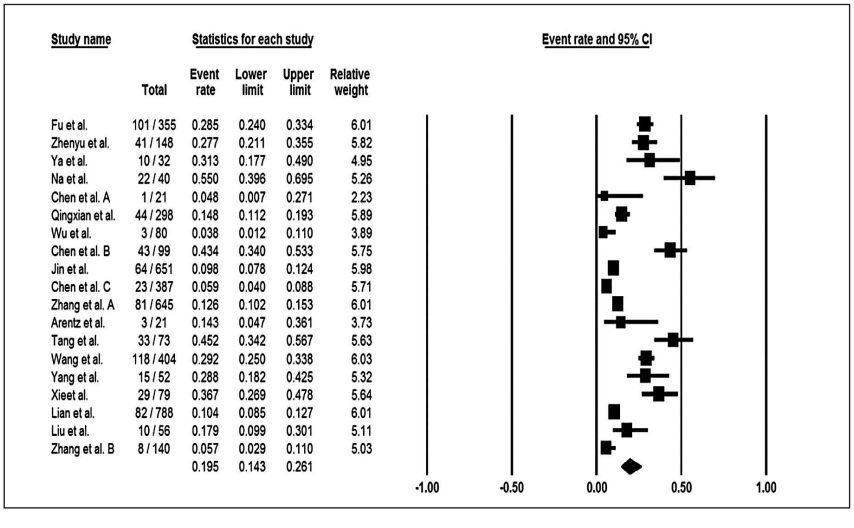

Figure 2 - Forest plots of the overall prevalence of liver injury among active COVID-19 patients.

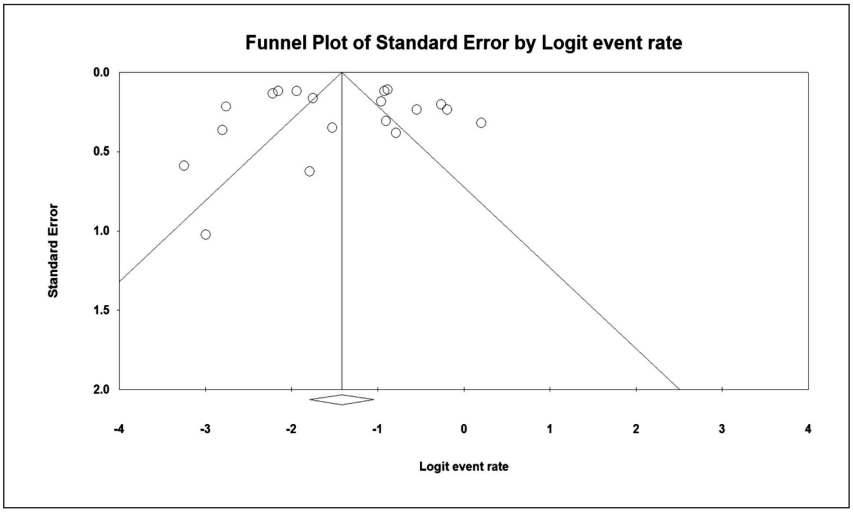

Figure 3 - Funnel plot of publication bias for the included studies (Active patients).

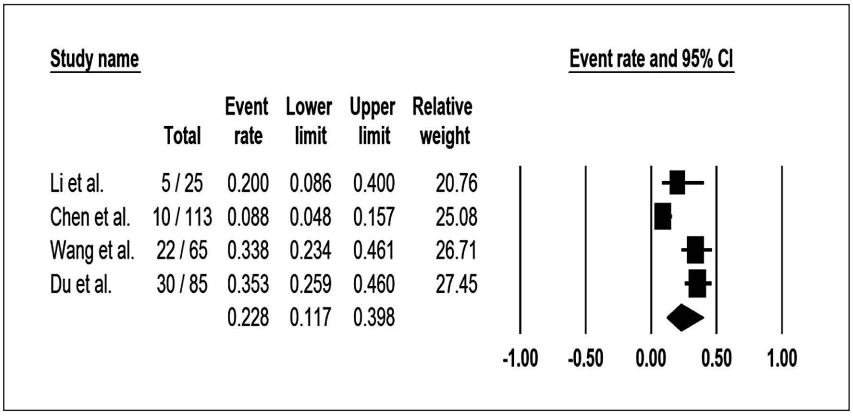

Figure 4 - Forest plots of the overall prevalence of liver injury among dead COVID-19 patients.

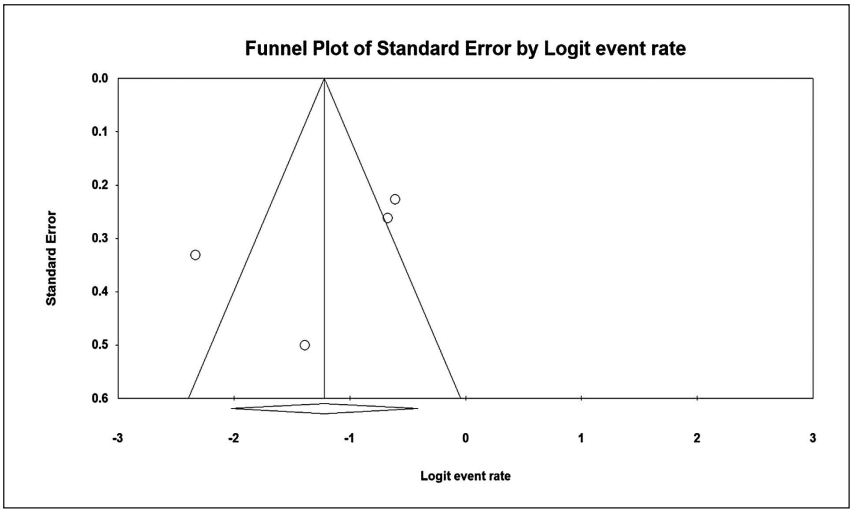

Figure 5 - Funnel plot of publication bias for the included studies (dead patients).

DISCUSSION

In this study, we assessed the liver injury and epidemiologic features of COVID-19 to gain a better insight into this complication caused by SARS-CoV-2. According to our analysis, the pooled prevalence of liver injury in these patients was 19.5%. The previous meta-analysis revealed that acute hepatitis was the most common complication in 13.3% of cases, followed by cardiac injury [30]. These results are lower than the rate reported in our study. The main reason for the discrepancy of liver injury may be due to a small sample size of the previous meta-analysis and the data was reported only in 3 studies, while 19 studies were analyzed in our study [30]. The range of liver injury among the included studies was between 3% and 55% [21, 25]. Liver damage has been considered as an important risk factor for severe outcomes and death in some viral infections including MERS and SARS [10]. Previous studies have shown that liver injury mainly is investigated using elevated the level AST, ALT, and Total Bilirubin followed by slightly decreased Albumin levels [31]. Findings from this meta-analysis and other studies supported the hypothesis that liver injury is the most frequently damaged outside of the respiratory system [10, 30].

SARS-CoV-2 uses ACE2 as an entry receptor to enter into a host cell in the lungs, kidneys, and heart. Previous studies showed that endothelial cells of the liver cells and bile duct cells abundantly express ACE2 [32]. Therefore, it has been shown that the liver is a potential target for SARS-CoV. Moreover, the previous finding suggested that the liver damage might be due to the damage to bile duct cells, but not liver cells by the virus infection in COVID-19 patients [10].

Another important finding is a significant heterogeneity in the pooled analysis. This heterogeneity may be due to different sample sizes, various populations studied, and different assessment criteria for liver injury; therefore, the results should be interpreted with caution.

The mechanisms of liver injury during SARS-CoV-2 infection remain mainly unclear. Several factors may contribute to liver injuries such as viral infection in liver cells or other causes including antibiotics, antivirals, and steroids, psychological stress, systemic inflammation induced by cytokine storm, and pneumonia-associated hypoxia induced by liver injury [33]. However, there is insufficient evidence for SARS-CoV-2 infected hepatocytes or virus-related liver injury in COVID-19 at present. In this regard, Fan et al. suggested that some drugs such as lopinavir/ritonavir should be prescribed with caution. In this regard, their results revealed that a significant proportion of hospitalized patients with impaired liver function had received lopinavir/ritonavir after admission [9].

The current study showed that the pooled prevalence of liver injury (22.8%) in death cases is slightly higher than the active cases. This finding showed that severe or death cases of COVID-19 have a higher percentage of liver injury compared to mild cases of COVID-19; this result is consistent with the findings in previous reports [26, 34].

According to a systematic review for sex distribution of COVID-19-related liver dysfunction by Feng et al., the proportion of infected men with liver injury was higher than that reported in infected women. Moreover, the age distribution of COVID-19-related liver dysfunction indicated that none of the children had abnormal serum liver enzymes and probably older age is associated with a higher likelihood of liver damage/dysfunction [1].

As one restriction of this study, interpretation of our meta-analysis findings might be limited by the small sample size.

In summary, the COVID-19 disease itself can result in severe and even fatal respiratory diseases and lead to ARDS and multiple organ failure. In this study, we reported the rate of liver injury caused by SARS-CoV-2 infection. The results of this systematic review highlight the importance of liver injury that may assist clinicians anywhere in the globe in controlling the COVID-19-related infection and complications. Moreover, the prevalence of liver injury can be higher in severe cases than in mild cases.

Competing interests
The authors declare that they have no competing interests.

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CT features of coronavirus disease 2019 (COVID-19) pneumonia: experience of a single center in Southern Italy

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SUMMARY

Purpose. The aim of this study was to report the radiological features of chest CT scan of patients with coronavirus disease 2019 (COVID-19) living in a town in Southern Italy where a significant outbreak of the disease occurred.
Methods. We revised the CT scan of 62 patients (34 male, 28 female, mean age 71+/-14 years) with clinical and laboratory signs of COVID-19, as assessed by positive SARS-CoV-2 RT-PCR testing. All patients underwent chest CT at the time of admission to the hospital. A semi-quantitative scoring system was used to evaluate the extension of the disease.
Results. Out of the 62 patients the main radiological findings were reticular pattern (29%), ground-glass opacities (24%), crazy paving pattern (11%) and consolidation (35%). Most of the lesions were bilateral (97%), posterior (95%) and located near pleura (50%) or lung fissures (45%), mainly involving the lower right lobe (56%) and lower left lobe (23%). Pleural thickening was observed in 72.6% of patients and pleural effusion in 18%. Median value of the score was 7.0 and was significantly higher in male than female (8.5 vs 6.0, p=0.03) and in patients with pleural thickening compared to those without this finding (8.0 vs 5.0, p=0.03)
Conclusions. We have observed patients with different stages of the disease. Lung score was significantly higher in male than female confirming the clinical observation of a worst prognosis in male subjects. Pleural thickening was frequently observed and significantly associated with a higher lung score suggesting a possible association with a more severe disease.

Keywords: COVID-19, Severe Acute Respiratory Syndrome Coronavirus 2, pneumonia, Tomography, X-Ray Computed.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly infectious disease of zoonotic origin caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1, 2]. This disease was firstly described in Wuhan, Hubei Province, China, and has rapidly spread to other countries [3, 4]. The World Health Organization (WHO) declared the outbreak to be a public health emergency on 30 January 2020 and recognized it as pandemic on 11 March 2020.
Fever is often the major and initial symptom of COVID-19, which may be accompanied by other aspecific symptoms such as dry cough, muscle ache, headache, sore throat, rhinorrhea [3-5]. Some patients experienced dyspnea and/or hy-

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poxemia usually one week after the onset of the disease and in severe cases quickly progressed developing an acute respiratory syndrome. In many cases, radiologic findings are the main precocious manifestation of the diseases and can give a great support for diagnosis, as reported in a wide range of infections [6-11].

SARS-CoV-2 has been reported to utilize angiotensin-converting enzyme-2 (ACE2) as cell receptor in humans [12], causing pulmonary interstitial damages followed by parenchymal changes. Chest imaging plays a pivotal role in the diagnosis and management of patients with COVID-19 and it has been reported that chest computed tomography (CT) examination has a sensitivity of 98% [13]. Chest CT images could show different imaging features or patterns in COVID-19 patients with a different time course and disease severity. The radiological findings of COVID-19 pneumonia have been reported in several papers mainly involving Chinese population. For these patients, a radiologic score able to precociously identify those with the highest risk of an unfavorable outcome can be useful, as we demonstrate in patients with other pathologic conditions [15-20].

Italy was the first western county to be extensively involved by COVID-19 pandemic. The disease has mainly diffused in the Northern regions of the country, but "hot spots" have also been detected in other regions. In this study we report the radiological findings detected at CT scan of COVID-19 patients living in Ariano Irpino, a town in Southern Italy where a significant outbreak of the disease occurred.

PATIENTS AND METHODS

We reviewed the chest CT scan of patients with suspected COVID-19 pneumonia evaluated at the Radiology Unit of Sant'Ottone Frangipane Hospital in Ariano Irpino (Italy) between March 5th 2020 and April 3th 2020. The study was approved by local ethics committee (Comitato Etico Campania Nord-Ovest)

The study group included 62 patients (34 male, 28 female, mean age 71 +/- 14 years) evaluated at the time of admission to the hospital. All patients presented the clinical and laboratory signs of COVID-19 infection, as assessed by positive SARS-CoV-2 RT-PCR testing. All patients underwent High Resolution Chest CT using the GE OPTIMA 64 Slice Tomograph and the images were examined by three independent radiologists with more than 10-year experience on chest CT imaging.

The lesions detected at CT scan were described as follow:

- *ground-glass opacities* (GGO, hazy areas with slightly increased density in lungs without obscuration of bronchial and vascular margin);
- *reticular pattern* (thickened pulmonary interstitial structures such as interlobular septa and intralobular lines that present as a collection of small linear opacities on CT images);
- *crazy paving* (thickened interlobular septa and intralobular lines with superimposition on a GGO background, resembling irregular paving stones);
- *consolidation* (homogeneous increased intensity of lung parenchyma that obscures the margins of underlying vessels); in some cases, an *air bronchogram* (pattern of air-filled bronchi on a background of opaque airless lung) was visible in the context of an area of consolidation;
- *vascular enlargement* sign (dilated small vessels around and within the lesions due to the damage and swelling of the capillary wall caused by pro-inflammatory factors);
- *air bubble sign* (small air-containing space in the lung which might be the pathological dilation of a physiological space, or a cross section of the bronchiolectasis, or associated with the process of consolidation resorption).
- *Bronchiectasis*
- *Pleural effusion*
- *Pleural thickening*

Distribution of the lung lesions was classified as pleural (involving mainly the peripheral region near to pleura), close to lung fissures and parenchymal (involving mainly the central region of the lung).

A semi-quantitative scoring system was used to quantify the extension of the disease [15,16]. Each of the 5 lung lobes was visually evaluated and scored from 0 to 5 according to its involvement: 0, no involvement; 1, <5% involvement; 2, 6-25% involvement; 3, 26%-49% involvement; 4,50%-75% involvement; 5, >75% involvement. The total CT score was the sum of the individual lobe scores and ranged from 0 (no involvement) to 25 (maximum involvement).

Statistical Analysis
Data were analyzed using statistical software (SPSS, version 22.0, IBM). Continuous variables were expressed as median and percentile distribution (25° and 75° percentile) and were analyzed using the Mann-Whitney *U* test.

RESULTS

Radiological lesions
The CT features of COVID-19 pneumonia are summarized in Table 1. The most frequent lesion was the reticular pattern (Figure 1a) detected in 50/62 patients (81%). Other lung radiological lesions observed at CT scan were GGO (Figure 1b) in 42/62 patients (68%), crazy paving pattern (Figure 1c) in 15/62 (24%) and consolidation in 33/62 (53%). In these last cases an air bronchogram (Figure 1d) within the consolidation area was visible in 15/33 (45%). Only in few patients a single lung lesion was present (only GGO in 6 patients, only reticular pattern in 5, only crazy paving in 5 and only consolidation in 3). In most patients (43/62, 69 %) more than one lesion was present, so we grouped the patients according to the main radiological findings (Table 2). Out of the 62 patients, the main radiological lesions were consolidation in 22 (35%), reticular pattern in 18 (29%), GGO in 15 (24%) and crazy paving pattern in 7 (11%). Most patients presented bilateral lesions (60/62, 97%) that were mainly detected in the posterior area of the lungs (59/62, 95%). Lesions were located frequently close to pleura (31/62, 50%) or lung fissures (28/62, 45%) and in only 3 cases (5%) the lesions were mainly parenchymal. The main lung lobes involved were the lower right lobe (35/62, 56%) and lower left lobe (14/62, 23%). In 8 patients (13%) the distribution of lesions was ubiquitous and in the remaining patients the main lung lobes involved were the upper right lobe (in 3 subjects, 5%) and middle lobe (in 2 subjects, 3%).
Other radiological lesions were air bubble sign (12/62, 19%, Figure 1e), brochiectasis (21/62, 34%), and vascular enlargement (55/62, 89%, Figure 1f). CT scan showed also pleural lesions. Out of the 62 patients pleural thickening (Figure 1a) was observed in 45 (73%) and pleural effusion in 11 (18%). Small mediastinal nodes (< 1 cm) were detected in all patients. Only a minority of patients presented other lung diseases. Chronic bronchitis was observed in 3 patients (5%), emphysema in 5 (8%) and lung nodule in 1 (2%). No patient presented pericardial effusion.

Table 1 - Radiological lesions detected at chest CT scan.

		Total patients 62
Parenchimal Lesions	Ground Glass Opacity	42 (67.7%)
	Reticular Pattern	50 (80.6%)
	Crazy Paving Pattern	15 (24.2%)
	Consolidation	33 (53.2%)
Pleural Lesions	Pleural Effusion	11 (17.7%)
	Pleural Thickening	45 (72.6%)
Other Radiological Findings	Air Bubble Sign	12 (19.4%)
	Bronchiectasis	21 (33.9%)
	Vascular Enlargment	55 (88.7%)
	Node <1 cm	62 (100%)
Other Lung Disease	Chronic Bronchitis	3 (4.8%)
	Emphysema	5 (8.1%)
	Lung Nodules	1 (1.6%)

Table 2 - Radiological lesions detected at chest CT scan grouped according to prevalent lesions and to lung distribution.

		Total patients 62
Main Radiological Lesion	Consolidation	22 (35.5%)
	Reticular Pattern	18 (29.0%)
	Ground Glass Opacity	15 (24,2%)
	Crazy Paving Pattern	7 (11.3%)
Distribution of Lesions	Bilateral	60 (96.8%)
	Posterior	59 (95.2%)
	Pleural	31 (50.0%)
	Lung Fissures	28 (45.2%)
	Parenchyma	3 (4.8%)
Main Lung Lobe Involved	Lower Right Lobe	35 (56.4%)
	Lower Left Lobe	14 (22.6%)
	Ubiquitous	8 (12.9%)
	Other Lobes	5 (8%)

Lung score
Lung score was used to quantify the severity of lung involvement. Median value of lung score was 7.0 (IQR 5.0-10.0) ranging from 1 to 22. As

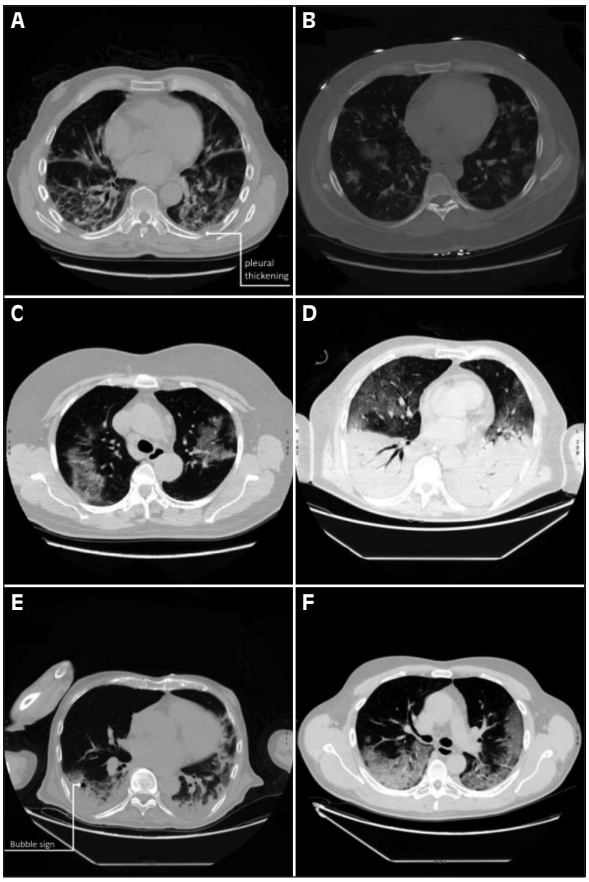

Figure 1 - Radiological lesions observed at chest CT scan of patients with COVID-19 pneumonia; 1a) reticular pattern associated with pleural thickening; 1b) GGOs; 1c) crazy paving; 1d) consolidation pattern with air bronchogram; 1e) consolidation with air bubble sign; 1f) crazy paving associated with vascular enlargement.

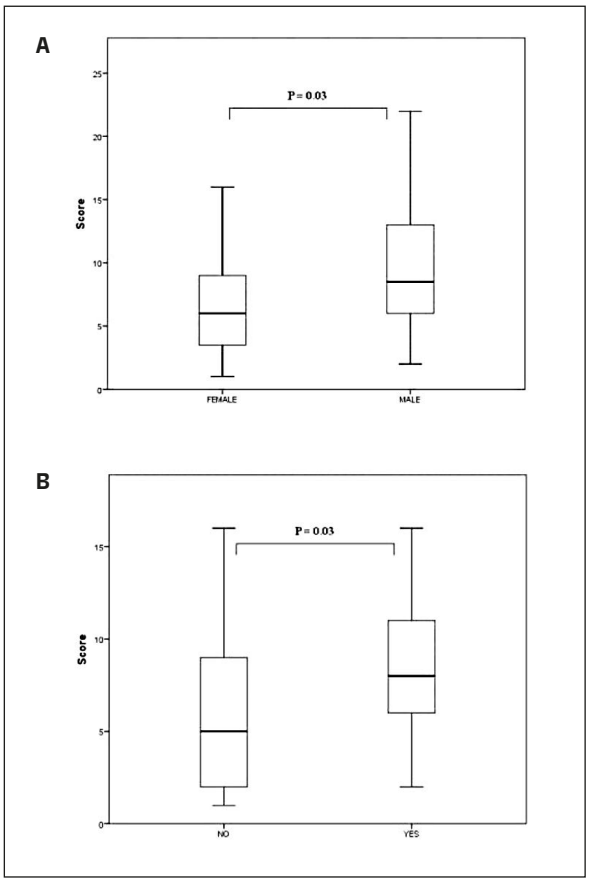

Figure 2 - Box-whiskers plot of CT lung score. Results are reported as median values (black lines), interquartile (25th-75th percentiles) range (boxes) and 10th-90th percentiles (whiskers). The statistical difference between groups was evaluated using the Mann-Whitney test. 2a) lung score was significantly higher in male than female; 2b) pleural thickening was significantly associated with a higher lung score.

shown in Figure 2a, lung score was significantly higher in male than female [respectively, median 8.5 (IQR 6.0-13.0) and median 6.0 (IQR 3.5-9.0); p=0.03)] Lung score was higher, even if not statistically significant in right lung compared to left lung [respectively, median 4.5 (IQR, 3.0-6.0) and median 3.0 (IQR 2.0-5.0); p=NS].

Pleural thickening was significantly associated with a higher lung score (Figure 2b). This score was significantly higher in subjects with pleural thickening than in those with no pleural lesion [respectively, median 8.0 (IQR 6.0-11.0) and median 5.0 (IQR 2.0-9.0; p=0.03).

DISCUSSION

Imaging plays a pivotal role in the diagnosis of COVID-19 and chest CT is strongly recommended in the early diagnosis, representing a tool to monitor the disease progression as assessed on the basis of a number of studies on the argument [21-31].

In the early phase, lesions are distributed along the sub-pleural areas or bronchi indicating the spreading of the lesions along the airway, starting with invasion of the alveolar epithelium of the cortical lung tissues, and extending gradually

from the periphery to the center. In this stage, single or multiple small GGOs infiltration, consolidation, and interstitial thickening could be seen. In the progressive phase, the number of lesions increases significantly and the GGOs and the consolidations coexisted and air bronchograms may appear [18].

In this study we have analyzed the CT scan of patients living in a small town where a significant outbreak of COVID-19 occurred. This town was immediately lockdown and considered red zone and all patients living in this area have been evaluated at our Hospital. We have reviewed all CT scan performed at the time of admission of the patients to the hospital and we have observed different stages of the disease. Only in few patients a single lung lesion was present, while in the majority of them we found mixed patterns that have classified according to the main lesion observed. The GGO pattern is believed to be the earliest lesion visible at CT scan and in the first radiological study of 21 patients by Chung et al. [32] was found in 57% of the patients. In other studies, this lesion has been described with a frequency ranging between 14% and 91% [18]. In our series we have observed the GCOs be the prevalent pattern in 67% of patients, in line to what reported in literature. The most frequent lesion observed in our series was the reticular pattern, which was detected in 50/62 patients (80%). Reticular pattern is another common lesion found at CT scan of patients with COVID-19 pneumonia [18]. Consolidation is another radiological sign frequently found and detected in 53 % of patients in our series. Consolidation is considered as an indicator of disease progression. It has been shown that lung involvement gradually increases to consolidation up to 2 weeks after disease onset [25].

In keeping with data reported in literature, in our series the lesions were bilateral in the majority of the patients (60/62, 96%) and located in the posterior area of the lungs (59/62, 95%). Although all lung segments can be affected, the lower lobes were mainly involved, with a significant preference for the lower right lobe compared to the left one (56% vs 22%). This may be due to the anatomic position of the right bronchus that is wider, shorter, and more vertical in direction than the left one and so in the early phase of the disease, the virus is more likely to invade the branches of the right inferior lobar bronchus.

We have evaluated the severity of lung involvement using a semi-quantitative scoring [24] already used to study patients recovering from severe acute respiratory syndrome (SARS). The total CT score could range from 0 (no involvement) to 25 (maximum involvement) and in this study, where chest CT scans were performed at the time of admission of patients, we observed values ranging from 1 to 22, with a median value of 7.0. These data indicate that we observed subjects with different stages of the disease, even if the majority presented a limited lung involvement at the time of the observation. Lung score was higher, even if not statistically significant in right lung compared to left lung (respectively median value 4.5 and 3.0) indicating a more severe involvement of the right site as discussed before. It was significantly higher in male than female (respectively median value 8.5 and 6.0, p value 0.03) and this data confirm the clinical observation of more severe disease and worst prognosis in male subjects.

Pleural thickening has been reported as radiological sign of COVID 19 pneumonia [31]. In this study we found that pleural thickening was observed in 45/62 (72 %) and was significantly associated with a higher lung score compared with subjects with no pleural thickening (respectively median value =8.0 and 5.0 Mann Whitney p value 0.03). These results suggest the possibility that this radiological sign may be associated with a more severe disease.

We have had the possibility to evaluate the radiological features of all cases of COVID-19 pneumonia occurred in a relatively small "red zone" at the time of admission to a single hospital and this represents the main strength of this work. On the other hand, now we do not know the clinical evolution of these patients and this represents the main limitation of the work

In conclusion we have reported the radiological findings observed at chest CT scan of patients with COVID 19 pneumonia living in a small town of Southern Italy, where a significant outbreak of the disease occurred. Reticular pattern, GGOs and consolidation were the main radiological signs with a prevalent bilateral extension and mostly located near pleura or lung fissures. Pleural thickening was also frequently observed and significantly associated with a higher lung score suggesting the possibility that may be associated with a more severe disease

Conflict of interest
None

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Le Infezioni in Medicina, Suppl. 1, 111-117, 2020

Cognitive load and performance of health care professionals in donning and doffing PPE before and after a simulation-based educational intervention and its implications during the COVID-19 pandemic for biosafety

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SUMMARY

Introduction: The Personal Protective Equipment (PPE) is essential to avoid the COVID-19 spread to health care workers. Its use can be difficult, posing a high risk of contamination, mainly during doffing, then with the risk of becoming infected.
Methods: We conducted a prospective before-and-after design that used clinical simulation as a research methodology in a clinical simulation center of Colombia. A simulation-based educational intervention with two cases related to COVID-19 was proposed in the emergency room and the intensive care unit. We conducted A workshop for donning and doffing of personal protective equipment (PPE) and a debriefing after the first case.
Results: In the pre-test, 100% of participants failed donning and doffing PPE, 98.4% were contaminated, only one-person did not contaminate out of. The mean cognitive load was high (7.43±0.9 points). In the post-test, 100% were successful in donning the PPE and 94.8% in doffing; only 9.8% were contaminated. The mean of the cognitive load was low (4.1±1.4 points), and the performance was high (7.9±1.1). Of the total, 73.8% of participants reported overload in the doffing. The most difficulties were in gown/overall, and N95 mask removal.
Discussion: The PPE donning and doffing is critical and may be changed significantly by active training. In responding to the current COVID-19 pandemic in 2020, activities of training in donning and doffing PPE would provide a means of training personnel, reducing the cognitive load and maybe the risk of contamination and infection of health care workers.

Keywords: SARS-CoV-2/COVID-19, knowledge, donning, doffing, PPE, Colombia, Latin America.

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INTRODUCTION

Emergent infectious diseases are a challenge to any health system [1]. The current pandemic of the Coronavirus Diseases 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), is the best recent example of this challenge[2, 3]. Originated in Wuhan, Hubei, China, in December 2019, this emerging infectious disease became a World Health Organization (WHO)-declared pandemic [4, 5]. Till May 13, 2020, this new infection has a worldwide distribution, with more than 4.34 million people infected and more than 296 thousand people dead [6, 7].

Many affected countries have had significant impairment among the healthcare workers. That has been the case of Italy, where up to March 15, 2020, reported COVID-19 in more than 22,000 people, with near 10% of them being health care workers [8]. That increased the health care crisis, the hospital collapse, and impose a high-stress load on the rest of health professionals [9].

In this setting, the Personal Protective Equipment (PPE) is essential to avoid the COVID-19 spread to health care workers [10]. However, its use can be difficult since it has multiple steps. Improper use of PPE poses a high risk of contamination, mainly during doffing [11]. Even more, complex procedures generate intrinsic and extrinsic cognitive load, which can increase the possibility of failure, and in this case, becoming infected [12].

The cognitive load theory assumes that working memory is limited, so complex tasks and experiences overflowing it. The cognitive load is divided into three types: intrinsic, extraneous, and germane. The intrinsic load refers to the difficulty of the task itself. Extraneous load is defined as the working memory that deals with solving situations that are not directly related to the task, and germane load is the intentional effort of working memory to do the task and trespassing the information from short term memory to long term memory [13, 14].

The recommendations of the Italian experience with COVID-19 emphasize the difficulty of managing this new disease, the need to maintain non-technical skills and to make use of checklists, cognitive aids, adequate PPE, and assisted donning and doffing [15].

For all these reasons, the present study aimed to assess the cognitive load and the performance of health care professionals in donning and doffing PPE before and after a simulation-based educational intervention.

METHODS

We conducted a prospective before-and-after design, that used clinical simulation as a research methodology, between February and March 2020, in a clinical simulation certified center of the Coffee-Triangle region, Colombia. A simulation-based educational intervention with two cases related to COVID-19 was proposed in the Emergency Room (ER) and the Intensive Care Unit. (ICU).

We conducted A workshop for donning and doffing of personal protective equipment (PPE) and a debriefing after the first case.

Scenarios

Scenario 1: A middle-aged man who returned from China five days before, develops cough, fever, and mental impairment. The intrinsic load was donning and doffing PPE; the extraneous load was her wife and the nurse's anxiety in the Emercency Room.

Scenario 2. A middle-aged man who returned from Italy, was in respiratory failure and septic shock and he needed orotracheal intubation and shock treatment. The intrinsic load was donning and doffing PPE; the extraneous load was in the ICU nurse´s mental model of critical ill management.

The scenarios were piloted, retested, and approved by the academic committee of the simulation center. The first scenario concluded if more than 80% of the team was contaminated, the second scenario with mechanical ventilation start.

The sample was constituted by physicians, nurses, and respiratory therapists from the emergency room and intensive care unit of third-level institutions of Armenia, Quindio, Colombia; recruitment was by open call.

Instruments

The 9-point Paas scale was applied to determine the cognitive load (1: very, very low - 9: very, very high) [16] Instruction in the concept of cognitive

load, and diligence of the scale was included within the workshop design. The U.S. Centers for Disease Control and Prevention, Atlanta (CDC) donning and doffing checklist was placed on the scale sheets where the participant place the step that seems most complex to them.

The checklist was be used for the placement and removal of the WHO personal protection equipment - CDC. It was be applied before and after the intervention. Each reviewer maked a general evaluation of performance with a scale like that of Paas of 9 items (1: Very, very bad - 9: very, very good) considering the use of equipment and maintenance of protection during simulated cases. The evaluators were instructed in the techniques of the appropriate use of personal protection equipment and completion of the checklist.

With the two scales, it was represented a graph in terms of efficiency in four quadrants: Efficient (high performance - Low cognitive load), Effective (high performance - high cognitive load), Inefficient (low performance - high cognitive load) and Dunning-Kruger effect (low performance and low cognitive load).

A Likert-type survey of 14 questions and five items (1: Totally disagree, 5, Totally agree) was sent in Google Forms® related to the perception of cognitive load during the simulated cases.

Statistical analysis was performed in SPSS 26, IBM®, the qualitative variables were summarized with proportions, and the quantitative variables with measures of central tendency and dispersion; statistical significance was considered only for $p<0.05$.

Ethics

The participation was voluntary. Health professionals were invited and informed about the characteristics and scope of the study. All the participants signed an informed consent form. This work did not represent any type of economic incentive for the participants or researchers. A committee of ethics in research approved the study.

RESULTS

A total of 61 healthcare workers participated in this study. Of them, 59% were women. The median age was 32 years old (interquartile range, IQR=26-43). Of them, 49.2% were physicians, 18% respiratory therapists, and 16.4% nurses. The median time of clinical experience among them was 8 years (IQR=2-17). Most participants were ICU (52.5%), and ER (26.2%) staff.

Of all participants, the 57.4% knew the PPE, and the 32.8% had previously used it.

In the pre-test, all the participants failed donning and doffing PPE; moreover, all were contaminated, except one that did not touch the infected patient and took distance from the simulated scenario. The mean cognitive load was high (7.43±0.9 points), and the performance very low (2.5±0.8).

In the post-test, 100% of participants were successful in donning the PPE and 94.8% in doffing; only 9.8% were contaminated. The mean of the cognitive load was low (4.1±1.4 points), and the performance was high (7.9±1.1). Of the total, 73.8% of participants reported an overload in the doffing. The most difficulties were in gown/overall, and N95 mask removal (Figures 1-2).

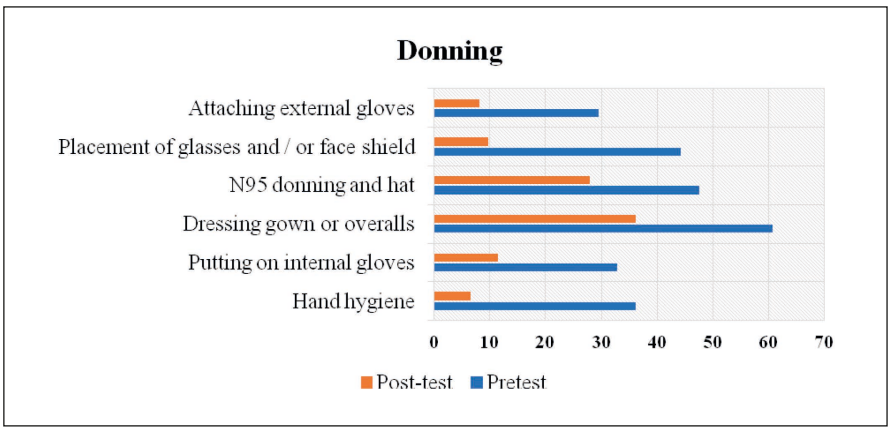

Figure 1 - Donning difficulties (%).

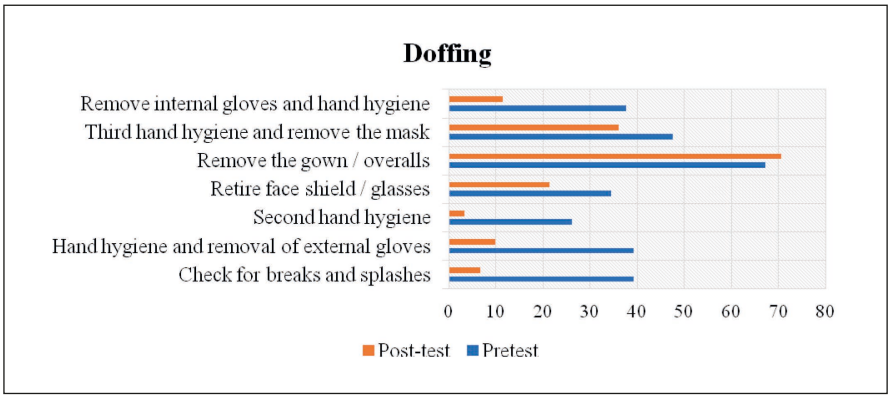

Figure 2 - Doffing difficulties (%).

No statistically significant difference was found in cognitive load or performance by gender, age, profession, or work area.

The relationship between performance–cognitive load in the pre-test, showed most participants in the inefficient quadrant; in the post-test, most participants were in the effective and efficient quadrants ($p<0.05$) (Figures 3-4).

The Likert scale showed reliability with Cronbach's alpha of 8.0. In the group of questions related to intrinsic load, there was a moderate-strong agreement on the overhead that a COVID-19 case management offers (40%-34.5% respectively), this includes donning and doffing. A moderate-strong agreement was also found in extrinsic loading that stress and the noise generated by the equipment increases the difficulty (38.2% and 38.2%, respectively). There was a strong agreement that stress and anxiety are factors that increase the difficulty of caring for the critically ill patient with COVID-19 and that doing assisted donning and doffing with the help of a verifier decreases the difficulty of the task, as well as individual and collective stress (54.5%) (Table 1).

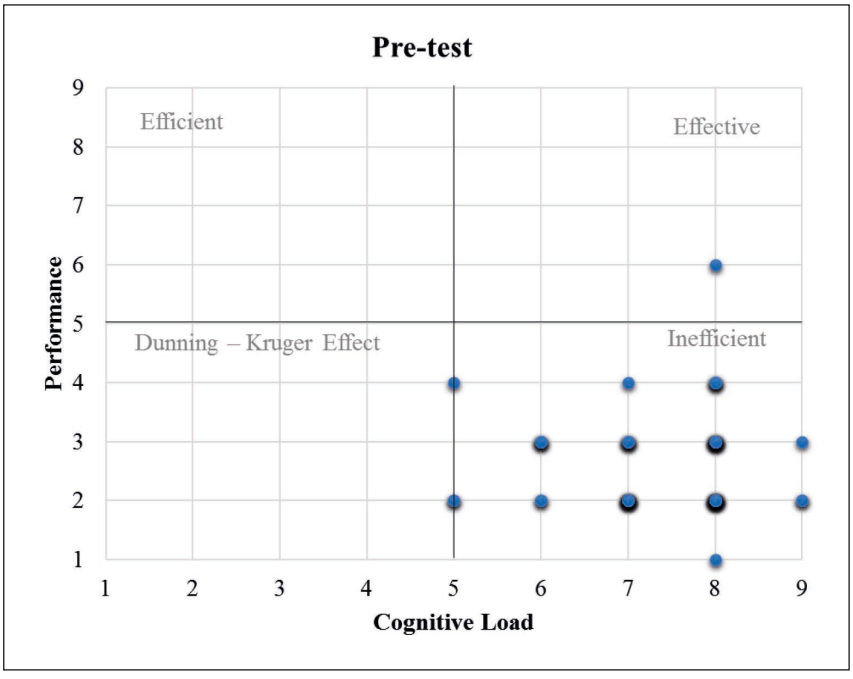

Figure 3 - Pre-test relationship between cognitive load and performance (points).

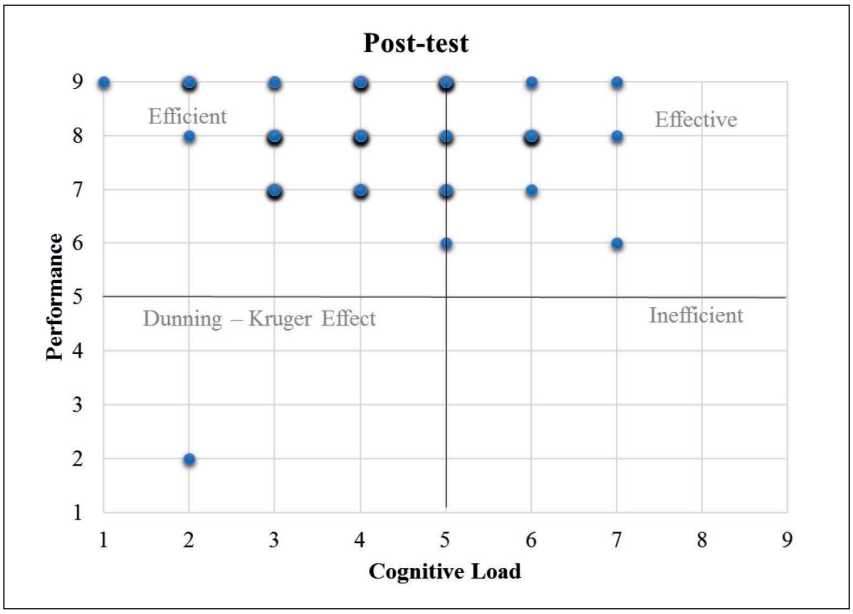

Figure 4 - Post-test relationship between cognitive load and performance (points).

Table 1 - Perception of donning and doffing difficulties.

	Steps	*Pretest %*	*Post-test %*	*p*
Donning	Hand hygiene	36.1	6.6	**0.0002**
	Putting on internal gloves	32.8	11.5	**0.0089**
	Dressing with gown or overalls	60.7	36.1	**0.0112**
	Putting on N95 respirator and hat	47.5	27.9	**0.0399**
	Placement of glasses and/or face shield	44.3	9.8	**<0.0001**
	Putting on external gloves	29.5	8.2	**0.0055**
Doffing	Breaks and splashes check	39.3	6.6	**<0.0001**
	Hand hygiene # 1 and removal of external gloves	39.3	9.8	**0.0004**
	Hand hygiene #2	26.2	3.3	**0.0009**
	Removal of face shield/glasses	34.4	21.3	0.1575
	Removal of gown/overalls	67.2	70.5	0.8450
	Hand hygiene #3 and removal of N95 respirator	47.5	36.1	0.2708
	Removal of internal gloves and hand hygiene #4	37.7	11.5	**0.0016**

In bold, p, significant differences between pretest and post-test.

DISCUSSION

Caring for critically ill patients is complex, due to the severity of the pathologies, the use of biotechnology and the emotional activation, which is related to increased cognitive load. One of the most critical issues with the pandemic caused by a biosafety threat agent, as is the SARS-CoV-2, is the appropriate use of PPE by health care workers during attending suspected or confirmed cases [17]. When working with infectious diseases with high risk of contagion , such as Ebola and COVID-19, the simple act of donning (putting on) and doffing (removing) PPE becomes a lifesaving

procedure not only for the medical staff but also for the thousands of people who depend on them [18]. However, with a higher level of protection, the level of complexity in donning and doffing is also higher [10], that can be associated with rise of the cognitive load.

During the current COVID-19 pandemic, the cognitive load seems critical for multiple clinical settings, such as the emergency room, surgical areas, or ICU, among others [19]. There, numerous biosafety breaches during donning and doffing may occur. The correct use of PPE is necessary to decrease the number of infected healthcare workers caring for patients with COVID-19 [20]. For these reasons, training on procedures and techniques, the surveillance, and retraining can help to control it, decrease cognitive load [12], and fear perception, increase the feeling of safety and performance in multidisciplinary teams [21, 22].

In Colombia, where we performed this study, the appropriate use of PPE is critical as more than 7,006 cases of COVID-19 have been confirmed till May 1, 2020, and 459 (6.5%) corresponded to healthcare workers, and 7 of them have died, included critical care and ER personnel [23].

Our study is the first to measure the impact of cognitive load on the performance of donning and doffing PPE in clinical simulation whit ER and ICU staff, who are the first line of care for critically ill patients with COVID-19, therefore, the most exposed and vulnerable to contagion. This study suggests that donning and doffing PPE is critical and may be changed significantly by active training with clinical simulation in terms of performance and decreased cognitive load.

In response to the current COVID-19 pandemic in 2020, activities of training, on-site, or even virtual, in donning and doffing PPE, would provide a means of training personnel, and in the case of virtual tools minimizing the amount of time and PPE used in training and ensuring social distancing. Then, also, the use of training videos to be tested to ensure completeness, accuracy, and clarity of actions have been proposed, however, training in a high-fidelity clinical simulation scenario, with the imposition of intrinsic and extrinsic cognitive load, is closer to real clinical practice, which cannot be achieved just by watching a video, in non-stress conditions [18].

Finally, it should always be remembered that there is a very high risk of contamination during doffing the PPE. Therefore another individual should watch the health care worker while donning and doffing the PPE and alert the person to any possibility of contamination [19].

CONCLUSIONS

Despite the knowledge of PPE, healthcare professionals do not carry out fully adequate donning and doffing. Donning and doffing of PPE generate high cognitive load, teams training in high fidelity clinical simulation minimizes the load and increases performance. We recommended assisted donning and doffing, strictly following the checklists.

Funding
None.

Conflict of Interest
None of the authors report conflict of interests.

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Clinical experience with therapeutic dose of Low-Molecular-Weight Heparin

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SUMMARY

A 71-year old gentleman with history of arterial hypertension treated with valsartan presented on was hospitalized at the Infectious Diseases Unit, University of Bologna (Italy) for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and received treatment with hydroxychloroquine 200mg bid (400 mg bid the first day), azithromycin 400 mg qd, thrombotic prophylaxis with enoxaparin 4000 UI qd and Venturi mask oxygen delivering FiO2 of 31%.
The case highlights the high frequency of coagulopathy in patients with moderate to severe cases of SARS-CoV-2 associated disease (COVID-19). After one week the patient significantly improved and the daily dose of enoxaparin was reduced and definitively discontinued four days later.
The case highlights the high frequency of coagulopathy in patients with moderate to severe cases of SARS-CoV-2 associated disease (COVID-19).
Considering the available information we believe that LMWH may represent a promising treatment for COVID-19 but further well-designed trials are needed to address these points.

Keywords: LMWH, COVID-19.

A 71-year old gentleman with history of arterial hypertension treated with valsartan presented on March 30th, 2020 to the Emergency Department of a tertiary teaching hospital in Italy for a 7-day course of fever, with a temperature of up to 38.9°C and chills. On examination, the temperature was 37.6°C, the blood pressure 150/90 mm Hg, the heart rate 124 beats per minute, the respiratory rate 23 breaths per minute, and the oxygen saturation 95% while the patient was breathing ambient air. Physical examination revealed diffuse coarse crackles at the lung bases, he was overweight (body mass index 27 kg/m^2, weight 79 kg) whereas the remainder was normal. Blood tests showed white blood cells count of 5670 per microliter with a lymphocyte count of 970 per microliter, creatinine 0.71 mg per deciliter, lactate dehydrogenase of 311 international units (IU) per liter, C-reactive protein of 6.7 mg per deciliter, normal D-Dimer value. Blood gas analysis performed while the patients was breathing in ambient air revealed partial pressures of oxygen (PaO2) of 61 mmHg carbon dioxide (PaCO2) 32 and the pH of 7.45 with a partial oxygen pressure to fraction of inspired oxygen (FiO2) (P/F ratio) of 290 mmHg. Chest high-resolution computed tomography (HRCT) showed presence of bilateral ground glass (Figure 1). The patient was then transferred to Infectious Disease Unit after performing a nasopharyngeal swab that resulted positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and received treatment with hydroxychloroquine 200mg bid (400 mg bid the first day), azithromycin 400 mg qd, thrombotic prophylaxis with enoxaparin 4000 UI qd and Venturi mask oxygen delivering FiO2 of 31%. After a period of defervescence, fever relapsed on April 2nd. He appeared dyspneic with a deterioration of blood gas analysis (P/F 264 mmHg). New blood test showed worsening of C-reactive protein (11.6 mg per deciliter), platelets count of

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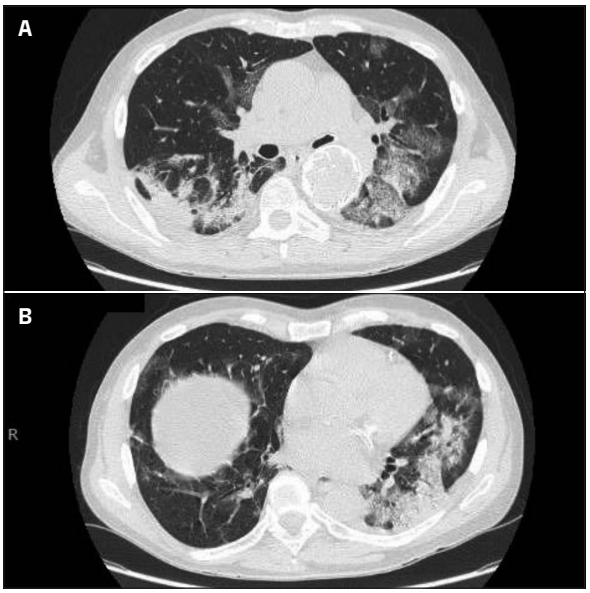

Figure 1 - High-resolution computed tomography performed on admission.

102.000 per microliter. D-Dimer was 2.1 mcg/mL (normal range 0.5 mcg/mL). The sequential organ failure assessment (SOFA) score was 4. A CT pulmonary angiography ruled out acute pulmonary but confirmed worsening of radiological findings [1,2]. Additionally, a venous doppler ultrasound excluded deep venous thrombosis (DVT). However, based on the risk of sepsis-induced coagulopathy (Table 1) he received treatment with daily 8000 IU of enoxaparin. Meanwhile the oxygen support was escalated to a helmet non-invasive ventilation with positive end expiratory pressure (PEEP) of 8 cm H2O with FI02 of 60% alternated to a reservoir mask delivering 15 liters per minute of oxygen. The patient was also screened for enrollment in a compassionate use clinical trial of tocilizumab but after improvement of clinical conditions on the next days the treatment was postponed. In fact, blood gas analysis performed on April 3rd and April 4th showed P/F ratios of 290 mmHg and 350 mmHg, respectively. On April 5th he was afebrile, supplemental oxygen was discontinued and he was discharged from hospital on April 9th. Daily dose of enoxaparin was reduced and definitively discontinued on April 13th. Additional naso-pharyngeal swabs were negative on April 11th and 12th. During a follow-up visit on May 5th he was afebrile, and all symptoms have

Table 1 - International Society Thrombosis and Hemostasis (ISTH) sepsis-induced coagulopathy (SIC) score.

Parameter	*Score*	*Range*
Platelets court (x mmc)	1	100.000-150.000
	2	<100.000
INR	1	1.2-1.4
	2	>1.4
SOFA score	1	1
	2	>2
Cut-off Value	>4	

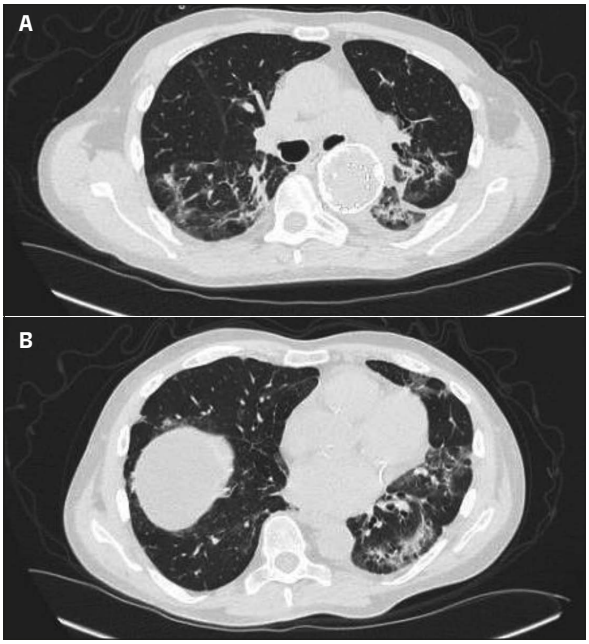

Figure 2 - High-resolution computed tomography performed 34 days after hospital admission.

resolved with the exception of cough, that was decreasing in severity. A new CT scan showed a significant improvement of infiltrates (Figure 2).

DISCUSSION

The case highlights the high frequency of coagulopathy in patients with moderate to severe cases of SARS-CoV-2 associated disease (COVID-19). A recent autoptic series of patients deceased for COVID-19 showed DVT in 7 out of 13 analyzed cases. In most of these cases, DVT was not clinical suspected and pulmonary embolism was deemed

the cause of death in 4 of them [3]. As a matter of fact that between 20 and 50% of hospitalized patients with COVID-19 show laboratory findings suggestive for coagulopathy. In addition, a D-Dimer value >1 µg/mL resulted an independent predictor of mortality (OR 18,42 95%IC 2.64-128.55; p=0·0033) in a recent Chinese study enrolling 191 hospitalized patients with COVID-19 [4]. Similar findings were observed in a study on critically-ill COVID 19 patients [5].

In a prospective study conducted in 4 French ICU units and including 150 patients with acute respiratory distress syndrome (ARDS) cause by SARS-CoV-2 managed with antithrombotic prophylaxis with heparin at daily dosage of 0.5 mg per kg major thrombotic complication were found in 64 cases. These were classified in pulmonary embolisms in 25 cases, ischemic strokes in 3 and DVT in 3 cases. Additionally, in 28 out of 29 patients (96.6%) receiving continuous renal replacement therapy experienced circuit clotting. Most patients (>95%) had elevated D-dimer and fibrinogen. Conversely, thrombocytopenia was detected only in 34% of patients and in about 80% normal levels of INR and aPTT were found. A comparison with non-COVID-19 ARDS patients (n = 145) confirmed that COVID-19 ARDS patients (n=77) developed significantly more thrombotic complications, mainly pulmonary embolisms (11.7 vs. 2.1%, p<0.008) and coagulation parameters significantly differed between the two groups [6].

In another Dutch case series authors were able to detect similar findings. Among 180 COVID-19 ICU patients, a composite outcome based on pulmonary embolism, DTV, ischemic stroke and myocardial infarction was reached by 31% of patients (95%CI 20-41). Additionally, a major thrombotic event was directly correlated with mortality (HR 5.4; 95%CI 2.4-12). Age (adjusted hazard ratio (aHR) 1.05/per year, 95%CI 1.004-1.01) and coagulopathy, defined as spontaneous prolongation of the prothrombin time >3 s or activated partial thromboplastin time >5 s (aHR 4.1, 95%CI 1.9-9.1), were independent predictors of thrombotic complications [7, 8].

These clinical features seen in clinical and autoptic case series may resemble that of macrophage activation syndrome and may explain the inconsistency between increment of D-Dimer, relatively low fibrinogen levels and normal or mild thrombocytopenia [9]. Therefore, during a severe infection of SARS-CoV-2 two different entities may co-exist or develop alternatively: the macrophage activation syndrome and disseminated intravascular coagulation. Anyhow, high D-Dimer levels should alert the clinician to a potential risk of coagulopathy and worse prognosis [10].

According to this background, low-molecular-weight heparin (LMWH) may assume a key therapeutic role for COVID-19 as also suggested by world health organization (WHO) guidelines [12].

Beyond its anticoagulant effects, there are several studies which have shown that heparin possesses various anti-inflammatory and immunomodulatory properties. The non-anticoagulant fraction of enoxaparin has also been shown in-vitro suppression of IL-6 and IL-8 release from human pulmonary epithelial cells. Moreover, *in vitro* and *in vivo* experimental studies have shown that human coronaviruses utilize heparin sulfate proteoglycans for attachment to target cells. Indeed, interaction between the SARS-CoV-2 Spike S1 protein receptor binding domain (SARS-CoV-2 S1 RBD) and heparin has been recently showed suggesting a role for heparin in the therapeutic armamentarium against COVID-19 [13]. Finally, retrospective studies showed a reduced 28-day mortality among COVID-19 patients with higher D-dimer or sepsis-induced coagulopathy (SIC) score treated with heparin treatment compared with no treatment [14].

To date, the only study that evaluated the potential benefit of a treatment based on LMWH is a retrospective cohort study on 449 patients with severe COVD-19 of which 99 received LMWH a Although overall mortality was similar in the whole cohort, a subgroup analysis showed a significant reduction of mortality among participants with SIC score ≥4 (40.0% vs 64.2%, *P*=.029), or among those with D-dimer 6-fold higher than normal range or above (32.8% vs 52.4%, *P* = .017) [15]. Despite these results several aspects of LMWH should be further clarified. First, it is not clear whether all patients could really benefit form treatment with LMWH, or it should be reserved to those with suspected coagulopathy. Second, the dosage of LMWH should be clearly defined. In fact, in most of the aforementioned studies major thrombotic events occurred even during standard prophylaxis with LMWH.

Considering this areas of uncertainty, guidelines of major scientific societies (American Society of Hematology, International Society of Haemostasis and Thrombosis) do not recommend treatment with >0.5 mg/kg qd unless a diagnosis of major thrombotic event is made [16, 17].
Considering the available information we believe that LMWH may represent a promising treatment for COVID-19 but further well-designed trials are needed to address these points.

Conflict of interest
None

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Vitamin C (ovi) D; An unexplored option!

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To the Editor,

The world is facing one of the biggest challenges in the form of COVID-19 infection which is now a pandemic. A high infectivity, no effective proven prophylaxis till date and lack of therapeutic options makes the control of COVID-19 a difficult task [1, 2]. Social distancing and hand hygiene are probably the best weapons at present. The role of vitamins in prophylaxis as well as in treatment of COVID-19 does not have a strong evidence yet. However, there are plausible mechanisms for benefit of various vitamins.

Vitamin C is an important anti-oxidant and enzymatic co-factor, and has been studied in sepsis and severe acute respiratory failure in high dose infusions (dose 50 mg/kg QID for 4 days) [3]. Mechanisms postulated for Vitamin C in sepsis include inhibition of endothelial cell proliferation and apoptosis, smooth muscle-mediated vasodilation, and endothelial barrier permeability [4]. However, recent evidence has shown no added benefit of Vitamin C to steroid in septic shock [5]. Vitamin C can have a promising role in pulmonary edema in COVID-19 by decreasing cytokine surge, neutrophil activation, epithelial damage and vascular injury, thereby improving ventilator free days and mortality [6]. Even as a prophylactic drug, Vitamin C has also been shown to reduce viral symptoms by about 85% in viral influenza [7]. It may have a similar role in COVID-19. Ongoing trials for Vitamin C use as prophylaxis and treatment in COVID-19 have been summarized in Table 1.

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Vitamin D deficiency has been documented to be associated with increased risk of viral infections. Replacement and correction of Vitamin D enhance cathelicidin and anti-inflammatory cytokines and suppresses T-helper 1 response thereby suppressing IL-2 and cytokine storm [8]. A trial in children has found reduction in risk whereas one trial in infants has found reduction in viral load in influenza by Vitamin D supplementation [9, 10]. A recent review has revealed that trials not finding the benefit of Vitamin D in influenza can be due to no baseline Vitamin D values and high vaccine coverage against influenza. It concludes that in view of a wide range of mechanisms, Vitamin D may have a prophylactic role in preventing viral infections like influenza [11]. Similarly, in literature, Vitamin D has been studied in enveloped viruses including dengue, Respiratory syncytial virus, Hepatitis C, H9N2 Influenza [8]. DPP-4/CD-26, a virulence marker for COVID-19, is an adhesion molecule for viral entry into host cells. Replacement of Vitamin D can suppress this molecule besides also inhibiting the pro-inflammatory cytokines. Prioritized supplementation of inpatients, nursing home residents, older adults, diabetes mellitus or immunocompromised and healthcare workers can be advocated if benefit can be elicited from current studies which are underway for COVID-19 (Table 1). Vitamin D also has an immunomodulatory action also which can potentially decrease lung injury in COVID-19 [12, 13].

Folic acid, a cheap and easily available oral vitamin, has been found to indirectly inhibit furin. Furin, an enzyme of the convertase family, causes activation of coronavirus by sequence-specific cleavage of the spike protein on the virus surface into S1 and S2 domains thereby helping it to enter the host cell [14]. Thus, there is a potential for its

use in prevention or treatment of early stages of respiratory disease.
To summarize, there is a huge potential for use of vitamins in aiding in prophylaxis and management of COVID-19 patients; in view of the plausible target pathophysiology, there is no economic constraints, easy availability and safety.

Table 1 - Summary of ongoing clinical trials on utility of vitamin in COVID-19.

Number	*Title*	*Interventions*	*Age*	*Allocation*	*Masking*	*Primary Outcome*	*Start Date*	*Expected Completion Date*	*Location*
1. NCT04334005	Effect of Vitamin D Administration on Prevention and Treatment of Mild Forms of Suspected Covid-19	25000 UI of Vitamin D supplement in addition to the standard treatment.	40 to 70 years	Randomized	Double (Investigator, Outcomes Assessor)	Composite of cumulative death (i.e. mortality) for all causes and for specific causes. [Time Frame: Through study completion, an average of 10 weeks]	April 10, 2020	June 30, 2020	Granada, Spain
2. NCT04264533	Vitamin C Infusion for the Treatment of Severe 2019-nCoV Infected Pneumonia: a Prospective Randomized Clinical Trial	12g Vitamin C+ sterile water for injection; total volume: 50 ml. 12 ml/h; infusion pumpq 12 h	≥18 years old	Randomized	Triple (Participant, Care Provider, Outcomes Assessor)	Ventilation-free days [Time Frame: on the day 28 after enrollment]	February 14, 2020	September 30, 2020	Wuhan, China
3. NCT04335084	An Open Label Phase II Pilot Study of Hydroxychloroquine, Vitamin C, Vitamin D, and Zinc for the Prevention of COVID-19 Infection	Drug: Hydroxychloroquine Dietary Supplement: Vitamin C Dietary Supplement: Vitamin D Dietary Supplement: Zinc	≥18 years old	Single Group Assignment	None (Open Label)	Prevention of COVID-19 measured by negative testing with RT-PCR [Time Frame: 24 weeks]	April 2020	July 2021	California United States,
4. NCT04323514	Use of Ascorbic Acid in Patients with COVID 19	10 gr of Vitamin C intravenously in addition to conventional therapy	Child, Adult, Older Adult	Single Group Assignment	None (Open Label)	In-hospital mortality [Time Frame: 72 hours]	March 13, 2020	March 13, 2021	Palermo, Italy
5. NCT04334512	A Study of Quintuple Therapy to Treat COVID-19 Infection (HAZCpaC)	Drug: Hydroxychloroquine Drug: Azithromycin Dietary Supplement: Vitamin C Dietary Supplement: Vitwamin D Dietary Supplement: Zinc	≥18 years old	Single Group Assignment	None (Open Label)	Successful treatment as determined by Negative Test and resolution of symptoms [Time Frame: 24 weeks] Safety of Quintuple Therapy [Time Frame: 24 weeks]	April 2020	April 2021	California, United States
6. NCT04326725	Proflaxis for Healthcare Professionals Using Hydroxychloroquine Plus Vitamin Combining Vitamins C, D and Zinc During COVID-19 Pandemia: An Observational Study	Drug: hydroxychloroquine (plaquenil) 200mg single dose repeated every three weeks plus Vitamin C including zinc once a day were included in the study	20 Years to 90 Years	Case-Control	NA	Protection against COVID-19 [Time Frame: 4 months]	March 20, 2020	September 1, 2020	Istanbul, Turkey

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Number	Title	Interventions	Age	Allocation	Masking	Primary Outcome	Start Date	Expected Completion Date	Location
7. NCT04323228	Anti-inflammatory/ Antioxidant Oral Nutrition Supplementation on the Cytokine Storm and Progression of COVID-19: A Randomized Controlled Trial	The intervention groups will receive daily oral nutrition supplement (ONS) enriched in eicosapentaenoic acid, gamma-linolenic acid and antioxidants. The composition of one can (8 fl oz) of the intervention-ONS includes: 14.8 g protein, 22.2 g fat, 25 g carbohydrate, 355 kcal, 1.1 g EPA, 450 mg DHA, 950 mg GLA, 2840 IU Vitamin A as 1.2 mg β-carotene, 205 mg Vitamin C, 75 IU Vitamin E, 18 ug Selenium, and 5.7 mg Zinc	18 Years to 65 Years	Randomized	Double (Participant, Care Provider)	Primary Outcome Measures: Change from baseline score of Nutrition risk screening-2002 (NRS-2002) at end of the trial] Change from baseline Serum ferritin level at end of the trial] Change from baseline serum Interleukin-6 concentration at end of the trial Change from baseline serum C-reactive protein concentration at end of the trial Change from baseline serum Tumor necrosis factor-β concentration at end of the trial Change from baseline serum monocyte chemoattractant protein 1 (MCP-1) at end of the trial [Time Frame: up to 3 months]	April 10, 2020	October 30, 2020	Riyadh, Saudi Arabia
8. NCT03680274	Lessening Organ Dysfunction with Vitamin C (LOVIT)	Vitamin C: 50 mg/ kg every 6 hours for 96 hours.	≥18 years old	Randomized	Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor)	Number of deceased participants or with persistent organ dysfunction [Time Frame: Both assessed at 28 days]	November 8, 2018	December 31, 2021	Quebec, Canada

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Conflicts of interest
There are no conflicts of interest.

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Infection prevention and control in blood purification centers during the COVID-19 epidemic: a single institution experience from Zhejiang, China

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To the Editor,

The 2019 novel coronavirus disease (COVID-19), which is distinct from Severe Acute Respiratory Syndrome - Coronavirus (SARS-CoV), Middle East Respiratory Syndrome - Coronavirus (MERS-CoV) and other influenza viruses, is a global pandemic of respiratory disease caused by coronavirus-2 (SARS-CoV-2) [1]. As of April 10, 2020, 1.619.495 cases have been cumulatively confirmed and 97.200 deaths have been documented worldwide [2]. About 20% of patients with COVID-19 develop a severe or critical disease with hypoxia or respiratory failure, and its overall case-fatality rate is approximately 2.3% [3]. Older age and co-morbidities, such as arrhythmia, hypertension and diabetes, are the critical risk factors for death of severe COVID-19 patients [4, 5]. Unlike the massive outbreak of COVID-19 in Wuhan City, a total of 1.268 patients with COVID-19 were diagnosed in Zhejiang Province from January to March 2020, including 24 cases in our hospital.

During the COVID-19 epidemic in Zhejiang Province in Eastern China, all elective surgeries were postponed and the outpatient clinics for stomatology and otolaryngology were temporarily closed, but the blood purification center remained functional in our hospital. Hemodialysis patients suffer from a primary disease such as chronic kidney diseases, systemic lupus erythematosus (SLE), diabetes, etc., as well as harbor a higher risk for cardiovascular diseases and infections [6, 7]. Hence, prevention and control of COVID-19 is especially important in the blood purification center. Herein, we summarized the experience in prevention and control of COVID-19 infection in our blood purification center, which can provide guidance for the prevention and practical work of blood purification centers.

Medical staff were trained in the prevention and control of COVID-19 infection through video conferencing using the Dingding software. For personal protection, medical staff were required to wear disposable caps, face shields or protective goggles, surgical masks, isolation gowns, medical protective gloves and disposable shoe covers while dealing with common hemodialysis patients. Medical protective clothing and medical protective masks were necessary besides the above protective equipments for close contact with suspected or confirmed COVID-19 patients. In addition, hospital staff were forbidden to have meals in the canteen. Meals ordered on mobile phone were sent to the hemodialysis room by the canteen staff to avoid crowding.

In addition to routine plasma disinfections three times a day, ultraviolet disinfection for 30 minutes was conducted at the end of hemodialysis work at our hemodialysis center. Bed units and surfaces of hemodialysis machines were disinfected using chlorine-containing disinfectant instead of dou-

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ble-chain quaternary ammonium salt disinfectant to efficiently kill the SARS-CoV-2. A designated nurse supervised and recorded the disinfection process, including disinfection of the floor, door knobs, etc.

Three levels of control for COVID-19 were conducted for hemodialysis patients. At the first level, patients and their caregivers had their body temperatures measured by thermometer guns, and their health QR codes on mobile app Alipay or WeChat were checked (the green code meant little chance of having been infected with COVID-19, the yellow and red codes meant travel to virus-hit areas in the past two weeks or a high risk of COVID-19). Only people with normal body temperature and green code were allowed to enter the blood purification center and other general wards. Otherwise, they were advised to go to a fever clinic through the special channel. At the second level, a special channel was set up for hemodialysis patients and their caregivers (one patient was allowed only one caregiver) and the latter must wait at a fixed place outside the blood purification center. At the third level, hemodialysis patients went into the buffer chamber, had their body temperatures measured again, implemented hand hygiene, removed the coat and wore surgical masks, disposable shoe covers and isolation gowns. Nurses and volunteers (medical staff volunteers from other departments in the hospital) assisted patients in accomplishing the above tasks and led them into the hemodialysis room. If a patient had fever, sore throat, cough, or other respiratory symptoms, he was sent to the isolation ward for hemodialysis for the next 14 days. Subsequently, chest CT and nucleic acid test were performed to exclude COVID-19.

In summary, under the strict management for prevention and control of COVID-19, no patient or medical staff suffered from COVID-19 at our blood purification center during the epidemic, except a suspected case who was excluded in the end.

Conflicts of interest

All authors declare no conflicts of interest related to this work.

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